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EYPS-01

Synthetic Peptides restore the Epithelial Sodium Channel Function in Pseudohypoaldosteronism Type 1B Mutants

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Pseudohypoaldosteronism type 1B (PHA1B) is a rare, life-threatening, salt-wasting syndrome, which presents in the first days of life with failure to thrive, vomiting, dehydration, low blood pressure; hyperkalemia, hyponatremia and metabolic acidosis suggest hypoaldosteronism, but plasma aldosterone and renin activity are high. This end-organ resistance to aldosterone is caused by various loss-of-function mutations in the amiloride-sensitive epithelial sodium channel (ENaC). Synthetic peptides (e.g. solnatide) mimicking the lectin-like domain (TIP) of the human tumor necrosis factor (TNF) has been shown to activate current through wildtype (WT) ENaC and ENaC carrying point mutations associated with PHA1B. In addition other types of mutations, which were described in PHA1B patients, were created *in vitro* and expressed heterologously in HEK-293 cells. The channel activity was studied using Patch-clamp technique and the expression using Western blots of biotinylated surface proteins. The membrane abundance varied considerably among the mutants; some were higher, others lower than WT, but all observed mutant ENaCs were at least present at the cell membrane, in our expression system. Nonetheless the current density of all tested mutations was decreased compared to WT likely to be the cause of the disease. Regardless of the type of mutation the peptides were able to restore the channel function of mutant ENaC to current density levels of WT or even higher. Our findings suggest that the synthetic peptides solnatide and its congener represent a promising new strategy to treat PHA1B, which hitherto has been treated only symptomatically.

EYPS-02

Different modulation of ion currents in hippocampal pyramidal neurons and NG108-15 cell line by delta opioid receptor antagonist naltrindole

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Naltrindole (NTI) represents a highly potent, selective non-peptide antagonist of delta opioid receptors (DOR). Part of its effects on neuronal excitability may be mediated by an effect on activity of voltage dependent ion currents. We compared effect of an acute application of NTI on ion currents in rat hippocampal pyramidal neurons, which express all subtypes of opioid receptors, and differentiated NG108-15 cells, which predominantly express DOR.

Hippocampal neurons were isolated from newborn Wistar rats and maintained in a primary culture up to two weeks. Measurements were done at Day 9-12 *in vitro*. Differentiation of NG108-15 cells cultured in a serum-free Dulbeccos modified Eagles medium was induced by an addition of 1 mM dbcAMP, and 1x N2 supplement for 7-11 days. Ion currents were measured by a whole-cell patch clamp. Concentration of NTI was 10 μ M.

NTI significantly inhibited sodium current in hippocampal neurons but did not affect it in NG108-15 cells. Similarly, calcium currents were inhibited in hippocampal neurons but not in NG108-15 cells. In contrast, NTI significantly inhibited both transient and sustained potassium current in NG108-15 cells.

In hippocampal neurons, inhibition of potassium currents was less prominent and was not statistically significant. Inhibition of individual ion currents developed slowly allowing us to presume that it was mediated by activation of intracellular signaling pathway rather than by direct interaction of NT1 with the channel protein.

In conclusion, inhibitory effects of NT1 in hippocampal neurons and in NG108-15 cells are complementary suggesting that they may be mediated through different signaling pathways including an interaction with different subtypes of opioid receptors.

EYPS-03

Investigation of the extracellular Ca^{2+} entry in mouse pancreatic ductal cells

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Introduction: Acute pancreatitis (AP) is the most common inflammatory disorder in the gastrointestinal tract with an overall mortality of 20-30% in severe cases. The treatment of AP is not resolved yet, urging the identification of novel drug targets. Toxic cellular Ca^{2+} overload was highlighted as a key event in pancreatic acinar and ductal cells during the pathogenesis of AP. In addition, the inhibition of Orai1 in pancreatic acinar cells markedly decreased the Ca^{2+} toxicity and the severity of AP. However, We have no information regarding the role of Orai1 in pancreatic ductal physiology or pathophysiology.

Methods: Wild type FVB/N mice were used for the isolation of pancreatic ductal fragments. The intracellular pH and Ca^{2+} level of the pancreatic ductal cells (PDC) were measured by microfluorimetry. The effect of selective Orai1 inhibitors provided by CalciMedica was evaluated.

Results: The tested compounds dose-dependently inhibited Ca^{2+} influx during the carbachol induced Ca^{2+} signal in PDC. Inhibition was complete at a concentration of 10 μM (CM-B: 99.87%, CM-C: 95.29%). Next, endoplasmic reticulum Ca^{2+} stores were depleted with cyclopiazonic acid and the inhibition of store-operated Ca^{2+} entry (SOCE) was investigated after the re-addition of extracellular Ca^{2+} . Under these conditions CM-B and CM-C significantly, but not completely, decreased SOCE in PDC (55.96% and 55.03% respectively). The removal of extracellular Na^{+} to abolish activity of the $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger had no effect on the inhibition of SOCE by CM-B or CM-C. We also showed that the inhibition of Orai1 has no effect on the basal secretion of HCO_3^{-} by PDC, which is the main physiological function of these cells.

Conclusions: We showed that Orai1 has a significant role in the Ca^{2+} signaling of PDC. In the next step we will evaluate the pathophysiological relevance of the channel.

EYPS-04

BLOCKAGE OF EXOSOME GENERATION REDUCES TAU PROTEIN CAUSED NEURONAL LOSS AND MICROGLIA PROLIFERATION

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Progressive neurodegeneration is associated with tau modifications leading to neurotoxicity and brain cell death. In addition, various abnormal tau monomers and aggregates accumulate in interstitial and cerebrospinal fluids. Extracellular tau might be toxic to brain cells. There is a growing evidence that exosomes may contribute to cell-to-cell transmission of pathogenic tau protein. Exosome uptake might be regulated by various factors such as protein kinase C (PKC) that controls sphingomyelinase activity involved in exosome generation. The aim of this study was to investigate toxic effect of extracellular

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EYPS

tau on brain cell culture and whether exosome and PKC inhibitors may suppress neurotoxicity of exogenous tau (2N4R isoform). Cultures of rat cerebellar granule cells (CGC) were treated with various type of monomeric or aggregated recombinant 2N4R tau plus/minus 1 μM Ro31-8220 (PKC inhibitor) and 13 μM GW4869 (exosome inhibitor) for 48 hours. Neuronal and microglial cells densities and viability were evaluated by fluorescence microscopy. Our results showed that tau protein 2N4R isoform at micromolar concentration slightly decreased viability of neurons, but caused significant neuronal loss (~70 %) and microglia proliferation (~200 %) in CGC. Neuronal loss and microglia proliferation were completely prevented by GW4869 that inhibits neutral sphingomyelinase. PKC inhibitor also blocked tau neurotoxicity and microglial proliferation in cell culture. 2N4R had similar effects independently of the preparation method. Our data suggest that neutral sphingomyelinase and PKC activation is required for 2N4R induced neuron necrosis, loss and microglia proliferation in CGC.

EYPS-05

TRPA1 and TRPV1 photosensitization by 7-dehydrocholesterol – connections to the Smith-Lemli-Opitz syndrome

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Question: Low activity of the 7-Dehydrocholesterol (7DHC) reducing enzyme by means of a loss of function mutation can lead to a 1000-fold increase in 7DHC plasma levels. This is the pathophysiological basis of the autosomal recessive Smith-Lemli-Opitz syndrome, characterized by congenital abnormalities, retardation, and exceptional hypersensitivity when exposed to sunlight. Focusing on the TRPA1 and TRPV1 ion channels, we investigated how elevated 7DHC levels cause a sensitivity to UVA light.

Methods: Calcium-based microfluorimetric assessment of the photosensitisation caused by 7DHC treatment and UVA light exposure was performed on transfected HEK293t cells and mouse DRG neurons. 7DHC-generated currents were recorded using the whole-cell patch clamp technique on transfected HEK293t cells. CGRP release from the isolated mouse trachea served as an index of 7DHC- and light-induced neuronal activation.

Results: Human TRPA1-transfected HEK293t cells exhibited significant calcium transients upon 7DHC and UVA exposure, as opposed to untransfected cells. 7DHC and UVA exposure alone activated cells, but the combination had a supra-additive effect. TRPV1 transfected cells lead to lower responses compared to TRPA1 in identical experiments. Considerable photosensitization occurred after 1-15 hours of 7DHC preexposure in cells expressing TRPA1 or TRPV1. TRPA1 antagonist A-967079 and TRPV1 antagonist BCTC significantly reduced 7DHC and UVA-induced responses. DRG neurons exposed to 7DHC for 4 hours yielded similar results and confirmatory evidence was obtained from the respective TRP channel knockout mice. Tissue CGRP release was also significantly increased due to 7DHC and light.

Conclusions: TRPA1 and, to a lesser extent, TRPV1 mediate 7DHC induced photosensitization to UVA light exposure.

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EYPS-06

Advanced-level analysis of spiking EEG activity potentiated by high dietary methionine: contribution of purinergic signaling

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Questions: Hypersynchronous firing of neuronal networks is a key feature of epilepsy manifested as spiking EEG activity. High methionine diet results in hyperhomocysteinemia and elevated susceptibility to epileptogenesis. Purinergic signaling has been recognized to be involved in a wide range of activities of the nervous system. The aim of this study was to investigate the effects of high dietary methionine on spiking EEG activity and involvement of ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) activity in the rat brain.

Methods: Male Wistar rats were used in this study. They were fed during 30 days either with standard (control group) or diet containing high level of methionine (7.7 g/kg, experimental group). EEG activity was recorded for 90 min upon homocysteine thiolactone (HCT) convulsive trigger dose at 512 Hz sampling rate. Number and duration of SWD were analyzed in EEG traces, by using visual inspection and fast-Fourier transform, continuous and discrete wavelet transform. Rats were decapitated and E-NTPDase activity was determined spectrophotometrically in synaptic plasma membranes isolated from the brain.

Results: Experimental and control group showed different patterns of brain activity. Rats on high dietary methionine had an increased number of SWD, as well as prolongation of individual SWD elicited by HCT compared to control group. Further analysis showed that the activity of E-NTPDase, an enzyme of purinergic signaling pathways, was higher in the brains of the rats from experimental comparing to control group.

Conclusions: These results suggest that ectonucleotidase pathway may play a contributory role in potentiation of spiking EEG activity by high dietary methionine/ hyperhomocysteinemia related to epileptogenesis.

EYPS-07

Platelet PI3K in Acute Lung Injury

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Platelets are anucleated blood cells, which are critical for hemostasis. However, increasing evidence indicates that activated platelets influence immune responses, as they interact with leukocytes. This interaction promotes leukocyte trafficking to inflammatory sites, e.g. induced by acute lung injury (ALI). As phosphatidylinositol 3-kinase (PI3K) is central in conducting platelet activation and neutrophils are important in ALI, we wanted to elucidate the effect of platelet PI3K on ALI.

Hence, we bred mice with a platelet-specific p85 α deficiency, a regulatory subunit of the PI3K. Platelet activation was examined by detecting surface activation markers via flow cytometry and platelet aggregation via light transmission aggregometry. To induce ALI we treated mice intra-tracheally with hydrochloric acid and analyzed pulmonary leukocyte influx by flow cytometry.

We found that platelets lacking p85 α had reduced CD62P and CD40L surface expression compared to wild-type platelets in response to AY-NH2 (PAR4 agonist) and convulxin (GPIV agonist). Further, platelet aggregation upon thrombin stimulation was diminished. Challenging platelet p85 α -deficient mice and wild-type littermates with ALI, we observed attenuated pulmonary leukocyte accumulation. Moreover p85 α deficiency provoked impaired platelet-neutrophil/ monocyte aggregate formation.

Our results indicate that PI3K is important for platelet activation. Lack of platelet PI3K reduced platelet-leukocyte interactions and therefore likely diminished leukocyte extravasation, may leading to ameliorated symptoms in ALI. Thus, platelet PI3K is not only crucially involved in platelet-mediated hemostasis, it is also essential for their immunological functions.

EYPS-08

The role of Aflibercept and Ranibizumab against oxidative stress in Retinal Pigment epithelium cells (ARPE-19). Mechanisms related to nitric oxide release and apoptosis, autophagy modulation.

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The Age-Related Macular Degeneration is the leading cause of severe and irreversible loss of vision in developed countries. In the mechanisms of action of the anti-VEGF agents the involvement of nitric oxide (NO), and the modulation of mitochondria function and of apoptosis/ autophagy have not been examined, yet. In the present study, we planned to evaluate the effects of Aflibercept and Ranibizumab in human retinal pigment epithelium cells (RPE; ARPE-19) cultured in physiologic/peroxidative conditions on cell viability/proliferation and mitochondria function. Mechanisms related to NO release, apoptosis/autophagy, and Akt and ERK1/2 expression/activation were examined, as well. RPE, either or not subjected to peroxidation were administrated Ranibizumab (0.025-0.20 mg/ml) and Aflibercept (0.025-0.50 mg/ml) for 1, 5 and 30 min. The modulation of NO release, cell viability/proliferation, oxidant/antioxidant system, mitochondrial membrane potential were examined by specific dyes. eNOS/iNOS, markers of apoptosis/autophagy and kinases activation/expression were analyzed by Western Blot. In RPE, Aflibercept and Ranibizumab increased NO release in a dose and time-dependent way in physiologic condition. In the presence of hydrogen peroxide both the anti-VEGF agents prevented the collapse of mitochondrial membrane potential and cell viability. In the presence of the NOS inhibitor, the effects on NO release were reduced or abolished. Those findings were accompanied by modulation of apoptosis/autophagy and of above kinases activation. The present results have shown protective effects elicited by Aflibercept and Ranibizumab on RPE undergone peroxidation through the modulation of NO release, apoptosis and autophagy.

EYPS-09

Influence of compression aids on baroreflex function in patients with cervical spinal cord injury

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Patients after cervical spinal cord injury (cSCI) often suffer from orthostatic hypotension (OH). Although vagal cardiac chronotropic effect is preserved in cSCI, baroreflex vascular tone regulation is impaired due to interrupted sympathetic pathways below the cSCI level. Patients usually use compression aids (CA, e.g. stockings, abdominal corset) to mitigate blood pressure (BP) drop during orthostasis. This study aimed to assess the influence of CA on baroreflex function in cSCI patients during orthostasis.

BP was continuously recorded in 9 cSCI patients during passive orthostasis without and with CA. Beat-to-beat systolic blood pressure (SBP) and inter-beat intervals (IBI) sequences were obtained from continuous non-invasive BP recording. Pulse pressure (PP) was evaluated as a mean of beat-to-beat differences between SBP and diastolic pressures. Closed loop of SBP IBI interaction was mathematically opened by bivariate autoregressive model; causal coherence (a measure of IBI and SBP synchronization) and gain estimating baroreflex sensitivity (BRS) were calculated in baroreflex direction (from SBP to IBI).

When CA were applied, coherence and PP were significantly increased as compared to orthostasis without CA ($p < 0.05$). CA application was also associated with an increase of BRS (borderline significant; $p = 0.059$). Patients reported less OH symptoms when wearing CA.

Use of CA increased venous return and consequently stroke volume expressed as an increase of PP in this study. This probably prevented the baroreflex sensitivity decrease during orthostasis. We suppose that positive influence of CA on blood pressure regulation during orthostasis weakened OH symptoms in cSCI.

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TEACHING SYMPOSIUM

TS-01

Physiology in a classical curriculum in the 21st century: Flexner 2.0

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The Flexner Report of 1910 resulted in an immense transformation of medical education in many parts of the world as scientific prowess became the holy grail of medical universities. In the utilitarian sense this change provided enormous therapeutical benefits to the patients. However it came with many unwanted side effects: (1) patients became seen as malfunctioning systems to be fixed without emphasis on treating the person; (2) less importance were given to the quality assurance of teaching; (3) medical education started to focus more on mortality abandoning morbidity; (4) publications were pursued so much that many became unreliable. These effects eroded the trust and respect of the medical profession and started to erode the stance of science as well. The newly available technological advances that enabled patients to quickly gain a superficial insight into any medical topic strengthened this trend as well. The same technological advances and the increased number of students put further pressure on the universities teaching methods. Clearly, all these necessitated a realignment of the flexnerian route.

Semmelweis University, which traditionally has an emphasis on teaching firm scientific reasoning, is addressing these challenges with a set of new policies but we argue that it is possible and even favorable to counter the flexnerian side effects with having the fundamental sciences still visible and influential. These departments are pivotal in teaching scientific thinking and decreasing their influence would be counterintuitive in an era when uncritical thinking is becoming widespread.

The lecture describes the steps being taken at Semmelweis and describes the position of Physiology in this Flexner 2.0 curriculum.

TS-02

Structural and functional integration of physiology teaching: the view from Cardiff

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Physiology teaching can benefit from the erosion of traditional boundaries between life science disciplines. The original Physiology department at Cardiff University now forms part of a multi-disciplinary School of Biosciences; this arrangement has generated opportunities for colleagues with different backgrounds and expertise to work together to develop physiology teaching.

Our medical curriculum has evolved from a traditional approach with clear distinctions between basic sciences and clinical training, through a systems-based curriculum, to, more recently, a fully integrated case-based approach, where scientists and clinicians work together to ensure that students' understanding of basic science and clinical skills are developed in a cohesive manner. This has provided an opportunity to highlight the relevance of physiology as a keystone of medicine and to embed physiological concepts and principles into clinical thinking. Our Physiology degree programme has also developed to reflect the interdisciplinary nature of modern physiology and the curriculum now benefits from the contributions of colleagues across the School of Biosciences. This arrangement supports provision of research-led teaching and allows individual staff to work collegially and teach to their strengths. Wider choice in the content of their degrees has also allowed Physiology students to tailor their degree to reflect their specific interests and led to wider inclusion of physiology components in related degree schemes.

This talk will address the benefits and limitations of teaching physiology within an integrated curriculum and will evaluate the consequences of this approach at Cardiff University.

TS-03

Organizational consequences of discipline-oriented versus integrated teaching

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Medical education in its classical form is teacher-oriented and organized by discipline. Subjects are taught in a non-integrative way with a clear separation between disciplines such as physiology and anatomy. The focus is on theoretical knowledge and not on skills or competences. In this system, departments are organized around disciplines, and they are responsible for the content and organization of their own disciplinary courses. The chair of a department is accountable for the quality of these courses, while (a member of) the board of the medical school monitors and supervises the content and quality of the whole medical curriculum.

Over the last decades, an increasing number of medical schools have changed their medical curriculum in such a way that theoretical and practical education is integrated. A well-known example for such an integrated approach is problem-based learning (PBL). PBL curricula are characterized by active, student-centered learning in a multidisciplinary setting. Education is no longer organized by single departments and disciplines, but by committees and planning groups consisting of staff members of different preclinical and clinical departments. Inevitably, this means more central governance and reduced autonomy of the departments. A central management team or structure is required to commission and oversee all educational activities that together constitute the curriculum and to monitor the educational performance of staff members of all participating departments. In some universities, this change is accompanied by a transition from discipline-oriented to integrated departments. The potential (dis)advantages and consequences of such a transition will be discussed during the presentation.

KEYNOTE LECTURE/ PLENARY LECTURES

KL-01

The stressed brain of rodents and humans

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Stress activates the sympathetic nervous system and the hypothalamus-pituitary-adrenal axis which results in the release of (nor)adrenaline and cortisol (corticosterone in rodents) respectively. This will change the function of all organs including the brain, from the molecular through cellular level, up to behavior. The effects occur in two time-domains: rapid non-genomic actions which behaviorally promote immediate self-centered solutions; and later, genomic actions which are beneficial from a perspective of the future. The response to stress in adulthood depends on the genetic background of individuals and can predispose them to resilience or vulnerability to stressful conditions. This is influenced - possibly through epigenetic modifications - by stressful events experienced across the lifespan but especially when these events occur during early development. In animal models, brief pharmacological interventions during puberty can counteract the effects of early life stress. Current studies investigate the critical windows for treatment efficacy.

PL-01

A FULLY ORGANIC RETINAL PROSTHESIS REINSTATES VISION IN BLIND ANIMALS

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The degeneration of photoreceptors in the retina is one of the major causes of adult blindness in humans. Unfortunately, no effective clinical treatments exist for the majority of retinal degenerative disorders. We report the fabrication and functional validation of a fully organic prosthesis made of P3HT as a photosensitive semiconductive layer, PEDOT:PSS as a conductive layer and silk fibroin as a biocompatible passive substrate for long-term *in vivo* subretinal implantation in the eye of Royal College of Surgeons rats, a widely recognized model of *Retinitis pigmentosa*. Electrophysiological and behavioral analyses revealed a prosthesis-dependent recovery of light sensitivity and visual acuity that persisted up to 6-10 months after surgery. The rescue of the visual function was accompanied by an increase in the basal metabolic activity of the primary visual cortex, as demonstrated by PET imaging. This was complemented by a modest and transitory inflammatory response that decreased soon after surgery with full preservation of retinal morphology. In parallel, the authors are moving ahead with the functional validation of a device optimized for implantation in a swine model of degenerative blindness. The experimental findings obtained so far highlight the possibility of developing a new generation of fully organic, highly biocompatible, low cost, and functionally autonomous photovoltaic prostheses for subretinal implants to treat degenerative blindness. This line of translational research is of clinical relevance in retinal degenerative diseases, characterized by an incidence of 1 in every 4000 people worldwide, for which there is currently no medical treatment.

PL-02

Endolysosomal two-pore channels: from genes to function

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Two-pore cation channels (TPCs) are members of the pore-loop cation channel family that are specifically expressed in the membrane of endolysosomal organelles. Over the last couple of years our laboratory has characterized the functional properties of the two mammalian TPCs (TPC1 and TPC2)

using imaging and patch-clamp approaches in intact lysosomes. Moreover, we have generated genetic mouse models to examine the roles of TPCs in a variety of physiological and pathophysiological settings. In my lecture I will give an overview on our recent work on TPCs. TPCs are broadly expressed in the body and play key roles in many processes, including cholesterol homeostasis, cancer cell migration and neoangiogenesis. Moreover, these channels are involved in the intracellular trafficking of several pathological viruses including Ebola. These roles make TPCs an attractive target for future drug development

PL-03

Exercise as Medicine – the role of myokines mediating muscle-organ cross-talk

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Physical activity represents a cornerstone in the primary prevention of at least 35 chronic conditions. However, over the past two decades, considerable knowledge has accumulated concerning the significance of exercise as the first-line treatment of several chronic diseases. Of note, today exercise has a role as medicine in diseases that do not primarily manifest as disorders of the locomotive apparatus, e.g. diabetes, cardiovascular disease, cancer, dementia and depression.

During the past decade skeletal muscle has been identified as an endocrine organ. We have suggested that cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert either autocrine, paracrine, or endocrine effects should be classified as *myokines*. The muscle secretome consists of several hundred secreted peptides. This finding provides a conceptual basis and a whole new paradigm for understanding how muscles communicate with other organs such as adipose tissue, liver, pancreas, bones, and brain and may influence the function and metabolism vis muscle-organ cross-talk. In addition, recent evidence suggests that myokines mediate both anti-inflammatory and anti-cancer effect.

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PL-04

Targeting Brain Circuits to Reverse Obesity and Type 2 Diabetes

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The 5-hydroxytryptamine (5-HT, serotonin) 2C receptor (*Htr2c*; 5-HT_{2C}R) agonist lorcaserin (Eisai Inc) is a new medication for obesity treatment that also improves type 2 diabetes in patients. However, the neural circuits mediating lorcaserin's therapeutic effects remain to be elucidated. We observed that preventing *Pro-opiomelanocortin* (*Pomc*) expression (*Pomc*NEO) within the arcuate nucleus of the hypothalamus (ARC) abolished lorcaserin's anorectic and glucoregulatory effects and that restoration of *Pomc* specifically within a subset of ARC neurons expressing 5-HT_{2C}Rs (*PomcHtr2c*) is sufficient to mediate lorcaserin's therapeutic effects. One receptor target of *Pomc* is the melanocortin₄ receptor (*Mcr4r*). We reveal that lorcaserin suppresses appetite and improves glycemia via downstream melanocortin₄ receptor (*Mcr4r*) activation. On a *Mcr4r* null background, the selective restoration of *Mcr4r*

function within ChAT neurons (*Mc4rChAT*) is sufficient to mediate lorcaserin's glucoregulatory, but not anorectic effects. Thus, our results reveal a divergence in the neurocircuitry underpinning lorcaserin's anorectic and glycaemic therapeutic effects.

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PL-05

Thyroid hormone determination of neural stem cell fate.

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During vertebrate evolution, thyroid hormone acquired multiple roles in orchestrating and optimizing physiological responses during development, particularly brain development. Much of our recent work has addressed how modulation of thyroid hormone availability affects neural stem cell biology and notably the determination of neuronal versus glial fates. Our model is the neurogenic niche of the adult mouse sub-ventricular zone (SVZ). Neural stem cells in the adult SVZ can generate neurons and glial cells, including oligodendrocytes, the myelinating cells that are crucial for proper brain function. Using mainly *in vivo* experimental approaches, we have shown that the active form of thyroid hormone, T3 and TRa1, directly represses *Sox2* (a key factor involved in stemness) favouring the appearance of neuroblasts and commitment to the neuronal lineage. Our current work addresses the question of whether and how thyroid hormone signalling plays a role in glial lineage and oligodendrocyte determination in the adult mammal. In contrast to neuronal commitment, we show that absence of thyroid hormone signalling (hypothyroidism) promotes oligodendrocyte commitment. Thus, neuronal *versus* oligodendrocyte precursor cell determination in the adult SVZ is the mirror image one of the other: T3/TRa1 together favour progression towards a neuronal phenotype whereas a T3-free window is required for glial cell fate determination. These findings have clear implications for treatment of neurodegenerative disease during ageing as well as for understanding the origin of certain neuro-developmental disorders.

SYMPOSIA

Symposium 01: Mitochondrial and cell membrane Ca²⁺ and Na⁺ signaling in health and disease

S01-1

Systematic identification of MCU modulators by orthogonal interspecies chemical screening

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The mitochondrial calcium uniporter complex is essential for calcium (Ca²⁺) uptake in mitochondria of all mammalian tissues, whereby it regulates energy metabolism and cell death. An ever-growing number of human diseases linked to dysfunctions of mitochondrial Ca²⁺ homeostasis qualify the uniporter as a target of broad pharmacological interest. However, at present we lack lead compounds for the selective regulation of its activity. Here, we introduce a high-throughput orthogonal, interspecies assay that identifies direct modulators of human MCU. Our strategy exploits a D-lactate- and mannitol/sucrose-based bioenergetic shunt that allows deconvolving false positive hits due to chemical impairment of mitochondrial membrane potential and upstream Ca²⁺ signaling pathways. Out of more than 600 clinically approved drugs, we identify a direct, selective inhibitor of human MCU. Our approach is a highly effective tool for MCU-specific drug discovery, and, more generally, for therapeutic targeting of mitochondria.

S01-2

Shaping cell motility and metabolism by coordinated Ca²⁺ and Na⁺ signals

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ORAI channel proteins (ORAI1/ORAI2/ORAI3) form highly Ca²⁺ selective channels which control a wide variety of physiological and pathophysiological functions. ORAI proteins are gated and regulated by the endoplasmic reticulum Ca²⁺ sensor proteins, STIM1 and STIM2 and contribute subunits to channels activated by either store depletion or by store-independent means. Mitochondrial Ca²⁺ homeostasis is crucial for cellular function and is controlled through Ca²⁺ uptake by the mitochondrial Ca²⁺ uniporter (MCU) and extrusion by the Na⁺/Ca²⁺ exchanger (NCLX). Here, we discuss how MCU and NCLX regulate distinct ORAI-mediated Ca²⁺ entry pathways across the plasma membrane and distinct downstream signaling pathways. We will also discuss the impact of altered STIM, ORAI and NCLX activity on mitochondrial function and cell migration in a model of invasive adenocarcinoma.

S01-3

Dynamic aspects of calcium-dependent regulation in mammalian isoform/splice variants of the sodium-calcium exchanger

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Three gene products of the sodium-calcium exchanger (NCX1, NCX2 and NCX3) and their multiple splice-variants are expressed in a tissue-specific manner to control Ca²⁺-dependent events. NCXs are strongly regulated by Ca²⁺ interaction with the regulatory CBD1 and CBD2 domains. NCX1 and NCX3 (but not NCX2) generate splice variants, where the splicing segment exclusively resides at CBD2. Recent findings reveal a unifying mechanism for decoding the allosteric signal upon Ca²⁺ binding, describing the Ca²⁺-dependent tethering of CBDs, where Ca²⁺ is "occluded" at the two-domain

interface. A slow dissociation of occluded Ca^{2+} results in NCX inactivation, where the exon-dependent interdomain interactions generate upto 100-fold differences in the Ca^{2+} off-rates and inactivation rates of NCX. This represents the editing mechanism for dynamic regulation of tissue-specific (cardiac, skeletal, brain, kidney, etc) NCX variants. Ca^{2+} binding to CBD1 rigidifies the backbone dynamics at the two-domain interface, where the strength, duration and remoteness of Ca^{2+} -induced rigidification is exon dependent. This Ca^{2+} -dependent rigidification is associated with dynamic shift of numerous conformational states in the absence of any global conformational changes ("population shift" mechanism). Thus, the exon-dependent conformational variances govern dynamic contributions of NCX variants to cell-specific affairs (excitation-contraction coupling, action potential duration, neurotransmitter secretion, etc). Collectively, NCX isoform/splice variants share a common mechanism for decoding the regulatory signal, where the splicing segment secondarily shapes dynamic features of NCX to match cell-specific oscillations in $[\text{Ca}^{2+}]_i$.

S01-4 (O)

Adrenergic stimulation leads to distinct intracellular Ca^{2+} and cAMP-dependent PKA responses in single rat astrocytes

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During the arousal and startle response, *locus coeruleus* neurons, innervating practically all brain regions, release noradrenaline (NA), which reaches brain cells, including astrocytes. Astrocytes, a subset of glial cells, express both α - and β -adrenergic receptors (ARs) and thus represent an important target for NA. Although devoid of electrical excitability, astrocytes respond to NA by activation of α - and β -ARs with increased cytosolic levels of secondary messengers Ca^{2+} and cAMP, i.e. cytoplasmic excitability. AR-activation controls many processes in astrocytes, including cell morphology and metabolism. It is known from biochemical studies that Ca^{2+} and cAMP signals in astrocytes can interact. However, it is presently unclear whether the temporal properties of the second messengers are time associated upon AR-activation. We used confocal microscopy to study AR agonist-induced intracellular changes in Ca^{2+} and cAMP in single cultured rat astrocytes by real-time monitoring of the Ca^{2+} indicator Fluo4-AM, and the fluorescence resonance energy transfer-based nanosensor A-kinase activity reporter 2, which reports the activity of cAMP via its downstream effector protein kinase A (PKA). We have observed that temporal profiles of the respective secondary messenger systems are distinct in astrocytes. While the activation of α_1 -ARs triggers Ca^{2+} oscillations within 10 s, the activation of β -ARs leads to a ~10-fold slower persistent increase in cAMP-dependent PKA activity devoid of oscillations. Moreover, the results revealed that β -AR activation in cultured astrocytes potentiates the α_1 -AR induced Ca^{2+} response and *vice versa*, indicating that the pathways control and tune the activity of each other at the single-cell level.

S01-5 (O)

Function and distribution of the mitochondria in pancreatic ductal epithelial cells

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Mitochondrial dysfunction is a hallmark of several disease pathogenesis including acute pancreatitis (AP). Our previous results suggest that mitochondrial damage is crucial in bile acid induced inhibition

of pancreatic ductal HCO₃⁻ secretion, however the details of mitochondrial function and dysfunction in pancreatic ductal epithelial cells (PDEC) is not known yet.

Guinea pig and Cyclophilin D WT and knock out (KO) mouse pancreatic ducts were used. Mitochondrial distribution was studied by electron microscopy (EM), membrane potential ($\Delta\psi_m$) was measured by confocal microscopy and pancreatic ductal HCO₃⁻ secretion by microfluorometry.

EM measurements revealed that the mitochondrial density is significantly higher on the apical side of the guinea pig PDEC compared to the middle or the basal segment in HEPES solution. The apical mitochondrial density increased further in CO₂/HCO₃⁻ buffered solution, or during the administration of 5 μM forskolin. This redistribution was also confirmed by the $\Delta\psi_m$ measurements as we detected increased TMRM fluorescence on the apical side of the PDEC during stimulation. The genetic KO of cyclophilin D significantly reduced the loss of $\Delta\psi_m$ and protected pancreatic ductal HCO₃⁻ secretion during the administration of 500 μM chenodeoxycholic acid.

Our results suggest that mitochondrial function has a central role in the function of PDEC presumably by providing ATP for fluid and ion secretion. The opening of MPTP seems to be crucial in the bile acid induced toxicity offering a potential therapeutic target in AP.

Symposium 02: Structure and function in islets of Langerhans in health and disease

S02-1

β cell diversity is required for normal islet function

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Aim: Insulin-secreting β cells are a heterogeneous islet population whose activity is guided by a subgroup of pacemakers termed hubs. Since normal islet function may depend on maintaining this diversity, we looked into the effects of overexpression-induced β cell maturity on gene expression, signalling and insulin secretion.

Materials and methods: Maturity in adult mouse islets was forced using an adenoviral polycistronic construct for *Ngn3*, *MafA*, *Pdx1* and *mCherry* (Ad3-NPM). Non-transduced islets (CT) served as controls. Gene expression was assessed by QRT-PCR. *Pdx1* and insulin were detected by immunohistochemistry. Ca^{++} , ATP and cAMP dynamics in live islets were assessed by Nipkow spinning disk microscopy. Glucose-stimulated (GSIS) and incretin-stimulated insulin secretion (ISIS) were HTRF-measured.

Results: Ad3-NPM increased the expression of *Pdx1* and *MafA*, without changing *Ngn3*. *Pdx1* overexpression was predominantly localized to the immature beta cells, reducing cellular heterogeneity. There was a marked decrease in the magnitude of Ca^{++} responses to glucose ($\Delta F=0.81$ vs 0.44 AU, CT vs Ad3-NPM; $P<0.01$), along with a reduction in β cell- β cell coordination and hub number (12.6 vs 5.6 % hubs, CT vs Ad3-NPM; $P<0.05$). Ad3-NPM islets showed an increase in the ATP/ADP ratio and a marked reduction of the cAMP. Basal insulin secretion was increased post-overexpression, with impaired GSIS and ISIS (7.5-fold vs 5-fold post-glucose and 98-fold vs 50-fold after exendin-4 stimulation; CT vs Ad3-NPM).

Summary: Disrupting β cell heterogeneity through forced overexpression weakens islet function and lowers insulin secretion. This suggests that cell diversity plays a critical role in islet performance.

S02-2

Induction of pancreatic beta-cell neogenesis

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The recent discovery that genetically-modified pancreatic alpha-cells can regenerate and convert into beta-like cells *in vivo* holds great promise for diabetes research. However, to eventually translate these findings to human, it is crucial to discover compounds with similar activities. Herein, we report the identification of GABA as an inducer of alpha-to-beta-like cell conversion *in vivo*. This conversion induces alpha-cell replacement mechanisms through the mobilization of duct-lining precursor cells that adopt an alpha-cell identity prior to being converted into beta-like cells, solely upon sustained GABA exposure. Importantly, these neo-generated beta-like cells are functional and can repeatedly reverse chemically-induced diabetes *in vivo*. Similarly, the treatment of transplanted human islets with GABA results in a loss of alpha-cells and a concomitant increase in beta-like cell counts, suggestive of alpha-to-beta-like cell conversion processes also in humans. This newly discovered GABA-induced alpha-cell-mediated beta-like cell neogenesis could therefore represent an unprecedented hope towards improved therapies for diabetes.

S02-3

The patterns of synchronicity and functional connectivity in islets of Langerhans

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Classical morphological and physiological approaches, such as gross inspection, light and electron microscopy, blood analyses and secretion assays, in concert with classical descriptive and inferential statistics, have taught us a lot about the structure and function of islets of Langerhans, revealing assemblies of coupled heterogeneous beta cells producing oscillations in metabolism, membrane potential, cytosolic calcium, and secretion of insulin that are probably crucial for oscillations in plasma insulin and normal glucose tolerance. Recently, high resolution *in situ* morphological and functional live cell imaging experiments, together with graph theoretical analyses and mathematical modelling, have revealed that heterogeneity, coupling, and oscillations are probably inseparably linked and necessary to ensure efficient synchronization, robustness, and adaptability of beta cell assemblies in the long run. These insights opened doors to modern network science in islet physiology and are forcing us to rethink what we know about the structure and function of islets of Langerhans and possibly other endocrine tissues, and are beginning to change our understanding of diseases, such as diabetes mellitus.

S02-4

Heterogeneity on all levels: insight into pancreatic islet function with modeling

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Hormone secretion from pancreatic islets results from complex regulatory mechanisms operating on multiple biological scales. On each of these levels, there is pronounced heterogeneity between the functional units. At the lowest subcellular level, the members of ion channel populations are exposed to uneven biophysical conditions, and secretory granules show heterogeneity in, e.g., positioning with respect to calcium sources and local protein abundance. At higher levels, there are cell-to-cell and

islet-to-islet differences in e.g. glucose sensitivity. Mathematical modeling has been used to investigate how the pancreas exploits such heterogeneity to create particular secretion patterns, while – at the same time – taming heterogeneous behavior to provide robust responses to changing glucose levels. Our recent modeling has suggested that cell-to-cell variation in combination with electrical coupling within the islets can lead to so-called functional small-world behavior as a result of wave propagation. Concerning the subcellular levels, I will show how to exploit heterogeneity in granule properties to quantify how e.g. local protein abundance controls exocytosis with advanced statistical methods. I will discuss how neglecting such subcellular heterogeneity may lead to misleading results concerning exocytosis or ion channel function. Finally, multi-scale modeling can be used to link the physiological levels to provide insight into how pancreatic secretion and cell function is influenced by subcellular and molecular events.

S02-5 (O)

Investigating SNAP-25b (Synaptosomal-associated protein 25) function in mouse islet physiology beyond its classical role in membrane fusion

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Questions

SNAP-25 is a core protein of the SNARE complex mediating stimulus-dependent release of insulin from pancreatic β cells. The protein exists as two alternative spliced isoforms, SNAP-25a and SNAP-25b, differing in 9 out of 206 amino acids but their specific roles in β cells remain unclear.

Results

We explored the effect of SNAP-25b-deficiency on glucose-stimulated insulin release in islets and found increased secretion both *in vivo* and *in vitro*. However, slow photo-release of caged Ca^{2+} in β cells within pancreatic slices showed no significant differences in Ca^{2+} -sensitivity, amplitude or rate of exocytosis between SNAP-25b-deficient and wild-type littermates. Therefore, we next investigated if SNAP-25b-deficiency affected Ca^{2+} handling in glucose-stimulated β cells using intracellular Ca^{2+} -imaging and found premature activation and delayed termination of $[\text{Ca}^{2+}]_i$ elevations. These findings were accompanied by less synchronized Ca^{2+} -oscillations and hence more segregated functional networks between individual β cells. Islet gross morphology and architecture were maintained in mutant mice, although sex specific compensatory changes were observed.

Conclusions

In summary, our observations suggest that SNAP-25b in pancreatic β cells, except for participating in the core SNARE complex, plays an important role in regulating insulin secretion by affecting Ca^{2+} dynamics.

Keywords: exocytosis, β cell, Ca^{2+}

Symposium 03: Cerebrovascular disorders: Pathophysiological and pharmacological approaches

S03-1

Post-acute effects of CDNF and MANF on brain plasticity and repair

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Ischemic- stroke remains among the leading cause of serious motor disabilities in developed countries. Initial hemiparesis affects 80–90% of patients; however, 45–60% still exhibit motor deficits in the post-acute stroke phase. Post-ischaemic endogenous responses of the central nervous system go in line with an enhanced responsiveness to rehabilitative and plasticity-promoting treatments, such as growth factors, opening a time window in which brain repair mechanisms may be reactivated successfully. Therefore, to stimulate post-ischemic functional recovery, promoting perilesional tissue remodelling and pyramidal tract plasticity are major challenges of post-acute ischemic stroke. In this context, cerebral dopamine neurotrophic factor (CDNF) and mesencephalic astrocyte-derived neurotrophic factor (MANF) differ from the other known growth factors by their smaller size of ~18kDa and their unique amino acid sequences. These differences indicate that they utilize distinct signaling pathways and hence, they may exert different effects than other known factors. They are currently among the most promising molecules for the treatment for Parkinson's disease besides neuroprotective activity in acute ischemic- stroke. In the present talk, effects of CDNF and MANF on inflammation, neurogenesis, angiogenesis, post-ischemic functional recovery, perilesional tissue remodelling and pyramidal tract plasticity will be reviewed. These studies were supported by grant of The Scientific and Technological Research Council of Turkey (TUBITAK). Project number: 114S402.

S03-2

Pericytes as an important target in Stroke and other Neurological Diseases' Pathophysiology

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The role of microcirculation and cells of blood-brain barrier in stroke pathophysiology is an important issue and pericytes are one of the important players. Brain pericytes have many important roles in blood flow regulation and understanding their physiological properties and contribution to neurological disease mechanisms is important for development of novel therapeutic options.

Pericytes are the cells found around the abluminal side of endothelium and located in between microvascular endothelia and basal lamina-astrocytes. These cells are found mainly at the precapillary arterioles, the capillaries and the postcapillary venules. They have contractile properties and regulate the blood flow at the microcirculation via constricting or dilating capillaries, precapillary arteriols and post capillary venules. They can be considered as continuation of arterial smooth muscle cells and can regulate blood flow according to neuronal needs and hence functional connection can be established. Other than blood flow regulation, they have other several important roles like maintenance of blood-brain barrier.

Pericytes are especially important for providing adequate microcirculatory supply according to needs of neuronal tissue and form one of the functionally important part of BBB and take role in neurovascular coupling. They perform this regulation through their contractile properties, playing important roles in acute injuries like stroke as well as chronic neurological diseases. Understanding the role and disease producing mechanisms of neurovascular unit elements in different neurological conditions will provide novel targets for future treatments.

S03-3

Neuroregenerative approaches using neural progenitor cells to counteract cerebral ischemia

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Treatment of cerebral ischemia (i.e., ischemic stroke) has made tremendous progress in recent years. With systemic (intravenous) thrombolysis being a well-established therapeutic means to counter ischemic stroke, endovascular treatment has become an additional and powerful tool for physicians as well. Yet, a majority of patients does not qualify for either treatment paradigms because of potential side effects and narrow time window. As such, additional treatment paradigms are urgently needed. With neuroprotective approaches having failed so far, the focus of experimental stroke research has switched towards neuroregenerative strategies. Endogenous neurogenesis persists in the adult mammalian brain, although it might contribute little to neurological recovery as both survival and differentiation rates of new-born neural progenitor cells (NPCs) are low. Yet, endogenous neurogenesis can be stimulated by various means, among which the transplantation of ectopic NPCs is feasible. The latter, however, need to have an appropriate extracellular milieu in order to exert their protective and regenerative potential. Modulation of the post-stroke extracellular milieu is achieved by various experimental strategies. We herein focus on the introduction of post-stroke conditioning, i.e., the induction of an additional non-injurious ischemia of either the brain (cerebral post-conditioning) or the periphery (remote post-conditioning). Pro-injurious and rescue pathways will be analyzed after post-conditioning in stroke mice, followed by a transplantation of NPCs derived from the subventricular zone of the lateral ventricles. Finally, the impact of the combined therapeutic approach after both post-conditioning and NPC transplantation on post-stroke neurological recovery will be analyzed in mice.

S03-4 (O)

Internal carotid artery blood flow response to isometric handgrip and head-down tilt in healthy volunteers.

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Questions We examined internal carotid artery (ICA) blood flow response to isometric exercise in the horizontal position and in head-down tilt position (HDT). During HDT, the increased central blood volume results in an increase in CO. Handgrip maneuver increases MAP through the exercise pressor reflex.

Methods Blood velocity in ICA (Doppler ultrasound) was recorded during rest and handgrip (30% of maximal voluntary contraction) randomized between horizontal and HDT by 10° in 13 healthy volunteers. Heart rate, MAP, CO, ETCO₂ were recorded. ICA blood flow was calculated beat-by-beat from velocity, the diameter of ICA, angle of insonation and instantaneous heart rate. Wilcoxon signed rank test evaluated the differences between conditions.

Results Results (median, 95% CI) show that ICA blood flow was preserved from rest (260, 189-304 ml/min) to handgrip (282, 200-323 ml/min) in the horizontal position, from rest to HDT (265, 190-287 ml/min) and to the combined handgrip and HDT (258, 182-295 ml/min). On the contrary, CO, MAP and HR increased significantly from rest to handgrip (CO: +15%, (p<0.001), MAP: +16%, (p<0.001), HR: +10%, (p<0.001)) and to HDT+handgrip (CO: +23%, (p<0.001), MAP: +17%, (p<0.001), HR: +15%, (p<0.001)). ETCO₂ did not change between conditions.

Conclusions ICA blood flow did not change with static exercise in the horizontal position nor during HDT (elevated CO) despite the significant increase in MAP. Sympathetic activation through the exercise pressor reflex may induce cerebral vasoconstriction which prevents cerebral hyperemia. Our findings suggest an intact static cerebral autoregulation which maintained cerebral blood flow despite the simultaneous increases in MAP and CO.

S03-5 (O)

The role of angiotensin -1 receptors in vasodilator responses of middle cerebral arteries in Sprague-Dawley rats

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Questions: This study aimed to evaluate the role of angiotensin 1 receptors in endothelium-dependant (flow-induced dilation (FID) and acetylcholine (ACh)) and endothelium-independent (sodium nitroprusside; SNP) vasodilator responses in cerebral resistance vessels of Sprague-Dawley rats. Additionally, the effects of AT-1R blockade on oxidative status and the role of oxidative stress on vasodilator responses was examined.

Methods: Healthy male Sprague-Dawley rats (N=6-7 per group) were fed low salt (0.4%NaCl; LS group) or LS+losartan (AT-1R blocker; losartan 40 mg/day in water ad lib) for 7 days. Middle cerebral arteries responses to ACh (10-6M), SNP (10-6M) and FID (FID established by increases in pressure gradient ($\Delta 10$ - $\Delta 100$ mmHg)) were studied in absence/presence of superoxide scavenger, TEMPOL (100 μ mol/l). Plasma angiotensin II (ANG II) levels were measured by ELISA and plasma antioxidant capacity (FRAP) and lipid peroxidation level (TBARS) were measured by spectrophotometry. Mean arterial blood pressure (MAP) was also measured.

Results: LS+losartan group exhibited significantly decreased vasodilation in response to ACh and FID, while SNP-induced dilation was preserved in both groups. FID was restored by addition of TEMPOL in chamber bath. LS+losartan group had significantly higher ANGII plasma concentration, higher TBARS and lower MAP compared to LS group. FRAP was similar between the groups.

Conclusions: Increased oxidative stress may be underlying impaired endothelium-dependant dilation responses. Results suggest important role of ANG II in maintaining arterial relaxation responses via AT-1 receptor activation in physiological conditions. **Support: Croatian Science Foundation grant #IP-2014-09-6380.**

Symposium 04: Current developments in the pulmonary circulation

S04-1

Sphingolipids - new players in pulmonary vasoconstriction and lung vascular remodeling

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Sphingolipids are a class of lipids that contain a backbone of sphingoid bases. Of late, sphingolipids, and specifically ceramide and sphingosine-1-phosphate (S1P) have emerged as important mediators of cell and organ homeostasis as well as key signaling molecules involved in the pathogenesis of cardiovascular and respiratory diseases. There is so far no known receptor for ceramide; however, ceramide activates a series of downstream signalling pathways by formation of ceramide-rich lipid rafts and caveolae which cluster receptor molecules and recruit intracellular signalling molecules. S1P acts as intracellular signalling molecule by mechanisms that are likely similar to those of ceramide. On the other hand, S1P signals as extracellular mediator via five cell surface G-protein coupled receptors (GPCRs) termed S1P₁₋₅.

Both S1P and ceramide have recently become implicated in the regulation of lung vascular tone and remodeling. Neutral sphingomyelinase, which generates ceramide from sphingomyelin at the outer leaflet of the cell membrane, mediates hypoxic pulmonary vasoconstriction (HPV) by promoting the recruitment of transient receptor potential canonical 6 (TRPC6) Ca²⁺ channels that are essential for HPV to caveolae in a process that surprisingly is dependent on cystic fibrosis transmembrane

conductance regulator (CFTR). Via activation of S1P₂ receptors, S1P concurrently activates both Rho kinase signaling and - via phospholipase C - TRPC6 which conjointly cause smooth muscle cell contraction. In chronic hypoxia, prolonged stimulation of S1P₂ promotes lung vascular remodeling by stimulating both smooth muscle cell proliferation as well as hypertrophy. As such, sphingolipids may present promising targets for the prevention or reversal of lung vascular remodeling and pulmonary hypertension.

S04-2

FoxO transcription factors in pulmonary hypertension: Pathophysiology and therapeutic implications

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Pulmonary hypertension (PH) is a progressive disease of multifactorial etiology, which has a poor prognosis. Variants of PH affect up to 100 million people worldwide. Increased proliferation and migration and resistance to apoptosis of pulmonary vascular cells play a major role in pathologic remodeling processes underlying different variants of PH. Forkhead box O (FoxO) transcription factors are key regulators of cellular proliferation, migration, differentiation and apoptosis by modulating and integrating multiple signaling pathways. We have recently observed that in pulmonary vessels and pulmonary vascular smooth muscle cells and adventitial fibroblasts of patients with different groups of PH (group 1: Pulmonary arterial hypertension; group 3: PH associated with lung diseases) and lungs with experimental PH, FoxO isoforms (FoxO1, FoxO3) are inactivated via phosphorylation and nuclear exclusion. In addition, we have identified the receptor tyrosine kinase (via PI3K/AKT), cytokine (via STAT3) and Hippo signaling as upstream pathways mediating FoxO's control of PH. Pharmacological inhibition and genetic ablation of FoxO1 in smooth muscle cells and FoxO3 in fibroblasts reproduced PH features *in vitro* and *in vivo*. Either pharmacological reconstitution of FoxO activity using paclitaxel/UCN-01, or reconstitution of the transcriptional activity of FoxO1 or FoxO3 by gene therapy, restored the physiologically quiescent vascular phenotype *in vitro*, linked to changes in cell cycle control and bone morphogenic protein receptor type 2 signaling, and reversed vascular remodeling and right-heart hypertrophy *in vivo*. Taken together our studies have provided strong evidence for the involvement of FoxO proteins in PH pathogenesis and FoxO-reactivation offers a potential therapeutic option for PH.

S04-3

Adapting to high altitude

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Highlanders are well adapted to live at high altitude, and so is the developing human fetus. In a prospective observational study on healthy term newborns in Peru (Puno at 3840m) that included novel non-invasive visualization of microcirculation we demonstrated that vessel density is elevated by 14% in neonates born to women living at high altitude as compared to babies born at sea level, most likely revealing an early adaptive mechanism to a highly hypoxic antenatal environment.

As regarding ascending mountaineers, adequate acclimatization time to slowly adjust to hypoxic conditions is one of the most important aspects. Thus, it is crucial to focus on the crosstalk between oxygen and iron homeostasis. To ensure that sufficient iron is provided for red blood cell production, hypoxia-induced soluble factors - such as the novel Epo-controlled erythroferrone that is expressed in erythroid precursor cells - reach the liver where they reduce expression of the iron hormone hepcidin. In turn, suppression of hepcidin allows both, elevated iron release from storage organs and enhanced absorption of dietary iron by enterocytes.

Living at moderate to high altitude leads to the elevation of hemoglobin (Hb) levels in humans but scarce information exists on the effect of lower altitude on erythrocyte production. We compared

several parameters including Hb values to the residence altitude of about 70'000 Swiss men aged 18-22 years. We observed a significant increase of Hb values for every 300 meters of augmented altitude the young Swiss men live at. Thus, even a modest increase in the residence altitude significantly elevates Hb values. Apart from gender, age, ethnicity and socio-economical effects, altitude should be considered when defining the Hb threshold for a given population even when residing at altitudes below 1000 m above sea level.

S04-4 (O)

Alveolar oxygen respiratory oscillations measured in arterial blood.

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The partial pressure of oxygen in arterial blood can increase during inspiration and decrease during expiration in the presence of a variable shunt fraction, such as with cyclical atelectasis, but it is generally presumed to remain constant within a respiratory cycle in the healthy lung. In our experiments, arterial oxygen partial pressure was measured continuously with a fast intravascular sensor in the carotid artery of anaesthetized, mechanically ventilated pigs, without lung injury. Here we demonstrate that the partial pressure of arterial oxygen shows respiratory oscillations in the uninjured pig lung, in the absence of cyclical atelectasis (determined with dynamic computed tomography), with oscillation amplitudes that exceeded 50 mmHg, depending on the mechanical ventilation conditions. These respiratory oscillations in the partial pressure of arterial oxygen can be modelled from a single alveolar compartment and a constant oxygen uptake, without the requirement for an increased shunt fraction during expiration. Our results are likely to contribute to the interpretation of arterial oxygen respiratory oscillations observed during mechanical ventilation in animal models of the acute respiratory distress syndrome.

S04-5 (O)

Brain-derived neurotrophic factor mRNA expression in peripheral and cerebral vessels : Impact of physical training

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Questions:

For a long time, the neuron was considered as the preponderant cellular source of cerebral brain-derived neurotrophic factor (BDNF). However, we recently showed that the cardiovascular system contains as much BDNF as the brain with a prominent expression in endothelial cells and that physical training (PT) increases BDNF protein levels in both peripheral (aorta) and cerebral vessels. In this context, the aim of the present study was to determine i) if these vessels expressed BDNF mRNA and ii) the impact of PT on BDNF gene expression.

Methods:

Experiments were performed on 2 groups of WISTAR male rats: sedentary and exercised. Exercise was induced by a treadmill run (18 m/min, 30 min/day) for 7 consecutive days. BDNF, eNOS (as a marker of shear stress) and Tie2 (a specific marker of endothelial cells) mRNA expressions were measured by RT-qPCR in peripheral (abdominal aorta) and cerebral microvessels.

Results:

BDNF mRNA was expressed in both peripheral and cerebral vessels correlated with endothelial cells enrichment (Tie-2). However, while PT significantly increased eNOS and BDNF mRNA levels in cerebral microvessels, it was without effect in aorta.

Conclusions:

The present study is the first to show that both peripheral and cerebral vessels expressed BDNF gene. The differential expression between peripheral and cerebral vessels in response to PT could be explained either by differences in endothelial cells enrichment and/or in shear stress at the surface of the endothelium (inversely proportional to the diameter), which are both higher in cerebral microvessels than in aorta.

Symposium 05: Exhale negativity-chloride currents in the cardiovascular system

S05-1

What keeps Cl⁻ out of equilibrium in the muscle cells of the cardiovascular system?

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In both vascular smooth muscle cells (SMC) and cardiomyocytes Cl⁻ is in disequilibrium across the membrane. While the resting membrane potential is about - 50 mV in SMC and about - 80 mV in cardiomyocytes the Cl⁻ equilibrium potential is about 25-30 mV in SMC and about 20-25 mV in cardiomyocytes. This means that increased Cl⁻ conductance in SMC leads to depolarization because the membrane potential of SMC is probably always negative to the Cl⁻ equilibrium potential. In the heart the effect of increasing the Cl⁻ conductance may either contribute to repolarization from membrane potentials positive to the Cl⁻ equilibrium potential or delay repolarization at membrane potentials negative to the Cl⁻ equilibrium potential.

In both muscles two transporters are responsible for transporting Cl⁻ in. One is the Cl/HCO₃⁻-exchanger (AE), which uses the energy of the HCO₃⁻ electrochemical gradient to transport Cl⁻ into the cells in exchange for HCO₃⁻. In the SMC the dominant AE is AE2 (the SLC4A2 gene product) while in the cardiomyocytes the dominant AE is AE3 (the SLC4A3 gene product). In addition to establishing the Cl⁻ disequilibrium these transporters also play an important role in regulation of the muscle pH. The other transporter of importance for the Cl⁻ disequilibrium is the Na,K,Cl-cotransporter (NKCC1) which is the SLC12A2 gene product. This transporter uses the energy in the electrochemical gradient for Na⁺ to transport Cl⁻ into the cells. In addition to its contribution to establishing the Cl⁻ disequilibrium this transporter is important for regulation the volume of the muscle cells.

S05-2

Calcium-activated chloride channels and vascular smooth muscle: AN(y)O1 know the answer?

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Arterial smooth muscle cells actively accumulate chloride ions (Cl⁻) so that activation of any Cl⁻ channel will lead to Cl⁻ efflux. The ensuing membrane depolarization increases the open probability of voltage-dependent calcium channels leading to smooth muscle contraction and reduced arterial diameter. Of the different types of Cl⁻ channel identified the most extensively recorded in vascular smooth muscle cells is the calcium-activated chloride channel (CaCC), which for many years had no molecular identity. Since 2008 ANO1 (also termed TMEM16A) has been identified as a component of CaCCs. ANO1 is present in vascular smooth muscle and ANO1-specific blockers alter vascular tone. However, there are a number of discrepancies and variations in the ANO1 story. This talk will review

the evidence for ANO1 (TMEM16A) as the molecular correlate for calcium-activated chloride channels and will highlight some of the interesting debating points in this field of research.

Techniques: Patch clamp electrophysiology, western blot, arteriography, transgenic animals

Keywords: Calcium-activated chloride channels, TMEM16A,

S05-3

Recent advances in research of cardiac calcium-activated chloride channels

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TMEM16A and/or Bestrophin-3 mediated Ca^{2+} -activated Cl^- current ($I_{\text{Cl(Ca)}}$) may cause cardiac arrhythmias but others showed $I_{\text{Cl(Ca)}}$ to be antiarrhythmic. True profile of $I_{\text{Cl(Ca)}}$ during an actual ventricular action potential (AP) is poorly understood.

Profile of $I_{\text{Cl(Ca)}}$ (studied as 0.5 mM 9-anthracene carboxylic acid (9-AC)-sensitive current under whole-cell AP voltage-clamp (APVC) conditions) contained an early fast outward and a late inward component in canine left ventricular cells. Both components were reduced by ryanodine, while fully abolished by nisoldipine and BAPTA. Setting $[\text{Ca}^{2+}]_i$ to 1.1 μM decreased, while Bay K8644, isoproterenol (ISO), and faster stimulation increased $I_{\text{Cl(Ca)}}$. The early outward component of $I_{\text{Cl(Ca)}}$ was larger in subepicardial than in subendocardial cells.

9-AC generated early afterdepolarizations (EAD) recorded with sharp electrodes at low stimulation rates and their incidence was higher in ISO. 9-AC increased short-term variability of repolarization and reduced phase-1 repolarization. 9-AC increased AP duration in a reverse rate-dependent manner in all cell types except subepicardial ones.

Whole-cell $I_{\text{Cl(Ca)}}$ density and normalized protein expressions of TMEM16A and Bestrophin-3 did not differ significantly among left ventricular cells of various origin. TMEM16A and Bestrophin-3 showed co-localization with one another and also with Cav1.2 channels in both canine and human left ventricular myocytes.

$I_{\text{Cl(Ca)}}$ activation requires Ca^{2+} -entry through neighbouring L-type Ca^{2+} channels but augmented by sarcoplasmic reticulum Ca^{2+} -release. $I_{\text{Cl(Ca)}}$ can be protective against cardiac arrhythmias by reducing spatial and temporal heterogeneity of cardiac repolarization and EAD formation.

S05-4 (O)

Cardioprotective Action of Intermittent Hypoxia on Left Ventricle Function in Type I Diabetic Rats

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Questions: Diabetic cardiomyopathy is defined as a kind of heart failure without arterial hypertension or coronary artery disease. It includes left ventricular hypertrophy and fibrosis without evidence of coronary artery atheroma. Since it is well known that cardioprotective effect of hypoxia takes place via increased stabilisation of hypoxia-inducible factor (HIF), inhere we aimed to increase HIF stabilization and examine a possible cardioprotective action of intermittent hypoxia on depressed left ventricle (LV) function in diabetic rats.

Methods: Male Wistar rats were randomly divided into four groups: 1) Control (C) 2) Diabetes (D) 3) Intermittent Hypoxia (IH) 4) Diabetes + Intermittent Hypoxia (D+IH). Single dose of STZ (50 mg/kg) is injected (i.p.) to 11-week old animals to establish Type I diabetes. IH groups were exposed to the hypobaric hypoxia at 69,3 kPa ($\approx 14\% \text{ O}_2$), 6 hours per day for 42 days. LV function and VEGF, PHD2, PHD3, MMP-2, MMP-9 protein levels measured. LV capillarization was evaluated histologically and redox status of serum and myocardial tissue samples were also examined.

Results: IH treatment of diabetic rats normalized the impaired LV function and restored the MMP protein level decrement and reduced the enhanced myocardial oxidative stress. IH treatment elevated VEGF protein level in both diabetic and non-diabetic rats, whereas it decreased PHD2 and PHD3 protein levels and increased left ventricular capillary density in only D+IH group.

Conclusions: Collectively, our data suggest that IH treatment of diabetic rats has a marked protective effect on LV function in diabetic heart via affecting HIF-VEGF related angiogenesis and cardiac redox status, and thereby inducing a regulation of cardiac remodelling due to increased MMP levels.

S05-5 (O)

Cardioprotection of the ischemic myocardium induced by preconditioning in the distant organ: the role of peroxisome proliferator-activated receptors

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Questions. Application of ischemic preconditioning (IPC) in humans is limited by technical requirements and duration, while "remote" IPC (RIPC) appears as more promising. Its mechanisms are not completely clear; signals from distant organs are suggested to be transduced to target ones via neural/humoral pathways or by means of systemic response. Activation of transcription factors PPAR induces both genomic and non-genomic PC-like effects associated with cell survival, antiapoptotic and antiinflammatory processes. This study aimed to elucidate the role of PPAR- α in the mechanisms of RIPC.

Methods. In anesthetized adult Wistar rats, RIPC was evoked by 3 cycles of 5-min inflation (200 mmHg)/5-min deflation of pressure cuff placed on the hind limb, with or without administration of PPAR- α antagonist MK886 (MK, 3 mg/kg i.p., prior to RIPC). In Langendorff-perfused hearts subjected to 30-min test ischemia/120-min reperfusion, infarct size (IS, TTC staining), postischemic functional recovery (LVDP) and occurrence of ventricular tachyarrhythmias served as the indicators of myocardial

injury. In parallel groups, hearts were examined for PPAR- α gene expression (RT-PCR) and PKC ϵ protein levels (WB).

Results. RIPC reduced IS (by 53%), severity of arrhythmias and improved LVDP recovery by 51% ($p < 0.05$). Levels of PPAR- α mRNA and PKC ϵ proteins were increased in the RIPC hearts (by 50% and 28%, respectively). All effects were reversed by pretreatment of rats with MK.

Conclusions. The results confirm the effectiveness of RIPC in protection against ischemia and suggest the role of PPAR- α as one of potential cardioprotective mechanisms implicating involvement of PKC ϵ . Grants VEGA SR 2/0201/15, APVV-0102-11, MAD-SAV-AV ČR 15-15, ITMS 26230120006

Symposium 06: Microvascular mechanisms under different pathophysiological conditions

S06-1

Cardiovascular peptides in coronary modulation: focus on Chromogranin-A and its derived peptides

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Coronaries undergo neurohumoral modulation elicited by adrenergic and cholinergic neurotransmitters, autacoids, and an increasing number of endocrine, paracrine, and autocrine peptides. These substances form complex networks that allow coronary adaptation to myocardial requirements. Recently, we described the cardiovascular profile of the protein Chromogranin A (CgA) and its peptides. CgA, co-stored and co-released with catecholamine in chromaffin granules, is expressed also in the heart. On the isolated rat heart, CgA biphasically affects the coronary pressure (CP), and induces negative inotropism and lusitropism. Rat coronaries are also influenced by the CgA-derived rat-CgA1-64 (rCgA) and catestatin (CST: CgA352–372), both showing cardio-inhibitory and anti-adrenergic actions. rCgA reduces CP, and counteracts Endothelin-1 (ET1)-induced vasoconstriction. Contrarily, CST increases CP under basal conditions and relaxes coronaries constricted by ET1. The mechanism that sustains the coronary effects of CgA and its fragments involves the nitric oxide (NO)-cGMP signaling. Of note, the CgA-derived C-terminal positive inotrope β -adrenergic-like Serpinin (CgA403-428) is without coronary effect. These data expand the knowledge on the integrated networks that allow coronary and myocardial modulation under basal and stressful conditions. Considering the emerging role of CgA and its peptides in cardiovascular diseases (e.g. Myocardial Infarction and Acute Coronary Syndrome), and their myocardial protection against Ischemia/Reperfusion injury, these findings are also of remarkable biomedical interest.

S06-2

High blood pressure-induced cerebrovascular failure leads to dementia

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Maintenance of an appropriate cerebral blood flow (CBF) has an important physical constraint because the brain is seated in the rigid cranium, where limited space available for volume expansion. To comply with this limitation there is a well-developed, high gain autoregulation of CBF. There are two important mechanisms responsible for the autoregulation of CBF coupled to changes in hemodynamic forces. One is the pressure-sensitive myogenic mechanism, whereas the other is the newly recognized flow-sensitive constrictor mechanism. In experimental genetic hypertension, we have found that cerebral arteries of a special, so-called stroke-prone rat, do not develop adaptation related to the upregulation of cytochrome P450 ω -hydroxylase-derived arachidonic acid metabolite, 20-hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) and corresponding increased constriction.

Also, in aged - but not in young - rats, Ang II-induced hypertension resulted in the impairment of blood-brain barrier, reduced capillary number, and learning disabilities. Extrapolating these finding to human, one can propose that alterations in these mechanisms may contribute to the development of cerebrovascular disorders, such as vascular dementia and Alzheimer disease-like syndromes. Support: Hungarian National Science Research Fund (OTKA) K 108444 and Hungarian Hypertension Society.

S06-3 (O)

Intestinal microcirculation during hemorrhagic shock and resuscitation

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Background. Hypotensive resuscitation has been proposed in hemorrhagic shock. However the optimal level of arterial pressure and oxygenation is debated. We investigated the relationships between mean arterial pressure, intestinal microcirculation and mucosal oxygen tension during hemorrhagic shock and resuscitation at different inspired oxygen fraction concentration.

Methods: Thirty-two mice were progressively exsanguinated and then transfused in MAP-titrated steps of 10 mmHg. Mice were randomized to four experimental groups: a control group in which sham mice underwent a laparotomy and three interventional groups with a common phase of exsanguination followed by progressive resuscitation at three different inspired oxygen concentrations (FiO₂) (15%, 30%, and 100%). Intestinal mucosal oxygenation (intestinal pO₂) and microcirculatory parameters were recorded at each 10 mmHg MAP step.

Results: During exsanguination, intestinal mucosal hypoxia (PO₂ \leq 20 mmHg) appeared at a MAP of 60 mmHg and MAP < 60 mmHg was associated with a high percentage of animal with intestinal hypoxia (\geq 32%). Combination of MAP and microcirculatory parameters was superior to MAP alone at predicting mucosal oxygenation. Inversely, During resuscitation with FiO₂ = 30%, the microcirculatory parameters increased linearly with MAP levels while they had a nonlinear relationship with intestinal pO₂. Hypoxia (FiO₂ = 15%) was poorly tolerated. In hyperoxic group (FiO₂ = 100%) intestinal pO₂ became significantly higher than baseline values as soon as 50 mmHg MAP.

Conclusions: . A MAP < 60 mmHg was associated with a high percentage of animal with intestinal hypoxia. Normoxic resuscitation (FiO₂ = 30%) was sufficient to restore intestinal pO₂.

S06-4 (O)

Acetylsalicylic acid (aspirin) induces endothelium-dependent, cyclic nucleotide-dependent vasodilation of uterine arteries.

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Human studies on the use of acetylsalicylic acid (aspirin, ASA) for the treatment of preeclampsia - a disease that affects 5-8% of pregnant women, and is associated with mortality and morbidity of both mother and fetus - are contradictory. The aim of this study was to investigate the effects of the ASA on resistance arteries from the systemic (splanchnic) and reproductive (uterus) systems of non-pregnant (NP), mid-pregnant (MP: day 14) and late pregnant (LP: day 20) rats. The drug was tested on isolated, cannulated arcuate uterine arteries, and on 3rd order mesenteric arteries using pressure arteriography. Following precontraction with phenylephrine, ASA dilated both uterine and mesenteric vessels in a concentration-dependent manner. Effects were similar in vessels from all groups at concentrations below 10⁻⁷ M; at higher concentrations, vasodilation decreased in uterine (but not mesenteric) arteries from LP animals. Moreover, vasodilation was significantly reduced (>80%) by removing the endothelium, or by inhibiting nitric oxide synthase with L-NAME ($p < 0.01$); cyclooxygenase inhibition (indomethacin) was without significant effect. Inhibition of the cyclic

nucleotides cAMP (ODQ) or cGMP (SQ) reduced ASA-induced uterine artery vasodilation by approximately 50 and 75%, respectively ($p < 0.05$). This is the first study to show a direct vasodilatory effect of ASA on uterine resistance vessels. Further, it supports low dose ASA therapy for preeclamptic pregnancy and suggests possible prophylactic or beneficial effects on the uteroplacental circulation.

S06-5 (O)

Salt-sensitive hypertension: role of vascular endothelial growth factor C and lymphangiogenesis

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Hypertension is generally recognized as a major risk factor for chronic kidney disease. Recent studies have shown that mononuclear phagocyte system cells exert homeostatic and blood pressure regulatory control via TonEBP/VEGF-C and NOS3 in cutaneous lymphatic capillaries, in salt-sensitive hypertension. Although renal lymphatic system has been implicated in renal inflammation in several kidney disease models; however, the implication of VEGF-C in renal lymphatic system in salt-sensitive hypertension remains unclear. The aim of this study is to unravel renal TonEBP/VEGF-C mechanism and its implication in salt-induced hypertension in mice. Thirty two BALB/c male adult mice were divided into four groups: sham, sham treated with VEGF-C and high-salt treated group with or without VEGF-C; VEGF-C was administered by subcutaneous injections. VEGF-C treatment reduced blood pressure and TNF- α plasma concentration in salt treated mice. In addition, both renal TonEBP and NOS3 expressions and activities were more pronounced in VEGF-C high-salt treated group. Skin lymphatic capillary density increased with high salt and to a greater extent with VEGF-C. Finally, kidneys from both high-salt and VEGF-C groups presented mononuclear infiltrates and increased lymphatic capillary density associated with renal impairment, as assessed by proteinuria; however, the latter was significantly ameliorated under VEGF-C treatment only. In conclusion, TonEBP/VEGF-C signaling pathway seems to exist in the kidney. It reduces blood pressure and preserves renal function in salt-sensitive hypertension via the induction of renal lymphangiogenesis and NOS3 expression. VEGF-C might constitute a potential treatment for hypertension and kidney function impairment.

Symposium 07: Recent advances in molecular physiology: metabolomics and beyond

S07-1

The Role of Metabolic Profiling in Cardiovascular Medicine

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Man is a complex ecosystem with thousands of biochemical processes working together through time to maintain health. In order to understand the biology of man and to intervene in an appropriate manner and time to prevent disease, we require knowledge of how the body works at the level of genes, proteins and metabolites. The metabolic phenotype can provide a window onto dynamic biochemical responses to physiological and pathological stimuli. Metabolic profiling strategies for analyzing biosamples, encompassing high-resolution spectroscopic methods (NMR spectroscopy, LC-MS, GC-MS etc) in combination with multivariate statistical modelling tools, have been shown to be well-suited to generating metabolic signatures reflecting gene-environment interactions with several applications in cardiovascular disease (CVD). Spectroscopic analysis has been applied across a wide range of studies with the aim of characterizing classes of disease, different physiological states or response to particular therapies and the natural extension is to derive predictive models for metabolic response from a baseline profile. The complexity and interactive nature of biological systems can

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Symposia

introduce confounding variation into the metabolic profile data. Methods for characterizing the metabolic consequences of biological processes associated with CVD will be discussed with particular emphasis on accommodating extraneous variation and optimizing biomarker recovery. Additionally a framework for predicting response to interventions at the individual level will be presented and examples drawn from a selection of laboratory and clinical studies.

S07-2

Gut microbiota and derived metabolites in metabolic disorders

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The human body is hosting at least 100 trillion bacteria, which constitute an extremely rich and diverse microbiota with a large fraction hosted by the gut. The human being is thus the result of a mutualistic association between gut microbiota and its own biology. An unbalance of the gut microbiota or dysbiosis has been demonstrated in a variety of tidily-linked human diseases, including metabolic (obesity, diabetes), cardiovascular or immuno-inflammatory disorders. A factor very frequently found related to dysbiosis is the loss of gut microbiome richness. Our team has shown that some obese people with a loss of gut bacterial richness, that had more cardio-metabolic risk factors (insulin resistance, dyslipidemia, increased low-grade inflammation in blood or tissues), improved less these risk factors with a restrictive diet but rich in fibers. This phenotype of loss of bacterial richness appears even worse in individuals with severe or morbid obesity. In addition, particular species, such as *Akkermansia muciniphila* and *Faecalobacterium prausnitzii*, are also correlated with better metabolic health. Dietary interventions or bariatric surgery can improve gut microbiome diversity and drastically change the gut microbiota ecosystem. The change of some bacterial species are linked to improved cardiometabolic risk factors. However, a lot remains to understand the bidirectional interactions between gut microbiote and host metabolism. Recent data from our laboratory showed that some metabolites associate with loss of gut bacterial richness. This presentation will review some recent discoveries in the field of cardiometabolic and nutrition-related diseases.

S07-3

Metabolomics approaches to study NAFLD

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Non-alcoholic fatty liver disease (NAFLD) is a major risk factor leading to chronic liver disease, liver failure, and Metabolic Syndrome. Here we integrate global transcriptome from human liver biopsies and metabolic fluxes measured across the human splanchnic vascular bed within a genome-scale model of human metabolism to chart liver metabolic activity and functionality in NAFLD. The genome-scale metabolic modeling reveals that NAFLD is associated with reduced metabolic adaptability on a network level, that is, excess liver fat accumulation puts increasing demands on the liver to adaptively regulate metabolic responses to maintain the basic liver functions. We also show that increased amount of liver fat induces mitochondrial metabolism, lipolysis and a relative increase of glycerol as a substrate for gluconeogenesis. Failure to meet excessive metabolic challenges coupled with the reduced metabolic adaptability may lead to a vicious pathogenic cycle leading to the co-morbidities of NAFLD.

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Symposia

S07-4 (O)

EFFECTS of TUMOR NECROSIS FACTOR ALPHA INHIBITION on STREPTOZOTOCIN-INDUCED MITOCHONDRIAL DAMAGE in PANCREATIC β - CELLS

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Although rheumatoid arthritis (RA), an autoimmune disorder, is effective on joints, it might affect other parts of body as well. The exact mechanism of RA is not understood. But, inflammation may be most important for the development of it and also some diseases e.g. diabetes mellitus. Type-1 diabetes is one of the most common chronic autoimmune disease characterized by the loss of insulin-producing beta cells. Tumor necrosis factor alpha (TNF- α) stimulates the inflammation response and apoptotic cell death. Prevention of TNF- α is considerably successful for the treatment of RA. Moreover, TNF- α may modulate hyperglycemia both RA and diabetes mellitus patients. The aim of this study was to investigate whether TNF- α inhibition by adalimumab, etanercept, or golimumab could prevent pancreatic beta cell apoptosis via mitochondrial damage and therefore could maintain insulin secretion. Human β -cell line (1.1B4) was used to conduct 5 groups as: control (C), Diabetes (D), Diabetes+Adalimumab (10 μ g/mL; DA), Diabetes+Etanercept (5 μ g/mL; DE), Diabetes+Golimumab (10 μ g/mL; DG). After diabetes induction by streptozotocin (20 mM), for 4 hours, cells were incubated with TNF- α inhibitors for twenty-four hours. Mitochondrial membrane potential (MMP), active caspase-8 levels, insulin secretion, and actin filament distribution were evaluated. Diabetes probably led to energetic stress by depolarization of MMP. On the other hand, TNF- α prevention ameliorated the MMP depolarisation. Caspase-8 activation in diabetic cells has been prevented by TNF- α inhibitors, therefore they were effective to protect the β -cells. Inhibition of TNF- α enhanced insulin secretion by the preservation of actin filaments; while the secretion was attenuated by diabetes. TNF- α inhibition might prevent the β -cells lose by decreasing apoptosis via restoration of MMP and might prevent the decrease in insulin secretion.

S07-5 (O)

Effect of hypoxia on adiponectin pathway in murine and cellular models: which involvement in COPD-associated cardiovascular risk?

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Introduction: Modulation of adiponectin plasmatic level (Adpl) was previously found to vary differently in COPD (Chronic Obstructive Pulmonary Disease) patients, probably due to the heterogeneity of the disease. Hypoxaemia is a component of respiratory diseases frequently observed in severe COPD. It initiates compensatory mechanisms mainly mediated by a family of transcription factors (Hypoxia Inducible Factors HIFs). Hypoxaemia was suggested to modulate Ad pathway. Due to its anti-diabetic, anti-inflammatory and anti-atherosclerotic properties, we postulate that alteration of Ad pathway could participate to metabolic troubles and cardiovascular co-morbidities in COPD patients.

Methods and Results: To better understand the specific impact of hypoxaemia on Ad pathway, we used a mouse model of chronic hypoxaemia (FiO₂ 10%, 8h/day). Exposure to hypoxaemia for 35 days resulted in (i) an increased level of high MW multimers, considered as the most active forms and (ii) a reduced Ad receptor (AdipoR1/2) abundance in muscle without any changes in their mRNA expression.

Since these alterations could impact the atherogenic risk, we evaluated AdipoR abundance in RAW murine macrophages exposed to low oxygen level (2%, 24h). After verification of HIF 1 α induction, we

demonstrated that exposure to hypoxia induced a reduced AdipoR1/2 level. This deregulation was associated with a reduced p-AMPK abundance, a regulator of metabolic homeostasis involved in Ad pathway. Investigations on hallmarks of cardiovascular risk are ongoing.

Conclusion: Hypoxaemia modifies Ad forms distribution and causes AdipoR modulation both *in vivo* and *in vitro*. These troubles could participate to pathophysiological mechanisms linked to COPD co-morbidities.

Symposium 08: Pain induced by local acidosis

S08-1

Pain induced by tissue acidosis

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The occurrence of local acidosis in inflammation is known since 1930. The metabolic acidosis is not sufficiently oxygenated but metabolically active tissue plays a pathophysiological relevant role in the brain, in the heart, in tumours, in intermittent claudication, in the eroded gastric mucosa and, more general, in inflammation. Hypoxia leads to a pH drop, the extent of which, even without synergism with other factors, may be sufficient to sensitize and activate nociceptive neurons and trigger pain. As molecular mechanisms, acid sensing ion channels (ASIC, 1997), the proton sensitive capsaicin receptor TRPV1 (1997) and more recently the chemo-sensitive ion channel TRPA1 was described (2014). For TRPA1 substantial species are known, and this extends to the exclusive pH sensitivity of the human TRPA1. Further, many ion channels have a reduced conductivity in an acidic medium, which could act excitatory e.g. in the case of potassium channels contributing to the resting membrane potential. Considering this, a study testing the discussed ion channel targets was conducted. Acidosis pain was induced by a three minute continuous intradermal infusion of an extracellular solution buffered at pH 4.3. Reported pain levels rapidly subsided after the end of infusion. However, coinfusion of TRPV1 antagonist BCTC, TRPA1 antagonist A-967079 or amiloride did not alter the pain levels, questioning the hypothesised targets at least in the skin. Further investigation of the few remaining targets, which still lack good pharmacological tools, is necessary to elucidate this essential mechanism causing pain.

S08-2

Oligodendrocyte acidification contributes to TRPA1-mediated damage in ischaemia

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Oligodendrocytes wrap myelin around axons to increase the conduction velocity of the action potential. Failure to myelinate, damage to myelin, or loss of oligodendrocytes leads to severe debilitating mental and physical impairment. Myelin damage during ischaemia was previously thought to be mainly by activation of oligodendrocyte ionotropic glutamate receptors, which allow a detrimental influx of calcium. By patch-clamping oligodendrocytes to record their currents, whilst imaging their intracellular ions with ion sensitive dyes, and applying solution which mimics the conditions found in ischaemia, we found that most of the glutamate-mediated current into oligodendrocytes is indirect and caused by a rise in extracellular potassium concentration. Oligodendrocytes respond to raised extracellular potassium concentrations by acidifying their cytosol; this in turn activates TRP channels. Evidence suggests that these proton-activated TRP channels are TRPA1 because the response is inhibited by specific TRPA1 channel blockers (HC 030031 and A965079), and genetic TRPA1 knock out.

The calcium influx through the proton-activated TRP channels leads to separation of myelin lamellae, which may contribute to the decrease in conductance of the action potential observed during ischaemia. As such TRPA1 represents a possible new therapeutic target that may decrease white matter damage in ischaemia.

S08-3

Pharmacological modulation of TRPA1 for the treatment of neuropathic pain and neurological disease modification

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TRPA1 is a calcium permeable nonselective cation channel that was initially thought to be exclusively expressed in peripheral peptidergic sensory neurons. However, subsequent studies have revealed a much wider tissue distribution of TRPA1 including principal neurons, astrocytes and oligodendrocytes in the CNS and in several other cell types in the periphery.

A large number of natural, synthetic and endogenous compounds activate TRPA1 through intracellular redox modulation. Intracellular calcium may activate and desensitize TRPA1. Calcium-dependent desensitization limits the amount of depolarization and calcium influx.

TRPA1 activation is a well-established mechanism to cause pain, itch and neurogenic inflammation in animals and human. A recent human genetic study suggests that excessive TRPA1 activation contributes to a number of neurological and non-neurological disease symptoms.

Several preclinical studies suggest that central TRPA1 activation is involved in the maintenance of neuropathic pain. Interestingly, sustained TRPA1 activation in experimental diabetes animal model was earlier shown to maintain persistent pain and contribute to development of peripheral neuropathy. TRPA1 antagonist attenuated neuropathic pain in phase 2 clinical trial. Elegant recent study showed that hypoxia-induced internal acidosis activates TRPA1 and contributes to oligodendrocyte toxicity and white matter injury.

Pharmacological TRPA1 modulation has potential for the treatment of neuropathic pain as well as disease-modification in numerous neurological conditions.

S08-5 (O)

Pain threshold evaluation requires to record the speed of stimulus intensity variation

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The term pain threshold refers to the measure of the intensity of a physical stimulus (usually applied by a tester) that evokes pain. To avoid the influence of the tester, there is a particular procedure, named autoalgotometry, in which the subject being evaluated applies and controls by himself the force against the autoalgotometer tip. The applied force is then recorded over time by a computerized device.

The aim of the present work was to evaluate possible correlations between speed of force variation and pain threshold in healthy subjects. Fifty healthy volunteers (21 males, 29 females, age 18-29 y) were evaluated for the pain threshold using the autoalgotometric procedure by applying fast- or slow-increasing forces on a computerized autoalgotometer tip (1.0mm in diameter) with their fingers and reaching a minimal or maximal pain intensity.

The results showed that there is a positive correlation between test speed and pain threshold measures. It was also found that male participants reached higher speeds compared to female

participants when asked to execute fast; accordingly, male participants showed higher pain thresholds (both for the minimal and the maximal pain intensity) compared to female participants in the fast tests. When the tests were executed slowly, the minimal pain threshold did not differ between males and females, but the maximal pain threshold was still higher in males compared to females.

These results highlights the importance of recording the speed of force application in a pressure pain-threshold evaluation and support the use of the autoalgotometric procedure for this purpose.

Symposium 09: Brute force and signaling: concepts in vascular mechanotransduction

S09-1

Piezo1 mechanical force sensor in the endothelium

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The sensing of physical forces arising due to blood flow is key in the maturation and remodeling of blood vessels required in embryonic development and adult life but the mechanisms by which the physical forces are sensed have been elusive. Is there a specific force sensing protein and, if so, how does it sense force and generate appropriate downstream signals? We have revealed how calcium ion-permeable non-selective cationic channels formed by assembly of Piezo1 proteins act as sensors of physiological force and determinants of vascular structure in both development and adult physiology (Li et al 2014 *Nature* 515, 279-282). Global and endothelial-specific disruption of mouse Piezo1 profoundly disturbed the developing vasculature and was embryonic lethal within days of the heart beating. The importance of Piezo1 channels as sensors of blood flow was shown by Piezo1 dependence of shear stress-evoked ionic current and calcium ion influx in endothelial cells and the ability of exogenous Piezo1 to confer shear stress sensitivity on otherwise resistant cells. Downstream of this calcium ion influx there was protease activation and spatial reorganization of endothelial cells to the polarity of the applied force. These and other new findings will be discussed. Supported by grants from the Medical Research Council UK and Wellcome Trust.

S09-2

Adjusting G-protein signaling to enable vascular smooth muscle cell phenotype changes during hypertension

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The increase in wall stress during chronic hypertension drives the remodeling of the media that eventually leads to arterial stiffening. Under these conditions, vascular smooth muscle cells (VSMCs) are exposed to an elevated level of biomechanical stretch which alters their phenotype. They stop maintaining their contractile and quiescent phenotype e.g. by inactivating the transcriptional coactivator myocardin and adjust signaling pathways to acquire a synthetic state. In this context, G-proteins such as Gq/11 may not only serve as transducers of biomechanical stimuli but also act as determinants of the VSMC phenotype switch. Their activity is tightly controlled by proteins known as regulators of G-protein signaling (RGS) which have the capacity to terminate the activity of G α -subunits. This lecture will focus on the role of RGS5 as determinant of the synthetic phenotype and RhoA activity of VSMCs exposed to biomechanical stretch or hypertension. While not much affecting blood pressure during homeostasis, RGS5 may in fact attenuate extensive arterial constriction in response to sympathetic Gq/11-mediated stimuli during hypertension and support RhoA-controlled adaptive arterial remodeling. Collectively, adjusting G-protein activity appears to be a prerequisite to orchestrate the responses of VSMCs to biomechanical forces evoked by hypertension.

S09-3

The response of the dysfunctional endothelium to elevated flow - implications for plaque disruption

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Atherosclerosis primarily occurs at bifurcations and curved sections of arteries, implicating local haemodynamics in the initiation and progression of disease. Endothelial mechanosensitivity, which translates the frictional force exerted by blood flow (shear stress) into a biological response, underlies this association. Endothelial cells in regions of the vasculature exposed to normal laminar flow adopt a quiescent anti-inflammatory phenotype that resists the development of atherosclerosis. This contrasts with cells exposed to disturbed flow, which triggers an increase in permeability, reduces the bioavailability of nitric oxide and amplifies the response to inflammatory mediators. As atherosclerosis develops, the endothelium overlying stenotic plaques can be exposed to very elevated shear stress, which depending on the degree of stenosis can be >15-fold higher than in non-diseased sections. The response of the endothelium to elevated shear stress has received little attention, despite stenotic plaques being more likely to suffer plaque rupture or endothelial erosion, the two principle causes of acute coronary syndromes. We particularly focus on endothelial erosion of plaques and have identified that endothelial erosion frequently occurs towards the throat of stenotic plaques, exposing the endothelium to elevated shear stress, and that elevated shear modifies endothelial behaviour. Histopathology has demonstrated an association of erosion with smoking, suggesting endothelial dysfunction may affect the response of the endothelium to elevated flow and contribute to detachment. In addition, our work implicates a hyperactivation of the antioxidant system as a potential contributor to this process.

S09-4 (O)

Endothelial cells are sensitive to shear stress via Wnt/Planar Cell Polarity pathway

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The aim of this study was to investigate the role of the Wnt/Planar Cell Polarity (PCP) pathway in the sensitivity of endothelial cells (EC) to shear stress. Experiments were done on carotid endothelium obtained from euthanatized wild type C57Bl6 mice, and on HUVEC cells cultured in fibronectin-coated capillaries and submitted, via a microfluidic device, to no flow, low "venous", and high "arterial" shear stress (0, 3 and 10 dynes.cm⁻², respectively), transfected with siRNA against Pdzrn3, Ror2 and Daam1. Cells were labelled for the nucleus (Hoechst) and the Golgi apparatus (Golphi4 and Golgin97 immunolabelling). Using confocal microscopy and video tracking, the following parameters were defined: cell polarity (nucleus lengthening and flattening), orientation (angle of the nucleus-Golgi vector with flow direction), and motility (velocity, tortuosity and directionality of cell movement). Comparisons were done using Kruskal Wallis and post-hoc Dunn testing, and chi2 test for angle distribution. In carotids, EC were polarized and oriented against blood flow direction. In vitro, CE polarity, orientation and motility were dependent on shear-stress intensity, with antidromic direction and motility for high shear stress, significantly altered by the inhibition of PDZRN3, ROR2 and DAAM1 expression. These results show that CE are spatially sensitive to venous and arterial shear stress, and that the Wnt/PCP actors PDZRN3, ROR2 and DAAM1 are implicated in this sensitivity.

S09-5 (O)

Investigating the role of Gβγ subunits in Kv7 dependent relaxations

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Questions: Kv7 channels are important regulators of vascular tone. Recently, native vascular Kv7 channels were shown to be regulated by Gβγ subunits. Here, we aim to establish the functional implications of G protein βγ subunits in Kv7 dependent relaxations of the rat vasculature

Methods: Relaxations to isoproterenol and calcitonin gene related peptide (CGRP) of the rat mesenteric and renal arteries were assessed by myography. Localisation of Kv7.4 and Gβ was studied using proximity ligation assay.

Results: Relaxations to isoproterenol in MA and RA, and CGRP in MA are Kv7 dependent. Relaxations to isoproterenol in rat renal, but not mesenteric arteries, were impaired in the presence of Gβγ inhibitors (gallein, M119K and Grk2i). Relaxations to CGRP in mesenteric arteries were sensitive to Gβγ inhibition. In MA myocytes treated with isoproterenol or CGRP there was an increase in Kv7.4-Gβ PLA puncta. Treatment of cells with gallein inhibited this increase, but did not affect basal puncta levels. In RA myocytes there is a higher level of basal Kv7.4-Gβ, but this does not increase with isoproterenol treatment. Gallein treatment decreases basal puncta levels in the RA.

Conclusions: Gβγ subunits are required for CGRP relaxations in MA and isoproterenol relaxations in RA, but not MA. The Kv7.4-Gβ interaction is implicated in mediating some vasorelaxant signals.

Symposium 10: Intracellular Ca²⁺-compartments in cardiac physiology and disease

S10-1

T-tubules in physiological and pathological intracellular Ca²⁺ dynamics

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Contraction of cardiomyocytes is dependent on sub-cellular structures called dyads, which are functional junctions between invaginations of the surface membrane (t-tubules) and the sarcoplasmic reticulum. Well-organized dyads enable efficient triggering of Ca²⁺ release during the action potential, and powerful contraction. Dyads are formed gradually during development, with progressive assembly of both t-tubules and sarcoplasmic reticulum and precise trafficking of Ca²⁺ handling proteins including the L-type Ca²⁺ channel and Ryanodine Receptor. During diseases such as heart failure, dyads are broken down with a reversion to an immature phenotype. Our data indicate that these alterations include both disorganization of t-tubules and dispersion of Ryanodine Receptor clusters; changes which reduce the efficiency of Ca²⁺ release. Elevated stress placed on the myocardial wall of the dilated, failing heart is a key trigger of disrupted dyadic structure as it signals reduced expression of the dyadic anchor junctophilin-2. However, other changes that occur during heart failure are compensatory, including the growth of new dyads in the longitudinal axis of the cell. Our data indicate that the membrane-bending protein BIN1 signals such dyadic growth. Thus, interventions which unload the heart and/or exploit the hearts inherent compensatory capacity to grow dyads can benefit heart failure patients.

S10-2

Mitochondrial redox regulation in heart failure

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In patients with heart failure (HF), defects in cardiac myocytes consist of alterations in calcium handling, energetic deficit and oxidative stress. Central to these defects are mitochondria, since they are the main source for cellular ATP, but also reactive oxygen species (ROS), and their function is tightly controlled by calcium. Over the past years, we have developed a concept that explains how in HF, an imbalance between ADP-induced acceleration of respiration and calcium-induced activation of the Krebs cycle results in net oxidation of mitochondrial pyridine nucleotides, where NADH and FADH₂ are required for ATP production at the respiratory chain and NADPH for the anti-oxidative capacity. While in systolic HF, the primary defects in calcium handling reduce mitochondrial calcium uptake, in hypertrophic cardiomyopathy, excessive ATP consumption increases ADP to accelerate respiration. The net oxidative stress accounts for necrosis, maladaptive cardiac remodeling, left ventricular dysfunction, arrhythmias and death. Therapeutic strategies aimed at re-equilibrating this imbalance may be useful to improve the treatment of patients with HF.

S10-3

Effect of Troponin Ca²⁺ Binding Properties on Myofibril Force Kinetics

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Ca²⁺ Troponin (Tn) interaction is often altered in myopathies and cardiomyopathies. We have engineered the Ca²⁺ binding properties of troponin C (TnC) to study the role of increased (I60Q sTnC, I61QcTnC) and decreased (M80Q sTnC, L48QcTnC) Ca²⁺ dissociation rate (k_{off}) on activation and relaxation of skeletal and cardiac muscle. Previously we reported that skeletal and cardiac myofibril force development kinetics (k_{ACT}) are not influenced by decreasing k_{off} from Tn, but are slowed by an increase in k_{off} (Kreutziger et al. 2008 JPhysiol. 586:3683-3700; Kreutziger et al. 2012 J Mol Cell Cardiol 50:165-174) at low [Pi] (5 μ M). The time to initiation of force (k_{lag}) following a rapid (~10ms) switch from pCa 9.0 to pCa 3.5 provides information about thin filament activation rate and our preliminary data suggest this rate may also be sensitive to k_{off} . In rabbit psoas and mouse ventricular myofibrils (15°C) k_{lag} is almost eliminated for M80Q sTnC and L48Q cTnC and significantly increased by I60Q sTnC and I61Q cTnC. Additionally, though k_{ACT} is similar for force increases from either full or partial activation, k_{lag} disappears when starting from partial activation. These experiments demonstrate a potential approach to study thin filament activation kinetics without the need for fluorescent probes attached to thin filament proteins that can affect their function.

S10-4 (O)

Spermidine feeding reduces high blood pressure and improves diastolic function in Dahl salt-sensitive rats

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Hypertension is an independent risk factor for the development of diastolic dysfunction and heart failure (HF). Caloric restriction (CR) reduces high blood pressures and the related decline in diastolic function. We hypothesized that dietary intake of natural CR mimetic spermidine (SPD) phenocopies the cardioprotective effects of CR. Dahl salt-sensitive rats (7-week-old males) were fed high-salt diet±3mM SPD (in drinking water) until the age of 14 or 19 weeks. We performed non-invasive blood pressure measurements, echocardiography, invasive hemodynamics, immunoblotting-based titin analysis, renal histology and chemical analytical methods. In humans, a correlational study was conducted between dietary spermidine intake and cardiovascular disease (N=800, Bruneck Study). The increased plasma level of SPD was associated with reduced blood pressure, arterial elastance, left ventricular (LV) mass, relative lung and liver weights as well as enhanced ventricular-vascular coupling and LV diastolic function in SPD-treated rats. SPD feeding resulted in reduced levels of ornithine (spermidine precursor) and greater bioavailability ratio of global arginine (the only source for the vasodilator nitric oxide), reduced plasma levels of TNF α , increased phosphorylation of the titin isoform N2B and the protein kinase G-dependent titin phosphosite S4080, coinciding with reduced cardiomyocyte stiffness. SPD also reduced hypertensive renal damage determined by lower urinary lipocalin-2 levels, fibrosis and glomerulosclerosis. In humans, higher SPD intake was associated with reduced blood pressure and risk of HF. Our results suggest that dietary polyamine spermidine ameliorates hypertension and prevents diastolic dysfunction.

S10-5 (O)

Towards the role of store-operated Ca²⁺ entry in skeletal muscle physiology

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Extracellular calcium (Ca) influx does not significantly contribute to muscle twitch generation but is involved in muscle development, fatigue resistance and ageing and contributes to the dysregulation of Ca in malignant hyperthermia, muscular dystrophy, and other types of myopathies.

Unfortunately, the currently available experimental techniques do not allow to sufficiently study these important processes, in particular under physiological conditions.

Here we report on a novel microscopy based technique that allowed us to track the very small Ca-fluxes across the sarcolemma at high sensitivity and temporal resolution overcoming previous experimental limitations. We found that physiologically stimulated skinned rat *extensor digitorum longus* muscle fibres exhibited a fast transient trans-sarcolemma Ca-flux upon each single action potential (AP). The flux was activated rapidly within 18 ms. Inhibition of the ryanodine receptor by tetracaine abolished sarcoplasmic reticulum (SR) Ca-release and largely reduced the sarcolemma Ca flux providing strong evidence for an underlying store-operated Ca entry (SOCE) mechanism. Varying the tetracaine concentration and thus the amount of Ca released from the SR revealed a distinct threshold of SR Ca release that was necessary for SOCE activation. In contrast, when the amount of Ca released from the SR was increased SOCE rose gradually to reach a maximum of about 3 μ M (with respect to cytosolic volume) during a single AP.

In conclusion, we provide the first measurements and description of SOCE during a single AP in mammalian skeletal muscle fibres.

Symposium 11: Pancreas: Physiology and disease

S11-1

Pancreatic cancer: a case of lost identity.

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Pancreatic ductal adenocarcinoma (PDAC) accounts for 95% of pancreatic cancer cases. Mainly due to its morphology, it was long considered to originate from normal ductal cells. However, the analysis of genetically engineered mouse models of PDAC revealed that acinar cells expressing an oncogenic mutant form of KRas can generate ductal lesions, through acinar-to-ductal metaplasia. Interestingly, acinar cells are sensitive to mutant KRas during embryo development, but become refractory in adult life, suggesting that differentiation represent a tumor-suppressive mechanism. Consistently, de-differentiation of acinar cells towards a progenitor-like phenotype is observed during pancreatitis, a major risk factor for PDAC development. Maintenance of full differentiation is crucial also during tumor progression. Attenuation of the epithelial and pancreatic phenotype in PDAC cells, accompanied by the acquisition of non-pancreatic and more mesenchymal features, correlates with increased metastatic potential and worse prognosis in patients. We have revealed that the transcription factor GATA6 is essential for the maintenance of the full differentiation of acinar cells, and inhibits the epithelial-to-mesenchymal transition in PDAC. As a consequence, GATA6 suppresses tumor initiation and progression, by locking epithelial cells in a well-differentiated state. In accordance to this hypothesis, GATA6 expression is lost in a subset of tumors with altered differentiation and worse outcome.

S11-2

Multiple roles of purinergic signalling in pancreas

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The pancreas is an organ with a central role in nutrient breakdown, nutrient sensing and release of hormones regulating whole-body nutrient homeostasis. Various cells — endocrine, exocrine, stromal and immune cells contribute to the integrated function of pancreas. Unfortunately, pancreas is also a site of several serious diseases including pancreatitis, cystic fibrosis, diabetes and pancreas cancer. The aim of our research is to gain understanding how various cells release ATP and utilize it as a short-range signal between various cells and how these processes are dys-regulated in various diseases. In pancreatic acini the intracellular ATP is accumulated in secretory zymogen granules by the vesicular nucleotide transporter. Following physiological stimuli, ATP is released by exocytosis into the lumen of pancreatic ducts. In pancreatic ducts ATP is released by other mechanisms and one of the triggers relevant to pancreatitis are bile acids. Within pancreatic ducts, ATP and adenosine act via specific purinergic receptors to regulate duct secretion by activating specific ion channels. Pancreatic damage or highly metabolically active cancer cells can lead to substantial release of ATP which can have detrimental effects. Our current studies address the role of the multifunctional purinergic P2X7 receptor in the pancreatic ductal adenocarcinoma. We find that both in duct cancer cells and fibrogenic pancreatic stellate cells, the purinergic P2X7 receptor has crucial functions in cell survival and behavior and may be considered as a relevant therapeutic target.

S11-3

Pharmacological targeting of cell type identity in the endocrine pancreas

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The pancreatic islets of Langerhans are composed of at least five distinct endocrine cell types that develop from a common progenitor cell under the direction of master regulatory transcription factors. Mouse genetics has shown that the mis-expression of specific transcription factors from other lineages causes the transdifferentiation of these cell types. For example, alpha cells convert into beta-like cells in vivo upon expression of the beta-cell factor Pax4 or upon loss of the alpha cell factor Arx.

Master regulatory transcription factors are powerful mediators of cellular transdifferentiation, yet their protein structures make them hard to target pharmacologically, to the extent that they are often deemed "undruggable". We use chemical, functional genomic and genetic screening to identify compounds and additional targets for the induction of insulin expression in alpha cells. Recently, we discovered the antimalarial compound class of artemisinins to impair alpha cell identity, by affecting GABA receptor signaling and glucagon secretion. Here we will describe the molecular mechanism of action of these compounds, as well as other protein targets in alpha cell transdifferentiation.

S11-4 (O)

Reversal of premature aging markers after bariatric surgery

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Background: Obesity is considered to be a major risk factor in developing cardiac disease. In addition, obese patients suffer from a premature aging phenotype including increased secretion of senescence associated secretory proteins (SASP) and reduced telomere length compared to healthy controls.

Methods: We enrolled 56 patients undergoing bariatric surgery. Blood samples were taken before and 24 months after surgery. Markers of premature aging including the SASP IL6 and PAI-1, and IL10 as well as telomere length and telomere oxidation were evaluated.

Results: Overall, patients showed a significant drop of body mass index. In addition plasma levels for IL6 and PAI-1 were significantly reduced after surgery. We found increased plasma levels for IL10. In addition, telomere length on average increased by 58% in the patient cohort (0.37 ± 0.28 a.u. before versus 0.59 ± 0.28 a.u. after bariatric surgery, $p < 0.001$). The telomere increase was accompanied by a reduction in the telomere oxidation index (2.86 ± 4.4 before versus 0.78 ± 0.56 after bariatric surgery, $p < 0.001$) indicating reduced oxidative stress for the telomeric region. This is further supported by an inverse correlation of telomere length with telomere oxidation at both time points ($r = -0.376$, $p < 0.001$ pre surgery and $r = -0.705$, $p < 0.001$ post surgery).

Conclusion: Our data indicate a significant reduction of the SASP IL6 and PAI-1 in plasma after bariatric surgery. We observed an increase in telomere length in this setting. However, given the reduction in oxidative stress at telomeric regions we speculate that the increased telomere length is not due to active elongation but due to reduced breakage caused by telomere oxidation.

S11-5 (O)

The impaired function of the plasma membrane Ca^{2+} pump causes Ca^{2+} overload and cell damage in CFTR knock out pancreatic ductal cells

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Introduction: The cystic fibrosis transmembrane conductance regulator (CFTR) has a major role in pancreatic ductal secretion and its genetic defects damage the pancreas. It is known that intracellular Ca^{2+} homeostasis is disturbed in bronchial epithelial cells in cystic fibrosis (CF), but the connection of CFTR and the intracellular Ca^{2+} signaling has never been suggested in pancreatic damage in CF before.

Aims: Our aim was to characterize the Ca^{2+} homeostasis of CFTR-deficient PDEC.

Materials&methods: Wild type (WT) and CFTR knockout (KO) mouse pancreatic ductal and acinar cells and human CF pancreatic cell line (CFPAC-1; ΔF508 mutant) were used for intracellular Ca^{2+} measurements. Mitochondrial membrane potential ($\Delta\Psi\text{m}$) and mitochondrial morphology was assessed in isolated pancreatic ducts. Immunofluorescent staining and quantitative PCR measurements were performed to detect changes of protein expressions.

Results: The plateau phase of the agonist-induced Ca^{2+} signal was elevated in CFTR-deficient PDEC, which was caused by decreased function of the plasma membrane Ca^{2+} pump (PMCA). The functional inhibition of CFTR has no effect on the PMCA activity. Similarly native CFPAC-1 cells and PDEC treated with siRNA to inhibit the expression of CFTR showed the same PMCA dysfunction. Viral transfection of CFPAC-1 with CFTR gene completely restored PMCA function. Sustained $[\text{Ca}^{2+}]_i$ levels decreased $\Delta\Psi\text{m}$ and induced cytochrome c release in CFTR KO PDEC without significant alterations in mitochondrial morphology.

Conclusion: Dysfunction of PMCA leads to disturbed Ca^{2+} homeostasis in CFTR-deficient PDEC and the consequent cellular Ca^{2+} overload impairs mitochondrial function which might contribute to the pancreatic damage in CF.

Symposium 12: Current trends in cell therapy for functional recovery of the diseased heart

S12-1 Cardiac Bone Marrow-Derived Cell-based Therapy associated with scaffold for Heart Repair

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The development of patched associating of bone marrow-derived cells (BMDC) and a substrate/scaffolds provided evidence of beneficial outcomes and optimal delivery approaches on myocardial infarction (MI). Our research focuses on both the importance of the cell origin and of the scaffold.

We have investigated in vivo a variety of scaffold associated with BMDC using a subchronic MI rat model and we report here our results.

In addition, because preclinical trials are usually performed with BMDC isolated from healthy donor although, for clinical application, BMDC isolated from infarcted patients are the main autologous cell source, we hypothesized that the therapeutic capacity of the implanted biological patch may vary with BMDC origin. We compared the regenerative potential of a biological patch composed of BMDC isolated from a healthy or infarcted donor as a treatment of MI in a rat model.

We report our results and give some insight on the patch presenting the best outcomes.

S12-2 Excitation-Contraction Coupling Plasticity in Pluripotent Stem Cell-Derived Cardiac Myocytes

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Calcium regulation by the sarcoplasmic reticulum (SR) is a fundamental property of heart muscle that ensures efficient excitation-contraction (EC) coupling. SR calcium cycling is fully functional in the adult healthy myocardium but poorly utilised during development and disrupted during cardiac disease, indicating that SR contribution is a highly regulated and plastic function. Leveraging on the naïve and plastic properties of human induced pluripotent stem cell-derived cardiac myocytes (iPSC-CMs), we studied the effects of multicellular patterns and extracellular matrix (ECM) on the development of SR calcium cycling. We found that culture with human fibroblasts affects the EC coupling machinery in iPSC-CMs. Our data suggest the importance of heterocellularity and the ECM in the development of specialised features, particularly EC coupling, of adult cardiac muscle in naïve cardiac cells. This is not only relevant for the applications of iPSC-CMs in translational medicine and cardiovascular research but also to understand and target the plasticity of the EC coupling machinery in physiological conditions and during cardiac disease.

S12-3 Generation and functional characterization of human induced pluripotent stem cell-derived pacemaker cell clusters

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Questions: Cell-based biological pacemakers aim to overcome technical limitations and potential side effects of electronic pacemaker devices. We sought to develop a novel approach for subtype-specific generation of pacemaker-type cells from human induced pluripotent stem cells (hiPSC).

Methods: hiPSC were differentiated into spontaneously beating clusters by co-culturing with visceral endoderm-like cells for 10-12 days and further culturing in a specified medium for up to 8 weeks, followed by cellular in-depth characterization.

Results: After 8 weeks of culture, clusters showed spontaneous beating rates (79.3 ± 3.1 beat/min, $n=10$) comparable to the human sinoatrial node (SAN) and were thus designated as pacemaker cell clusters (PCC). They exhibited abundant expression of pacemaker hallmark genes (*Tbx3*, *Tbx18*, *HCN4*), while myocardial markers were downregulated, indicating nodal-type differentiation. Whole-cell voltage-clamp recordings showed action potentials (APs) with nodal- or atrial-like characteristics, while ventricular-like APs were not detected. Treatment with ivabradine resulted in significant rate lowering and stimulation with isoproterenol increased rate demonstrating chronotropic rate response. When co-cultured with neonatal rat ventricular myocytes (NRVM), PCC determined beating rate of co-cultures that significantly exceeded rate of NRVM monolayers.

Conclusion: We developed a virus-free, selective protocol that produces spontaneously beating cell clusters with SAN characteristic from hiPSC, providing an *in vitro* platform for disease modelling, drug testing and the future generation of patient-specific biological pacemakers.

S12-4 (O)

Monomeric adiponectin modulates nitric oxide release and calcium movements in porcine aortic endothelial cells in normal/high glucose conditions.

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Questions: Perivascular adipose tissue can be involved in the process of cardiovascular pathology through the release of adipokines, namely adiponectins. Monomeric adiponectin was found to increase coronary blood flow in anesthetized pigs through increased nitric oxide (NO) release and the involvement of adiponectin receptor 1 (AdipoR1). The present study was planned to examine the effects of monomeric adiponectin on NO release and Ca²⁺ transients in porcine aortic endothelial cells (PAE) in normal/high glucose conditions and the related mechanisms. METHODS: PAE were treated with monomeric adiponectin alone or in the presence of intracellular kinases blocker, AdipoR1 and Ca²⁺-ATPase pump inhibitors. The role of Na⁺/Ca²⁺ exchanger was examined in experiments performed in zero Na⁺ medium. NO release and intracellular Ca²⁺ were measured through specific probes. RESULTS: In PAE cultured in normal glucose conditions, monomeric adiponectin elevated NO production and Ca²⁺. Similar effects were observed in high glucose conditions, although the response was lower and not transient. The Ca²⁺ mobilized by monomeric adiponectin originated from an intracellular pool thapsigargin- and ATP-sensitive and from the extracellular space. Moreover, the effects of monomeric adiponectin were prevented by kinase blockers and AdipoR1 inhibitor. Finally, in normal glucose condition, a role for Na⁺/Ca²⁺ exchanger and Ca²⁺-ATPase pump in restoring Ca²⁺ was found. CONCLUSIONS: Our results add new information about the control of endothelial function elicited by monomeric adiponectin, which would be achieved by modulation of NO release and Ca²⁺ transients. A signalling related to Akt, ERK1/2 and p38MAPK downstream AdipoR1 would be involved.

S12-5 (O)

TRPC – NFAT signaling inhibition mediates the cardiac anti-fibrotic effect of polyphenols

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Cardiac fibroblasts (CF) play a major role in myocardial fibrosis. Transient receptor potential canonical (TRPC) channels are non-selective calcium channels present in CF and implicated in cardiac remodeling. However, limited understanding of these cells impedes the development of potential therapies for cardiac fibrosis. Polyphenols have been shown to improve health and to decrease the incidence of heart disease progression. The aim of our study is to assess the effect of polyphenols on the TRPC3-mediated signaling in CF and their potential anti-fibrotic role. Isolated rat CF exhibited increased NFAT activation that was inhibited by polyphenols and cyclosporine. This inhibitory effect was also noted on cell proliferation and migration as well as on inflammation and fibrosis-associated markers, in a non-cytotoxic manner. These cellular events were caused by a decrease in basal cytosolic calcium, along with a run down in angiotensin II/OAG calcium entries. TRPC3 protein decrease was responsible of the reduction in calcium entries; indeed, blocking specifically TRPC3 resulted in a similar calcium entry block. In addition, a cross-talk between NFAT and TRPC3 was demonstrated, and blocking either one was behind the polyphenols anti-fibrotic effect. *In vivo*, in an L-NAME hypertension rat model, polyphenols were able to reduce cardiac hypertrophy and normalize fractional shortening, as confirmed by histopathology and plasma stress biomarkers. This functional improvement was correlated with the decrease in TRPC3-NFAT signaling pathway activation at the level of CF. In conclusion, these findings suggest that TRPC-NFAT inhibition by polyphenols may represent an important cardiac anti-fibrotic therapeutic strategy.

Symposium 13: Place navigation in dynamic world in healthy and disordered brain: focus on cognitive coordination and behavioral flexibility

S13-2

Spatial memory and cognitive control and flexibility deficits in animal models of schizophrenia and obsessive-compulsive disorder

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Spatial memory is an ubiquitous mechanism for localizing spatial goals in the environment. Cognitive control refers to the ability to select relevant and appropriate sensory stimuli and behavioral actions. Animals and humans face abundance of incoming information, some relevant or important, and other useless or even distracting. Based on experience, subjects must attend to the former and ignore the latter in order to choose the appropriate behavior. Both spatial navigation and cognitive control are significantly impaired in several disorders of the central nervous system. This lecture will discuss deficits in these processes in schizophrenia and obsessive-compulsive disorder and show a convergence of preclinical studies done in rodents and clinical studies with patients and healthy controls. This work was supported by AZV grants 15-34524A and 17-30833A, GACR grants 17-04047S and 16-13399S and GACR center of Excellence P304/12/G069. Institutional support for IPHY was provided by RVO: 67985823. Institutional support for NIMH-CZ was provided by the project "Sustainability for the National Institute of Mental Health", under grant number LO1611, with a financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the NPU I program.

S13-3

Impaired cognitive coordination and behavioral flexibility in first episode schizophrenia patients: navigation in dynamic environment.

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The assessment of cognitive functions represents a crucial step in the diagnostics and in therapy of mental disorders such as schizophrenia. In order to produce test methods applicable in comparative studies we have designed a human analogue of an animal task performed on a rotating arena that demonstrated its sensitivity towards cognitive changes observed in animal models of schizophrenia. The novel virtual Carousel Maze Task (vCMT) is aimed at spatial learning and cognitive coordination processes. In contrary to the original avoidance paradigm in animals, human subjects are performing in a preference task. The virtual analogue thus requires them to find and remember several hidden goal positions placed on an open arena rotating in a rectangular room. These target positions are bound either to the rotating arena or to the stable room reference frame. The vCMT task is composed of four phases, representing carousel maze variants with growing difficulty level. First-episode schizophrenia patients show in comparison to matched healthy controls deficit of spatial cognition and mental flexibility tested in dynamic environment of the vCMT task. Performance impairment observed in patients supports cognitive deficit assessed using standardized paper-pencil tasks. Our results correspond well with studies testing animal models of schizophrenia in comparable real task variants. Based on our findings, we propose the vCMT task as a screening for spatial cognition in translational clinical studies.

The study was supported mainly by Ministry of Health of the Czech Republic, grant nr. AZV 17-30833A and by the project nr. LO1611 with a financial support from the MEYS under the NPU I program.

S13-4 (O)

The relationship between heart rate variability and symptoms severity in children with autism spectrum disorders

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Introduction: Diagnosis of autism spectrum disorders (ASD) is based exclusively on expert observation and assessment of behavior and cognition, not etiology or biological marker. Although diagnosis of ASD is based on behavioral assessment, various physiological measures, such as heart rate variability have also been used to look for the neurological or autonomic dysfunctions underlying ASD. The aim of this study was to determine the relationship between heart rate variability and severity of autism, including symptoms observed in boys with autism spectrum disorder.

Methods: Study sample included children 35 with Autistic Disorder and 21 neurotypical children matching chronological age. To assess severity of disorder and symptoms of autism, Autism Diagnostic Observation Schedule- second revision was performed by trained professional. Heart rate variability parameters, measured in supine position in duration of 3 minutes were further analyzed.

Results: Statistically significant differences between children with autism spectrum disorder and neurotypical children in heart rate variability parameters were observed. In addition, we found significant negative correlation between overall standardized ADOS-2 score and Social affect score with heart rate variability parameters representing the function of parasympathetic nervous system.

Conclusion: Our results suggest atypical autonomic function in children with autism spectrum disorder. Moreover, the study supports the relationship of abnormal autonomic regulation with social skills and overall severity level of autism spectrum disorders.

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S13-5 (O)

Interacting Networks for Time Perception and Working Memory

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Questions: Time is an important concept which determines most human behaviors, however questions remain about how time is perceived and which areas of the brain are responsible for time perception. The aim of this study was to evaluate the relationship between time perception and working memory in healthy adults.

Methods: Functional magnetic resonance imaging (fMRI) was used during the application of a visual paradigm that comprises of four different tasks: Control, time perception, working memory and dual tasks. During different conditions, participants (n = 15, eight male) responded according to the instructions.

Results: The results showed activations in right dorsolateral prefrontal and right intraparietal cortical networks, together with the anterior cingulate cortex (ACC), anterior insula and basal ganglia (BG) during time perception. On the other hand, working memory engaged the left prefrontal cortex, ACC, left superior parietal cortex, BG and cerebellum activity. Both time perception and working memory were related to a strong peristriate cortical activity. On the other hand, the interaction of time and memory showed activity in the intraparietal sulcus (IPS) and posterior cingulate cortex (PCC).

Conclusions: The PCC might play a major role as a connection hub between lateralized frontoparietal networks and subcortical brain regions like AIC and BG. Altogether, results support a

distributed neural network based model for time perception and that the intraparietal and posterior cingulate areas might play a role in the interface of memory and timing.

Symposium 14: Cardiovascular oscillations: from signal to physiological interpretation

S14-1

Simultaneous characterization of sympathetic and cardiac arms of the baroreflex during incremental head-up tilt

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Questions. During orthostatic challenge venous return is reduced, as a result of blood pooling in the legs, and cardiac output tends to decrease, thus resulting in a possible arterial pressure (AP) drop and consequent orthostatic syncope. To counteract this tendency sympathetic and cardiac baroreflex controls are activated leading to tachycardia and peripheral vasoconstriction respectively. We tested the hypothesis that cardiac baroreflex sequence analysis (Bertinieri G et al, J. Hypertens. 3, S79-S81, 1985) can be extended to characterize sympathetic arm of the baroreflex as well.

Methods. We extended the traditional sequence approach devised to typify the cardiac baroreflex from spontaneous fluctuations of systolic AP (SAP) and heart period (HP) to the analysis of diastolic AP (DAP) and sympathetic discharge variabilities. In a group of 12 healthy young humans we recorded integrated muscle sympathetic nerve activity via microneurography technique from peroneal nerve, invasive AP from the radial artery, electrocardiogram (lead III) and respiratory movements during incremental head-up tilt with table inclination sequentially increased from 0 to 60°.

Results. The absolute value of cardiac and sympathetic baroreflex sensitivity decreased in proportion to the challenge, while the percentage of both cardiac and sympathetic baroreflex sequences increased. Moreover, the fraction of DAP variations evoking opposite-sign changes of sympathetic discharge and that of SAP variations evoking same-sign HP changes augmented, thus indicating a significant cardiac and sympathetic baroreflex activations.

Conclusions. Sequence analysis can be exploited for the simultaneous characterization of cardiac and sympathetic arms of the baroreflex in healthy humans.

S14-2

Cardiorespiratory interactions are responsible for both mechanical and nervous cardiovascular oscillations

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Questions:

Cardiovascular oscillations are part of a healthy circulation. Respiratory sinus arrhythmia (RSA) is a major part of heart rate variability. We investigated if pulmonary stretch receptors or central feed forward mechanism is the main cause of RSA.

Methods:

In 19 young and healthy subjects we measured RSA during spontaneous breathing and supported

ventilation by non-invasive intermittent positive pressure ventilation (NIV). Supported ventilation provides similar stretch to pulmonary receptors during inspiration, but reduces the subjects initiation of breathing and therefore reduces the central feed forward mechanism. RSA was quantified by Fourier spectral analysis as the area under the curve at 0.15-0.40 Hz.

Results:

RSA was reduced by 60% (95% confidence interval 48%-74%) during NIV as compared to spontaneous breathing. In 13 subjects, RSA was reduced by more than 50 % during NIV, indicating that central feed forward mechanism was the main contributor to RRSA. In 3 subjects, RSA was unchanged during NIV, indicating that pulmonary stretch receptors were the main contributor to RSA. In 3 subjects, RSA was reduced by 30-40% during NIV.

Conclusion:

In a majority of healthy subjects, RSA was caused by the central feed forward mechanism. A minority of the subjects had pulmonary stretch receptors as their main cause of RSA.

S14-3

Beat-to-beat QT interval variability and autonomic activity.

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The QT interval of body surface ECG reflects the depolarization and repolarization processes across the ventricular myocardium. Ventricular repolarization duration fluctuates from beat to beat, giving rise to QT interval variability (QTV). During the last two decades, QTV has received increasing clinical interest as elevated QTV has been demonstrated in patients suffering from various cardiac conditions, including myocardial infarction and dilated cardiomyopathy. Significant research efforts have been undertaken to elucidate mechanisms that underlie the beat-to-beat fluctuations in QT interval and evaluate its clinical significance. One of the physiological variables that has been repeatedly linked to QTV is the activity of the sympathetic nervous system.

In this talk I will review the evidence for relationship between QTV and sympathetic nervous system activity as reported in the literature and summarize our results obtained from the direct comparison between measures of sympathetic activity and QTV.

S14-4 (O)

Light at night increases blood pressure response to norepinephrine in hypertensive rats

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Artificial light at night (ALAN) is a problem of modern society and may contribute to health problems including cardiovascular diseases. We evaluated effects of low light intensity (2-3 lux) applied during the dark (D) phase for 5 weeks on systolic blood pressure (BP), heart rate (HR), core body temperature (BT) and locomotor activity (LA) in normotensive (WT) and spontaneously hypertensive (SHR) rats measured by telemetry. During the control week and weeks 2 and 5 of ALAN exposure, rats were administered by norepinephrine (NE) during both the light (L) and D phases. Responses were expressed as area under the curve. Under control conditions, NE significantly ($p < 0.001$) increased BP in SHR (L: 338 ± 13 , D: 284 ± 13 ; $p < 0.01$) in comparison to WT (L: 217 ± 15 , D: 162 ± 16 ; $p < 0.01$). After 2 weeks of ALAN exposure, differences in BP between L and D disappeared in both strains. After 5 weeks of ALAN exposure, differences in L and D BP response were restored in SHR (L: 397 ± 13 , D: 318 ± 23 ; $p < 0.001$) and WT (L: 248 ± 16 , D: 178 ± 16 ; $p < 0.001$). In SHR we observed continuous increase in BP response ($p = 0.03$) after ALAN exposure. HR and BT responses to NE were significantly related to the strains (HR $p = 0.01$; BT $p = 0.02$) and time of NE administration (HR $p < 0.001$; BT $p < 0.001$), while LA did not differ between strains ($p = 0.66$) and weeks ($p = 0.32$).

In conclusion, we observed circadian changes in BP response to NE, which was increased in SHR in comparison to WT. Short-term ALAN exposure diminished circadian difference in BP response, which was restored after long-term exposure to ALAN in both strains of rats. Importantly, BP response significantly increased after ALAN exposure in hypertensive as compared to normotensive rats.

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S14-5 (O)

Angiotensin II promotes K_v7.4 channels degradation through reduced interaction with HSP90

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Questions. Angiotensin II (Ang II) modulates vascular smooth muscle cell (VSMCs) contractility and the expression of potassium (K⁺) channels. Among them, voltage gated K⁺ channels belonging to K_v7 family modulate arterial contractility and mediate the responses to several endogenous vasorelaxants. In particular, K_v7.4 protein is down-regulated in several arterial beds in different models of hypertension. In this study we evaluated the effects of Ang II on vascular K_v7.4 expression and function.

Methods. Whole mesenteric artery (MA), as well as isolated VSMCs from Wistar rats were incubated with 100nM Ang II. Subcellular localisation of K_v7.4 subunits was assessed by immunofluorescence experiments. RNA and protein levels were measured by quantitative PCR and western blot. Functional effects were evaluated by wire myography. Proximity Ligation Assays were performed to measure protein:protein interactions.

Results. Ang II reduced K_v7.4 localisation at the plasma membrane in VSMCs, and decreased protein expression in MA without a concomitant reduction of mRNA levels. In addition, Ang II impaired the vasorelaxation produced by the K_v7.4 activator ML213 in pre-contracted MA. Proteasome-inhibitor MG132 prevented Ang II-induced reduction of K_v7.4 protein levels and function. Ang II decreased the number of interactions of K_v7.4 with the chaperone protein HSP90, and increased the interaction with the E3 ubiquitin ligase CHIP. Inhibition of HSP90 with 17-AAG reduced K_v7.4 protein levels and increased its interaction with CHIP.

Conclusions. Ang II alters K_v7.4 protein stability by decreasing its interaction with HSP90. This determines K_v7.4 degradation via the proteasome, possibly by an increased activity of CHIP.

Symposium 15: The cellular and molecular mechanisms controlling skeletal muscle plasticity

S15-1

Cellular and molecular mechanisms controlling muscle mass and metabolism

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The cellular basis of age-related tissue deterioration remains largely obscure. The ability to activate compensatory mechanisms in response to environmental stress is an important factor for survival and maintenance of cellular functions. Autophagy is activated both under short and prolonged stress and is required to clear the cell from dysfunctional organelles and altered proteins. The removal of mitochondria via mitophagy requires an efficient mitochondrial shaping machinery. We report that autophagy and mitochondrial dynamics in muscles declines with ageing and that both are reactivated by exercise. Exercise stimulates mitochondrial fusion via the transcription factor TFEB independently

of PGC1a but does not affect autophagy. TFEB induces the expression of genes involved in mitochondrial biogenesis, fatty acid oxidation and oxidative phosphorylation. This coordinated action optimizes mitochondrial substrate utilization, thus enhancing ATP production and exercise capacity. Finally, both fusion and fission when specifically blocked in muscles shorten life span of animals but only OPA1 deletion results in multiple organs senescence. Mitophagy is important to prevent mitochondrial dysfunction and oxidative stress. Mitochondrial dysfunction and oxidative stress directly affect acto-myosin interaction and force generation. Therefore, mitochondrial quality control is activated by exercise and is critical for muscle function and when impaired it systemically reverberates to whole organism affecting animal health and ageing.

S15-2

The control of skeletal muscle insulin sensitivity and protein turnover in disuse and inflammation

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Immobilisation results in non-inflammatory mediated muscle atrophy and insulin resistance. Post-absorptive and post-prandial rates of muscle protein synthesis (MPS) are suppressed under such conditions, and of sufficient magnitude to wholly account for muscle mass losses in humans. Importantly, under both conditions the phosphorylation state of mTOR pathway proteins cannot explain these immobilisation induced deficits in MPS. This, in combination with a lack of robust evidence for increased muscle protein breakdown during immobilisation, has led to the suggestion that the aforementioned decline in MPS is the primary determinant of muscle atrophy in humans, which is not the case in rodent hind-limb unloading. Immobilisation also results in the development of whole body and muscle insulin resistance in people within 1-5 days, which suggests that a lack of muscle contraction *per se* is the main physiological driver of this dysregulation, which will be discussed.

Systemic and muscle level inflammation are also widely reported to be drivers of skeletal muscle atrophy and insulin resistance, although the mechanistic basis of these events is also poorly understood. Attention will be given to the role of muscle cytokine mediated dysregulation of muscle AKT signalling, activation of FOXO transcription factors, and upregulation of FOXO downstream target genes, which have been linked directly to the inhibition of MPS and mitochondrial pyruvate oxidation, and increased muscle proteolysis.

Collectively, these observations highlight the current lack of understanding of the dominant cellular and molecular mechanisms dysregulating muscle protein turnover and insulin sensitivity in immobilised (non-inflammatory) and inflammatory states, and also whether they are at all additive in effect.

S15-3

Skeletal muscle cell populations and regeneration

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The presence of resident stem cells (satellite cells) in skeletal muscle affords a potential to regenerate fully after injury and yet the incidence of injury recurrence suggests that muscle repair is often complete. A better understanding of the contribution of different cell types and their interactions is therefore required if appropriate treatment strategies are to be developed. Traditionally, studies on muscle regeneration in humans have used maximum voluntary eccentric muscle contractions to induce damage. However this model only rarely leads to myofibre necrosis, so regeneration as such is limited. We have worked with eccentric contractions induced by neuromuscular electrical stimulation, where necrosis of approximately 25% of myofibres is apparent and thus serves as a model for studying necrosis and repair (Saclier *et al.*, 2013; Mackey *et al.*, 2016; Mackey *et al.*, 2017). This is

characterised 1 week post injury by phagocytosis of necrotic myofibres by macrophages, 9 times baseline levels of myogenic cell content, adipocyte infiltration and a doubling in the number of fibroblasts surrounding the muscle fibres. Fibroblast number increases further at 4 weeks post injury, when many fibres are still undergoing regeneration. The role of inflammation in this context will also be discussed.

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S15-4 (O)

Functional state of muscle mitochondria in patients with preclinical cognitive deficiency

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Sedentary ageing accelerates the risk of neurodegenerative and metabolic diseases. Here we examined whole body metabolism & muscle mitochondrial functions in association with cognitive state in seniors with mild cognitive impairment.

Methods: Insulin sensitivity was examined by euglycemic hyperinsulinemic clamp, Resting Energy Expenditure (REE) & metabolic substrate preference (RQ) by indirect calorimetry (Ergostik, Geratherm). Cognitive functions were assessed with MMSE, CogState and Memtrax and functional state of muscle (*m. vastus lateralis*) mitochondria by O2k high-resolution respirometry (Oroboros, n=14).

Results: We found that muscle mitochondrial oxidative phosphorylation (OXPHOS) capacity was negatively associated with age ($R=-0.574$, $p=0.032$) & BMI ($R=-0.548$, $p=0.042$). Maximal noncoupled respiration rate also decreased with age ($R=-0.549$, $p=0.042$) and rotenone induced inhibition of NADH-linked mitochondrial respiration was negatively associated with the reaction time (Memtrax, $n=14$, $R=-0.557$, $p=0.037$). Moreover, there were positive associations of mitochondrial fatty acid oxidation rate with short-term memory (CogState, $n=14$, $R=0.616$, $p=0.019$) and whole body metabolic dynamics (Δ REE) with both, coupled ($n=8$, $R=0.797$, $p=0.018$) and noncoupled ($n=8$, $R=0.717$, $p=0.045$) mitochondrial respiration rate.

Conclusions: We clearly showed that functional state of muscle mitochondria is linked with age, BMI, whole body metabolic state, as well as with the cognitive functions in seniors with mild cognitive impairment.

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S15-5 (O)

Effects of eccentric and concentric trainings on brain-derived neurotrophic factor (BDNF) signaling in cognition-related brain regions

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Question:As compared to concentric (CON) contraction, eccentric (ECC) contraction offers the advantage to combine high muscle forced with a low energy cost. Thus, ECC training is being increasingly recognized as a promising strategy to improve functional capacity in restrained cardiorespiratory patients. Surprisingly, while aerobic training demonstrates positive effect on cognition through elevation of BDNF levels in the frontal cortex (FC) and hippocampus (H), changes in BDNF signaling between CON and ECC training have never been investigated.

Methods:BDNF and synaptophysin were measured (Western blot) in FC and H in sedentary and treadmill-trained (30 min/d for 1 week) adult male Wistar rats. The treadmill was inclined at -10% and +5% to generate ECC and CON contraction, respectively. Velocity (m/min) was lower for ECC (12) than CON (14) training, so that exercise intensity was about 50% of the maximum aerobic speed in both modalities. The measurements were performed 24h after the last session of treadmill.

Results:BDNF and synaptophysin levels were significantly higher in trained than sedentary rats irrespective of the region examined and the levels did not differ between CON and ECC trainings.

Conclusion:These data reveal that moderate aerobic training-induced BDNF levels elevation in cognition-related brain region is independent on the mode of contraction. These data support the idea that ECC training to improve muscle strength is not disadvantageous for cognition.

Symposium 16: Exciting mechanisms of neuroglial excitability

S16-1

IONIC SIGNALLING AND ASTROGLIAL FUNCTION

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Despite a common ontogenetic origin, neurons and astroglia are fundamentally different in their excitability. Neuronal excitability, which is generally defined as electrical excitability, is determined by the existence of a specific complement of voltage-gated ion channels (Na⁺ channels, K⁺ channels, and, to a lesser extent, Ca²⁺ channels) in the plasmalemma. Depolarization of the neuronal plasma membrane (resulting from a sensory or synaptic input) to a certain threshold activates these channels, which in turn generates regenerative action potentials that propagate throughout excitable membranes and underlie long-range nerve impulse conduit by axons. Astrocytes are electrically non-excitable and unable to generate plasmalemmal action potentials due to a very low density of voltage-gated channels in their plasma membranes in association with the high resting K⁺ permeability. Nevertheless, glial cells are excitable, in the sense of actively responding to information from their surroundings. The substrate for glial excitability is associated with spatially and temporally controlled fluctuations in concentration of intracellular ions, mainly calcium and sodium, which in turn regulate and control numerous molecular cascades contributing to glial physiological processes.

S16-2

ADRENERGIC ACTIVATION OF ASTROCYTES SHAPES CALCIUM AND cAMP SIGNALLING AFFECTING CELL MORPHOLOGY AND GLYCOLYSIS

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A number of recent studies revealed that astrocytes are the main target of the *locus coeruleus*-noradrenergic nerve terminals that supply noradrenaline widely throughout the central nervous system. *Locus coeruleus* activation triggers a global central nervous system response, associated with arousal, which affects many processes including metabolism and memory formation. Astrocytes respond to noradrenaline via G-protein coupled adrenergic receptors with increased levels in cytosolic Ca²⁺ and cAMP. Here we report that temporal characteristics of the adrenergic Ca²⁺ and cAMP excitability in astrocytes differ. Activation of α_1 -adrenergic receptors triggers periodic Ca²⁺ oscillations within 10 s, while the activation of β -adrenergic receptors leads to a ~10-fold slower tonic rise in cAMP/PKA activity, but devoid of oscillations. We report that these two pathways synergize to generate the optimal cellular and global CNS response to *locus coeruleus* activation. In addition to signaling we will also address how adrenergic excitability in astrocytes regulates glucose availability, morphology and cellular edema. *In vivo* and *in vitro* imaging of astrocytes revealed that adrenaline reduces hypotonicity-induced cellular edema in cortical astrocytes. Adrenaline via cAMP-signalling reduces hypotonicity-induced cytosolic Ca²⁺ excitability, which may prevent astrocyte swelling. These findings reveal new targets for the treatment of cellular edema in the central nervous system.

S16-3

Protein astrogliopathies in human neurodegenerative diseases and aging

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Neurodegenerative diseases are characterized by progressive dysfunction and loss of neurons associated with depositions of pathologically altered proteins showing hierarchical involvement of brain regions. Protein astrogliopathy (PAG), including deposition of amyloid- β , prion protein, tau, α -synuclein, and very rarely transactive response DNA-binding protein 43 (TDP-43) is not unprecedented or unusual in neurodegenerative diseases. Morphological characterization of PAG is considered, however, only for the neuropathological diagnosis and classification of tauopathies. Astrocytic tau pathology is seen in primary frontotemporal lobar degeneration associated with tau pathologies (FTLD-Tau), but also in the form of aging-related tau astrogliopathy (ARTAG). Importantly, ARTAG shares common features with primary FTLD-Tau as well as with the astroglial tau pathologies that are thought to be hallmarks of a brain injury related tauopathy known as chronic traumatic encephalopathy. Supported by experimental observations, the morphological variability of PAG might reflect distinct pathogenic involvement of different astrocytic populations. PAG might indicate astrocytic contribution to spreading or clearance of disease-associated proteins, however, this might lead to astrocytic dysfunction and eventually contribute to the degeneration of neurons.

S16-4 (O)

Lysophosphatidic acid activates peripheral glial cells

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Chronic pruritus is a frequently observed symptom in hepatobiliary disorders. The intensity may range from minor irritation to substantially disabling, resulting in a dramatic reduction of the quality of life. Bile salts, endogenous opioids and serotonin have been discussed as pruritogens in cholestasis. However, for these substances plasma concentrations of patients are not correlated to the itch intensity. In contrast, lysophosphatidic acid (LPA) and its generating enzyme autotaxin were associated with itch intensity in cholestatic patients (Kremer, 2010). Using microfluometry we were able to demonstrate, that LPA18:1 elevates cytosolic free calcium concentrations in cell cultures from sensory ganglia. The majority of cells activated by LPA18:1 were identified not as neurons but as satellite glial cells. The magnitude of LPA-responses was inversely correlated with calcium responses to potassium and capsaicin. Besides satellite glial cells also Schwann cells responded to LPA18:1. Human and murine TRP channels expressed in HEK293t cells showed at best a marginal involvement of TRP channels in cellular responses to LPA, the respective glia cells of TRPV1 and TRPA1 knockout mice showed no relevant reduction in LPA-induced responses. In summary, we found an activation of peripheral glial cells by LPA18:1. Modulation of glial cells can alter neuronal function, which poses the question of a glial component in the regulation or even the generation of cholestatic itch.

S16-5 (O)

Pharmacological modulation of the fusion pore of exo- and endocytotic vesicles in cultured rat astrocytes

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In the brain, astrocytes participate in gliotransmission via vesicle-based mechanisms. To better understand the role of glial exo-/endocytotic mechanisms in brain physiology, pharmacological agents are often employed. Ketamine exerts a multitude of neuropharmacological effects of much interest and we have shown that ketamine plays a role in glial exocytotic release and the fusion pore. Furthermore, we have demonstrated that ketamine is also involved in fusion pore modulation during endocytosis with high-resolution capacitance measurements. Such electrophysiological measurements enable direct investigation of the fusion pore formed during vesicle fusion/fission events. In addition to the electrophysiological cell-attached patch-clamp method, we employed super-resolution structured illumination microscopy to examine vesicle interactions with the plasmalemma in cultured rat astrocytes with which we further investigated the role of dynamin. Dynamin is a structural GTPase involved in vesicle scission from the plasmalemma during endocytosis and new evidence is emerging that implicates a role of dynamin in vesicle exocytosis as well. Using the dynamin modulators DynoleTM-34-2, DyngoTM-4a, and RyngoTM-1-23, as well as the fluid-phase markers dextrans, we have revealed that dynamin modulation, similar to ketamine, interrupts vesicles at the plasmalemma, resulting in an accumulation of vesicles that remain attached to the plasmalemma via a narrow fusion pore that lapses into a state of repetitive opening-closing. Increased dextran uptake into exocytotic vesicles after dynamin inhibition indicated prolonged retention of these vesicles at the plasmalemma. Such pharmacological effects on the geometry and kinetics of the fusion pore during vesicle fusion/retrieval provide insights into gliotransmission release and (re)uptake that likely modulate synaptic activity.

Symposium 17: Monocyte subsets in cardiovascular biology

S17-1

Monocyte subsets in man and mice

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Monocytes/macrophages serve a variety of functions in immune response and inflammation including phagocytosis, antigen presentation and cytokine production. Monocytes arise from bone marrow precursors, they are released into blood, where they circulate for 1-2 days and then can continue into tissue to generate macrophages. Heterogeneity was first described in man using flow cytometry with CD14 and CD16 antibodies. This approach defines classical CD16-negative monocytes and CD16-positive monocytes that consist of non-classical and intermediate monocytes. While constituting 10% of all monocytes in homeostasis the CD16-positive monocytes can increase in number in inflammation. Conversely, some anti-inflammatory therapies including glucocorticoid treatment will selectively decrease the CD16-positive monocytes. Monocyte subsets in mice are defined as Ly6C++ CD43+ and Ly6C+ CD43++ cells. Comparative transcriptomics show that these subsets are similar to human classical and non-classical monocytes, respectively. Also, the mouse Ly6C+ CD43++ monocytes, similar to human non-classical monocytes, express higher levels of MHC class II and of pro-inflammatory cytokines. They are, however, not identical, in that in man TREM-1 and CXCR4 receptors are selectively found on classical monocytes while in the mouse they are selective for non-classical monocytes. These data suggest that studies in mouse models may assign roles to classical and non-classical monocyte subsets that are different to their role in human health and disease. Human monocyte subsets have been analyzed with respect to transcriptome, miRNAome and DNA methylation status and these data show unique interactions leading to differential gene expression in non-classical monocytes. Also, in man, but not in the mouse, non-classical monocytes show a selective expression of the slan-marker and the advantages of this marker will be discussed.

S17-2

The role of monocyte subsets in atherosclerosis

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Atherosclerosis is considered to be an inflammatory process in which monocytes and monocyte-derived macrophages play a key role in both initiation and progression of the disease. Circulating monocytes can be divided into three distinct subtypes according to their surface expression of CD14 and CD16. Classical monocytes (CM; CD14++CD16-) account for approximately 90% of all circulating monocytes. CD16-positive monocytes namely intermediate monocytes (IM; CD14++CD16+) and non-classical monocytes (NCM; CD14+CD16++) show a pro-inflammatory phenotype, exhibit an increased production of inflammatory cytokines upon stimulation and are elevated in chronic inflammatory diseases. Furthermore, the CD16+ monocyte population was shown to be expanded in patients suffering from stable coronary artery disease (CAD) and correlated with intima-media thickness and BMI in apparently healthy adults. In a study involving more than 900 patients undergoing elective coronary angiography, the proportion of IM predicted future cardiovascular events. We were able to demonstrate that monocyte subset distribution was associated with associated with HDL subfractions and small dense LDL (sdLDL). In addition, we could demonstrate that patients with stable CAD and elevated levels of Lipoprotein(a) (>50mg/dL) have an increased proportion of circulating IM.

S17-3

Monocyte subsets in cardiac disease and repair

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Monocytes play pivotal roles in innate immunity and inflammation. Once in circulation, they provide non-specific host protection against foreign pathogens being equipped with a multitude of receptors for their prompt detection and mechanisms of their elimination. The receptors involved in innate responses are not antigen or pathogen-specific, but they rather detect certain molecular patterns, that can be present on different types of bacteria or viruses. However these, so called pattern recognition molecules, can also interact with human tissues, leading to release of inflammatory cytokines, reactive oxygen species, and matrix metalloproteases, thus contributing to cardiovascular remodeling and pathology. Monocytes are phagocytes and have a range of scavenger receptors, e.g., involved in internalization of cholesterol. Whilst these activities are intrinsically beneficial, they play a major role in atherogenesis via foam cell formation. Monocytes possess unique high developmental plasticity, which is an ability to transform into a variety of cell types after their migration to tissues. Monocytes can differentiate into different types of macrophages, but also give origin to cells of other lineages, including myofibroblasts, and at least under experimental conditions, even cardiomyocytes. Monocytes also represent a major pool endothelial progenitors, being involved in angiogenesis. Importantly, monocytes are diverse and include several functional subsets. Classical monocytes, Mon1, are proinflammatory, and typically their high counts are related to poor outcomes. The roles of more recently discovered Mon2 and Mon3 are increasingly better understood and their properties suggest their potential in tissue repair.

S17-4 (O)

Injured renal epithelium cell fate and inflammation are controlled by *de novo* expressed Notch3

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Injured epithelium is crucial for macrophage activation and progression of inflammation. Notch3, a novel mediator of renal disease, is *de novo* expressed by suffering cells. In the acute kidney injury model of ischemia reperfusion Notch3 was found in tubular and infiltrating macrophages. To examine Notch3 role in epithelial cell phenotype and whether ectopic expression of Notch3 can induce an inflammatory response, we generated mice overexpressing Notch3 intracellular domain (N3ICD) in tubules. One month of N3ICD overexpression induced a mild inflammatory response without affecting renal function and structure. However, after ischemia/reperfusion, mice overexpressing N3ICD showed exacerbated infiltration of inflammatory cells and severe tubular damage. Co-culture of N3ICD overexpressing tubules and macrophages activated the latter. Inversely, Notch3 knock-out (KO) mice were protected against ischemia reperfusion showing minimum inflammation and preserved renal structure. Isolated macrophages from Notch3 KO mice showed reduced migratory capacity and low levels of pro-inflammatory cytokines. Chromatin immunoprecipitation identified NF-κB as the principal inducer of Notch3 in ischemia/reperfusion. In summary, our data indicate that Notch3 expression is induced by NF-κB in injured epithelium and infiltrating cells. This activation sustains a pro-inflammatory environment that attracts activated macrophages to the site of the injury leading to rapid deterioration of renal function and structure. Targeting Notch3 may provide a novel strategy against parenchymal damage and inflammation.

S17-5 (O)

Endothelial IκB Kinase 2 in Atherosclerosis

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Endothelial cell activation via the transcription factor NF-κB constitutes one of the first steps in atherogenesis, leading to the recruitment of leucocytes to the arterial wall. We established a conditional transgene mouse model mimicking chronic endothelial inflammation to elucidate the underlying mechanisms linking inflammation and atherosclerosis. This was achieved by crossing mice with an inducible, endothelial-specific Cre recombinase with a strain bearing constitutive active IKK2 (calKK2) downstream of a loxP-flanked stop cassette. calKK2 expression was induced by tamoxifen injection at five consecutive days. Aortic mRNA levels of the activation markers E-selectin, ICAM-1 and VCAM-1 were significantly upregulated after induction of calKK2. Lymphocyte and granulocyte numbers were found to be significantly reduced in the circulation, with a parallel increase in aortic draining lymph nodes. To monitor potential changes in aortic gene expression due to inflamed endothelial cells, we performed RNA-sequencing of wildtype and calKK2 aortas. Evaluation of the results with *Ingenuity Pathway Analysis* software revealed that the most upregulated pathways were associated with B- and T-cell signaling. To investigate the role of calKK2 in the development of atherosclerosis, calKK2-mice were crossed on an ApoE deficient background and fed a cholesterol-rich diet for 10 weeks. *En face* preparations of isolated aortas showed increased atherosclerotic plaque areas in calKK2-mice, implying an aggravation of the atherogenic process. In summary, endothelial expression of calKK2 led to endothelial cell activation resulting in an accelerated development of atherosclerosis, which was associated with increased infiltration of B and T cells

Symposium 18: Retina Degeneration: New technologies for the bionic retina

S18-1

The new Retina Implant Alpha AMS: How does it work and what can blind patient see?

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Purpose: Assessing safety and efficacy of a technically advanced subretinal electronic implant in end-stage retinal degeneration (RD) in an ongoing prospective clinical multicenter trial.

Methods: The new RETINA IMPLANT Alpha AMS (Retina Implant AG, Reutlingen, Germany) with 1600 pixels providing a visual field diameter of 15 deg, was implanted subretinally in 15 blind RD-patients (age 55.2 +/- 10.2 y, mean +/- SD). Functional outcome measures included 1) screen-based tests of light perception, light localization, grating acuity and Landolt C-rings; 2) grey level discrimination; 3) activities of daily living (ADL) .

Results: Implant-mediated light perception was observed in 14/15 patients. During the observation period (12 months) implant mediated localization of visual targets was possible in 13/15 patients. Grating acuity was 0.1 cpd (cycles per degree) in 4/15; 0.33 cpd in 5/15; 1.0 cpd in 2/15 and 3.3 cpd in 1/15 patients. Best visual acuity assessed with Landolt C- ring was 20/546 and 20/1111. Improvements (power ON vs. OFF) of ADL table tasks were reported by 13/15 patients. Results were overall stable during observation period. The majority of adverse events (AEs) were transient and mostly of mild to moderate intensity, and all were treated successfully.

Conclusions: Psychophysical and subjective data show that RETINA IMPLANT Alpha AMS is reliable, well tolerated and partially restores visual functions in the majority of patients. Compared with previous Implant Alpha IMS, longevity of the new Implant Alpha AMS has considerably improved with a similar efficacy profile as Alpha IMS. Alpha AMS has meanwhile been certified as a commercially available medical device, in Germany reimbursed by the public health system. Providing centers have been recruited in several European countries.

S18-2

Subretinal prosthesis and optogenetic therapy: Functional validation on the primate retina.

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Retinal prostheses were shown to provide useful vision for the detection of contrasted objects. However, they do not allow face recognition, text reading and independent locomotion. We have here explored the potential of subretinal photovoltaic retinal implants and optogenetic therapy. Photovoltaic implants are activated locally by infrared light whereas optogenetic therapy relies on visible activation of a microbial opsin expressed in residual neurons by gene therapy.

Photovoltaic retinal implants were first validated on the ex vivo blind primate retina obtained by shaving off the photoreceptors with vibratome sectioning. On this model, some retinal ganglion cells (RGCs), sending normally visual information to the brain, responded reliably to only one of the 100µm-wide units (3-4 mW.mm⁻² at 4ms). In vivo, the implants induced photoreceptor degeneration by separating the retina from its underlying metabolic supplier, the choroid. Behavioral tests indicated that the infrared sensitive implant was able to restore visual perception following one unit activation.

In optogenetic therapy, microbial opsins were successfully expressed in RGCs at the perifoveal ring. These cells were activated by intense light within the spectral range of the expressed microbial opsin. The required light duration was compatible with video rate stimulation allowing thereby pattern recognition.

These results demonstrate that photovoltaic subretinal implants can provide visual perception with a single unit (100µm) resolution. Alternatively, optogenetic therapy can provide cellular resolution in the perifoveal ring by direct RGC activation. The two strategies for restoring vision are due to enter in clinical trials next year.

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S18-3

A fully organic retinal prosthesis restores vision in a rat model of degenerative blindness

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The degeneration of photoreceptors in the retina is one of the major causes of adult blindness in humans. Unfortunately, no effective clinical treatments exist for the majority of retinal degenerative disorders. In this work we report on the fabrication and functional validation of a fully organic prosthesis for long-term in vivo subretinal implantation in the eye of Royal College of Surgeons rats, a widely recognized model of retinitis pigmentosa. Electrophysiological and behavioural analyses revealed a prosthesis-dependent recovery of light sensitivity and visual acuity that persists up to 6–10 months after surgery. The rescue of the visual function was accompanied by an increase in the basal metabolic activity of the primary visual cortex, as demonstrated by positron emission tomography imaging. Our results highlight the possibility of developing a new generation of fully organic, highly biocompatible and functionally autonomous photovoltaic prostheses for subretinal implants to treat degenerative blindness.

S18-4 (O)

Chromatin Shannon entropy in peripheral blood lymphocytes increases after UV-induced DNA damage

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Questions: Recently, many new mathematical algorithms have been suggested as useful in quantification of structural changes in cell nuclei during physiological processes. One of them is Shannon entropy which is able to evaluate the level of information disorder in various medical signals. Our work suggests that this parameter significantly increases in lymphocyte chromatin after exposure to UV radiation.

Methods: Human peripheral blood with the addition of RPMI was incubated at 37°C, and exposed to a 5 minute pulse of UV light at a dose which in conventional circumstances induces apoptosis. 30 minutes after exposure, the cells were fixed in methanol and stained using DNA-specific Feulgen technique. Shannon entropy analysis was performed on digital micrographs of treated (n=50) and untreated (control, n=50) chromatin structures.

Results: The average value of chromatin Shannon entropy increased after UV exposure, and the difference between the experimental and control group of cells was statistically highly significant (p<0.01). Receiver operating characteristics analysis showed an excellent discriminatory ability of Shannon entropy in distinguishing damaged from normal chromatin structures.

Conclusions: Shannon entropy analysis is potentially a sensitive method in detection of fine structural changes in chromatin during UV-induced DNA damage. Shannon entropy is potentially a valuable parameter applicable in cell physiology research.

Keywords: Cell; ROC analysis; method

S18-5 (O)

Data Driven Graph-Theoretical Reconstruction and Quantification of 2D and 3D Tree-Like Biological Structures

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Advances in biomedical imaging and the corresponding computational approaches have significantly contributed to our understanding of biological systems. Capturing the relevant information from organs or tissues can rely on conceptually very different techniques encompassing x-rays (micro computed tomography: μ CT), sound (ultrasound), magnetic field (magnetic resonance imaging: MRI), radioactive pharmaceuticals (nuclear medicine: PET) or optical light (confocal laser scanning microscopy: CLSM). Due to the ever increasing amount of data that is produced by different imaging techniques, advanced computational tools are required to ensure automated, consistent and accurate extraction of structural features. In the present contribution we shall describe a novel and efficient approach for an automated reconstruction of 2D or 3D tree-like structures, such as vasculatures. Our method is based on graph-theoretical concepts which makes it very generic and can be applied to different setups. To demonstrate the performance of our methodology we present a quantitative geometrical and topological characterization of the vascular network in an insect wing recorded with light microscopy and a 3D arterial vasculature in a mouse kidney scanned with a μ CT device.

Short Talks 1

ST1-1

G-protein mediated regulation of TRPM3 channel activity

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TRPM3 proteins form non-selective Ca^{2+} -permeable cation channels sensitive to the endogenous steroid metabolite pregnenolone sulfate and to temperature increase. TRPM3 channel activation results in enhanced glucose-induced insulin release and heat-evoked nociception, but it is unclear how channel activity is regulated. In addition, alternative splicing generates a variety of TRPM3 proteins with distinct amino acid compositions and cell-type specific expression patterns. The functions and modes of regulation for most of these splice variants have not been elucidated so far. Using electrophysiological techniques and Ca^{2+} -imaging we show that GPCR activation inhibits TRPM3 channels. In nociceptor neurons, activity of endogenous TRPM3 channels was inhibited by μ -opioid receptor activation, a G*q*/o-coupled process. However, direct action of G*q*/o-subunits on TRPM3 channels could be excluded through overexpression experiments. By contrast, overexpression of β/γ -subunits of heterotrimeric G-proteins strongly inhibited TRPM3 activity. Additionally, co-immunoprecipitation experiments indicate that G β and TRPM3 interact directly. Furthermore, we show that TRPM3 splice variants lacking exon 17 can not be inhibited by GiPCR activation, providing evidence that TRPM3 inhibition is dependent on highly specific protein-protein interactions. By mutational analysis we characterize this putative G β -TRPM3 interaction site.

Together, our data show that TRPM3 channels are subject to well-known intracellular regulatory mechanisms that allow fast and potent regulation of channel activity and the resulting calcium influx indicating physiological importance

ST1-2

Role of KCa3.1 channels in glioblastoma induced angiogenesis

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Human glioblastoma multiform (GBM) is a highly malignant brain tumour characterized by elevated angiogenesis. Intermediate conductance calcium activated potassium channels (KCa3.1) is overexpressed in human GBM tissue compared to healthy human normal brain, and the involvement in angiogenesis process is not yet investigate. To verify this aspect, we study the effects of TRAM-34, selective inhibitors of KCa3.1, in ex-vivo model based on graft of GBM cell on the chorioallantoic membrane (CAM) of chick embryo by evaluating morphometric, histologic and gene expression markers. The TRAM-34 reduce vascularity when applied in the area near to GBM implant, whereas have not effect, when applied in the normal developing CAM area. Gene expression analysis confirm an anti-angiogenic effect of TRAM-34 in CAM-GBM *ex vivo* model. We further studied the effects of angiogenic factor SDF-1 (Stromal Derived Factor-1, ligand of CXCR4) and anti-angiogenic agents such as LY294009 (an inhibitor of PI3Ks) and silver nanoparticles (AgNPs) on KCa3.1 current expressed in GBM cell lines (U251 and GL-15). Utilizing the patch clamp technique, we observed that short pre-incubation of SDF-1 increased KCa3.1 current, whereas LY294009 and AgNPs reduced the same current. By utilizing fibroblast models (NIH-3T3 wt and NIH-3T3 EAG1-G440s expressing cell), we study the effect of hypoxia in KCa3.1 current regulation. Hypoxia upregulate endogenous KCa3.1 current when co-expressed with proangiogenic EAG1 gene, whereas is not able to regulated KCa3.1

current in wt model. We propose that KCa3.1 channel is involved in signalling of GBM angiogenesis and can represent a new anti-angiogenic target in GBM therapy.

ST1-3

Model of brain cellular edema in the study of neuroprotection by methylprednisolone

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Intracranial hypertension threatens the life by compression of the vital centers or by the loss of cerebral perfusion pressure. A common form of intracranial hypertension is brain edema. Aim of this work was to complete and test an experimental model of brain edema in rats and use it to study neuroprotective effects of methylprednisolone.

Brain edema in rats was induced by water intoxication. Distilled water in a amount corresponding to 20% of the animal weight was given in three doses intraperitoneally (i.p.) together with desmopressin (0.032 mg/kg). Another group of water intoxicated rats received methylprednisolone i.p. (100mg/kg). A standard CT scans of the brain were obtained and density mean values were determined. Signs of myelin disintegration were studied at histological sections in hippocampus and in cerebral cortex. Laboras apparatus (Metris B.V.) was used to analyse behavioural pattern. Intracranial pressure was measured under general anaesthesia using intraparenchymal microsensor (Opsens Medical).

Induced brain edema resulted in a lower density at CT, in increased intracranial pressure, it was accompanied with various forms of myelin disintegration and decreased locomotor activity. Administration of methylprednisolone proved to have protective effect against the elevation of intracerebral pressure as well as for the structural and behavioral changes.

Experimental model of brain edema has the CT characteristics of clinical cases, brings an increase of intracerebral pressure and structural and functional impairment. Neuroprotective effect of methylprednisolone was observed in histological and behavioral studies, and namely in the reduction of intracranial pressure.

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ST1-4

The role of hyaluronan-based brain extracellular matrix (bECM) in stabilization of neural network activity via regulation of GluR1-containing AMPA receptor synaptic pool

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Question

Recruitment or deletion of GluR1-containing AMPA receptors (GluR1-AMPA) to postsynapses is common to changes in synaptic strength. We studied if enzymatic digestion of hyaluronan-based bECM influenced neuronal network activity and synaptic expression of GluR1-AMPA.

Methods

Primary hippocampal cultures from C57BL/6J mice (P18) were used. Hyaluronic acid was removed by 50 U/ml hyaluronidase. AMPARs cell surface expression was assessed by Western blot and

immunostaining. Changes in gene expression were tested by 8x6000K whole-genome mouse chip (Agilent Technologies). Neural network activity was recorded by the microelectrode array system (Multi Channel Systems). Patch-clamp recordings were used to estimate mEPSCs and mIPSCs amplitude and frequency.

Results

We showed that enzymatic digestion of hyaluronan-based bECM causes seizure-like activity correlating with significant increase in synaptic GluR1-AMPA, as was shown immunocytochemically and by Western blot. Transcriptional analysis indicated a change in the expression of calcineurin group genes. The frequency of mEPSC and mIPSC was higher 48 hours after hyaluronidase exposure with the reverse effect at the 72 hour point.

Conclusions

Our results show that hyaluronidase treatment leads to upregulation of GluR1-AMPA, which correlates with an increase in mEPSC frequency. Increased excitatory transmission and excessive calcium influx through GluR1-AMPA may induce seizure-like activity. Transcriptional data suggested that hyaluronidase exposure causes changes in expression of calcineurin-linked genes responsible for the diffusion of synaptic receptors and synaptic plasticity modulation.

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ST1-5

Identification of the biomarkers for low and high grade astrocytoma patients outcome prognosis based on the analysis of gene activity and function

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Astrocytomas – one of the most prevalent brain tumours arising from the brain cells astrocytes and according to the WHO are classified in 4 groups of malignancy. Notwithstanding grading of astrocytoma (Ast), the outcomes of clinically similar patients are rather different. Therefore molecular examination of Ast tumours is necessary to improve accuracy and efficiency of diagnostics, prognostics and treatment.

The aim of this study was to identify the set of molecular markers to improve the current methods applying to diagnose Ast tumour and patient outcome.

The study included 138 different malignancy Ast tumours (14 grade-I, 45 grade-II, 30 grade-III and 49 glioblastomas). mRNA expression analysis was performed using quantitative real-time Reverse-Transcription PCR, protein level was estimated using Western blot technique. Methylation status of genes promoters was detected by MS-PCR followed by DNA bisulfite treatment. Functional investigations of the targets were performed on U87 glioblastoma cell line applying cells migration and invasiveness, viability and proliferation assays followed by exogenous overexpression of targets.

The present study involved 20 genes with potential value for diagnostics. After the extensive analysis of the genes mRNA, protein expression and methylation in tumours tissue, we found 5 very promising targets – NDRG2, AREG, RUNX3, SEMA3C, NPTX2 as biomarkers for astrocytoma typing and prognosis, which were confirmed by the functional analysis.

ST1-6

Does the activity of the proteasome decline during human ageing and in the brains of Parkinsons disease patients?

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The pathological hallmarks of Parkinsons disease (PD) are the loss of dopaminergic neurons of the substantia nigra pars compacta (SNpc), and the deposition of aggregated α -synuclein in Lewy bodies. A reduction in the activity of the ubiquitin-proteasome system (UPS) could limit removal of ubiquitinated α -synuclein and thereby contribute to PD proteinopathy. Age is the principal risk factor for neurodegenerative diseases such as PD. Depleted UPS activity in the brain could arise during normal human ageing, and/or under pathological conditions. To examine the influence of human ageing on brain UPS activity, we quantified UPS chymotrypsin activity within the prefrontal cortex of *post-mortem* human brain tissue over the age range of 21-84 years. 6 human control subjects were assessed for each decade between the second and ninth decades. Furthermore, to examine regional vulnerability of the SNpc region to loss of UPS function, UPS chymotrypsin activity was quantified in *post-mortem* tissue from the prefrontal cortex, caudate nucleus, cerebellum, hippocampus, corpus callosum, thalamus, and SNpc in 10 PD patients and 10 age and sex matched control subjects. Chymotrypsin activity was measured via cleavage of a N-succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin peptide and fluorimetry. Our results show that although there are moderate fluctuations between decades, there was no significant decline in UPS activity during human ageing. Differences in UPS activity between brain regions was evident for control and PD brain tissues, and these will be discussed.

ST1-7

Kynurenic acid and its amid analogue could be possible drug candidates for controlling the activity of opioid system.

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Previously, we have shown that the N-methyl D-aspartate (NMDA)-receptor antagonist kynurenic acid (KYNA) and its analogue KYNA1 do not bind directly to mu, kappa and delta opioid receptors *in vitro*. On the other hand, chronic administration of KYNA and KYNA1 resulted in region (cortex vs. striatum) and opioid receptor-type specific alterations in G-protein activation of mouse brain homogenates. Here we describe for the first time the acute effect of KYNA and KYNA1 on opioid receptor function with the possible involvement of the NMDA receptor.

The acute 30 minutes *in vivo* KYNA1 and KYNA treatments altered opioid receptor G-protein signaling or ligand potency depending on the opioid receptor type and brain region (rat cortex vs. striatum) using [³⁵S]GTP γ S binding assays. Pretreatment with the NMDA receptor antagonist MK-801 impaired or reversed the effects of KYNA1 and KYNA. These results suggest an NMDA receptor mediated effect. After acute 30 minutes treatment HPLC measurements revealed a similar KYNA1 and a higher KYNA plasma concentration compared to cerebrospinal fluid concentrations. Finally, KYNA, KYNA1 and MK-801 showed comparable results in opioid receptor G-protein activity and ligand potency with acute *in vivo* treatments when they were administered *in vitro* for 30 minutes on isolated cortex and striatum slices.

We previously demonstrated that KYNA1 and KYNA acutely altered opioid receptor function *in vivo* and *in vitro* through the NMDA receptor depending on the opioid receptor type and brain region. This study may lead to a new, indirect approach to influence opioid receptor signaling.

ST1-8

A relation between mitochondria and epileptiform discharges

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Mitochondria have been linked to epilepsy by virtue of the production of reactive oxygen species (ROS). Mitochondria are the major physiological source of ROS but their role in epileptogenesis is unclear. Epileptogenesis involves an abnormal type of electrical activity, termed the paroxysmal depolarization shift (PDS). The PDS differs from normal action potential by a depolarized shoulder lasting for several hundreds of milliseconds, mediated by L-type calcium channels (LTCCs). Here we investigated the link between mitochondria and epileptiform activity by studying the effect of paroxysmal depolarization shifts (induced by bicuculline + the LTCC agonist Bay K8644) on mitochondrial distribution, motility, morphology and bioenergetics in neuronal networks formed from dissociated hippocampal cells. We found that PDS reversibly affected the mitochondria in the soma and the proximal neurites by changing the mitochondrial morphology and by inhibition of the movement of up to 90%. At the same time, the neurons appeared energetically stressed. The occurrence of PDS led to a transient calcium influx into the mitochondria and a decrease in mitochondrial membrane potential. The application of the ATP-synthase inhibitor oligomycin, further depolarized the mitochondria, indicating that during the epileptiform activity, ATP synthase reverses and mitochondria act as consumers of ATP. Measurements using the superoxide-indicator MitoSOX demonstrated the formation of ROS, which may result from enhanced mitochondrial respiration. Hence, PDS activity appears to severely compromise mitochondrial function. Taken together, our data illustrate a relation between PDS formation and mitochondrial dysfunction.

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Short Talks 2

ST2-1

Reduction of fractal complexity in lymphocyte chromatin architecture during oxidopamine - induced apoptosis

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Questions: Oxidopamine is a known neurotoxin which, in some concentrations, was shown to be capable of inducing apoptosis in lymphocytes. Many aspects of this process, especially concerning changes in chromatin architecture in early stages of programmed cell death, remain unclear.

Methods: Peripheral blood samples, incubated at 37°C with the addition of RPMI, were treated with oxidopamine (300 mM). 120 minutes after the treatment, the samples were smeared on microscope slides, and stained using DNA-specific Feulgen technique. Fractal analysis of chromatin structure was done in National Institutes of Health (NIH, Bethesda, MD) software, similarly as in our previously published studies. In order to evaluate changes in fractal complexity, average values of chromatin fractal dimension and lacunarity were calculated.

Results: There was a statistically highly significant ($p < 0.01$) reduction of chromatin fractal dimension in oxidopamine-treated lymphocytes. Receiver operating characteristics analysis demonstrated that fractal dimension is a sensitive parameter in identifying the treated cells. Chromatin fractal lacunarity significantly increased ($p < 0.05$) in oxidopamine-treated cells.

Conclusion: Proapoptotic agent oxidopamine may induce a significant reduction in chromatin fractal complexity. Fractal analysis as a method may be capable of detecting discrete changes in chromatin architecture during early stages of apoptosis.

Keywords: DNA, Hydroxydopamine, Fractal dimension

ST2-2

Streptozotocin-induced diabetic rats the effect of *Ganoderma Lucidum* polysaccharides on oxidative damage in the liver.

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In this study, we studied the effects of exogenic application of *Ganoderma lucidum* polysaccharide on oxidative stress and blood glucose levels using induced diabetics rats. Therefore we used 60 Wistar albino rats aged 4-5 months and divided them into 6 groups, with 10 rats in each group. We did not apply any control to control groups and *Ganoderma lucidum* polysaccharide, whereas with a trial group animals we applied an intraperitoneal injection of 50 mg/kg dose of Streptozotocine. Among diabetic control animals we created the groups to which we applied as follows 60, 120 and 180 mg/kg of polysaccharides. During the entire experiment the rats were fed *ad-libitum*. In addition, to synchronize the diabetic control group during the study, they were given the physiological saline orally. During the study we applied *Ganoderma lucidum* polysaccharides exogenously (60, 120, 180 mg/kg) and it did not affect the level of total antioxidant in diabetes significantly ($p > 0.05$), however with a dose of 180 mg/kg the total oxidant level in pancreas and liver tissues was reduced significantly and has been considered as crucial fact. The collected data supported our view on histopathologic examination. Based on all the information, we used one type of fungus *Ganoderma lucidum* polysaccharides which did not influence the blood glucose level in diabetes, whereas with regard to LDL and total oxidants it played a significant role in reducing its levels.

ST2-3

PODOCYTE-EXPRESSED STAT5 CONFERS PROTECTION DURING EXPERIMENTAL GLOMERULONEPHRITIS AND ADRIAMYCIN NEPHROPATHY IN MICE

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Glomerular diseases are a leading cause of chronic kidney failure and the podocyte is one of the main target of these diseases. We previously evidenced a protective role for a podocyte-expressed immune receptor such as the common gamma chain (γC) during glomerulonephritis. We also found that STAT5, a transcriptional factor classically described and activated downstream γC in T-cells is upregulated in podocytes during glomerulonephritis. Hitherto, STAT5 role in podocyte remains unclear. Using mice with a podocyte-specific deletion of *Stat5*, we analyzed the role of STAT5 in two experimental models of glomerular diseases. First, during crescentic glomerulonephritis, podocytic-STAT5 deficient mice developed increased proteinuria compared to their wild-type littermates. Second, during adriamycin induced-nephropathy, the absence of podocyte STAT5 leads to increased albuminuria and severe podocytic injuries in comparison to controls. Moreover podocytic lesions were associated with loss of podocyte differentiation markers such as nephrin especially in podocytic-STAT5 deficient animals. Renal T-cells and macrophages infiltration were not affected by the deletion

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Short Talks 2

of podocytic STAT5. Taking together, our results suggest a yet unsuspected protective role of podocytic γC /STAT5 signaling during glomerular diseases.

ST2-4

Exercise restores diabetes-mediated contractile dysfunction of isolated rat seminal vesicle

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The aim of this study was to investigate possible effects of exercise on diabetes-mediated contractile dysfunction of seminal vesicle in rats. Diabetes was induced by intraperitoneal streptozotocin injection, single dose 60 mg/kg, in male adult Sprague Dawley rats. Diabetes was confirmed by blood glucose level measurement from tail vein. Rats were divided into control, diabetes, low intensity exercise (0.5 km/h 30 min run on treadmill) and high-intensity exercise (1 km/h, 60 min) was performed for 8 weeks ($n = 7$ in each group). At the end of 8 weeks *in vivo* protocol, animals were sacrificed and isolated seminal vesicles were suspended in an organ bath containing physiological saline, pH 7.4, continuously bubbled with 5% CO_2 and 95% O_2 (37°C). Isometric contractions in response to noradrenaline and electrical stimulation was recorded using force-displacement transducer. Data were statistically analysed.

Diabetes caused an increased contractile response, compared to contractility of strips from respective control group, of seminal vesicle strips to both noradrenaline (90 μM) and electrical field stimulation (EFS). And exercise, in an intensity-dependent manner, significantly attenuated this increased contractile response to noradrenaline and EFS in terms of both peak amplitude and area-under contractile curve.

Data from this functional *in vitro* organ bath study indicates that chronic exercise restores the diabetes-induced hypercontractility of seminal vesicle, suggesting that exercise may provide beneficial effects on seminal vesicle contractility-related male fertility impairment secondary to diabetes.

ST2-5

High intensity interval training in cardiac rehabilitation: A randomized controlled trial investigating platelet function

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Questions:

Exercise training is a cornerstone of cardiac rehabilitation (CR) programs. However, the exercise-intensity eliciting maximal beneficial adaptations remains controversial. Since platelets play a key role in atherosclerosis, the aim of this study was to compare effects of *high-intensity interval training (HIT)* with *moderate-intensity continuous training (MCT)* on platelet function.

Methods:

At the beginning of CR, patients with coronary artery disease were randomized to either HIT or MCT with identical net energy expenditure, performed on bicycle ergometers. Both groups performed 4 training sessions per week over a period of 12 weeks. Maximal oxygen consumption (VO_{2max}) and parameters of platelet function were assessed before training, after 6 and 12 weeks. Primary endpoint was platelet reactivity measured as the half-maximal effective dose (EC_{50} in μM) of platelet agonist TRAP-6 in terms of P-selectin expression after 6 weeks of training, quantified by flow cytometry.

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Results:

70 patients were randomized to HIT or MCT. There were no significant baseline differences between groups regarding VO_2max (HIT vs. MCT: 22.9 vs. 23.1 ml/min/kg, $p > 0.5$) or platelet reactivity (6.59 vs. 6.63 μM , $p > 0.5$). The overall increase of VO_2max after 6 weeks was 2.5 ml/min/kg ($p < 0.0001$) without any group differences ($p > 0.5$). However, HIT had greater effects on parameters of platelet function than MCT, including the primary endpoint: The EC_{50} of TRAP-6 (P-selectin expression) was higher after 6 weeks of HIT (7.60 vs. 6.74 μM , $p < 0.01$), indicating lower platelet reactivity in response to HIT compared to MCT.

Conclusions:

HIT seems to be more effective than MCT in reducing platelet reactivity in patients undergoing cardiac rehabilitation.

ST2-6

Partial Loss of A20 exacerbates IFN γ dependent Transplant Arteriosclerosis through De-Regulation of IFN β .

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Inflammation including activation of the IFN γ /STAT1 axis is central to vascular remodeling associated with transplant arteriosclerosis (TA). Strategies to interrupt IFN γ -mediated JAK-STAT signaling in the vasculature are hence particularly attractive. In this study, we investigated the impact of loss of the NF- κ B inhibitory protein A20 on IFN γ signaling in the vasculature.

Silencing of A20 in vitro in EC and SMC led to a significant super-induction of bona fide IFN γ dependent atherogenic genes through upregulation of STAT1 expression. A20 controlled STAT1 expression independent of its NF- κ B inhibitory function but by affecting basal subthreshold levels of its upstream inducer, IFN β .

In vivo, we performed totally mismatched (C57BL/6 to BALB/c) aortic to carotid vascular transplantations in mice. Partial loss of A20 as in A20 heterozygote (HET) allografts induced a significant aggravation of TA lesions in A20 HET versus WT allografts, as evaluated by intima over media ratios. Intriguingly, this phenotype was reverted by ectopic expression of a dominant negative STAT1 isoform in A20 HET allografts.

Altogether, these data uncover an important physiologic role for A20 as a regulator of pathologic vascular remodeling of TA, in part thanks to its novel inhibitory effect on IFN γ signaling and independent of its NF- κ B inhibitory function. These results are clinically relevant in light of A20 SNPs associated with decreased expression and function.

ST2-7

Regulation of Two-pore Domain K⁺ Channels by Natural Effectors and Pharmacological Agents

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Two-pore domain K⁺ (K2P) channels represent a large family of K⁺ channels that are important regulators of cellular electrical excitability throughout the human body. They are involved in a large

variety of biological processes including e.g. nociception, neuroprotection and hormone secretion as well as in disease states like pain, migraine, arrhythmias or depression. K2P channels are finely regulated by a wide range of natural stimuli, including signalling lipids, membrane tension, extracellular/intracellular pH, temperature, peptides and voltage but also by pharmacological agents like volatile anaesthetics or antidepressants. Recently a number of new potent K2P channel activators have been reported, though their effector mechanisms are only poorly understood. In this study we report a lower resolution X-ray structure of the TREK-2 K2P channel cocrystallized with the activator compound BL-1249 uncovering a conserved binding site at the pore entrance of the side fenestrations, which are present in several K2P channels. Systematic cysteine mutagenesis scans, MD simulations and blocker competition experiments suggest that this drug-binding site is functional in most K2P channels, accommodates chemically diverse compounds including many known channel activators and controls the selectivity filter gate in a subtype specific manner. In K2P channels with a low intrinsic activity occupation of the novel drug-binding site results in activation, whereas those with high intrinsic activity were inhibited. These results establish a conserved but chemically promiscuous drug interaction site that defines the diverse pharmacology of K2P K⁺ channels.

ST2-8

The Effects of Adropine Application in Rats on Nutrient Intake and Water Consumption

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Aim: Adropine hormone, which is encoded by the gene that is associated with energy homeostasis, is a metabolic peptide whose expression is determined in many tissues mainly in the hypothalamus and the liver. We aimed to determine the effects of atropine hormone at different doses on nutrition behavior and water consumption by investigating some peripheral and central signals.

Methods: 40 male Wistar Albino rats were used in our study. The study was designed as the control group, the Sham Group (300 μ l distilled water), 4 μ g/kg adropine and 40 μ g/kg adropine groups. The injections were made for 10 days. The adiponectin, peptideYY (PYY), glucagon-like peptide (GLP), Oxyntomodulin (OXT) and ghrelin levels were evaluated with the ELISA Method; and the expressions of Agouti-related peptide (AgRP) connected with food intake in the arcuate nucleus of the hypothalamus, Neuropeptide Y (NPY), Proopiomelanocortin (POMC) and Cocaine amphetamine-regulating transcript (CART) neurons were evaluated with immunohistochemical method. The One-Way ANOVA was used in statistical analysis. All results were given as mean \pm SD.

Results: Significant decreases were determined in food consumption, weight gain, and water consumption in the groups which received high-dose adropine. In the groups, which received high-dose adropine when compared with the Control Group, the adiponectin, PYY, GLP and ghrelin levels increased at a significant level ($P < 0.05$); however, no changes were determined in the OXT levels. There were no changes in the expression of the AgRP, NPY, POMC and CART neurons in all groups.

Conclusion: These results showed that the effects of adropine hormone on nutrient intake behaviors occurred over the peripheral hormones rather than central signals.

This study was supported by Atatürk University Scientific Research Projects Unit (Project No: 2015/281,2015/39)

POSTER SESSION A

A01: Cardiac physiology

A01-1

CARDIOVASCULAR PARAMETERS, MOOD BEHAVIOUR AND ATMOSPHERIC PRESSURE

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The aim: To investigate the cardiovascular parameters correlated with bioclimatic indexes and mood status.

Material and method

There were investigated 20 healthy students aged 20.87 ± 0.99 years, with BMI 20.54 ± 3.08 , nonsmokers, with a moderate daily physical activity. Using an individual chart the students monitored their cardiovascular parameters (heart rate, blood pressure) and mood status in the morning and evening. The determination was performed in periods with growing atmospheric pressure in December 2015 and January 2016. The monitoring was performed in two different stress conditions, low stress (during the holiday season) and high stress (during exam period).

Results and discussions

The cardiovascular parameters progressively increased when the atmospheric pressure increased at a rate of 10hPa.

Daily variations of the cardiovascular parameters showed that the parameters increase in the evening ($r=0.863$, strong correlation).

When atmospheric pressure increased (from 1012.31 ± 0.47 hPa to 1034.5 ± 1.54 hPa) the heart rate increased with 7%, TAS increased with 9.5% and TAD increased with 10%. The increase of the blood pressure can be explained by vasoconstriction induced by negative temperatures which occurred in high atmospheric pressure. The increase of the atmospheric pressure was associated with the decrease of the behavioral mood with a tendency for sadness and depression.

During the exam period all the cardiovascular parameters increased, and this can be explained by cardiac sympathetic stimulation.

All the variations were in normal limits, and this can be explained because the subjects were young and healthy.

Conclusions

The increase of atmospheric pressure was correlated with the increase of cardiovascular parameters in young, healthy subjects and with the decrease of mood.

A01-2

BLOOD PRESSURE MODIFICATION AND STUDENTS LIFESTYLE

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The increasing obesity among young people, smoking, coffee, alcohol associate with stress represent risk factors of high blood pressure.

We proposed to study blood pressure variation at students correlated with their lifestyle.

Were asked 27 students, 15 boys and 12 girls, about their lifestyle, coffee consumption, alcohol, smoking. Blood pressure was determined in the morning, afternoon, between November and February. During the session period determinations were made before and after the exams.

Coffee consumption was increased at girls (75%) comparative with boys (46.66%); alcohol consumption (41%); smoking at 53.33% boys and 33.33% girls. At the group of boys, during exams, a moderate increased systolic blood pressure 124.66 ± 11.44 mmHg and diastolic blood pressure 74 ± 8.42 mmHg was noticed, in the afternoon. At girls group before the exam systolic (113.33 ± 11.14 mmHg) and diastolic blood pressure (72.08 ± 5.82 mmHg) increased.

Blood pressure modification showed a response from the activation of the sympathetic vegetative system induced from the exam stress, associated with the effect of coffee consumption, which is higher at girls comparative with boys.

We recommend periodical check of blood pressure at youths that have high values, informing the youth about the risk of the negative factors, and about the modification of their lifestyle to a healthy life.

A01-3

Reversed ratio of peripheral monocyte subsets in spontaneously hypertensive rats

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Phenotypic and functional heterogeneity of peripheral blood monocytes has been recognised in humans and other species. In order to better understand a physiological significance of monocyte subsets and their role in disease conditions, changes of monocyte subpopulations in various disease models need to be investigated. Therefore, the present study aimed to characterize two main subpopulations of blood monocytes and evaluate their daily variability in spontaneously hypertensive (SHR) and normotensive Wistar (WKY) rats. Blood monocytes were analysed by flow cytometry and defined as CD43lowHis48high and CD43highHis48low, which are homologous to classical and non-classical monocyte subsets in humans, respectively. Animals were blood sampled 3 hours before the end of the passive (ZT09) and active phase (ZT21). The higher number and percentage of monocytes was found in SHR as compared to WKY rats and at ZT09 than ZT21. Both classical and non-classical monocyte subsets were increased in SHR and the reversed ratio of classical to non-classical monocyte subsets was revealed in SHR (0.62 ± 0.03) as compared to WKY rats (1.53 ± 0.09). Moreover, non-classical monocyte subsets of SHR displayed higher expression of signal regulatory protein alpha than those of WKY rats suggesting their altered phagocytic regulation. In conclusion, non-classical monocytes were dominantly elevated in SHR and thus can play an important role in the pathomechanism of hypertension. Moreover, our data suggest preferential use of this ratio rather than the absolute number of classical and non-classical monocyte subsets to evaluate cardiovascular risk. Supported by grants VEGA 1/0557/15 and APVV-0291-12.

A01-4

Baroreflex sensitivity: an algebraic dilemma

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Two main experimental approaches can be used to assess the baroreflex sensitivity (BRS): closed-loop (CL), which relies on spontaneous fluctuations of arterial pressure (AP); and open-loop (OL), which involves external mechanical or pharmacological interventions on a steady AP. CL defines the BRS as the mean slope of the linear regression between R-to-R interval (RR) or heart rate (HR) and AP; OL, instead, as the maximum negative slope of the logistic relationship between HR (or AP) and the induced changes in carotid sinus pressure. We provide algebraic demonstration that, either in CL or in OL, using RR is not tantamount to using HR as dependent variable in the analysis of BRS, although one is the reciprocal of the other. In CL, if we define BRS as the positive slope of the relationship between RR and AP calculated on consecutive beats characterised by homologous changes in the two variables, the slope of the negative relationship between HR and AP is not the BRS. In OL, if we use RR instead of HR, the BRS changes quantitatively and depends on other parameters, whereas the centring pressure computed with RR differs from that computed with HR. These contradictions should be somehow resolved and opening a debate on this issue would be a way, in our vision, to proceed toward their resolution.

A01-5

Vasopressin V1a receptors are present in the carotid body and contribute to the control of breathing

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Questions: Peripheral chemoreflex triggered from carotid bodies (CBs) in response to hypoxia, hypercapnia or acidity results in increase in ventilation and elevation of arterial blood pressure. Pressor effect of vasopressin (AVP) is mostly mediated by vasopressin type 1a receptors (V1aRs); however, little is known about the role of AVP and V1aRs in the control of breathing. The aim of our study was to evaluate how AVP affects ventilation and if the effects are mediated by V1aRs. **Methods:** We evaluated ventilatory and hemodynamic responses to intravenous AVP before and after blockade of V1aRs in urethane anesthetized normotensive male Sprague-Dawley rats. We also checked the responses to local administration of AVP close to the CBs. Finally, we did immunostaining of CBs for V1aRs and tyrosine hydroxylase, a marker of chemoreceptor cells. **Results:** Intravenous AVP decreased minute ventilation (MV) and respiratory frequency (RR) and it increased arterial blood pressure (BP). Systemic blockade of V1aRs prevented these changes. Local administration of AVP close to the CBs increased MV and RR without significant changes in the BP. Immunostaining of the CBs revealed V1aRs in glomus cells type 1. **Conclusions:** AVP and V1aRs are involved in the regulation of breathing. V1aRs are present on chemosensory cells of the CBs and stimulation of the CBs locally with AVP increases ventilation, which is contrary to the depressing effects on ventilation by systemically administered AVP.

A01-6

Effect of voluntary lung hyperinflation on central blood volume

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The purpose of our study was to evaluate the amount of blood that is translocated from the heart and pulmonary circulation (central blood volume) in trained breath-hold divers during glossopharyngeal insufflations (GPI), a maneuver that serves to increase lung volume above maximal values.

Cardiac magnetic resonance imaging including first-pass perfusion with gadolinium was determined in twelve breath-hold divers at rest and during the easy going phase of apnea with GPI.

With GPI, the lung volume increased by 0.8 ± 0.6 L ($11 \pm 7\%$, range 3-24%) above the total lung capacity (6.9 ± 1.4 L). The pulmonary transit time for gadolinium remained unchanged at 7.5 ± 2.2 sec, and pulmonary blood flow decreased by 2783 ± 1820 mL ($43 \pm 20\%$). Hence the pulmonary blood volume and the central blood volume decreased by 354 ± 176 mL ($47 \pm 15\%$) and 531.5 ± 248 mL ($46 \pm 14\%$), respectively.

Voluntary pulmonary hyperinflation leads to an almost 50% decrease in pulmonary and central blood volume.

A01-7

Sympatho-vagal balance is higher in nurses following night-shift works

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Aims: Heart rate variability (HRV) is a technic used for non-invasive indirect determination of autonomic nervous system activity. Stressful conditions affect both time- and frequency-domain of HRV parameters. Night-shifts in health professionals poses great risks for health outcomes and therefore the current study, aimed at measuring HRV parameters in nurses who had night-shift works.

Materials and methods: HRV was measured by 5-min electrocardiographic (ECG, Poly-spectrum) recordings in nurses who had night shifts in the last three days ($n=13$) and controls who had normal daytime works ($n=10$). All ECG recordings were carried out in the mornings immediately following the commencement of daytime working hours (08:30 h). Time-domain (standard deviation of normal-to-normal beats, SDNN) and frequency-domain (low frequency-LF, high frequency-HF, LF/HF ratio) were calculated by the software compatible with ECG. Data were analyzed by general linear models within MINITAB statistical software.

Results: SDNN, LF and HF did not differ between the groups ($P>0.05$) but LF/HF ratio was higher in nurses who had night-shifts than the control group who had regular sleep-wake cycle ($P=0.044$).

Conclusion: LF/HF ratio is reported to reflect degree of sympatho-vagal balance. Therefore, higher LF/HF ratio in the nurses having night-shifts suggests increased sympathetic activity. This appears to be consistent with distressing conditions faced by health-care providers.

A01-8

High blood pressure in spontaneously hypertensive rats is accompanied by altered cardiovascular reflexes and changes in the expression of TNF, interleukin 10, and their receptors in the brainstem

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Questions

Neuroinflammation is associated with development of arterial hypertension. Tumor necrosis factor (TNF) and interleukin 10 (IL-10) are key pro- and anti-inflammatory cytokines involved in neuroinflammation. In the study we determined if brain expression of TNF, IL-10 and their receptors TNFR1 and IL-10R differs between spontaneously hypertensive (SHR) and normotensive (WKY) rats.

Methods

In adult male SHR (n=12) and WKY (n=12) rats, we noninvasively measured systolic blood pressure (SBP) and collected brains and blood. We used enzyme-linked immunosorbent assays to determine concentration of TNF, IL-10 and their receptors TNFR1, IL-10R in the hypothalamus (HTS), rostral ventrolateral medulla (RVLM) and nucleus of the solitary tract (NTS). We also pharmacologically evaluated baroreflex and peripheral chemoreflex in WKY (n=6) and SHR (n=6) rats under urethane anesthesia.

Results

In comparison to WKY rats, SHR rats had: 1) higher SBP, lower gain of the baroreflex and greater pressor response of the chemoreflex; 2) higher expression of TNF and IL-10 in RVLM and NTS; 3) higher expression of TNFR1 and lower expression of IL-10R in NTS. TNF, TNFR1, IL-10 and IL-10R in the HTS and serum did not differ between SHR and WKY rats and were lower than in the brainstem.

Conclusions

Our results show that hypertension and pro-hypertensive changes in cardiovascular reflexes are accompanied by altered expression of TNF, IL-10 and their receptors in the brainstem of SHR rats.

A01-9

Intrabrain administration of TNF and interleukin 10 differently affect arterial blood pressure in normotensive and spontaneously hypertensive rats

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Questions

A growing body of evidence suggests that pro- and antiinflammatory cytokines in the brain play an important role in the regulation of cardiovascular system. Tumor necrosis factor (TNF) and interleukin 10 (IL-10) are archetypal cytokines with pro- and anti-inflammatory properties, respectively. The aim of our study was to evaluate effects of intracerebroventricularly (ICV) administered TNF and IL-10 on blood pressure (BP) in normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats.

Methods

After insertion of arterial and brain cannulae, we ICV administered bolus of either 0.9% NaCl (control), TNF (200 ng), or IL-10 (200 ng) to adult male SHR (n=18) and WKY (n=18) rats and then we were continuously recording BP for 120 minutes. All procedures and recordings were carried out under urethane anesthesia. Gathered BP data was analyzed using additive mixed model (random intercept model with fixed effect for rat strain and nonparametric smooth function of time for each group).

Results

TNF treatment resulted in a gradual increase of BP of SHR rats, while the pressure of WKY rats remained stable. For both control and IL-10 groups decreasing trends were observed, however in case of SHR rats IL-10 administration resulted in a greater decrease of BP than in WKY rats.

Conclusions

The results of our study indicate that SHR rats show enhanced response to ICV administered TNF and IL-10, which suggests that the cytokines contribute to the hypertensive phenotype.

A01-10

Disturbances in mitochondrial metabolism of energy substrates in left ventricle of patients with type 2 diabetes

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Diabetes mellitus type 2 (DM2) is associated with a greatly increased risk for cardiac disease, originating both from vascular causes (ischemic heart disease) and metabolic disturbance itself (diabetic cardiomyopathy). Myocardial metabolism of DM2 patients was thus far studied using indirect approaches *in vivo*, and direct methods in human atrium *in vitro*, with some controversial findings regarding fatty acid utilization. However, no studies exist that directly investigated a mitochondrial substrate utilization in left ventricular tissue obtained from living patients.

Mitochondrial fatty acid (palmitoylcarnitine) and carbohydrate (pyruvate) oxidation were measured in permeabilized left ventricular fibers obtained from patients undergoing coronary artery bypass grafting surgery, whom either had DM2 or were not diabetic (Ctl).

There was no difference between the two groups in the oxidation of pyruvate (10 mmol/l). However, mitochondrial oxidation rate of palmitoylcarnitine (40 μ mol/l) in DM group was significantly decreased. There was no difference in activity of citrate synthase or pyruvate dehydrogenase, and no difference in expression of individual complexes of electron transfer chain.

In conclusion, the novelty this study is the direct observation that left ventricle of patients with DM2 exhibits significantly decreased oxidation rate of fatty acids, while the oxidation of carbohydrate is unaffected. This is associated with unchanged mitochondrial content and expression of mitochondrial respiratory chain complexes. Such mitochondrial disturbances can result in excessive intracellular accumulation of lipids in diabetic hearts, which is thought to contribute to pathogenesis of diabetic cardiomyopathy.

A01-11

Amplification of peripheral arterial pressure as a marker of cardiovascular risk

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Questions

Aging is known to be one of the underlying mechanisms of arterial stiffness. The aim of our study was to clarify the difference in peripheral pulse pressure amplification between young adults and middle aged persons.

Methods

Thirteen individuals aged 20 \pm 0.3 y/o and 16 persons aged 60 \pm 2 y/o were studied. Central aortic

pressure was estimated using applanation tonometry (Sphygmocor device, AtCor Medical, Sidney, Australia) at supine position following 10 minute rest.

Results.

The amplification ratio (the ratio between peripheral and central pulse pressure) was significantly higher in the young group as compared to the adults (1.7 ± 0.03 vs. 1.2 ± 0.05 , $p < 0.001$). We evidenced a negative correlation between the amplification ratio and the corrected for heart rate 75 bpm augmentation index ($r = -0.59$, $p < 0.03$ in the young group, $r = -0.36$, n.s. in the adults). In the young persons the larger amplification of peripheral systolic and pulse pressure might be assumed to result from the delayed return of the reflected pulse wave as compared to the older studied persons. Since this parameter was negatively and statistically significantly correlated to the augmentation index the amplification ratio was dependent on the most important index of arterial stiffness. In the aged persons the stiff arterial wall determined the earlier return of the reflected pulse wave in systole and the augmentation of the central systolic and pulse pressure. Consequently the amplification ratio in this age group got lower than in the young and the negative correlation with the augmentation index was reduced.

Conclusions. Our results supported the idea that the amplification ratio could be utilized as an additional and informative index of arterial stiffness.

Key words: applanation tonometry, amplification ratio, cardiovascular risk

A02: Vascular physiology

A02-1

Relationship between peripheral regional blood flow and heart rate recovery at one minute (HRR1) after constant-load exercise in lower-limb ischaemia.

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Background: Conditions that may influence heart rate recovery 1 minute after exercise (HRR1) are still incompletely defined. We hypothesized that the importance (both local severity and regional diffusion) of peripheral skeletal muscle ischaemia is associated with low HRR1.

Design and Methods: In 529 patients with suspected or confirmed peripheral vascular disease not receiving beta-blockers (61.4 ± 11.3 years old), we retrospectively studied the relationship of HRR1 to exercise-induced changes in transcutaneous oxygen DROP index (limb changes minus chest changes from rest). The sum of DROP indices observed on both calves and both buttocks (DROP_{tot}) provides the unique opportunity to estimate both the severity and the diffusion of exercise induced ischaemia on the right and left side simultaneously. It was used during a constant-load treadmill test (3.2 km/h; 10% grade) to classify patients in quartiles, the fourth quartile representing the more ¹important ischaemias.

Results: There was an inverse relationship between quartiles of DROP_{tot} and HRR1, even after adjustment for heart rate reserve (Delta HR: end-exercise minus resting heart rate), age (\leq or >60 years), gender, body mass index, treadmill maximal walking distance and ankle brachial index: adjusted $R = 0.629$; $P < 0.0001$.

Conclusion: During constant-load treadmill testing, DROP_{tot}, an index of the importance₁ of exercise-induced lower-limb ischaemia, correlates with HRR1. Whether HRR1 is improved in proportion of DROP_{tot} improvement in patients undergoing surgery or rehabilitation for peripheral artery disease is a fascinating issue for future studies.

A02-2

One week of high salt dietary intake increased peripheral blood monocytes' intracellular hydrogen peroxide and peroxynitrite level in young healthy women

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Aim: Previously we demonstrated that high salt dietary intake (HS diet) impairs endothelial function in both micro- and macrocirculation in young healthy women, even in the absence of blood pressure changes. This study aimed to evaluate one week of HS diet effects on reactive oxygen species (ROS) production in human leukocytes.

Materials and Methods: Healthy women ($N=15$, age: 20 ± 2) were taking HS diet (about 14g of NaCl/day) for 7 days. ROS production was measured in monocytes and lymphocytes from peripheral blood using flow cytometry (FACS Canto II, BD). Dichlorofluorescein diacetate (DCF-DA) was used to detect baseline and stimulated (phorbol 12-myristate 13-acetate (PMA)) intracellular hydrogen peroxide (H_2O_2) and peroxynitrite production, before and after HS diet. Data were expressed as the median fluorescence intensity (MFI).

Results: 24h-urinary Na excretion and calculated salt intake confirmed that subjects conformed to the diet protocols (NaCl, g/day: pre HS 4.9 ± 2.2 vs. post HS 12.2 ± 5.8 , $P=0.003$). HS diet significantly increased basal ROS production in monocytes compared to pre HS diet measurements (DCF-DA MFI pre HS 4.27 ± 0.91 vs. post HS 4.96 ± 1.3 , $P=0.024$). There was no significant difference in ROS production in lymphocytes before and after HS diet (DCF-DA MFI pre HS 2.01 ± 0.17 vs. post HS 1.95 ± 0.12 , $P=0.184$). PMA stimulation significantly increased ROS production in lymphocytes and monocytes before and after HS diet protocol.

Conclusion: The results of present study demonstrated that HS diet leads to leukocytes activation and increased oxidative stress which may contribute to endothelial activation and dysfunction even in the absence of blood pressure changes in young healthy persons. (HRZZ IP-2016-06-8744)

A02-3

Short-term high-salt intake causes increased oxidative stress in young healthy women

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Questions: Our previous studies have demonstrated that one week of high-salt (HS) intake causes significant impairment in microvascular reactivity in young healthy women. We speculated that one of the possible reasons for this impairment caused by HS intake may be increased oxidative stress. The aim of this study was to determine whether one week of high-salt intake effects oxidative status in young healthy women.

Methods: Healthy, normotensive (systolic pressure 113 ± 12 mmHg, diastolic pressure 73 ± 7 mmHg) women ($N=15$, age: 20 ± 2) were included in 2 weeks long protocol; at the first week subjects were taking low-salt diet (≈ 2.3 g of NaCl/day) 2nd week subjects were taking HS diet (≈ 14 g of NaCl/day) for 7 days. Oxidative stress was assessed via detection of lipid peroxidation in serum, measured by TBARS method (Thiobarbituric Acid Reactive Substances) using spectrophotometry. Venous blood samples were taken on the first day of HS protocol and on the last day of HS protocol.

Results: 24h-urinary Na excretion and calculated salt intake confirmed that subjects conformed to the diet protocols (NaCl, g/day: pre HS 5.3 ± 2.8 vs. post HS 11.2 ± 5.6 , $P=0.003$). One week of HS diet

significantly increased lipid peroxidation in serum compared to measurement before HS protocol (TBARS: pre HS 0.54 ± 0.10 vs. Post HS 0.60 ± 0.09 , $P=0.035$).

Conclusion: Our results have demonstrated that even one week of HS intake significantly alters oxidative status in young healthy women, by increasing lipid peroxidation. This finding supports our hypothesis that impaired oxidative status may be one of the factors included in the development of microvascular dysfunction caused by short-term HS intake in healthy, normotensive subjects. (HRZZ IP-2016-06-8744)

A02-4

Hyperthyroidism and vascular function: the impact of local and systemic mechanisms

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Hyperthyroidism might represent a model to study vascular effects of thyroid hormones *in vivo*. Thus, we aimed to assess the contribution of local and systemic factors to cutaneous microvascular reactivity in 31 hyperthyroid patients with Graves disease and 30 age-matched healthy controls. Simultaneously, electrocardiogram (ECG, for assessment of the heart rate (HR) and heart rate variability (HRV) by spectral analysis), digital artery blood pressure, the microvascular laser Doppler flux (LDF) and the corresponding skin temperature (T_{skin}) were measured. Vascular reactivity was assessed by inducing postocclusive reactive hyperaemia (PORH) to challenge local vascular mechanisms and cooling of the contralateral hand to challenge the sympathetic nervous system.

Patients exhibited significantly increased baseline LDF ($p<0.05$, Mann Whitney (M-W) test) but not T_{skin}, increased HR ($p<0.001$, t test) and systolic blood pressure (psystol) ($p<0.05$, t test) as well as increased low frequency to high frequency ratio (LF/HF) of HRV ($p=0.002$, M-W test). The time to peak of PORH was shorter (tpeak, $p=0.02$, M-W test) and the duration of PORH longer (tdur, $p=0.02$, t-test) in patients. The decrease of LDF during contralateral cooling was significantly smaller and the return to baseline LDF faster in patients in spite of higher relative psystol increase ($p<0.05$, ANOVA).

Hyperthyroidism profoundly impacts the control of skin microcirculation: higher baseline LDF and altered parameters of PORH imply an increased vasodilator capacity. Less pronounced response of LDF to indirect cooling in hyperthyroidism in spite of higher sympathetic nervous system activity implicates an important contribution of local mechanisms to vascular control.

A02-5

Acute exhausting exercise session affects endothelium-dependent, but not endothelium-independent vasodilation in professional rowers

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Aim: We previously reported that a single exposure to exhausting training (AE) impaired endothelium-dependent vasodilation in skin microcirculation of professional rowers. Present study aimed to test if AE affect endothelium-independent vasodilation in skin microcirculation, or if microvascular reactivity impairment is limited to endothelial dysfunction.

Methods: 20 professional rowers participated in this study. Blood pressure, heart rate, body mass index, waist to hip ratio, fasting lipid panel, plasma glucose and C reactive protein (CRP) were measured in all subjects. They all underwent AE. Blood lactate levels, arterial blood gas analysis and

acid base status were measured before and after AE. Cutaneous microvascular blood flow was assessed by Laser Doppler Flowmetry in response to iontophoresis of sodium nitroprusside (SNP) (endothelium-independent vasodilator) before and after AE.

Results: All rowers were normotensive, lean males, with normal lipidogram, glucose and CRP blood levels. Serum lactate significantly increased and metabolic acidosis occurred after AE. Systolic blood pressure and heart rate significantly increased during AE. The SNP-induced dilation was not significantly changed after AE and was similar to pre AE measurements (SNP% increase before AE 1017 ± 280 vs. after AE 1089 ± 253 , $P=0.132$).

Conclusion: This study demonstrated that AE did not provoke any change in endothelium-independent dilation in rowers. Taken together with our previous finding of impaired ACh-induced dilation in AE, present data suggest that AE affects microvascular function in professional rowers through its adverse impact on endothelial function.

Support: VIF2015-MEFOS-05 and Osijek-Baranja County grant 2014-2016.

A02-6

THE EFFECT OF A SHORT TERM HIGH SALT DIET ON CEREBROVASCULAR REACTIVITY IN RESPONSE TO BREATH HOLDING TEST IN YOUNG HEALTHY SUBJECTS

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Questions: There is a paucity of data on the effect of high salt diet on cerebral vessels dilation mechanisms in response to various stimuli in humans. Present study aimed to evaluate the effect of HS diet on middle cerebral artery (MCA) reactivity to breath holding test (BHT) in healthy human. Methods: Healthy women (N=6) and men (N=5) age 20 ± 1 were included in the study. 3x measurements were performed: 1) before any diet intervention, 2) after 1 week of low salt (LS) diet ($< 2.3g$ kitchen salt/day), and 3) after 2 week of HS diet ($> 11.2 g$ kitchen salt/day). Transcranial doppler signal (TCD) of the MCA in response to BHT (in duration of 30 seconds) was recorded. Blood pressure was recorded simultaneously by Finapres. Results: Diastolic cerebral blood flow (dCBF) and velocity time integral (VTI) were significantly reduced after HS diet compared to the first measurement. R-R interval, heart rate, systolic cerebral blood flow, cerebral perfusion pressure, cerebral vasomotor reactivity and cerebral pulsatility index did not show statistically significant difference. HS diet did not significantly affect any of arterial blood pressure values. Increased 24h-urinary Na⁺ excretion after HS diet confirmed that subjects conformed to diet. Conclusions: One week of HS diet can impair the cerebrovascular reactivity in human, even without affecting blood pressure values. Support: University of Osijek research grant, INGI-2015-16

A02-7

Investigation of Relations Between GSTT1 Polymorphism and Lower Extremity Varix

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Varicose veins of the lower extremity (VVLE) are a frequently encountered vascular disorder in the general population, but etiology of VVLE has not yet been fully explained. This study is planned to answer at least part of the question of whether the vein wall loses function and/or the deficiencies in its function are caused by deficiencies in genetic defense systems. For this purpose, the GSTT1 gene polymorphism encoding the enzyme GSTT1 responsible for the metabolism of cytotoxic agents, which plays a physiological role in initiating the detoxification of potential alkylating agents, has been investigated. Patient group blood samples were taken during the surgery of who had primary varixes of the saphen vein (n=30). Control group blood samples were taken during the graft surgery of the patients who had coronary arterial graft operation (n=30). 5 ml of peripheral blood samples were taken into an EDTA tube and samples were stored at -80°C until DNA isolation. DNA isolation was performed with PCR Preparation Kit and the obtained DNA were used for polymorphism analysis in GSTT1 gene (qRT-PCR). % 1 agarose gel was prepared depending on the product length. Samples were run on gel. A band of 459 bands was obtained for GSTT1. The appearance of this band on electrophoresis shows that the genotype is intact, that is the T wild genotype. There was no statistically significant relationship between the GSTT1 null genotype in control group with patient group.

A02-8

The relationship between soluble lectin-like oxidized low-density lipoprotein-1 and carotid intima-media thickness in patients with diabetes mellitus without cardiovascular diseases

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Background: Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is regarded as a central element in the initiation of endothelial dysfunction (ED). LOX-1 has been implicated as a key causative of a number of cardiovascular diseases (CVD). Measurement of soluble LOX-1 (sLOX-1) may provide a novel diagnostic tool for the prediction of ED. Since ED is a very early step in atherogenesis, we investigated whether sLOX-1 could be a novel diagnostic tool for the prediction of ED in patients with type 2 diabetes mellitus (DM) without CVD. We evaluated relationship of serum sLOX-1 with carotid intima-media thickness (CIMT). **Methods:** The three groups; DM with CVD (Group I), DM without CVD (Group II) and control were comprised. CIMT were measured on ultrasonography images. Serum oxidized LDL (oxLDL), sLOX-1 levels and paraoxonase-1 (PON-1) activity were measured from blood samples. All statistical comparisons were performed using the analysis of variance was used to compare multiple-group means. **Results:** OxLDL, sLOX-1 levels were significantly higher in the Group I and Group II than in the control. sLOX-1 levels were significantly higher in the Group I compared with the Group II. PON-1 activity was significantly lower in the Group I and Group II groups than in the control. There were no significant differences between the Group I and Group II. CIMT were significantly higher in the Group I and Group II than in the control but were significantly higher in the Group I compared with the Group II. There was a significant positive correlation between sLOX-1 and

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Poster Session A

CIMT in Group I and Group II. **Conclusions:** sLOX-1 levels could be strong biomarker for determining early endothelial damage in DM patients without cardiovascular diseases.

A02-9

Non-invasive estimation of arterial stiffness in healthy and asthmatic children - comparison of the methods: a pilot study.

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Questions

Asthma is an inflammatory disease that affects not only lungs but other organ system, e.g. a cardiovascular system. Arterial stiffness is a subclinical predictor of cardiovascular diseases. Its measurement is carried out by applanation tonometry (European countries) with such main parameters as pulse wave velocity (PWV) and augmentation index (AI) as well as measurement of Cardio-ankle index (CAVI) outside Europe.

The aim of our study is to estimate arterial stiffness in asthmatic and healthy children using two methods of its measurement and compare them.

Methods

We measured asthmatic children (group A, 52 respondents) and control group (C, 71 respondents) of the same age, systolic (SBP), diastolic (DBP) blood pressure and body mass index (BMI).

For each respondent we measured SPB, DPB, PWV, AI and AI standardized on pulse height and heart rate (AIx75) on the dominant side with Sphygmocor device (AtCor Medical, Australia). All measurements were calibrated by oscillometric blood pressure measurement on the brachial artery (Omron, HEM-907-E, Japan) on the same side. The CAVI was measured with VaSera device (Fukuda Denshi, Japan). The Statistic 13 software was used for statistical analysis.

Result

There were significant differences in AIx75 (3.0±10.9 vs. -2.0±10.6; p<0.05), PWV (6.6±1.1 vs. 7.2±1.4; p<0.05), CAVI (4.5±0.9 vs. 4.9±0.6; p<0.05) between A and C, and significant correlation between PWV and CAVI (p<0.05).

Conclusion

We can conclude that asthma bronchiale and its treatment changed the properties of vessels in children comparing to the healthy group. Our study shows that both methods reflect the changes in arterial stiffness.

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Poster Session A

A02-10

Vitamin D deficiency impairs geometrical structure and function of cerebral arteries

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Questions: Vitamin D deficiency is a global problem, which can lead to several pathophysiological consequences including cardiovascular diseases. Our goal was to examine the possible effect of vitamin D status on the geometry and function of cerebral arteries.

Methods: Four week old male Wistar rats were either fed with vitamin D deficient diet or received per os vitamin D supplementation in addition to the conventional rat food. Physiological parameters (body weight, arterial blood pressure, heart rate, serum sex hormone levels) and 25-hydroxyvitamin D levels were measured during the study. After 8 week of treatment the rats were decapitated and the anterior cerebral artery was removed. Geometry, biomechanical properties, smooth muscle tone and endothelial relaxation capacity of the isolated vessel segments were measured using pressure microangiometry.

Results: Vitamin D deficient diet did not change the mean physiological parameters, but caused significantly lower serum 25-hydroxyvitamin D levels. Vitamin D deficiency decreased the relaxed inner radius of arteries, increased the wall thickness / inner radius ratio and wall cross sectional area. The tangential wall stress was significantly lower in the vitamin D deficient group. In addition, vitamin D deficiency increased the myogenic as well as uridine 5'-triphosphate induced tone and impaired bradykinin induced relaxation.

Conclusions: Vitamin D deficiency causes inward hypertrophic remodeling in cerebral arteries, changes in the biomechanical properties and diminishes the endothelium-dependent relaxation capacities, which can potentially lead to disorders of the cerebral circulation.

A02-11

ARTERIAL STIFFNESS IN OBESE ADOLESCENTS – A RELATION TO VASCULAR RESISTANCE AND SYMPATHETIC NERVOUS SYSTEM ACTIVITY

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Introduction: Obesity is a risk factor of atherosclerosis development. For the early atherosclerotic changes assessment, the estimation of arterial stiffness using CAVI (Cardio-ankle vascular index) is used. Several recent studies paradoxically found an inverse relationship between CAVI and BMI (body mass index) in adolescents indicating lower stiffness in obesity. The aim of our study was to ascertain if this finding can be assigned to the difference in peripheral vascular resistance (PVR) possibly related to the changed sympathetic nervous system activity in obese adolescents.

Methods: In 21 obese (15.44 ± 2.8y, BMI: 30.51 ± 2.0kg.m⁻²) and 21 non-obese (16.13 ± 2.41 y., BMI: 20.81 ± 2.1kg.m⁻²) gender and age matched adolescents CAVI was measured together with PVR (PVR = 80* (mean MBP/mean CO)), where MBP (mean blood pressure) was measured on the beat-to-beat basis (Finometer, FMS, Netherlands) and CO (cardiac output) was measured using impedance cardiography (CardioScreen 2000, Medis GmbH, Germany). As a sympathetic activity

index, the magnitude of low frequency (0.04 – 0.15 Hz) oscillations in systolic blood pressure (LF SBP) was used.

Results: A significantly lower CAVI (p = 0.001) was found in obese group. In addition, we found both PVR (p = 0.002) and sympathetic nervous system activity index LF SBP (p = 0.006) significantly lower in obese group compared to controls.

Conclusion: In accordance with recent studies, we observed lower CAVI in obese adolescents. Our results indicate that this paradoxical result could be attributed to the lower PVR probably as a result of the lower vascular sympathetic activity in obese group.

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A02-12

About the human vascular response to hyperoxia

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The skin is an adequate model to explore a wide range of functional aspects of microcirculation, allowing noninvasive assessments. The normobaric oxygen breathing (NOB) is commonly used as a provocation test, although the mechanisms involved in its vascular response still need clarification. Our aim was to look deeper into the microvascular response to NOB using a combination of several noninvasive techniques. A group of 20 healthy subjects (20.3 ± 2.6 y.o.) was included after giving informed written consent. After acclimatization, subjects performed the NOB test while sitting, which consisted of three phases - 10 min breathing room atmosphere (I), 10 min breathing 100% oxygen through a facemask (II), and 10 min again breathing room atmosphere (III). Measured variables, recorded on both feet, included blood flow by laser Doppler flowmetry (LDF) and photoplethysmography (PPG), transcutaneous (tc) O₂ pressure, electrodermal activity (EDA), and transepidermal water loss (TEWL). Blood pressure (BP) and heart rate (HR) were measured with a digital device, and respiratory rate (RR) and depth (RD) with pneumography. Nonparametric statistics were applied (p<0.05). During phase II tcPO₂ increased significantly, creating hyperoxia, and revealed two distinct increment velocities. A consistent significant bilateral perfusion decrease was noted with both LDF and PPG. BP increased significantly, EDA increased bilaterally and HR decreased, both without significance. TEWL increased significantly in both feet, suggesting a change in epidermal water dynamics. These results suggest the utility of the combined use of these techniques for the noninvasive assessment of the vascular response to hyperoxia.

A04: Endocrine, neuroendocrine and metabolism

A04-1

Arginase-II promotes tumor necrosis factor-α release from pancreatic acinar cells causing β-cell apoptosis in aging

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Aims: Aging is associated with insulin resistance and pancreatic dysfunction. Our previous studies demonstrated that arginase-II (Arg-II) deficiency protects mice against atherosclerosis, vascular aging and obesity-associated type 2 diabetes. It has been reported that Arg-II is expressed in pancreas of rodents and humans. However, functions of Arg-II in regulation of pancreatic β-cells and in age-associated glucose intolerance are not known.

Design & Methods: The WT and Arg-11^{-/-} offspring from hetero/hetero cross were interbred to obtain WT and Arg-11^{+/+} mice, respectively. Pancreatic cell apoptosis was evaluated by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining.

Results: Here we show that targeted disruption of Arg-11 improves glucose tolerance as a result of increased insulin secretion without significant change in insulin sensitivity as compared to age-matched old wild type (WT) mice, which is associated with larger pancreatic islet size and higher β -cells mass in the old Arg-11^{-/-} mice. Arg-11 is mainly expressed in acinar cells and upregulated with aging in female WT mice with concomitant enhanced TNF- α release from the pancreatic acinar-cells leading to apoptosis of the pancreatic β -cells. Moreover, conditioned medium of isolated acinar cells from old WT mice enhances apoptosis of cultured β -cells in vitro, which is reduced by neutralizing antibody against TNF- α .

Conclusions: In this study, we demonstrate an age-associated Arg-11 upregulation in pancreatic acinar cells, which promotes TNF- α release through p38mapk, leading to β -cell apoptosis, insufficient compensatory insulin secretion, and glucose intolerance in mice in a gender-specific manner.

A04-2

EFFECTS OF MELATONIN OR GHRELIN TREATMENT ON ANGIOTENSIN II - INDUCED INTESTINAL MOTILITY IN DIABETIC RATS

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Objective: The irregular intestinal function is a common diabetic disorder, connected with the progression of the disease. It has been found that due to their antioxidant properties, hormones melatonin (MLT) and ghrelin (GHR) favorably affect many diabetic complications. This study aims to assess the effects of short - term MLT or GHR treatment on intestinal motility of rats with streptozotocin (STZ) - induced diabetes.

Methods: Male Wistar rats were divided as follows: controls and 3 diabetic groups: 1st treated only with a single STZ injection; 2nd and 3rd - single STZ injection followed by a 7-day period of either MLT or GHR application, respectively. The experiment lasted 42 days. In the end, intestinal preparations were influenced by Angiotensin II (AngII). The obtained contractions were analyzed with specific software.

Results: The preparations of jejunum and ileum from 1st diabetic group developed a powerful response to Ang II (amplitude 1.46 \pm 0.10g and 3.31 \pm 0.29g, respectively) compared to control preparations (1.08 \pm 0.12g and 2.20 \pm 0.11g, respectively). On the contrary, the large intestine of STZ-treated animals had a weaker reaction to Ang II. 7-day administration of MLT or GHR affected the amplitude and duration of Ang II-induced small intestinal contractions. The response to Ang II of the large intestine from the 2nd and 3rd group was not ameliorated.

Conclusion: The beneficial effect of MLT and GHR on the hyperactivity of the small intestine was probably due to antioxidant action and down-regulation of Ang II receptors in the smooth musculature. On the other hand, Ang II-mediated contractile activity of the large intestine was seriously impaired by diabetes and could not be enhanced by a short-term application of these hormones.

Acknowledgments: The study was supported by Grant 22/2014, Trakia University, Bulgaria.

Keywords: melatonin, ghrelin, diabetes

A04-3

L-Arginine has dual effect on Electrical and Calcium Activity in Mouse Beta Cells in Tissue Slices.

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L-arginine affects insulin secretion from pancreatic beta cells in a glucose-dependent manner, but the mechanism of its action is multifactorial and not dependent on energy metabolism of the amino acid. Arginine effects described in the literature are: a) depolarization of beta cells during electrogenic entry, b) functioning as a nitric oxide (NO) donor, increasing insulin secretion or suppressing it through inhibition of phosphofructokinase-dependent lowering of [ATP], c) concentration-dependent cGMP and non-cGMP mediated dual effects of NO, or d) activation of cAMP pathway.

We studied combined effects of arginine (1 or 10 mM) and glucose (3, 6, 9, 12 mM) on electrical activity and intracellular calcium oscillations [Ca²⁺]_i using acute pancreatic tissue slices.

Administration of 1 or 10 mM arginine resulted in either depolarization and increase in the frequency of electrical bursts or in membrane hyperpolarization with cessation of electrical activity. The latter effect was more frequently observed in lower glucose. Recording [Ca²⁺]_i oscillations in tissue slices assesses behavior of many cells in a given islet simultaneously. Addition of arginine revealed induction (in otherwise non-stimulatory glucose) or an increase in frequency of [Ca²⁺]_i oscillations, even up to the point of continuously elevated [Ca²⁺]_i, or a decrease in [Ca²⁺]_i without recognizable oscillations, corroborating the findings obtained by electrical activity recordings. Strikingly, these two opposite effects were observed in beta cells from the same islet. Both electrical and [Ca²⁺]_i recordings demonstrate that the effects of arginine are dependent on glucose but distinct from the mechanism of action of glucose.

Our findings confirm a possibly inhibitory influence of arginine on electrical and calcium activity of at least some beta cells, predominantly at lower glucose concentrations.

A04-4

METABOLIC SYNDROME AMONG ADULT POPULATION IN KIRKUK

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Metabolic syndrome is defined by a constellation of interconnected physiological, biochemical, clinical, and metabolic factors that directly increases the risk of cardiovascular disease, type 2 diabetes mellitus, and all cause mortality. The aim was to Study of metabolic syndrome in adult persons in Kirkuk in single center according to international diabetic federation. This single centre study was performed in an out patient clinic in kirkuk over a period of one year where 100 obese persons(52 male , 48 female [not pregnant]. Their mean age was 47.69 \pm 10.88 year. Obese cases were submitted to biochemical and physical examinations. According to the international diabetic federation criteria(2005) of 100 obese patients, after investigation , was found that 67 patients (34male , 33female) have metabolic syndrome abdominal obesity seen in all the patients followed by high triglyceride then high blood pressure(BP) next Low HDL cholesterol lastly high blood sugar. The study showed highest incidence between [41---50y age] and no increase in the incidence of MS with increase in BMI [Grade 1=19 MS Grade 2=31MS Grade3=17MS] . Male and Female40—59y age were about five times as likely as those 20—39y age to meet the criteria of MS. Prevalence of risk factors showed, highest incidence of central obesity[100percent], high triglyceride[54percent],high BP[45percent],low HDL cho[40percent],high BS[17percent].High incidence of metabolic syndrome among population, higher CAD and HF in MS cases than in non MS cases, highest age incidence of MS between [41-50y],high triglyceride in most of the patients.

A04-5

EFFECTS OF APELIN LEVELS, APELIN GENE POLYMORPHISM AND APELIN RECEPTOR GENE POLYMORPHISM TO METABOLIC CONTROL FOR CHILDREN WITH TYPE 1 DIABET

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Questions: Apelin is a new adipocytokine produced by fat tissue. There are many hypotheses between insulin resistance and hyperinsulinemia with apelin. Insulin resistance is discussed not only as a risk factor for type 2 diabetes but also as a risk factor for type 1 diabetes (T1DM). In this study, apelin level, apelin and apelin receptor (APJ) gene polymorphism; the effect on metabolic control in T1DM and healthy children was investigated.

Methods: 100 T1DM and 100 healthy children aged 1-18 years were included in the study. Apelin level was studied by ELISA, apelin and APJ gene polymorphisms (rs2235306, rs2235312, rs3115757 and rs11544374 (G212A), rs948847 (A445C), rs2282625) using real time PCR. Apelin level, apelin and APJ gene polymorphisms, HbA1c levels were evaluated together with the presence of complications.

Results: Apelin levels were 22.2 pg/ml (min.5.7 - max.206.1) in the T1DM group, 31.69 pg/ml (min.8.1 - max.169.9) in the control group and 0.042 p-value. Only the G212A apelin receptor gene polymorphism was significantly different in T1DM and control group (p = 0.039). There was a statistically significant difference in the presence of rs2235312 gene polymorphism between children with T1DM and non-polymorphic mean HbA1c (average of 4 HbA1c levels observed in the last one year) (p = 0.026).

Conclusions: Children with T1DM, apelin levels were significantly lower than in healthy controls. Only G212A apelin receptor gene polymorphism was detected in T1DM but there was no correlation between apelin level and polymorphism. The mean HbA1c was high in the presence of rs2235312 gene polymorphism in children with T1DM.

Keywords: apelin, polymorphism, type 1 diabetes

A04-6

Cyclic AMP Enhances Beta Cell Network Activity in Mouse Pancreatic Slices through PKA-dependent pathway

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Insulin secretion from beta cells is triggered upon increase in $[Ca^{2+}]_c$ and can be further amplified by cAMP, which has been previously described to act through PKA- or Epac2-dependent pathways. Since the precise mechanism of action is not fully understood we assessed the $[Ca^{2+}]_c$ dynamics in beta cell populations with electro- and opto-physiological approaches combined with the acute tissue slice technique, supported by network-based analyses. In the absence of forskolin, substimulatory glucose concentration failed to increase $[Ca^{2+}]_c$, while stimulatory glucose concentration evoked a transient calcium increase followed by synchronized high frequency Ca^{2+} oscillations. Addition of forskolin to the substimulatory glucose concentration triggered a delayed high frequency Ca^{2+} oscillations. In high glucose concentration the addition of forskolin further increased the frequency of Ca^{2+} oscillations. Despite a modest decrease in durations of individual oscillations, the relative active time increased by more than 50 %. Furthermore, the beta cell functional networks become denser in

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the forskolin regime, suggesting a higher degree of synchronicity. To determine which of the two aforementioned pathways was responsible for augmented Ca^{2+} oscillations, the same sets of experiments were performed on pancreatic slices from mice lacking the Epac2 protein. In this case, a qualitatively very similar behaviour was observed compared with WT littermates. These results corroborate previously published data describing that phosphorylation of several targets by PKA is responsible for the cAMP-augmented Ca^{2+} oscillations in pancreatic beta cells.

A04-7

Western Diet-Induced Early Dysfunction of Mouse Pancreatic Beta Cells

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To study stimulus-secretion coupling *in situ*, we combined electrophysiological, optophysiological, and novel complex network approaches with the acute mouse pancreas tissue slice preparation. At least four parameters of the beta cell response to glucose are affected by glucose concentration: (i) recruitment of beta cells (i.e., more cells are activated in higher glucose), (ii) advancement of their response (i.e., earlier response in higher glucose), (iii) enhancement of their activity (i.e., changes in oscillation frequency and duration), and (iv) coupling between cells (i.e. better coupling in higher glucose). We made use of a mouse model of western diet-induced obesity and type 2 diabetes to study the calcium response, calcium-secretion coupling, as well as coupling between different cells at different glucose concentrations. We fed western-diet (WD, composed of 40 % fat and 34 % sucrose) for 8 weeks to 12 weeks old male C57BL/6 mice. This resulted in increased body weight, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, impaired glucose tolerance, and insulin resistance. Glucose stimulation elicited calcium responses in beta cells that were qualitatively similar in WD and lean littermates, exhibiting high frequency oscillations superimposed on slower basal oscillations. 8 weeks of WD decreased the frequency of the high frequency oscillations (i.e. smaller enhancement) and the level of synchronicity between beta cells at medium glucose concentrations (9 mM). In summary, we found evidence that already after 8 weeks of WD intervention early dysfunction of beta cells is detectable.

A04-8

THE METABOLIC SYNDROME IN HYPERTENSIVE ELDERLY PATIENTS

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The aim of this study was to evaluate the metabolic syndrome prevalence in hypertensive elderly patients.

We investigated 348 patients (56.2% male) in County Hospital Timisoara (2016) with a mean age of 69.2±9.7 years. All patients were evaluated for personal history, anthropometric and biochemical parameters (arterial pressure, blood glucose, body mass index and lipid profile).

Hypertension was presented in 57.1% of patients and a high triglycerides level (353.27 ± 54.96 mg/dl) was detected in 64% of patients. 59.7% of patients were obese (BMI higher than 30 kg/m²). BMI was correlated with total, LDL – cholesterol values and also with the parameters of glucose metabolism (p<0.001). In study group, 47.8% of patients with MS presented type II mellitus diabetes. The prevalence of metabolic syndrome was 58.6% in those above the age of 72 years and 47.3% among 65 to 71 years old.

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These correlations pointed out the fact that is necessary in hypertensive elderly patients to control the life style and risk factors which can develop the metabolic syndrome.

A04-9

Glucose-Stimulated Beta Cell Calcium Dynamics in Acute Pancreas Tissue Slices from C57BL/6 Mice

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Previously, we had used confocal microscopy to characterize glucose-stimulated calcium dynamics in acute mouse pancreas tissue slices of the outbred NMRI strain. Since many genetic and diet-induced animal models of diabetes rely on the C57BL/6J background, we set out to characterize the beta cell response in this strain of mice. Functional multicellular calcium imaging was used to simultaneously record a large number of beta cells from all layers of islets. We studied the effect of stimulation with varied glucose concentrations on (i) the delay between glucose exposure and response (advancement), (ii) the number of cells that respond to glucose exposure (recruitment), (iii) the duration and frequency of [Ca²⁺]_i oscillations (enhancement), and (iv) the degree of coactivity between cells (synchronicity).

The delay in response shortened with increasing glucose concentrations. Activation of first beta cells started in 7 mM and the majority of cells were active already in 8 mM glucose. The frequency of [Ca²⁺]_i oscillations was increasing between 7 mM and 10 mM glucose and decreasing for higher stimulus concentrations. The durations as well as synchronicity of oscillations, however, were always found to increase with increasing glucose.

This study provides an upgrade on previous findings using single stimulatory glucose concentrations, showing progression of beta cell response to varied glucose concentrations, and characterizes the C57BL/6J background, providing a useful reference for studies using this mouse model.

A04-10

Inhibition of NMDA receptors provokes qualitative changes in intercellular communication patterns among pancreatic beta cells: Novel insights from multilayer network approaches

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Insulin-secreting beta cells in islets of Langerhans form a complex syncytium with non-trivially interconnected elements that are intrinsically nonlinear, heterogeneous, and driven by different oscillatory subsystems. Intercellular coupling mechanisms are the necessary substrate that ensures a coordinated activity and hormone secretion and are incompletely understood. To explore the collective behavior of these microorgans we combined advanced high spatio-temporal resolution confocal imaging with network science. Specifically, we used the multilayer network formalism for the characterization of calcium waves and communication patterns in islets. Along these lines, we investigated whether inhibition of NMDA receptors modifies beta cell synchronizability. Namely, antagonists of NMDA receptors, such as dextromethorphan, have recently been shown to prolong the durations of intracellular calcium oscillations which leads to an enhanced insulin secretion and have therefore been suggested as novel antidiabetic drugs. Our results indicate that in experiments with long-term exposure to stimulatory levels of glucose, the NMDA receptor blockade elongates the

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synchronous behavior of beta cell populations in comparison to glucose-stimulation only. Moreover, individual intercellular calcium events that are examined by means of network layers, were found to obey different organizing principles when the islets were treated with NMDA receptor antagonists. The proposed multilayer network approach is a new theoretical concept that provides novel insights into the complex intercellular signalization patterns in multicellular systems, which could not be obtained with conventional methodological tools.

A04-11

Metabolic and behavioral consequences of cola intake during pregnancy

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Introduction: Observational studies have shown that intake of sugar-sweetened beverages during pregnancy is associated with the risk of gestational diabetes. Thus, it may have adverse metabolic and behavioral consequences on the offspring. However, experimental evidence for these adverse effects is lacking. The aim of our study was to describe behavioral and metabolic effects of prenatal cola and high-sugar diet on the offspring. **Questions:** Does prenatal intake of cola and high-sugar diet lead to postnatal metabolic or behavioral disturbances? **Methods:** Pregnant CD-1 mice were randomized into three groups: cola, cola + high sugar diet and controls. Pregnant females had *ad libitum* access to either decarbonated Coca-Cola or tap water and to high-sugar diet or control diet. Renal, metabolic, as well as behavioral phenotyping was performed in the offspring in adulthood. **Results:** *Ad libitum* access to cola led to a higher caloric intake compared with the control group in pregnant mice. Nevertheless, the groups differed neither in weight gain, nor in renal functions. Fasting glycaemia was higher in the cola + high sugar diet. Prenatal cola and/or high-sugar diet did not affect the metabolism of the behavior of the offspring. **Conclusions:** The results of the present study did not confirm a causal relationship between cola intake and gestational diabetes. No evidence was found for any behavioral consequence of prenatal cola or high-sugar diet intake. The interpretation of animal studies focusing on gestation is limited by the short length of gestation in mice compared to humans.

A04-12

Effects of Selenium Supplementation on Cytokines in Experimental Hyperthyroidism

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Background: Even though selenium is an essential trace element, higher doses might have toxic effects. Selenium has regulatory effect on endocrine functions, role in antioxidant defense and also performs function in inflammatory incidents. So it may be evaluated that selenium has important efficiency in organism. Hyperthyroidism occurs due to excessive production of thyroid hormone by the thyroid gland. Cytokines play an important role in a thyroid tissue. In our study it has been investigated that if selenium supplementation has any effect on inflammatory cytokines as TNF-alpha and IL-6 in experimental hyperthyroidism.

Method: Hyperthyroidism was induced in wistar albino rats by oral intake of L- thyroxin (0.4mg/100g fodder). Animals were exposed to different doses of selenium (0.5 mg Na₂SeO₃/kg and 1 mg Na₂SeO₃/kg) for 30 days. TNF-alpha and IL-6 levels were determined by ELISA. The hyperthyroid group values were compared with control and hyperthyroid groups with selenium supplementation.

Findings: TNF-alpha and IL-6 levels were found higher in hyperthyroid group comparing to control group (p<0.01 and p<0.05, respectively). A significant decrease of TNF-alpha and IL-6 levels in hyperthyroid groups with 1 mg Na₂SeO₃/kg supplementation were measured comparing to hyperthyroid group (p<0.01 and p<0.05, respectively). It may be said that selenium supplementation in

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hyperthyroidism may have regulatory effect in inflammatory system and it possibly can be used in treatments for hyperthyroidism.

A06: Respiratory physiology

A06-1

The effect of ML204, a blocker of TRPC4/5 on cholinergic responses in mouse bronchus

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The TRPC3 is expressed in animal and human airways, where it has been implicated in mediating contraction in response to cholinergic agonists (1). Although other members of the TRPC family are also expressed in airway, their potential roles have not yet been investigated. As there is strong evidence that TRPC4 is involved in mediating cholinergic contractions in murine intestinal smooth muscle (2), we tested if the same might be true in airway smooth muscle. Rings of 2nd and 3rd order bronchi from mice were mounted in organ baths for in vitro tension recording. Concentration-effect relationships were recorded for carbachol (CCh, 100 nM – 10 µM) before and after exposure to ML204 (3 or 10 µM), a selective blocker of TRPC4 & TRPC5 channels (3). ML204 was effective at blocking the responses, especially at the lower concentrations of agonist (100 & 300 nM). Similar results were obtained when responses were evoked by electrical field stimulation (EFS) of intramural nerves at frequencies of 0.5 – 8 Hz, where the responses to the lower frequencies were greatly reduced by the drug. When the CCh and EFS responses were repeated in bronchi from mice lacking in functional TRPC4 channels (TRPC4 KO), they were similar to those in wild type mice. Moreover, when ML204 was applied in the TRPC4 KO preparations, it blocked CCh and EFS evoked contractions as in wild type. We conclude that the 1) TRPC4 channels are not essential for mediating bronchial contractions in response to cholinergic stimuli and 2) the blocking effect ML204 was mediated either by blocking TRPC5, or via a non-specific mechanism.

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A06-2

Radon in the exhalation air of patients in radon therapy

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Question: With the collection of time-resolved data of radon activity in the exhaled air of voluntary probands exposed to radon (Rn) in treatment facilities in Gastein, a model of the distribution of Rn222 inside different body compartments shall be established. **Methods:** During Rn therapy patients are exposed to Rn rich thermal water that diffuses through the skin into body tissues and the blood stream. Rn is transported by the blood to the lungs, where it will be exhaled. The challenge was to determine low radon activity concentrations in the exhaled air and to monitor the fast temporal changes of Radon activity concentrations in the exhaled air while bathing. Probands in bathtubs inhaled fresh, radon free outdoor air (<50Bq/m³) through a hose attached to a tight mask. The exhaled

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air was sampled in two min to six min intervals and stored in gas-tight metallized bags for subsequent measurement with Lucas cell technology. **Results:** Rn concentrations in bathtubs between 710 and 955 kBq/m³ for a total exposure of 20 minutes were applied. Immediately after the onset of the exposure, the Rn concentration in the exhaled air sharply increased and reached up to 8300 Bq/m³ by the end of the exposure. Normalization of the results to the Rn activity concentration in water and to the weight of the test persons yielded similar results for all persons between 72 and 108 Bq/(m³.kg/MBq/m³). **Conclusion:** The uptake of radon via the skin, its transfer to the blood, its subsequent distribution among human organs via the blood stream and its final exhalation through the lungs is simulated by the multicompartiment model RADMOD. In this model, human organs and tissues are represented by nine compartments, which are connected through the arterial and venous blood compartments.

A06-3

Correlation between Muscle Mass loss and spirometric abnormalities in COPD

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Introduction: Loss of Muscle Mass (MM) and strength developed by Limb and respiratory muscle often observed in advanced stage of COPD reduce physical capacity and lead to disability. These abnormalities were attributed to a myriad of factors, including mainly dyspnea, systemic inflammation, energy imbalance, corticosteroid medications and oxidative stresses.

Aim: the principal objective of this study was to evaluate the correlation between Muscle Mass reduction (MM) and spirometric abnormalities in COPD patients.

Methods: Our cohort study included 46 volunteer male COPD patients. Interrogation, slow and forced spirometries, bronchial obstruction reversibility and MM measurement (bioelectric impedance) were performed in our study group.

Results: The average values of age and percentage of MM were respectively 60±19 years and 35.5±3.8%. Spirometric data interpreted conforming to GOLD 2017 recommendations revealed that 76 % of obstructive defects are classified in stage 3 and 4. Significant correlations (p<0.05) were found between MM and these following parameters: difference between Low and Forced Vital Capacity as indicator of trapping air volume and FEV1 post bronchodilator as indicator of severity of airway limitation.

Conclusion: According to previous data, this study confirmed that MM loss is strongly correlated to severity grade of COPD and will be considered as indicator of disability. Early diagnosis, respiratory rehabilitation and dietary management must be systematically into COPD management strategies in our country to improve quality of life of patients and reduce healthcare cost.

A06-4

BODE index: an interesting survival prediction tool in obesity?

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Introduction: Obesity is a worldwide epidemic disease associated with systemic complications and increased mortality and morbidity. In respiratory system, accumulation of Fat Mass particularly in thorax and abdomen impairs respiratory mechanics and may cause dyspnea and intolerance to exercise in severe cases.

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Aim: The aim of this study was to investigate correlations between Body Fat Mass (BFM) and the multiparametric BODE index (Body Mass Index, Airflow Obstruction, Dyspnea and Exercise capacity Index) commonly used in respiratory chronic diseases as a reliable prognosis tool value.

Methods: This prospective study was conducted between January and August 2016 and included forty obese women volunteers without associated respiratory disease. Detailed questionnaire, MMRC dyspnea severity scale, Fat Body Mass percentage measurement (bio-electrical impedance analysis), forced spirometry and Six Minutes walk Test (6MWT) were performed in all patients.

Results: In this study group mean values of age, FM percentage and BMI were respectively 45 ± 12 years, $38.1 \pm 6.2\%$ and 36.5 ± 6.5 kg/m². Data revealed significant correlations between Body FM percentage and theses following parameters : FEV1, BMI, 6MWT distance, stage of dyspnea (MMRC) and than BODE index ($p < 0.05$).

Conclusion: Our data demonstrated that FM was strongly related to all parameters of BODE index. Thereby, we suggest that this multidiparametric grading system could be considered as a reliable obesity survival prediction tool.

A06-5

Immature lungs exposed to endotoxin: the effect of exogenous surfactant/polymyxin B

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The effect of surfactant therapy in combination with antibiotic polymyxin B (PxB) in a double-hit model of neonatal lung injury created by intratracheal instillation of LPS in premature newborn rabbits was evaluated.

The behaviour of the modified porcine surfactant (Curosurf®; Chiesi Farmaceutici, Parma, Italy) was tested after exposure to LPS (*E.coli*, 055:B5) and PxB in the captive bubble surfactometer. In the animal model, 27-days old rabbits received intratracheally saline (control) or LPS (500 µg/kg b.w.) and were ventilated with 100% oxygen. After 30 min, animals with LPS received no treatment, or Curosurf (200 mg/kg) without or with 3% PxB; controls received the same dose of surfactant. Animals were ventilated with tidal volumes 6-7 ml/kg for further 2 hrs. Lung compliance, lung gas volumes (LGV), alveolar expansion and lung histology were evaluated.

Addition of 5% LPS to Curosurf at 3 mg of phospholipids/ml increased the surface tension (gmin) from 0.6 ± 0.1 mN/m to 15.2 ± 0.9 mN/m and addition of 1-3% PxB to surfactant/LPS mixture restored gmin to low values. Animals treated with LPS had lower lung compliance and LGV in comparison to the other groups (all $p < 0.001$). Treatment with Curosurf/PxB, but not with Curosurf only, restored LGV. Addition of PxB to surfactant increased the alveolar expansion and reduced the number of inflammatory cells.

In neonatal model of RDS exogenous surfactant gives positive response even in simultaneous exposure to LPS, when enriched by PxB.

A06-6

Is the p-glycoprotein polymorphism a risk factor for smoking dependence

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Questions: Cigarette smoking is one of the most common addictions worldwide and leads health problem. Lots of smokers want to quit but not successful all of them. Genetic variants may be effects nicotine dependence and treatment of smoking cessation. Thus, the aim of this study was to investigate the association between p-glycoprotein (p-gp) polymorphisms and smoking cessation in a Turkish population.

Methods: 158 cigarette smokers and 52 nonsmoker healthy volunteers were included in the study. We determined the p-gp C3435T gene polymorphisms in all subjects. Varenicline was given those want to quit smoking for treatment of smoking cessation.

Results: In our study, smoking cessation success is also high (%57). There was no significant difference among control, quit smoke and smokers groups in terms of genotype distribution. Both CC genotype and c allele were found high in quit smoking group compare to smoker. But it is not significant. In control and quit smoking groups, the TT gene positivity was found 19% and 22% respectively, while the TT gene positivity increased to 32% in smoker group. FTND score was found highest in TT genotype individuals in this study. There was a positive correlation between TT genotype and FTND. It is 1.8 times more difficult to quit smoking in TT genotypes.

Conclusions: Our results suggest that p-gp did not show resistance to varenicline treatment. Failure of varenicline treatment in TT genotype individuals may be due to the high level of dependence. We think that p-gp gene may be associated with smoke dependence and this polymorphism may influences smoking cessation therapies.

Keywords: polymorphism, smoking cessation, varenicline

A06-7

Diagnosis strategy of Asthma-Chronic Obstructive Pulmonary Disease Overlap Syndrome

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Diagnosis of Asthma-Chronic Obstructive Pulmonary Disease Overlap Syndrome (ACOS) is difficult because of clinical and spirometric similarities between asthma and COPD. High level of fractional exhaled nitric oxide (FeNO), a marker of eosinophilic airway inflammation, was recently proposed to confirm ACOS.

This study included files of patients addressed to Pulmonary Function Test Laboratory from November 2015 to February 2017 for asthma or COPD suspicion and which have problematic spirometry were included: forced expiratory volume in one second (FEV1) over forced vital capacity (FVC) after bronchodilator < 0.7 and significant improvement after bronchodilator of FEV1 and/or FVC. FeNO measurement was carried. A sample group underwent eosinophils blood counting.

Thirty-nine patients were enrolled (33 males). All subjects were active or passive cigarette smokers or exposed to other noxious particles or gases. Study population was divided into 2 groups: patients suspected to have asthma G1 (n=24, 18 males, median age=55 \pm 10.52) and patients suspected to have COPD G2 (n=15, 15 males, median age=59.93 \pm 10.78). Obstruction seemed to be more marked in G2 and reversibility on FEV1 higher in G1, without statistical significance. In fact, FEV1

improvement was $20.89 \pm 9.06\%$ and 339.74 ± 173.57 ml. Mean FeNO was 13.48 ± 11.08 ppb and 8.53 ± 5.84 ppb respectively in G1 and G2. FeNO was increased in 4 patients from G1 (33.5 ± 10.21 ppb). FeNO was positively correlated with FEV1 improvement after bronchodilator in percentage ($p=0.001$) and in milliliter ($p<0.001$). Among G1, fifteen subjects had eosinophils counting which was positively correlated to FeNO ($p=0.032$).

This preliminary study highlights that FeNO associated to clinical features, spirometric reversibility test and eosinophils counting may be reliable in ACOS diagnosis. More studies are needed, with a larger sample, to confirm these data.

A06-8

Evaluating Of Systemic Inflammatory Biomarkers As A Result Of Intermittent Hypoxia In Obstructive Sleep Apnea Syndrome

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It is aimed investigate the relationship between obstructive sleep apnea syndrome (OSAS) and inflammation. In this study, the relationship between daytime sleepiness determined by applying epworth sleepiness scale and inflammatory markers were evaluated. Also, the relationship between these markers and the clinical parameters was investigated. TNF- α , TNF-R1, PPAR- α and NF- κ B levels were measured in fasting blood after polysomnography (PSG) in 31 moderate OSAS (Apnea Hypopnea Index (AHI)<30), 29 severe OSAS (AHI>30) and 30 healthy controls (AHI<5). ESS and AHI were significantly higher in the severe OSAS group than in the moderate OSAS group, minimum oxygen saturation was significantly lower. CRP, TNF- α , sTNF-R1 and NF- κ B were significantly higher in the OSAS group than in the control and PPAR- α were significantly lower. CRP, TNF- α , sTNF-R1 and NF- κ B were significantly higher in the severe OSAS group than in the moderate OSAS group and PPAR- α were significantly lower. Positive correlation was found between NF- κ B and AHI, ESS in the entire OSAS group. Negative correlation was found between AHI and PPAR- α . ROC analysis of biochemical parameters were examined. NF- κ B had the highest specificity (75%) and sensitivity (76.7%). It is important to look at symptoms of OSAS diagnosis as well as inflammatory markers. Serum NF- κ B levels can become routine laboratory parameters with a cheap, easy, non-invasive ELISA method with high sensitivity and specificity to confirm findings with PSG.

A06-9

Asthma and bronchiectasis : Spirometric features

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Asthma is a chronic respiratory disease due to bronchial inflammation. Bronchiectasis are permanent and irreversible increase of bronchial caliber due to various etiologies. Asthma and bronchiectasis can be associated. Can bronchiectasis be considered as an aggravating factor of asthma?

Retrospective study comparing patients with asthma and patients with both asthma and bronchiectasis, confirmed by thoracic CT scanner. Patients were interviewed and underwent forced spirometry with reversibility test.

Fourty non-smoking patients were included and divided in 2 groups. Group 1 (G1) including asthmatics (n=22, age=44 \pm 16, sex-ratio=0.37) and groupe 2 (G2) including asthma and bronchiectasis

patients (n=18, age=47 \pm 19, sex-ratio=0.8). Various spirometric profiles were found: normal (n=18 in G1, n=7 in G2), proximal obstructive ventilator defect (n=5 in G1, n=5 in G2), distal obstructive ventilator defect (n=8 in G1, n=2 in G2) and tendency to restriction (n=1 in G1 and n=4 in G2). Mean FEV1/FVC was 76 \pm 18% in G1 and 68 \pm 21% in G2. Mean FEV1 was 87 \pm 14% in G1 and 69 \pm 28% in G2. Mean FVC was 87 \pm 14 in G1 and 78 \pm 22% in G2. Mean FEV1 improvement was 2.5% and 55 ml in G1 and 3% and 60 ml in G2. Mean FVC improvement was 1% and 60 ml in G1 and 2% and 40 ml in G2.

Obstruction was more marked in patients with both asthma and bronchiectasis without statistical significance. However, there were no significant differences in reversibility test. Other studies are needed to enlarge the studied population.

A06-10

Spirometric and Six-minute walk test findings in pulmonary sarcoidosis

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Introduction: Six-minute walk test (6MWT) is a reliable tool for the objective evaluation of functional exercise capacity. It is commonly proposed in various chronic diseases management, such as pulmonary sarcoidosis.

Aim of the study: To study the relationship between clinical, spirometric and 6MWT features in pulmonary sarcoidosis.

Methods: Retrospective study carried on patients files addressed to Pulmonary Function Laboratory over a period extending from November 2015 to February 2017. Patients underwent forced spirometry and 6MWT.

Results: The study involved 42 patients. The mean age was of 53 \pm 11 and the sex-ratio was 0.23. Different stages of sarcoidosis were found: stage 1 (n=2), stage 2 (n=31), stage 3 (n=8) and stage 4 (n=1). Various spirometric profiles were observed: obstructive ventilatory defect (n=5), tendency to restriction (n=7) and normal profile (n=30).

The mean 6-minute walk distance (6MWD) was 447 \pm 80 meters namely 71 \pm 12% of predicted values. 6MWD was normal in 3 cases (mean=97.33%), moderately reduced in 34 cases (mean=71.58%) and severely reduced in 5 cases (mean=48.6%). Lowering in oxygen saturation was observed in one patient (stage 2) who had a moderately reduced 6MWD. There was a positive correlation between 6MWD and Forced Vital Capacity (FVC) in liters ($p=0.0005$) and with Forced Expiratory Volume in the first second (FEV1) in liters ($p=0.0022$). There were no significant correlations between 6MWT data and stages of sarcoidosis.

Conclusion: In light of this preliminary study, some spirometric parameters seem to be predictive of 6MWD. More studies are needed to enlarge the sample and deepen the investigations.

A06-11

Increased ragweed exposure and air pollution are associated with subsequent respiratory allergies to indoor and outdoor allergens in children

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Questions: Ragweed (*Ambrosia artemisiifolia*) is found in extremely high amounts in the Western part of Romania, and its pollen is highly allergenic, inducing severe allergic symptoms in a large proportion of the exposed population. Timisoara is a city in this part of Romania with increased air pollution. This study aims to evaluate the association between air pollution, pollen exposure and allergic manifestations in children.

Methods: A cohort of patients with symptoms of respiratory allergies (n = 1380) that presented at the Allergy Outpatient Clinic Timisoara in 2015 and 2016 was evaluated for allergic sensitization by clinical examination and skin prick test with aeroallergens. Air pollution with particulate matter with a diameter of less than 10 μm (PM10), which includes house dust mite particles, was also measured for 2015.

Results: 76 children under 12 years of age were identified with allergic rhinoconjunctivitis induced by ragweed pollen. Out of these, 88.16% were between 5 and 12 years of age. Most patients (81.57%) were polysensitized to both outdoor (ragweed, timothy, birch pollens) and to indoor (34.21% to house dust mites) allergens. Only 6 patients below 5 years old were polysensitized. In 2015, air pollution with PM10 exceeded normal values in 27 days.

Conclusions: Exposure to high concentrations of allergens, especially ragweed pollen allergens, and to high PM10 concentrations in infancy appears to increase the risk of polysensitization and allergic rhinoconjunctivitis in children under 12 years. These results support the hypothesis that high levels of ragweed pollen can induce sensitization and the development of allergic symptoms early in life, even if the period of exposure is less than 3 months per year.

A07: Gastrointestinal physiology

A07-1

Sinapic acid heals experimentally induced colitis in rats on behalf of its anti-inflammatory effects

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Sinapic acid (SA), a natural hydroxycinnamic acid, has been shown to have anti-oxidant effects in several other studies; however, its effects on colitis have not been investigated yet. The aim of the study is to show the effects of SA on experimentally induced colitis model. Wistar-Albino rats (n=10/group) were used. On 1st day, 30 mg/ml TNBS in 40% ethanol was administered to Colitis group and SA group intrarectally (ir) and treated with olive oil or SA (20 mg/kg in olive oil) per oral for 3 days. Control group received saline ir and treated with olive oil (1 ml/kg per oral) for 3 days. On the 4th day, all rats were decapitated. Tissue wet weight index (WI), malondialdehyde (MDA), glutathione (GSH) levels, myeloperoxidase (MPO) activity, and total oxidant and antioxidant status (TOS and TAS) were measured in colonic tissues. In addition, colonic tissues were examined macroscopically and microscopically. Tumor necrosis factor (TNF)- α is measured in serum. Results were analyzed by the ANOVA and Tukey's Kramer tests. The macroscopic and microscopic scores and WI of colitis group is significantly higher when compared with the control group (p<0.001) and SA treatment reduced these parameters (p<0.01). Increase in the colonic MDA levels, MPO activity and TNF- α levels in rats with colitis were attenuated by SA (p<0.05, p<0.01 and p<0.001 respectively). The GSH depletion in colitis group is prevented by the SA treatment (p<0.05). While TOS was higher in the colitis group compared to the control group (p<0.001), decreased TAS in the colitis group is improved by SA (p<0.01). This study indicates that SA may exert anti-inflammatory effects in a colitis model on rats and thus may open up a new research path to the treatment of colitis.

A07-2

CHRONIC LESIONS IN TRINITROBENZENE-SULFONIC ACID COLITIS

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Aim: to establish a standard model of colitis in the rat by studying the chronology of the installation of inflammatory lesions.

Materials and methods: Forty male Wistar rats, weighing 320–400 g, were recruited and divided into 4 groups of 10 animals in each. 5 rats served as control and 5 rats received TNBS. Groups were categorized based on the delay between TNBS administration and the day of sacrifice to macroscopic and histological evaluation. We euthanized the control and inflamed rats at 3, 7, 15 or 30 days after ethanol or TNBS administration in groups 1, 2, 3 and 4, respectively.

Results: Evaluation of macroscopic showed that there was no significant difference between TNBS-treated rats in all groups. However, colitis severity of groups 1 and 4 was higher than that of groups 2 and 3. All groups with TNBS treatment produced severe symptoms and macroscopic lesions. In all TNBS groups, severe and intense transmural inflammation, epithelial change and ulceration were observed and the difference was significant compared to the control group. The comparison between TNBS treated rats was observed noticeable inflammation, erosion and extensive lesions in group 1 but mucosal architecture was normal without irregular crypts and granulation tissue. The chronic lesions with modifications of mucosal architecture were observable in groups 2, 3 and 4. The difference is more significant between groups 1 and 3 (p=0.005).

The difference between group 1 and groups 2, 3 and 4 is important with more acute lesion in group 1 and more lesions of mucosal architectures and hyperplasia in groups 2, 3 and 4

Conclusion: With a single dose of TNBS, we demonstrate that the ideal experimental model to mimic IBD with chronic lesions would be the seventh and fifteenth day after TNBS administration with a maximum of macroscopical and histological chronic lesions.

A07-3

ANTI-INFLAMMATORY EFFECT OF *LENTISC* OIL IN EXPERIMENTAL COLITIS

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Aim: to investigate the anti-inflammatory effect of the *Pistacia Lentiscus* oil in TNBS-induced experimental colitis model.

Materials and methods: Colitis was induced in three groups of 5 Wistar rats by instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS). 5 rats received Lentisc oil 2 months before colitis induction (preventive group), 5 rats received the oil on the day of colitis induction (curative group) and 5 control rats. Lentisc oil was extracted from the ripe fruit of the plant by the cold press method and was analyzed by spectro-chromatography. Lentisc oil has been inserted with a standard diet at the dose of 30mg oil/100g of food/rat.

Results: The lentisc oil sample is composed mainly of: Oleic acid 47.96%, Palmitic acid 27.94% and Linoleic acid 20.22%. There was a statistically significant between control rats and treated rats with lentisc oil concerned body mass, bleeding index and diarrhea. Histological examination revealed a clear difference between the control and preventive groups with disappearance of erosion, decreased of cryptitis, irregular crypts and crypt loss in the preventive group. Curative group showed a significant decrease of ulceration, hyperplasia, cryptitis, irregular crypts and crypt loss compared to the control group. There was an attenuation of inflammation in the preventive group compared to the curative group without statistically significant.

Conclusion: Lentisc oil administration could provide a protective effect on intestinal inflammation in colitis rats induced by TNBS mainly when it is administered at a young age in preventive mode. This beneficial effect would involve a modification of arachidonic acid metabolism.

A07-4

Esophageal anomalies in chest pain-suffering patients with a normal coronary catheterization

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Questions:

Chest pain is frequent in the general population. A coronary cause is eliminated in 30% of cases. Apart from heart-based causes, chest pain may have pulmonary, bone, muscular or esophageal origins. The main cause of an esophageal-originating chest pain is the gastro-esophageal reflux.

The aim of our study is to determine the frequency and type of esophageal anomalies in chest pain-suffering patients with a negative cardiological diagnosis. To this end, we examine standard esophageal manometric and 24-hour pHmetric data.

Methods:

We carried out a 6-year retrospective study of patients standard esophageal manometers and 24-hour pHmeters to determine chest pain, using a normal coronary catheterization.

Results:

During 6 years, 33 patients were studied: 15 males and 18 females, with a mean age of 47,5 years. Examining esophageal manometers, motor disorders were found in 26 patients, i.e. 78,78% of cases, unspecific motor disorders (21 cases), and diffused spasms disease (2 cases). We found a nutcracker esophagus (1 case), and a major hypertonicity of the lower sphincter of the esophagus (1 case). A gastro-esophageal reflux was found at pHmeter in 19 patients (57, 57 %) with a good symptomatic validity in 30, 3% of cases.

Conclusions:

The gastro-esophageal reflux is the most frequent anomaly during a pseudo-angina chest pain, which needs carrying out a 24-hour esophageal pHmeter to detect a gastro-esophageal reflux associated or unassociated with a good symptomatic validity.

A07-5

Stress monitoring on gastrointestinal smooth muscle by electromyography

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Questions: Stress has short- and long-term effects on the functions of the gastrointestinal (GI) tract and may lead to the manifestation of different symptoms in the GI tract such as alterations in gastric-, and gut motility. Our aim was to use smooth muscle electromyography to detect stress-related GI motility disorders on rats, in vivo.

Methods: Subcutaneous abdominal electrodes were implanted into rats. The basal myoelectric signals were recorded in awake rats without movement restriction. Then the measurement was repeated under immobilization stress. The experiments were also carried out under haloperidol and diazepam treatments. Plasma samples were collected to measure the corticosterone and drug levels by ELISA kit and HPLC, respectively.

The myoelectric signals were analyzed by fast Fourier transformation. The software filtered the electric signals from the heart and the brain. The frequency of the electric activity was characterized by cycle per minute, the magnitude of the activity was expressed as power spectrum density (PsD).

Results: Immobilization stress increased the PsD in all sections of the GI tract. Haloperidol (1 mg/kg) ceased, while diazepam (5 mg/kg) decreased the stress induced activities. These alterations were in parallel with the change in corticosterone level. The presence of administered drugs was proved in plasma or brain.

Conclusions: There is a strong correlation between the alterations of PsD values and the stress hormone level. Smooth muscle electromyography seems to be proper to detect the stress level via GI tract. Our method may serve as a new, non-invasive tool for investigation of stress-related diseases in GI tract.

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A07-6

The relation between helicobacter pylori and Iron deficiency anaemia in Sulaimani city

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Helicobacter pylori infection is responsible for many digestive system disorders and have been implicated in some extra-gastric diseases, such as iron-deficiency anemia (IDA) and vitamin B12 deficiency. So, this study was designed to found any relation between Iron deficiency anaemia and *Helicobacter pylori* in sulamania city/iraq. The present study used eighty females patients infected with *H.pylori* and forty healthy. Patients were divided into four groups according to the age. *H.pylori* was diagnosed serologically by using anti-H. pylori IgG antibodies Elisa kit. After that, hemoglobin(Hb) ,mean cell volume(MCV), leucocytes (WBCs) counts, platelets counts, serum ferritin and total iron binding capacity (TIBC) were measured. Hb-levels and MCV-levels in all patients showed significant decreased ($P < 0.05$) compared with all control groups. While, the results of WBC and platelets counts showed no significant changes in all patient groups compare with all control groups. Serological tests, S.ferritin in all infected groups showed significant decreased ($P < 0.05$) compared with all control groups. While, TIBC levels showed significant increase in patient groups compare with control groups. Thats mean there is a relation between *Helicobacter pylori* infection and iron deficiency anaemia in Sulaimani city.

A07-7

Transient receptor potential melastatin 2 functional characterization in mouse pancreatic acinar cells

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Introduction: Aberrant intracellular Ca^{2+} signaling is the hallmark of acute pancreatitis (AP) inducing mitochondrial damage, intraacinar digestive enzyme activation and cell death. Thus prevention of toxic cellular Ca^{2+} overload is a promising therapeutic target. The transient receptor potential melastatin 2 (TRPM2) is a non-selective cation channel that plays major role in oxidative stress induced cellular Ca^{2+} overload in different cell types. Although likely, its role in pancreatic acinar cells and the pathogenesis of AP was not investigated yet.

Aim: Our aim was to characterize the functional activity of TRPM2 in pancreatic acinar cells.

Methods: In our experiments pancreatic acinar cells (PAC) were isolated from wild type (WT) and TRPM2 knockout (KO) mice with enzymatic digestion. The changes of the intracellular Ca^{2+} level was measured whit fluorescent microscopy using FURA2-AM.

Results: The intracellular Ca^{2+} signals evoked by 100 μ M carbachol were not different in WT and TRPM2 KO PAC. On the other hand, 1 μ M H_2O_2 induced significantly higher intracellular Ca^{2+} elevation in WT PAC compared to the TRPM2 KO. In Ca^{2+} free extracellular solution the Ca^{2+} signal in response to 1 μ M H_2O_2 was markedly reduced in WT PAC confirming that H_2O_2 activates dominantly extracellular Ca^{2+} influx.

Discussion: Our result confirmed the functional activity of the TRPM2 channel in pancreatic acinar cells. In our further investigations we aim to clarify the pathogenetic role of TRPM2 in AP.

A07-8

The effect of primary sensory neuron desensitization on experimental acute pancreatitis models

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Introduction: Pain is a predominant symptom of acute pancreatitis (AP). Pain sensation is thought to be mediated by primary sensory neurons expressing transient receptor potential vanilloid 1 (TRPV1), an ion channel nociceptor. TRPV1 is considered to participate in neurogenic inflammation and thus to have a major role in the pathogenesis of inflammatory disorders. Therefore, we aimed to examine if desensitization of TRPV1 neurons affects the severity of experimental AP in rats.

Methods: Four weeks before the induction of AP, the primary sensory neurons of male Sprague-Dawley rats were desensitized by resiniferatoxin (RTX, injected intraperitoneally (i.p.) at doses of 30 μ g/kg, 70 μ g/kg and 100 μ g/kg), an agonist of TRPV1. AP was induced by i.p. administration of 3g/kg L-ornithine or 4x20 μ g/kg caerulein, 3% 1ml/kg Na-taurocholate was administered intraduodally. Rats treated with L-ornithine/caerulein/Na-taurocholate and/or RTX were compared to their respective saline-treated controls. To determine AP severity, laboratory and histological parameters were measured.

Results: Compared to controls, desensitization caused increased serum and pancreatic amylase, myeloperoxidase activities, pancreatic water content and heat-shock-protein 72 expression in L-ornithine-induced AP, while the extent of necrosis increased in desensitized animals injected with Na-taurocholate. Desensitization ameliorated inflammation in caerulein-induced AP compared to the group without RTX pretreatment. Desensitization in itself did not significantly influence any of the measured parameters compared to the control group.

Conclusion: Primary sensory neuron desensitization had distinct effects on the severity of different AP models. It exacerbated necrotizing AP, but alleviated edematous AP.

A08: Behavioral and cognitive neuroscience

A08-1

Physical Exercise Performed to Chronic Social Isolated Rats Regulate Anxiety Behavior Without Improving Learning

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Questions: The contribution of early life stress to the development and maintenance of anxiety-like behaviors and learning are unclear. Also physical exercise may utilize the negative effects of stress and anxiety-like behaviors. To more clearly understand the relationship between them, we studied effects of exercise on anxiety and learning/memory on long term social isolation stressed rats.

Material and Methods: Male Wistar rats (n=32) 3 weeks old were randomly divided into; control (C), social isolation stress (SI), social isolation stress + exercise (SE), control + exercise (CE). Social isolation involved removing the animal from the home cage, and placing it into an isolated cage during

14 days. After that, physical exercise procedure was initiated through 4 weeks. For evaluate anxiety-like behavior we used elevated plus maze and open field test; for learning and memory morris water maze test was applied. The results were analyzed by SPSS 11.5 statistic software.

Results: In open field test, compare to the other groups exercised rats total distance moves higher ($p < 0.05$). In elevated plus maze test, there is a significant difference in the number of open arm entries in the intergroup evaluation ($p < 0.05$). CE group has a greater number of open arm entries when compared to C ($p < 0.05$). CE and SI rats spent more time in open arm compare to the C group ($p < 0.05$). There was no difference in learning and memory between groups.

Discussion: The way social isolation stress is applied and its duration affects learning and memory functions in different ways. There is a potential anxiolytic effect of long-term moderate treadmill exercise on social isolated rats according to our results. But we couldnt find differences between groups for learning and memory.

A08-2

Effect of Riluzole on Social Behavior and Anxiety in Valproic Acid-Induced Autism-Like Rat Model

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Autism spectrum disorder (ASD) is a neurodevelopmental syndrome causes severe disabilities on the individual, their family and the community for lifelong. Several genetic, neuroanatomic and biochemical factors have been considered for etiology. Up to now, neither specific etiologic factor nor effective treatment has been identified. Therefore we aimed to investigate the behavioral effects of riluzole which is an antigitamate agent, in rat model of ASD.

Valproic acid was applied (for only rats not in control/naive intact group) on the 12.5th gestational day of Wistar albino rats to create autism-like model. 24 newborn male rat pups divided into 3 equal groups; pure control, autism-like model nontreated and autism-like model plus riluzole treated. Riluzole (10 mg/kg. p.o) was administered in the 3rd postnatal week for 2 weeks.

Open field and three-chamber behavioral tests applied to 6 weeks of age. Both sociability and social preference indices with strangers in the three-chamber social interaction test were significantly lower in the autism-like rats. In the open field test, riluzole group spent less time on the periphery. This result showed that riluzole increased social interaction and decreased anxiety levels.

Our results suggest that a neuromodulating antigitmatergic agent riluzole seems to have some effects on behavioral symptoms of ASD in an animal model. Further studies are needed to reveal the role of riluzole in medical treatment of individuals with ASD.

A08-3

Investigation of the Process of Response Activation by Using a Visual Go-Nogo Task with Varying Task Difficulty

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Introduction: All living creatures adapt themselves to an ever-changing environment and adjust or maintain their goals accordingly. It is important to elicit a response activation to the task related stimuli and a response inhibition to the irrelevant stimuli for proper executive functioning. In this study, we aimed to analyze the relationship between the go stimuli difficulty and the response activation in the brain.

Methods: The event-related potential (ERP) record was taken from 21 healthy male volunteers (19-21 years) from 30 electrode locations (10/20 system). According to their similarity with nogo stimuli, three types of go stimuli designed as three level of difficulty and presented with probability of 0.6 in a visual go-nogo task. The amplitudes and latencies of ERP components in the averaged responses to each go stimulus groups were measured and analyzed by repeated measures of ANOVA.

Results: Both the reaction time and the omission errors significantly increased with the increment in difficulty level of the go stimuli ($p = 0.001$, $p = 0.001$). There is also a significant difference in terms of the amplitudes ($p = 0.001$) and latencies ($p = 0.001$) of P3 potentials among the three go groups. The P3 amplitudes decreased ($p = 0.043$, $p = 0.001$) and latencies prolonged ($p = 0.001$, $p = 0.001$) as the difficulty level of the go stimuli increased.

Conclusion: Our results consistent with the idea that P3 latency increases when categorization of the stimulus becomes more difficult. On the other hand, although more difficult stimuli are assumed to mobilize more processing effort or resources, P3 amplitude depresses with decreased stimuli discriminability.

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A08-4

Time-dependent changes in behavioural and molecular parameters after postweaning social isolation

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Questions: Several studies indicate that postweaning isolation rearing may affect nitric oxide (NO) production. During isolation rearing, rodents are deprived of social interactions that are critical for behavioural and neurobiological development. The aim of our study was to determine time-dependent impact of social isolation on behavioural and biochemical parameters in Wistar Kyoto rats. **Methods:** We used 13 and 32 weeks old males Wistar Kyoto rats. At day 21 postnatal, the animals were randomly divided into two groups. In the first group, rats were reared singly (isolation reared, IR) and in the second group, rats were reared 3 per cage (socially reared, SR). After 10 or 29-weeks of isolation rearing, behaviour in the open field as well as the acoustic startle response, its habituation and prepulse inhibition were assessed. In the cerebellum, the activity of NO synthase (NOS), protein expression of NOS isoforms and superoxide dismutase 1 (SOD1) were measured. We also determined concentration of conjugated dienes (CD), as the marker of tissue oxidative damage. **Results:** The number of entrance to the central zone in the open field test was significantly decreased only after 29 weeks of isolation. Habituation of the acoustic startle response was impaired in IR rats only after prolonged social isolation. Both 10-week and 29-week social isolation, led to increased CD concentration in the brain cortex. Total NOS activity and nNOS protein expression were significantly decreased in IR rats compared to SR rats only after 29 weeks of isolation. **Conclusions:** Our results indicate that duration of social isolation plays important role in the development of behavioural and biochemical changes. These alterations were more evident after longer period of social isolation.

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A08-5

EFFECT OF PROTEIN CARBONYLIZATION ON COGNITIVE FUNCTIONS IN DIABETIC RAT MODEL

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Background/Aim: Neuropathy due to diabetic complications causes structural and functional impairments in brain tissue, causing cognitive functions to deteriorate. We aimed to elucidate the mechanism of neuropathy in STZ-induced diabetic rats and to investigate the effects of ALA administration on brain tissue from biochemical and physiological aspects.

Materials and Methods: Forty Wistar albino male rats control group, STZ group ALA and STZ + ALA were divided into four groups. Single dose 50 mg / kg STZ ip to create diabetes. ALA was administered orally daily for six weeks at 100 mg / kg / day. Cognitive functions were assessed by MWM during the last week of treatment. Brain tissues of the sacrificed rats were divided into hippocampus, cortex, hypothalamus and striatum regions structures for PC determination.

Results: The changes in cognitive functions assessed by MWM were deteriorated according to the control and ALA group in the STZ group, whereas the results were improved according to the STZ group in the STZ+ALA group ($p < 0.05$). PC levels increased in the STZ group, and decreased in the STZ+ALA group compared to the STZ group ($p < 0.05$).

Conclusions: Distorted balance of oxidant-antioxidant in the rat brain tissue caused increased pc level in rat brain tissue, resulting in cognitive dysfunctions in the STZ-induced diabetes model. ALA is effective for ameliorate cell damage and cognitive functions in brain tissue by antioxidant and neuroprotective effect.

Key words: Diabetes Mellitus, Streptozotocin, Alpha Lipoic Acid, Neuropathy, Brain

A08-6

Effects of Chronic Caffeine Consumption on Cognitive Performance and Hippocampal Gene Expression on REM Sleep Deprived Rats

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Questions: Sleep deprivation is very common issue among the young people. Caffeine is a mediator that consuming with drinks in our daily live. In the literature, there is no study that explains relation between caffeine and sleep deprivation by the molecular level. We studied the possible effects of chronic caffeine consumption on learning and memory and the gene expression related with learning and memory on sleep deprived rats.

Material and Methods: Male Wistar rats (n=50) 8 weeks old were randomly assigned into five groups: control (C), caffeine (CF), sleep deprivation (SD) and caffeine/SD (SD +CF), pedestal control (PC). Sleep deprivation model was applied to rats by Plexiglass water tank. For learning and memory, Morris Water Maze Test (MWM) was used. RT-PCR technique was used for Grin1 (NR1), Grin2a (NR2A), Grin2b (NR2B) gene expression by Fluidigm Access Array. The results were analyzed by SPSS 11.5 statistic software.

Results: In MWM, SD +CF rats spend less time to reach the platform significantly ($p < 0.05$). There is a significant difference in the number of swimming velocity and path length to reach platform in the intergroup evaluation ($p < 0.05$). But the last day there was no difference in learning and memory between groups. For Grin1, Grin2a, Grin2b gene expression in CF, SD, SD+CF group, there is significant difference according to the control gene ($p < 0.05$).

Discussion: The chronic duration of caffeine consumption down regulated genes expression that related to learning and memory. According to the memory test, chronic caffeine consumption didn't regulate cognitive functions. Together with these findings, we can say that, chronic consumption is not effective tool for compensate the negative effects of sleep deprivation.

A08-7

Effects of Treadmill Exercise on Hippocampal Dependent Learning and NMDA Subunit Gene Expression on Social Isolated Rats

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Questions: Social isolation due to the use of technology, could affect younger age groups and the resulting stress can cause various diseases such as cardiovascular problems, obesity, psychiatric disorders will form an important risk factor in the coming years. Our goal is to investigate the potential effects of exercise on NMDA subunit gene expression in social isolation rats.

Methods: Male Wistar rats (n=32) 3 weeks old were randomly divided into; control (C), social isolation stress (SI), social isolation stress + exercise (SE), control + exercise (CE). Social isolation procedure was applied to the rats everyday between 08-14:00 hours for 6 weeks. Exercise procedure began at third week of isolation and initiated through 4 weeks. After exercise protocol, learning performance was evaluated by novel object recognition test. At the end of all experiment, rats were decapitated and their hippocampus tissue was isolated. RT-PCR technique was used for Grin1 (NR1), Grin2a (NR2A), Grin2b (NR2B) gene expression by Fluidigm Access Array. The results were analyzed by SPSS 11.5 statistic software.

Results: According to the new object recognition test, there is significant difference at 60 minute section in social isolation group compare to the control ($p < 0.05$). NMDA subunit gene expression is down regulated at SI and SE group ($p < 0.05$).

Conclusions: In the light of all these results, exercise didn't compensate the negative cognitive effects of social isolation. Due to the type of exercise, cognitive functions are affected differently. Because of being not voluntary type of exercise that we used, we couldn't show the ameliorate influences of exercise on stress responses.

Key words: Social isolation, exercise, learning, NMDA

A08-8

The brain-tumor related protein podoplanin regulates synaptic plasticity and hippocampus-dependent learning and memory

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The brain-tumor related protein podoplanin regulates synaptic plasticity and hippocampus-dependent learning and memory

Podoplanin is a cell-surface glycoprotein constitutively expressed in the brain and implicated in human brain tumorigenesis. The intrinsic function of podoplanin in brain neurons remains however uncharacterized. Using an established podoplanin-knockout mouse model and electrophysiological, biochemical, and behavioral approaches, we investigated the brain neuronal role of podoplanin. *Ex vivo* electrophysiology showed that podoplanin deletion impairs dentate gyrus synaptic strengthening. *In vivo*, podoplanin deletion selectively impaired hippocampus-dependent spatial learning and memory without affecting amygdala-dependent cued fear conditioning. *In vitro*, neuronal overexpression of podoplanin promoted synaptic activity and neuritic outgrowth whereas podoplanin-deficient neurons exhibited stunted outgrowth and lower levels of p-Ezrin, TrkA, and CREB in response to nerve growth factor (NGF). Surface Plasmon Resonance data further indicated a physical interaction between podoplanin and NGF. This work proposes podoplanin as a novel component of the neuronal machinery underlying neuritogenesis, synaptic plasticity, and hippocampus-dependent memory functions. The existence of a relevant cross-talk between podoplanin and the NGF/TrkA signaling pathway is also for the first time proposed here, thus providing a novel molecular complex as a target for future multidisciplinary studies of the brain function in the physiology and the pathology.

A10: Renal physiology

A10-1

Properties of Cell Surface P2X₇ Receptors in Chronic Kidney Disease

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Questions: Chronic low-grade inflammation is common in chronic kidney disease (CKD) patients. The P2X₇ receptor (P2X₇R) is increasingly recognized as an important cell surface regulator of several key inflammatory molecules. P2X₇R activation by extracellular ATP results in opening the cation channel followed by forming a non-specific pore. The aim of the study was to examine the P2X₇R function and expression in CKD.

Methods: The study involved 20 healthy volunteers and 20 non-diabetic patients with stage 2-3 CKD. Cytosolic Ca²⁺ measurements were performed by Fluo-3 fluorimetry in isolated peripheral blood mononuclear cells (PBMCs). To determine the P2X₇R function, a selective antagonist (AZ11645373) and the agonist (BzATP) were used. The function of P2X₇ pores was measured by ethidium uptake at basal conditions, and with BzATP stimulation or AZ11645373 inhibition. The expression of surface P2X₇Rs was evaluated by flow cytometry using the antibody (anti-P2X₇ extracellular).

Results: Cytosolic Ca²⁺ concentration ([Ca²⁺]_i) was increased in PBMCs of CKD patients when compared with healthy subjects. The agonist of P2X₇Rs, caused a sustained increase in [Ca²⁺]_i in both groups, but the effect was smaller in patients. The application of P2X₇R antagonist led to reduction in [Ca²⁺]_i. The permeability of P2X₇ pores in PBMCs of CKD patients was significantly increased in comparison with healthy volunteers. The expression of surface P2X₇Rs was 1.5-fold greater on PBMCs from CKD patients.

Conclusions: These results indicate altered P2X₇R channel and pore function and increased P2X₇R expression already in early stages of CKD.

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A10-2

Role of arginase-II in regulation of water balance

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Aim

Arginase-II (Arg-II) is highly expressed in kidney with its most abundant expression in proximal tubule epithelial cells and also in collecting duct cells. However, the function of Arg-II in kidney remains largely unknown. In the present study, we aim to investigate the role of Arg-II in regulation of vasopressin-regulated water channel protein aquaporin 2 (AQP2) in collecting ducts and the impact on water balance.

Methods and results

In cultured mouse collecting duct cell line mCCDcl1, desamino-d-arginine vasopressin (dDAVP), a synthetic vasopressin receptor V2-agonist, stimulated expression and membrane translocation of AQP2 as expected and upregulated Arg-II levels as assessed by immunoblotting and/or immunofluorescence staining. Silencing Arg-II further enhanced AQP2 expression and membrane translocation in response to dDAVP. Conversely, overexpression of native or an inactive Arg-II mutant suppressed the effects of dDAVP. In agreement with these findings in vitro, total and membrane-associated AQP2 levels were significantly higher in Arg-II-deficient (Arg-II^{-/-}) than wild-type (WT) mice, suggesting a negative regulation of AQP2 by Arg-II. Furthermore, the total and membrane-associated AQP2 levels in WT mice were increased by water deprivation paralleled with elevated Arg-II level in collecting duct cells, decreased urine volume and increased urine and plasma osmolality. Arg-II^{-/-} mice showed more pronounced water preservation effect under the water deprivation condition.

Conclusion

Arg-II in collecting duct cells influences water balance through negative regulation of AQP2 expression and membrane translocation independently of its L-arginine:ureahydrolase activity.

A10-3

USE OF ELECTROMAGNETIC FIELD SHIELDING FABRIC FOR PRENATAL CARE

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Questions: Electromagnetic (EM) shielding fabrics have been marketed by several manufacturers within the past few years. But, their efficacies in protecting living organisms against harmful effects of EM noise have yet to be established. This study aims to accomplish this task and investigates the EM protectivity in prenatal care with experiments performed on pregnant rats exposed to radio frequency (rf) field at a cell phone frequency.

Methods: Pregnant rats were divided into three groups: Sham (n=3), EM (n=3) and EM+fabric (n=3). These rats were placed around a 20 cm circle on the platform and their heads were positioned closed to a linear antenna positioned at the center of the platform. A shielding fabric was shaped as a curtain and placed to separate the rats in the EM and EM+fabric groups. The rats in the EM+fabric group were protected from the field by the fabric, but those in the EM group were unprotected. The rats were exposed to 900 MHz rf field (~200 mV/m at the head region) for 1 hr in each day throughout their pregnancies. Immediately after the birth, infants were sacrificed and kidneys were removed for histological analysis with H&E, Periodic Acid-Schiff and Masson's Tricrom staining. Bowman capsule and glomerular diameters, proximal tubule inner diameter, cortex and medulla thicknesses and basal membrane damage were examined and compared.

Results: Kidney structure and morphology of the prenatal rats were greatly affected by exposure to the EM field. Glomerular basement membrane continuity was interrupted. The diameters of Bowman capsule and proximal tubule were increased. Both cortex and medulla thickened. But, these effects were significantly less in the rats placed behind the fabric.

Conclusion: Shielding fabric protects the prenatal kidney against the potential harms induced by the EM field.

Key words: Electromagnetic field, kidney, shielding fabric

A10-4

CFTR as a regulator of the epithelial–mesenchymal transition

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Epithelial-mesenchymal transition (EMT) is an essential process in normal embryogenesis and is also activated during wound healing, cancer metastasis or organ fibrosis. EMT is characterized by the loss of epithelial phenotype correlated to the gain of mesenchymal markers and has also been linked to an increase in intracellular ROS level. Because we previously demonstrated that the cystic fibrosis transmembrane conductance regulator protein (CFTR) expressed in renal tubular epithelial cells is not only a chloride channel but is also involved in the cellular redox level modulation, we sought to examine the effects of the selective inhibitor of CFTR (CFTRinh-172) in the context of EMT process. We have shown, in vitro, that the pleiotropic growth factor TGF- β 1 stimulated EMT in renal proximal tubule epithelial cells, as evidenced by changes of cell morphology and EMT markers expression, assessed at both gene and protein level (downregulation of E-cadherin and upregulation of α -smooth-muscle actin, vimentin, fibronectin and N-cadherin). TGF- β 1 also increased ROS production and decreased the intracellular concentration of glutathione (GSH, the major antioxidant in renal cells). NAC, a precursor of GSH, inhibited TGF- β 1 EMT induction. Interestingly, CFTRinh-172 (5 μ M) prevented TGF- β 1-induced ROS production, GSH depletion and attenuated EMT response. These results suggest that CFTR, by its ability to modulate ROS levels, plays a critical role in EMT.

A10-5

Title Kidney regulation of inorganic pyrophosphate plasma level: Impact of chronic kidney disease.

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Questions. Chronic kidney disease (CKD) is ranked in 5 different stages according to glomerular filtration rate (GFR) and associated to arterial calcification. Plasma level of inorganic pyrophosphate ([PPi]pl) is a potent anti-calcifying factor. In contrast, inorganic phosphate plasma level ([Pi]pl) favors arterial calcification in CKD. Renal Pi handling is well characterized in CKD but not for PPi and the link between [PPi]pl and [Pi]pl in CKD is still unclear. In this pilot study we assessed [PPi]pl and [PPi]pl/[Pi]pl at diverse CKD stages. **Methods** [PPi]pl was determined in patients with moderate CKD, 6 weeks after kidney transplantation (stage t3b, n=10); moderate CKD without transplantation (stage 3b, n=9); end-stage CKD treated by hemodialysis (stage 5d, n=10) and controls (CTRL, n=29). [Pi]pl and calcium ([Ca]pl) levels were determined by standard techniques. [PPi]pl was measured by an enzymatic assay. Data were compared using unpaired test and Spearman tests. **Results and conclusion** [PPi]pl was not different between 5d and controls (0.90 \pm 0.45 vs 0.87 \pm 0.36 μ mol/l, ns). [PPi]pl was lower in t3b than in 3b (0.70 \pm 0.22 vs 1.01 \pm 0.32 μ mol/l, p = 0.03). [PPi]pl/[Pi]pl ratio is not statistically different between patients with CKD 3b, t3b and 5d. There was a correlation between [PPi]pl and [Pi]pl in t3b and 3b (r=0.63, p<0.005) but between this ratio and [Ca]pl in all CKD stages.

Our data are not in agreement with 2 independent reports showing decreased [PPi]pl in 3b and 5d as compared to CTRL. This suggest that [PPi]pl is linked to [Pi]pl independently from GFR, [Ca]pl and likely from renal excretion of Pi, which is lacking in 5d. This pilot study provide new perspectives in the calcifying background linked to CKD. **Key words**: chronic kidney disease, pyrophosphate, phosphate

A10-6

Effect of Resveratrol Application on Lipid Peroxidation in Experimental Renal Ischemia-Reperfusion Injury in Rats

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The purpose of this study was to investigate how resveratrol administration in rats affected experimental lipid peroxidation in renal ischemia-reperfusion injury.

A total of 48 rats were used and the animals were divided into 5 groups. 1.Sham, 2.Renal Ischemia, 3.Renal Ischemia + Reperfusion, 4.Resveratrol + Renal Ischemia, 5.Resveratrol + Renal Ischemia + Reperfusion. Resveratrol at a dose of 60 mg / kg / day for 3 weeks was applied in Group 4 and 5. Plasma-kidney MDA and erythrocyte-kidney GSH levels as well as histological changes in kidney tissue were determined.

In our study, the highest plasma and renal MDA levels and the lowest erythrocyte and renal GSH values were obtained in renal ischemia and renal ischemia reperfusion group. At the same time, increased histopathologic changes were observed in kidney tissue in renal ischemia and renal ischemia reperfusion group. Resveratrol application resulted decrease in plasma and kidney MDA levels but an increase in erythrocyte and renal GSH levels in group 4 and 5. Resveratrol application prevented renal ischemia and renal ischemia and corrected histopathologic changes in kidney.

The findings of our study suggest that increased tissue damage in renal ischemia/ ischemia-reperfusion in rats, suppression of antioxidant activity and histopathological changes are prevented by resveratrol application.

A10-7

The effects of relaxin on myoglobinuric acute kidney injury in rats

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Questions: Myoglobinuric acute kidney injury (mAKI) is a uremic syndrome that appears because of the damage in skeleton muscle cells and getting into circulation of inner-cell elements related to traumatic and non-traumatic reasons, namely rhabdomyolysis. We aimed to determine the relaxin level and its effects and effect mechanisms on kidney damage and functions in mAKI. **Methods:** Rats were deprived of water for 24 hours, serum physiologic (SF) was given to the first and second group of rats, hypertonic glycerol (%50) solution was injected into each back leg muscles with equal level of 8 ml/kg to the third and fourth groups of rats. Subcutaneous phosphate buffer solution (PBS) were injected on the 1st, 6th, 12th, and 18th hours in the 1st and 3rd group of rats. Subcutaneous 5 μ g/kg of relaxin were injected to 2nd and 4th group of rats in the same time basis. The Mann Whitney U test was used. **Results:** In our study, in the 3rd group, mAKI, there was statistically significant increase in serum urea, creatinine, potassium, nitric oxide (NO) and kidney malondialdehyde levels. We observed a significant decrease in kidney glutathione and NO levels. **Conclusion:** Relaxin treatment did not significantly change our investigated parameters in AKI model. We believe that more extensive studies

should be carried out on the dose of relaxin, its time and route of administration on the parameters of renal blood flow, renal functions.

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A10-8

Renal proximal tubular cells under the influence of the female hormone cycle

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Questions

Studies in human patients and animals have revealed that females are less susceptible to renal injury than males. Therefore we searched for potential influences of the female hormone cycle on basic renal functions.

Methods

Urinary excretion of the proximal tubular cell-derived marker proteins Fructose-1,6-bisphosphatase and Glutathione-S-transferase alpha was determined by an enzymatic assay or ELISA and normalized to urinary creatinine. alpha 1-Microglobulin, Albumin, Immunglobulin G, Estrone-3-glucuronide, Pregnanedione-3-glucuronide, and Luteinizing hormone were quantified in the urine samples by enzyme immunoassays.

Results

Healthy ovulating women showed transiently increased urinary excretion of Fructose-1,6-bisphosphatase and Glutathione-S-transferase alpha correlated with decreases of estrogen levels after ovulation or at onset of menses. Male probands and postmenopausal women, by contrast, showed consistently low levels over a comparable time period. The recurring, transiently higher rate of enzymuria in ovulating females might be sign of a periodical, temporally limited enhancement of proximal tubular cell turnover. Renal plasma protein handling appeared to be unaffected, since changes in urinary alpha 1-Microglobulin, Albumin or Immunglobulin G excretion could not be detected.

Conclusions

The study provides a first indication for the novel concept that proximal tubular tissue architecture might undergo periodical adaptations phased by the female reproductive hormone cycle. A recurring renewal of proximal tubular epithelium could provide enhanced repair capacity resulting in a higher resistance of women to renal injury as compared to men.

A10-9

Immunosuppressant dosing accuracy. Residual drug concentration versus estimation of the area under the curve

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This study aimed to assess the immunosuppressant dosing accuracy by performing drug blood monitoring and determining C0 and AUC.

Cyclosporine (CsA), tacrolimus, everolimus, and sirolimus concentration-time profiles of whole blood, and mycophenolate mofetil (MMF) concentration-time profile of whole blood serum was measured 12 h after the first dose. Mycophenolic acid in serum was determined by HPLC; other immunosuppressants (ISs) in the blood were determined by LC-MS. AUC were calculated by the Bayesian estimation and 3 point LSS.

In total C0 and AUC was determined in 614 kidney recipients. All patients graft life aged > 1 year.

In this study we have evaluated dosing accuracy by determining C0 and AUC compliance within ISs therapeutic ranges and found that cyclosporine treated patients AUC exposures were within the therapeutic range more often than C0 values. Similar results were obtained by analyzing other ISs AUC and C0 values compliance within therapeutic ranges. Slightly different results were obtained by analyzing MMF concentrations in serum, where C0 values were more often within the therapeutic range than AUC exposures.

Analyzes of C0 and AUC compliance within the therapeutic range also showed that CsA is administered at low doses more often, while other ISs are administered at low doses more often when assessing AUC versus C0. Except tacrolimus, this is given in too large doses.

Study results showed that determination of C0 and AUC give different results and explain why we should not consider only one parameter but should take into account both. These results also indicated that kidney recipients are treated by very low doses of immunosuppressants, except when tacrolimus is administered, this is given in too large doses.

A10-10

Serum fibroblast growth factor-21 is associated with renal sinus fat increment independently of total intraabdominal obesity

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Pathways through which obesity might cause renal disease are not well understood. Recent studies have associated ectopic lipid accumulation in the kidney with obesity-related renal disease. Human studies indicate that circulating levels of fibroblast growth factor-21 (FGF21) increased in obese individuals. FGF21 was found to closely associated with renal dysfunction in end-stage renal disease subjects. We hypothesised that renal sinus (RS) fat volume may be independently associated with increased level of FGF21.

The study included 110 subjects (60/50 F/M; age of 39.8±5.8). CT images were captured and RS fat accumulation was measured using the 3D-Doctor software. Both kidneys and RS fat were measured, and ratio left kidney sinus fat/left kidney (LS/LK) and right kidney sinus fat/right kidney (RS/RK) were calculated. Intraabdominal (IA) fat volume was measured at the level of renal hilus. FGF21 serum level was detected by ELISA assay. *Partial rank correlation was used to adjust the association between LS/LK, RS/RK and FGF21 after accounting for the IA fat volume. According on sex-specific 75th percentiles of FGF21 levels all measurements were divided into two groups.*

FGF21 correlated with both LS/LK and RS/RK ratios ($r=0.50$, $p<0.001$; and $r=0.45$, $p<0.05$). There were significant ($p<0.05$) increment of both LS/LK (0.0075; (0.0019; 0.0145) and 0.0142 (0.0077; 0.0332))* and RS/RK (0.0027; (0.0003; 0.0051) and 0.0084 (0.0017; 0.0311))* ratios when data were divided according on sex-specific 75th percentiles (234.32 pg/ml) of FGF21.

Taken together, these results suggest that serum FGF21 level may be increased in individuals with reduced renal function because of the increased fat accumulation in the renal sinuses.

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A10-11

The relationship between Saxagliptin and renal ischemia/reperfusion: A morphological approach

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Saxagliptin is a DPP-4 inhibitors and this compound include a precaution regarding increased risk of heart failure, particularly in patients with pre-existing cardiovascular or renal disease. This study is designed to determine the possible protective effect of Saxagliptin on the kidney I/R injury. Adult male Sprague-Dawley rats were used in this study. In the sham group, right kidneys of the animals were dissected. In the I/R group, right kidney was dissected and ischemia of 45 min was performed, and then reperfusion was applied for 24 h. In the treatment groups, two different doses of saxagliptin (2 and 10 mg/kg) was orally by gavage at the beginning of the ischemia unlike the I/R group. After 24h, all rats were sacrificed and renal tissues were taken for histological examinations. The renal tissues were fixed in 10% formalin solution, and then embedded in paraffin. The paraffin blocks were cut 5 µm and stained with haematoxylin and eosin (H&E). Histological examination showed normal kidney structure in the control group. In I/R group, the kidney sections appeared with variable changes and marked injury. These changes were dilation of the tubular lumen, hemorrhage and inflamatuar cell infiltration, hidropic degeneration, prominent hemorrhage between the tubules and glomerules, epithelial atrophy and cell desquamation in the tubules, vacuolization of tubular epithelial cells, and casts in tubular lumen compared to the kidney samples from the control group. All treatment groups showed reduced renal injury when compared with I/R group. But IR+ 10 mg/kg group exhibited significantly improved histological appearance compared to the I/R + 2 mg/kg group. The observations indicate that saxagliptin may have some effects on renal functions by affecting renal morphology.

A10-12

Clinical and urodynamic neurogenic bladder secondary to myelomeningoceles (MMC)

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Introduction : MMC is the most commune and viable of neural tube defect (NTD). Neurogenic bladder is among the most severe complication of MMC. Our aim is to study the clinical and urodynamic profile of bladder dysfunction in patients with MMC. Material and patients: We reviewed the records of 28 patients with neurogenic bladder secondary to MMC, followed between January 2014 and March 2017. Clinical and urodynamic data were studied.

Results:

The records of 28 patients (20 males and 8 females) were reviewed. The average age was 10,5 years ranging from 2 years and 25 years. Associated anomalies were found in all cases. Clinical manifestations of urinary disorders were found in 100% of cases. Urinary incontinence, enuresis showed the most frequent clinical manifestation (22 cases). Repeated episodes of urinary tract infection (UTI) were found in 44.38% patients. Only two patients presented renal failure. Hydronephrosis and vesico-urethral reflux were diagnosed in 8 of them. A diverticular bladder was detected in 6 cases. Reduced capacity was found in 18 of cases. eighteen cases showed hypocompliant bladder. Overactive detrusor and sphincterdyssnergia were noted respectively in 20 and in 16 of cases. Ten patients had important vesicle residues.

Conclusion:

Neurogenic bladder secondary to MCC have various clinical and urodynamic profile. Urodynamic studies must be performed earlier to evaluate the bladder functioning in order to prevent renal failure.

A13: Skeletal muscle physiology

A13-1

The effects of Zinc and Melatonin on Muscle Ischemia-Reperfusion Damage in Rat

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Ischemia-reperfusion is lead to damage in cell or tissue due to insufficient blood flow stream in tissue or organs. The aim of present study was to determine the effect of zinc, melatonin and zinc + melatonin supplementation for 3 weeks on muscle tissue MDA and GSH levels. This study was performed on 38 male Wistar-Albino rats.

Experiments groups were designed as sham-control, ischemia-reperfusion (I/R), zinc + I/R, melatonin + I/R and zinc + melatonin + I/R. Ischemia-reperfusion was induced by left femoral arter occlusion (1 hour) and reopening (1 hour). At the end of experiments tissue samples were analysed for MDA and GSH.

MDA levels in I/R groups increased significantly. Zinc and melatonin supplementation reduced MDA, however increased GSH levels.

The results of present study show that increased lipid peroxidation in muscle tissue by ischemia-reperfusion may be prevented by zinc and melatonin supplementation.

A13-2

Radon Registry Study

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Objective

Curative Radon (Rn) treatments for patients who suffer from inflammatory and degenerative diseases of the musculoskeletal system or chronic ailments of the skin and respiratory system have a long tradition in the Gastein Valley. Different clinical studies demonstrated that Rn therapy can cause a significant reduction of pain as well as a significant enhancement of functionality.

Methods

The purpose of the Radon Registry Study is to evaluate the modification of health related parameters before and after cure treatment, as well as three, six and nine months later. Those parameters will be collected by quality of life, pain and disease activity questionnaires. Simultaneously the received physical therapies and Radon treatments will be evaluated.

The main target is the identification of correlations between the cure treatments, applied Rn intensity and the improvement of patients health status. Patients that fulfil defined inclusion criteria and suffer from Osteoarthritis, Rheumatoid Arthritis, Ankylosing Spondylitis or Back Pain, can participate in the Radon Registry Study.

Results & Conclusion

The first study participants were included back in March 2016. Until now the analysed data of the questionnaires, reveal that the parameters for quality of life and pain show a significant improvement after cure in all indications. Similar data are illustrated in the disease-specific questionnaires. These preliminary data exhibit that the Rn cure treatment adduces a positive effect in the investigated parameters. In the long term the comparison of cure effectiveness against duration, type and intensity of treatments should bring an insight into the way Rn acts in patients.

A13-3

THE EFFECTS OF STRESS ON THE ACTION POTENTIAL OF SKELETON MUSCLES

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INTRODUCTION-PURPOSE: Its effects and harms on human body have been studied in real terms in recent years, and it has been concluded that stress has more negatively affected all the systems in human body biochemically, histologically and physiologically.

In the current study, the effects of exam stress on students' skeleton, chewing, swallowing, and temporal and biceps muscles.

MATERIAL-METHOD: 20 male and 20 female university student volunteers participated in the study. Recording BioPac mp100 device and surface electrode as electrode were used.

First, before the exams action potentials of the right and left masseter, right and left temporal, right and left digastric, right and left biceps muscles of the student volunteers were recorded through Biopac mp 100 device. Later, towards the end of fifteen days of exams, the action potentials of the same muscles were recorded again.

Through four different movements of resting, tightening, chewing and swallowing of right and left masseter chewing muscles, right and left temporal, and right and left digastric muscles helping swallowing, and finally right and left biceps muscles, and lifting a certain weight with biceps muscles, EMG recording was performed.

RESULTS: There was a significant correlation with the ANOVA test between the data of the male students before the exams and the data of the male students after the exams with the ANOVA tests ($p < 0.01$).

There was a significant correlation between female students ($p < 0.03$). The result is that the stressed muscles cause a decrease in the action potential millivolt, in other words, it produces less power.

KEYWORDS: Stress, Action potential, Chewing and swallowing muscles

A13-4

Functional evaluation in post-viral myositis

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The purpose of this study is to evaluate the clinical utility of electromyography (EMG) in the positive diagnosis of post-viral myositis. We investigated 32 patients in County Hospital Timisoara (2017). The myositis appearance is determined by viral infection. The clinical signs and the functional investigations were considered. All patients were evaluated for personal history and biochemical parameters. The clinical aspects of the disease were expressed by joints and muscles pain, reduced mobility, asthenia and fever.

The electromyography (bilateral vast medial and anterior tibial muscles) aspect revealed normal and low amplitude and duration of unit motor potentials in 82.7% of patients and normal recruitment pattern. Polyphasic potentials present for bilateral anterior tibial muscles in 29.4% of patients. All these aspects revealed a myogenic aspect of EMG but in 78.5% of patients with good prognosis.

The efficiency of treatment with specific anti-inflammatory agents is expressed by the decrease of symptomatology, optimization of the lab blood tests and the aspect of electromyography.

A13-5

Cartilage Marker Plots for Monitoring of Osteoarthritis Patients. A Pilot study

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Osteoarthritis (OA) is the most frequent cause of pain in the ageing population. Currently there are no disease-modifying OA drugs available. Treatment is limited to pain reduction, improvement of joint mobility/functionality and delay of disease progression or joint replacement in severe cases. The knowledge of systemic biomarkers, which reflect the ongoing situation in the affected joint(s), would facilitate a fast assessment of improvement or aggravation of disease during treatment.

Starting from October 2016 patients ($n=26$) with OA of one or both knees were enrolled in a randomized, controlled pilot study in the Bad Gastein Health area. They attended a health regimen comprising conventional physical therapies (control group ($n=13$)) and 8 additional visits to the radon gallery in the intervention group ($n=13$). Blood and urine samples were taken during the therapy and three and six months after the therapy to evaluate long-term effects. A disease-related questionnaire (WOMAC), the EQ-5D health questionnaire and a numeric rating scale for the assessment of pain were also given out. In May 2017, the study will be completed, providing us with blood, urine samples and questionnaire data from OA patients over five time points. Anabolic and catabolic cartilage biomarkers will be quantified in the samples by ELISA. These data will be used for the creation of cartilage marker plots to represent prevailing changes in the balance of cartilage metabolism during the cure regimen. For comparison, the same biomarkers are analyzed in urine, blood samples and primary chondrocytes of OA patients undergoing total knee arthroplasty. Radiographic analysis and macroscopic assessment will be correlated to levels of biomarkers to define their validity.

A13-6

The Novel Adipokine Vaspin is Associated with Increased Adiposity in Humans and Impacts on Human Skeletal Muscle Insulin Signalling.

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Introduction

Vaspin is a novel adipokine associated with insulin resistance in mouse models. However, neither the expression nor function of vaspin has been well characterised in humans. This study aimed to determine the relationship between vaspin adipose tissue expression and adiposity in humans, and to explore its effect on primary human myotube insulin signalling pathways.

Methods

Subcutaneous adipose tissue and skeletal muscle was obtained from n=8 lean (BMI 18-24) and n=9 obese subjects (BMI>30), undergoing elective total hip replacement surgery (NRES 14ES1044). Primary myotubes were isolated from muscle and differentiated over 8 days into myotubes. Vaspin expression, and its secretion, from adipose tissue was quantified by qRT-PCR and Western blotting. Primary human myotubes were pre-incubated with recombinant human vaspin (100ng/ml) for 24h and then stimulated \pm insulin (30nM), or were acutely stimulated with vaspin for between 5-15 min. The effect on AKT activation (thr308) was determined by mesoscale analysis and Western blotting.

Results

Vaspin secretion was detected from both lean and obese adipose tissue. Vaspin mRNA expression was significantly higher in obese adipose tissue, comparison to lean ($p<0.01$) and positively correlated with body-weight ($p<0.01$ $r^2=0.37$) and BMI ($p<0.01$ $r^2=0.44$). Stimulation of primary human myotubes with recombinant vaspin activated AKT in a time dependent manner ($n=3$), and appeared to blunt the effect of insulin.

Discussion

The secretion of vaspin from adipose tissue, its association with increased adiposity and its effect on myotube AKT activation suggests vaspin could be a novel mediator of skeletal muscle insulin sensitivity.

A13-7

Energy production and transfer in oxidative muscles of mice with deleted wolfram (wfs1) gene.

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Introduction: To study the mechanisms of Wolfram syndrome, we used wfs1-deficient mice, models of this syndrome. We assessed function of mitochondria, the changes in activities, amounts and functional coupling between mitochondria and enzymes involved in the transport of energy in oxidative muscles of wfs1-deficient mice.

Methods: Samples of heart and *musculus soleus* of wfs1 deficient and wild type mice were used. Mitochondrial function was assayed by high resolution oxygraphy of permeabilized muscle fibers. Enzyme activities were determined spectrophotometrically and LFQ intensity of proteins was evaluated by nano-LC-MS/MS analysis of muscle homogenates.

Results: Compared with controls ADP-stimulated state 3 respiration did not change in heart, but it decreased in the *musculus soleus* by 34% ($p<0.004$). In wfs1-deficient mice functional coupling of adenylate kinase and mitochondria in heart decreased by 39% ($p<0.05$), but in *musculus soleus* did

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not change. Compared to wild-type, in *musculus soleus* and in heart of wfs1-deficient mice total activity of adenylate kinase did not change. Nano-LC-MS/MS analysis showed that relative amounts of mitochondrial 2-oxoglutarate dehydrogenase and succinyl-CoA ligase subunit alpha decreased in *musculus soleus*, compared to the wild type respectively 1.69 ($p<0.005$) and 2.08 times ($p<0.0002$). Relative amount and activity of citrate synthase in homogenates of muscles did not change.

Conclusion: Wfs1-deficient mice are characterized by a decreased mitochondrial oxidative phosphorylation due to mitochondrial disorders in *musculus soleus* and by impaired functional coupling between adenylate kinase and mitochondria.

A13-8

Increased proton leak and expression of mitochondrial proteins in white skeletal muscle of mice with deleted wolfram (wfs1) gene.

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Introduction: Mice models of Wfs1 deficiency allow to study the mechanisms of human Wolfram syndrome. Our aim was to assess the changes in function of mitochondria and proteins of energy metabolism in glycolytic muscle of wfs1-deficient mice.

Methods: Samples of *musculus rectus femoris* of wfs1-deficient and wild-type mice were used. Oxygraphy was done on permeabilized fibers. Homogenates were analysed by Real-time PCR method, spectrophotometry and nano-LC-MS/MS.

Results: Compared with wild type, in wfs1-deficient *m. rectus femoris* proton leak and citrate synthase activity increased by 73% ($P<0.001$) and 46% ($P<0.05$), respectively. UCP2 mRNA level was in wfs1-deficient *m. rectus femoris* 2.6 times ($P<0.05$) higher than in wild-type muscle. The amounts of mitochondrial succinate dehydrogenase iron sulphur subunit and cytochrome b-c1 complex subunit 1 were in *m. rectus femoris*, compared with the wild type, respectively, 2.08 ($P<0.0002$) and 1.61 times ($P<0.003$) larger. Compared with controls, in *m. rectus femoris* of wfs1-deficient mice total activities of creatine and adenylate kinase decreased by 34% ($P<0.01$) and 48% ($P<0.02$), respectively. The amounts of mitochondrial sarcomeric creatine kinase in wfs1-deficient *m. rectus femoris* increased 3.16-fold ($P<0.001$) and mitochondrial adenylate kinase 2 (AK2) 2.09-fold ($P<0.01$) compared with wild-type muscle.

Conclusion: Wfs1-deficient mice are characterized by a larger amount and activity of mitochondrial proteins in *m. rectus femoris*. The proton leak in this muscle was increased probably due to a larger amount of UCP2. Despite the drop in total activities of creatine and adenylate kinase in *m. rectus femoris*, the amounts of mitochondrial isoforms of these enzymes increased.

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Poster Session A

POSTER SESSION B

B01: Cardiac physiology

B01-1

Chronobiological aspects of general anesthesia in rat myocardial electrophysiology

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Questions: In the present time, there is no literature evidence regarding the effect of general anesthesia on basic electrophysiological myocardial parameters and their dependence on circadian rhythmicity or the light-dark (LD) cycle. To review the initial state of electrophysiological parameters that may predict the development of heart rhythm disorders in spontaneously breathing pentobarbital (P) - 40mg/kg, ketamine-xylazine (K/X) - 100mg/kg + 15mg/kg and zoletil (Z) - 30mg/kg anesthetized rats. **Methods:** The study was performed using female Wistar rats after adaptation to an LD cycle (12h light:12h dark). RR, PQ, QT, QTc intervals and QRS complex were recorded and analyzed from the II bipolar lead for their dependence on the LD cycle. **Results:** The longest RR (K/X light 242 ± 4 , dark 220 ± 20 ms vs. P light 169 ± 16 , dark 178 ± 34 ms and Z light 172 ± 25 , dark 219 ± 57 ms) and QT interval (K/X light 84.3 ± 15 , dark 90.7 ± 7.4 ms vs. P light 73.5 ± 15.4 , dark 76.0 ± 9.7 ms and Z light 79.4 ± 12.3 , dark 70.7 ± 10.4 ms) under K/X anesthesia in both of the light periods. The longest PQ (Z light 51.8 ± 5.4 , dark 46 ± 3.4 vs. P light 44.2 ± 7.7 ms, dark 45.3 ± 4.6 and K/X light 46.8 ± 12.3 , dark 36.4 ± 6.9 ms) and QTc interval (Z light 200.7 ± 28 , dark 160.7 ± 33.2 ms vs. P light 197.7 ± 40.9 , dark 190.7 ± 26.6 ms and K/X light 176.1 ± 25.8 , dark 197.5 ± 17 ms) durations occurred under Z anesthesia in the light period. **Conclusions:** From a chronobiological perspective, the most significant predisposition toward the development of ventricular arrhythmias originating from disorders of impulse production and conduction occurred under Z anesthesia in the light period. Those resulting from disorders in the dispersion of refractory periods occurred under K/X anesthesia in both of the light periods.

B01-2

Physiological and biochemical alterations of experimental systolic heart failure in mice overexpressing a serotonin receptor in the heart

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We have generated mice with cardiac specific overexpression of a physiologically relevant (inotropy, tachycardia) serotonin receptor, namely the 5HT4-receptor (TG) and studied them in comparison with their control littermates (WT). Injection of lipopolysaccharides (LPS) induced systolic heart failure in TG and WT starting 3 hrs post injection as judged from reduced ejection fractions in M-mode echocardiography of left ventricles in intact animals. However, at 7 hrs (were cardiac tissue was frozen) the decline in EF was less in TG than WT. This heart failure was accompanied by a huge increase in the mRNA (as assessed by quantitative polymerase chain reaction) of LPS binding protein (LBP) and toll like receptor 4 (TLR4) in TG but not WT ($p < 0.05$). Interestingly, LPS induced a decrease in mRNA of NFkB in WT but an increase in TG, whereas the mRNA of IkBalpha was to a similar extent increased by LPS in WT and TG. Moreover, the mRNA coding for the overexpressed human 5HT4 receptor was greatly downregulated ($n=3-4$ each, $p < 0.05$) in hearts of TG after LPS treatment. Hence, one can conclude that 5HT4 overexpression, in part, protects the cardiac inotropic function against LPS by interference with the signal transduction of LPS. LPS is suggested to reduce the stability of the mRNA for the transgenic 5HT4 receptor. It remains to be elucidated whether similar mechanisms might be operative in septic heart failure in patients.

B01-3

Uniaxial strain of cardiac tissue parallel to impulse propagation slows conduction more than in the perpendicular direction: untangling the effects of stretch on tissue resistance

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Background: Slow conduction resulting from altered myocardial properties is arrhythmogenic. These altered properties can be caused by deformation, which modifies tissue resistance, membrane capacitance and ion currents. Our aim was to differentiate the changes in conduction velocity (CV) induced by uniaxial strain applied parallel vs. perpendicular to impulse propagation.

Methods: Murine foetal cardiomyocyte strands were cultured on custom stretchable microelectrode arrays over a row of 6 electrodes spaced 1 mm apart. CV was determined from unipolar electrograms. Uniaxial strain (5%), either parallel to propagation (orthodromic) or perpendicular (paradromic), was applied for 1 min and accurately controlled by imaging a grid of markers during steady-state pacing (cycle length: 250-400 ms).

Results: Both strains induced immediate and reversible changes of CV. In material coordinates, 5% orthodromic and paradromic strain changed CV by $-2.3 \pm 0.3\%$ and $-1.0 \pm 0.5\%$, respectively (slowing). In observer coordinates, the corresponding changes were $+2.3 \pm 0.3\%$ (acceleration) and $-1.4 \pm 0.4\%$ (slowing). Because the cultures were isotropic, the difference in CV change between orthodromic and paradromic strain ($-1.3 \pm 0.7\%$ in material coordinates) isolates the effect caused solely by changes in tissue resistance. Thus, during orthodromic strain, tissue resistance accounted for about 55% of the CV change while other influences (e.g., stretch-activated channels) contributed 45%.

Conclusions: Potentially arrhythmogenic changes in cardiac CV caused by acute strain do not only depend on strain itself but also on the orientation of strain relative to impulse propagation. This dependence is due to different effects on tissue resistance.

B01-4

The selective late sodium current inhibitor GS967 reduces modifications of ventricular fibrillation activation complexity induced by mechanical stretch.

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Myocardial stretch produces detrimental changes in electrophysiological properties such as myocardial refractoriness, conduction velocity and heterogeneity, which in turn can modify the activation pattern and complexity during ventricular fibrillation (VF). An increase in Na^+ influx by activation of the Na^+/H^+ exchanger is one of the underlying mechanisms. Nevertheless, the role of the late Na^+ current (I_{NaL}) on stretch-induced modifications is not well known. We investigated the effect of the I_{NaL} blocker GS967 on the stretch-induced changes of the activation complexity during VF.

Methods

Langendorff technique for isolating and perfusing rabbit heart was used ($n=10$). Analysis of the myocardial activation complexity during induced VF (maintaining coronary perfusion) by high-resolution epicardial mapping was performed in control conditions and under GS967 (0.03, 0.1 and 0.3 μM) effects, prior to stretch and at min 3 during stretch. Activation maps were constructed in each

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situation and classified into 3 categories based on its complexity (low: I, intermediate: II, high: III) to determine complexity index=(n° maps I x 0.1 + n° maps II x 1 + n° maps III x 2)/ total n° maps. An ANOVA test was used (p<0.05).

Results

Myocardial stretch significantly increased the complexity of ventricular activation in control conditions (1.18 ± 0.26 vs 1.49 ± 0.33) and under 0.03 µM. However, complexity did not increase during stretch under GS967 at 0.1 and 0.3 µM, and it was significantly lower than in control conditions (control: 1.49 ± 0.33; 0.1 µM: 1.28 ± 0.42; 0.3 µM: 1.26 ± 0.32).

Conclusion

The inhibition of I_{NaL} with GS967 attenuates the stretch-induced electrophysiological effects responsible for the increased complexity of myocardial activation.

B01-5

Role of the late sodium current on ventricular refractoriness and electrophysiological heterogeneity modifications induced by acute local stretch. A study in isolated rabbit heart.

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Some heart pathophysiological mechanisms act by means of mechanoelectric feedback, as it is the local stretch of ventricular myocardium, which produces electrophysiological disturbances. Ionic currents and exchangers underlie the stretch changes, but the role of late sodium current (I_{NaL}) is not well known. We investigated, in isolated rabbit heart, the effects of GS967, I_{NaL} blocker, on ventricular refractoriness and electrophysiological heterogeneity modifications by stretch.

Methods. In ten Langendorff-perfused and fibrillating rabbit hearts, ventricular fibrillation (VF) was recorded with multiple electrodes on the left ventricle to determine: 1.The fibrillatory cycles (VV) and the percentile 5 of the VV (VVP5), as refractoriness indexes, and 2. Coefficient of variation of VV (CVVV) as heterogeneity index. VF was induced by pacing maintaining coronary perfusion. Stretch was produced by a device into the left ventricle. Measurements were made prior to the stretch (basal) and at the 3rd min of stretch, in control situation and after GS967 (0.03, 0.1 and 0.3 µM). Differences between 3 min of stretch and basal were determined to the VVP5 in control and after GS967. A repeated measures ANOVA test was used (p<0.05).

Results. VV decreased after stretch in control, and after GS967, 0.03 and 0.1 but not 0.3 µM. VVP5 decreased in control and after GS967 at all concentrations, but the differences between 3 min of stretch and basal were significantly less after 0.3 µM than control. CVVV tended to increase during stretch in control and GS967, 0.03 µM (p=0.09) but not after GS967 at 0.1 and 0.3 µM.

Conclusion. I_{NaL} seems to be implicated in the refractoriness and heterogeneity changes produced by myocardial acute local stretch.

B01-6

The effects of paced breathing on heart rate variability parameters

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The current study aimed to examine heart rate variability during controlled breathing at resonance frequency.

Methods. ECG parameters were recorded noninvasively in 87 young healthy volunteers (19–25 years old) breathing for 5 min at 4 different respiratory rates of 4.5, 5, 5.5, 6 and 6.5 breaths/min. Breathing at resonance frequency (RF) was manifested by highest peak in spectrogram. Heart rate variability features were analyzed using linear (time domain and frequency domain) and nonlinear methods. Nonlinear analysis of HRV was performed by using Poincare plot parameters (SD1, SD2), Sample Entropy and detrended fluctuation analysis (alpha, alpha1, alpha2).

Results. The mean value of RF was 5,67±0,07 breath/min. Time domain parameters (SDNN, RMSSD), were significantly higher during breathing at RF. LF, SD1, SD2 were significantly higher during deep breathing than that during spontaneous breathing (1736,91±599,41 ms2 vs 8391,41±777,48 ms2; 39,49±2,80 ms vs 51,19±2,94 ms and 78,11±3,43 ms vs 131,60±5,33 ms). SampEn, alpha and alpha 2 were significantly lower during deep breathing test than that at rest (1,65±0,03 vs 0,20±0,02; 0,835±0,016 vs 0,542±0,016; 0,840±0,019 vs 0,396±0,019). Alpha 1 was higher during RF breathing than that during spontaneous breathing (1,021±0,029 vs 1,506±0,019). There was no difference in heart rate and HF parameters during controlled and spontaneous breathing.

Conclusions. The results of the current study suggest that paced breathing alter linear and nonlinear dynamics of heart rate. Heart rate variability analysis could be effective in automatically detecting functional state during paced breathing.

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B01-7

Mechanisms of beta-adrenergic regulation of bioelectric activity in murine pulmonary veins myocardium

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Aim. Pulmonary vein (PV) myocardial tissue has been recently considered as a main source of a supraventricular arrhythmias, including atrial fibrillation (AF), due to ectopic automaticity or re-entrant conduction. It has also been demonstrated that sympathetic or adrenergic stimulation is critical for PV-derived ectopy and AF initiation. The mechanisms of adrenergic PV ectopy remain poor understood. The aim of the present study was to investigate mechanisms of a bioelectric activity changes in murine PV myocardium under adrenergic stimulation.

Methods. Mice (male, 20-30 g) were anaesthetized, multicellular preparations of the left atrium (LA) or PV were dissected and perfused at 37°C with Tyrode solution. Resting membrane potential (RMP) and spontaneous action potentials (AP) were recorded with use of standard microelectrode technique.

Results. Norepinephrine (NE, 10 µM, n=6), β-adrenoreceptor (β-AR) agonist isoproterenol (ISO, 10 µM, n=7), phosphodiesterase (PDE) inhibitor IBMX (10 µM, n=9) caused significant RMP hyperpolarization and induced series of spontaneous AP in murine PV (in 90% of cases). α-adrenoreceptor antagonist prazosin (5 µM, n=5) was unable to cease NE effects, while β-AR blocker propranolol (5 µM) completely abolished effects of NE (n=5) and ISO (n=5) in mice PV. In addition, NE- (n=6), ISO- (n=6) and IBMX-induced (n=6) spontaneous AP were suppressed by Ca²⁺-antagonist nifedipine (10 µM).

Conclusions. Murine PV myocardium is highly prone to ectopic firing and strongly depend on β -AR stimulation. Cyclic AMP accumulation and Ca^{2+} transmembrane current stimulation may underlie β -AR induced spontaneous activity in murine PV. This study is supported by Russian Science Foundation 14-15-00268 grant.

B01-8

Effect of mesenchymal stem cells administration on electrophysiological and contractile properties of ventricular myocardium in clinically relevant porcine model of sepsis

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Sepsis represents a serious life-threatening disease with high mortality. Previous studies have shown beneficial effect of mesenchymal stem cells (MSC) in rodent septic models. Our study aimed to test effects of possibly beneficial MSC administration on cardiac function in clinically relevant large animal model with high translational potential.

The domestic pigs were divided into 4 groups, control (C; n = 6), septic (S; n = 6), and control (MC; n = 7) and septic with MSC application (MS; n = 6). All animals were anaesthetized, mechanically ventilated and instrumented, 6 hours after instrumentation sepsis was induced in septic groups (S, MS) by fecal peritonitis. In MC and MS group MSC (1 million/kg) were administered 6 hours after sepsis induction. One day after sepsis induction the *in vivo* part of the experiment was terminated and hearts were examined. Membrane potential and contraction force were measured in trabeculae from right ventricles. Calcium transients and sarcomere length were determined in cells isolated from left ventricles.

Sepsis was associated with a depression of cardiac contraction and shortening of action potential duration. MSC application did not affect either electrophysiological or contractile properties in both control and septic animals. The data indicate that MSC application in sepsis does not exert direct cellular effects in cardiac muscle, neither beneficial nor detrimental.

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B01-9

Heart-rate variability did not affect subsequent night sleep parameters and cortisol awakening response

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QUESTIONS: Changes in sleep duration and sleep disturbance have been reported to affect hypothalamic-pituitary-adrenal axis (HPA) and the autonomic nervous system (ANS) activities. However, the effect of ANS activity on sleep parameters and HPA activity in the subsequent night and morning is not known. The aim of the current study, was, therefore, to assess the effects of heart rate variability on subsequent night sleep parameters and cortisol awakening response (CAR).

METHODS: Electrocardiographic recordings were carried out for 5 min for determination of HRV in healthy medical students (n=48). They were allowed to sleep in their normal routines in following night. Sleep diaries were filled for sleep parameters (Karolinska Sleep Diary and Questionnaire, Pittsburgh Sleep Quality Index). Salivary samples were taken at 0, 15, 30 and 60 min post-awakening for measurement of CAR. Cortisol concentrations were measured in the salivary samples by enzyme immunoassay.

RESULTS: Majority of the participants (80%) had time-domain variables within the normal range and they did not have sleep disturbances. Time- and frequency-domain parameters of HRV during the morning did not correlate with sleep parameters (time, duration, disturbed sleep, awakening problems) or CAR (mean, area under the curve) in the next day (p>0.05).

CONCLUSIONS: The results of the current study suggests that, under the conditions which does not have profound effects on ANS activity, neither sleep parameters nor next morning cortisol responses are affected by HRV. Additionally, a quality night sleep might counterbalance both the possible effects of previous days autonomic pressures and next mornings cortisol responses.

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B01-10

Simultaneous electro-optical endocardial and epicardial mapping of mechanoelectric feedback by left ventricular stretch in the isolated rabbit heart. An experimental validation of a custom-made endocardial balloon array with volume control.

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Introduction. Acute effects of myocardial stretch by mechanoelectric feedback (MEF) have not been explored in detail regarding endo-epicardial modifications at different rates and rhythms of activation. In this study, a miniaturized noncontact, multielectrode array (MEA) balloon was developed for ENDO mapping and volume-controlled left ventricular (LV) stretching (STR). Our aim was to study the modifications produced by moderate STR on stimulated restitution properties using this dual-sided approach.

Materials and Methods. Ten Langendorff-perfused NZW rabbit hearts were included. ENDO mapping was performed by means of a miniaturized MEA-balloon with 13 unipolar isolated pure-steel electrodes uniformly distributed. The device was connected to a volume-controlled circuit. High-resolution EPI optical mapping was performed using di-4-ANBDQPPQ and 7.5uM blebbistatine at

530fps. Dual-sided mapping was completely synchronized. STR was generated increasing 1.5-2 mL the physiological volume of the LV. We stimulated the hearts from the ENDO at 250, 200, 180, 160, 140 and 120 ms, before and during STR. Activation-recovery intervals (ARI) and action potential durations (APD, 30-90%) were analyzed. Repeated measures one-way ANOVA was used ($P < 0.05$).

Results. ARIs of ENDO electrograms highly correlated with APD measures at 80% of repolarization. We found a significant decrease in APD above 90% during STR at the faster rates (105.27 vs 100.21 at 140 ms; 103.46 vs 95.52 at 120ms, $p < 0.01$). Interestingly, we observed a non-significant biphasic frequency dependent effect in restitution in the epicardium. No differences were found in ENDO ARI before and after STR. Arrhythmia acceleration was observed during STR (13.63 vs 16.88 Hz, $p < 0.05$).

Conclusion. A new miniaturized MEA-balloon revealed an APD shortening and restitution biphasic response likely implicated in MEF modifications under STR.

B01-11

The effect of CHAMBER-REST on electrophysiology of the heart in young people

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Introduction. The aim of the study was to determine the effect of Chamber-Rest therapy on electrocardiographic parameters in young people. It is a therapeutic method based on a stay in a complete darkness to improve health status of people living under constant stress. Aside from improving the psychological status, this method could also affect vital functions including electrophysiology of the heart.

Methods. 14 students (19 to 26 years) were placed individually in a special room with maximal darkness for 96 hours. The room met the requirements for a comfortable stay in a quiet place. The participant received food and drinks as requested and did not use any device emitting light or showing the time. The first measurement was performed the day before starting the therapy. The next measurement was taken 30 minutes after completing the therapeutic session, followed by two more measurements in the fourth and the seventh day after exiting the dark room. The measured variables included PQ, QT and QTc intervals as well as the heart rate based on the 2nd bipolar lead of the ECG.

Results. The heart rate was significantly lower in the day of completing the stay, as well as in the fourth and the seventh day. The QT interval was significantly prolonged in the day of completing the stay, and the rest of ECG intervals remained unchanged.

Conclusions. 96 hours of darkness therapy lowered the heart rate of young people. This effect is beneficial because higher heart rate is associated with an increased risk of cardiovascular disease. The prolongation of the QT interval is a marker for development of ventricular arrhythmias. The QTc interval remained unchanged, therefore the predisposition to the emergence and progression of ventricular arrhythmias was not lowered.

B01-12

SYSTOLIC TIME INTERVALS: EFFECT OF MENTAL ARITHMETICS

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Basic systolic time intervals (STI) – left ventricular ejection time (LVET) and preejection period (PEP) – are affected by multiple factors including cardiac sympathetic activity. The aim of our study was to assess the behaviour of LVET and PEP during supine rest and mental arithmetic task (MA) together with other cardiovascular measures potentially influencing STI.

50 young healthy volunteers (28 f, 22 m, 18.7 years \pm 1.9) participated in this study. LVET, PEP, HR (heart rate), SVR (systemic vascular resistance) and VI (velocity index – index of cardiac contractility) have been measured continuously using impedance cardiography (CardioScreen® 2000) and photoplethysmographic volume-clamp method (Finometer Pro). Arterial stiffness (CAVI – Cardio-ankle vascular index) was examined at rest by VaSera VS-1000.

PEP and LVET significantly decreased compared to preceding rest during MA ($P \leq 0.002$), whileas SVR and HR significantly increased ($P \leq 0.003$). At rest, LVET correlated negatively with CAVI ($P = 0.025$) and HR ($P < 0.0001$); PEP was negatively related to VI ($P = 0.007$). Correlating a change in assessed measures (delta values) between MA and preceding rest we found a positive correlation between Δ LVET and Δ VI ($P = 0.018$) and a strong negative correlation with Δ HR ($P < 0.0001$). Δ PEP correlated positively with Δ SVR ($P = 0.020$) and negatively with Δ HR ($P = 0.003$).

Cognitive load was associated with a decrease of both STI. Significant correlations indicate that cardiac sympathetic activity is not the only factor influencing STI. Vascular properties and HR changes should be considered when interpreting STI as sympathetic activity indexes.

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B01-13

Heart rate variability of premature neonates from 28 weeks of amenorrhea to term equivalent as responses to painful or stressful cares in Neonatal Intensive Care Unit.

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The purpose is to investigate the variations of Heart Rate Variability (HRV) indicators during painful or stressful events felt by premature neonates. Furthermore, HRV indicators may follow different patterns depending on patient immaturity.

A routinely invasive procedure (considered as painful) followed by a cleaning and a diaper changing (considered as stressful) are processed on neonates with heart rate recording. Tachogram is segmented into 5 periods: T1 (baseline), T2 (painful event), T3 (break), T4 (stressful event) and T5 (resting) and a Fast Fourier Transform is performed to obtain Low Frequencies (LF: 0.04 - 0.15 Hz) and High Frequencies (HF: 0.15 - 2 Hz) HRV indicators. ANOVA are performed on LF and HF variables with intra effect of periods ($n = 104$) and inter effects of sex, Corrected Age (CA, level of immaturity), type of painful event (short or long lancing, on heel or hand) and break duration (5-30 minutes).

There is no global effect of sex, type of painful event and break duration on LF or HF means, without any interaction with periods. There is a very significative global effect of periods on LF and HF, which are the lowest on T2, low on T4, intermediate on T3, high on T5 and the highest on T1. There is a very significative global effect of CA on LF and HF. The less immature the group of infants is, the higher are LF and HF. The CA variable interacts significantly with periods on LF and HF. The less immature the group is, the stronger are the variations of LF and HF depending on periods.

Painful and stressful cares alter HRV of premature neonates. Sympathetic and vagal tones seem to increase with maturity. Less immature neonates seem to show an activity of their autonomic nervous system more efficient and suitable to stimulations, whatever their nature.

B02: Vascular physiology

B02-1

Effect of sexual dimorphism on the role of perivascular adipose tissue -derived chemerin in regulation of vascular tone of porcine coronary artery.

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Questions: Researches during the past decade have highlighted the functional role of perivascular adipose tissue (PVAT) in regulating the contractility of the underlying vascular smooth muscle cell layer. However, the mechanisms underlying these observations are little understood. Chemerin, an adipokine derived from adipocytes, has been identified as a potential vasoconstrictor. Therefore, the objective of this study were to determine the impact of chemerin on the vascular tone of porcine coronary arteries (PCAs) and to identify any sex differences in this adipose-vascular coupling.

Methods: Contractions in isolated segments of coronary arteries were determined using isometric tension recording system. mRNA expression of chemerin and its receptor (ChemR23) were measured using real time-PCR. Effect of chemerin on NADPH oxidase (Nox) activity in PCA homogenates was assessed using lucigenin-enhanced chemiluminescence.

Results: Chemerin-9 caused a significantly higher vasoconstriction in PCAs from females in comparison with males (females: 18.4 ± 7.1 , n=6; males: 8.6 ± 1.1 , n=9). Similarly, chemerin-9 enhanced Nox activity in female PCA but not in males (females: control 15.1 ± 3.1 , chemerin-9 19 ± 3 , n=6; males: control 22.3 ± 3.8 , chemerin-9 17.6 ± 1.8 , n=5). Chemerin mRNA was expressed in the PVAT, and the ChemR23 was expressed in PCAs, although there were no sex differences (chemerin: females: 1.43 ± 0.22 , n=10; males: 1.38 ± 0.17 , n=10) (ChemR23: females: 1.13 ± 0.1 , n=10; males: 0.9 ± 0.11 , n=8).

Conclusions: These results indicate that chemerin may have a role as a PVAT-derived contractile agent in female PCA only. This sexual dimorphism could be explained by the difference in the signalling of chemerin in PCAs rather than the expression of chemerin or its receptor in PVAT and PCA, respectively.

Key Words: sex differences, coronary artery tone, perivascular adipose tissue.

B02-2

Modulation of meningeal and medullary blood flow upon noxious stimulation of rat cranial dura mater

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Questions: Meningeal blood flow is controlled by vasoactive substances, among which calcitonin gene-related peptide (CGRP) released from trigeminal nociceptive nerve fibers is the main vasodilatory endogenous mediator. We aimed to clarify if noxious stimulation of the cranial dura mater modulates meningeal blood flow along with the blood flow in the medulla oblongata that contains the spinal trigeminal nucleus as projection site of trigeminal nociceptive afferents.

Methods: Meningeal blood flow was simultaneously recorded at the exposed rat parietal dura mater and the surface of the exposed spinal medulla using laser Doppler flow probes. The cranial dura mater was stimulated by electrical pulses of C-fiber strength and by topical application of capsaicin. The trigeminal ganglion was anesthetized by lidocaine injected by a needle, which was guided through the infraorbital canal.

Results: Electrical pulses (1 ms, 10 Hz, 8-12 V) increased meningeal blood flow by about 70 % and medullary flow by about 29 % within 5 min of stimulation. Blockade of vascular α -adrenoceptors by topical application of phentolamine (100 μ M) was followed by facilitated meningeal blood flow responses upon stimulation. Capsaicin (10-7 M) applied onto the dura increased meningeal blood flow by 15 % on average. Anesthesia of the trigeminal ganglion abolished the increases in medullary blood flow.

Conclusions: Both meningeal and medullary blood flow values are increased upon noxious stimulation of the cranial dura mater, likely mediated by CGRP. The absence of medullary blood flow responses after anesthesia of the trigeminal ganglion suggests that the blood flow is increased due to activation of afferent terminals in the spinal trigeminal nucleus.

B02-3

The vasoactive role of nitric oxide and hydrogen sulphide in adult spontaneously hypertensive rats

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According to our previous study in young spontaneously hypertensive rats (SHR) we could suppose a participation of nitric oxide (NO) and hydrogen sulphide (H_2S) in possible inherent adaptive strategy of conduit arteries in condition of sustained hypertension. The aim of this study was to confirm or refuse the compensatory mechanisms in developed phase of hypertension in SHR with an emphasis on manifestation of the NO and H_2S signalisations.

In the experiments 17-20-weeks-old Wistar rats and SHR were included. Systolic blood pressure (sBP) was measured by plethysmograph method and vasoactivity of isolated thoracic aorta (TA) was recorded by sensors of changes of isometric tension.

We observed an increased sBP and hypertrophy of myocardium in SHR. The contractile response of TA to exogenous noradrenaline was reduced in SHR due to inhibition effect of endogenous NO. In SHR impaired endothelial functions were confirmed, however through a prevalence of vasoconstrictors produced by cyclooxygenase but not as a result of reduced NO synthesis. Dual effect of H_2S donor (Na_2S) was showed in both strains; however an increased maximal vasorelaxant response was proved in SHR. Moreover, acute inhibition of NO production increased the relaxant phase of Na_2S effects. On the other hand, application of Na_2S modulatory dose (40 μ mol) increased the release of NO from exogenous NO donor, nitrosoglutation (0.5 μ mol/l) in Wistar rats but not in SHR.

The data confirmed that SHR disposed with adaptive mechanisms including NO and H_2S systems and their interaction (acute NO deficiency potentiated vasorelaxant effect of H_2S). These effects could provide compensation of the increased vascular tone in adulthood.

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B02-4

Expression of cellular machinery responsible for acetylcholine synthesis, transport and degradation in rat aorta

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Questions: Despite lack of parasympathetic innervations in blood vessels, acetylcholine (ACh) exerts vasodilatory effect mediated by endothelial M3 receptors. However, no information about the possible source of ACh acting on vascular endothelium is available. In line with recent knowledge that ACh could be synthesized and secreted by other than neuronal cells, we hypothesized participation of the non-neuronal cholinergic system. We therefore investigated the presence of cellular machinery responsible for ACh synthesis and re-uptake, as well as ACh degradation enzymes in rat aorta

Methods: We used thoracic aorta from 5 Wistar rats and proceeded with the technique of RT-qPCR.

Results: mRNA of the principal enzyme responsible for ACh synthesis - choline acetyltransferase was absent in rat aorta but an alternative enzyme - carnitin acetyltransferase was detected. High-affinity choline transporter 1, together with vesicular acetylcholine transporter, were expressed at very low levels. We detected expression of organic cation transporters, mostly OCT2 and OCT3 that are able to transport choline across the plasma membrane. We found only low acetylcholinesterase but high butyrylcholinesterase expression.

Conclusions: These data suggest that rat aorta possesses protein machinery for ACh synthesis, transport and degradation and thus ACh could be produced *in situ* or in proximity to the site of action.

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B02-5

The role of NO-H₂S interaction in vasoactive responses of rat and human isolated arteries

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Nitric oxide (NO) and hydrogen sulfide (H₂S) can interact and create new specific substances. However, the vasoactive effects of new products of NO-H₂S interaction have not been characterized yet. The aim was to evaluate the vasoactive action of NO-H₂S interaction in isolated thoracic aorta of adult normotensive (Wistar) and spontaneously hypertensive rats (SHR) and in renal artery isolated after nephrectomy of patients with arterial hypertension. Changes in isometric tension after administration of H₂S donor (Na₂S), NO gas bubbled solution (NOs), NO donor (nitrosogluthathione GSNO) and NOs-Na₂S, GSNO-Na₂S mixtures were evaluated. In Wistar NOs-Na₂S mixture revealed lower and slower vasorelaxation compared to NO and Na₂S alone. On the other hand, GSNO-Na₂S mixture induced higher and faster vasorelaxation compared to GSNO and Na₂S alone. In SHR the maximal vasorelaxation induced by GSNO-Na₂S mixture was similar and the half-time of return of the total relaxation was faster compared to vasorelaxation induced by GSNO alone. Nevertheless, whereas the half-time of return of the total relaxation induced by GSNO-Na₂S mixture was similar in Wistar and SHR, its triggering (reaching of the maximum) was slower in SHR. In patients suffering arterial hypertension GSNO-Na₂S mixture induced higher and faster vasorelaxation compared to GSNO and Na₂S alone. The concomitant dyslipidemia increased the maximal relaxation induced by Na₂S-GSNO mixture but the time of vasorelaxant effect remained unchanged. Results confirmed that product/s of NO-H₂S interaction trigger original vasoactive signal pathways with heterogeneity

independently on (i) the source of NO donor, (ii) origin of tissue (rat, human), and (iii) on the presence of pathological conditions (hypertension, dyslipidemia).

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B02-6

Effect of melatonin on blood pressure and fibrosis enlargement in the heart and aorta in experimental metabolic syndrome

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Objectives: Melatonin is involved in many signaling processes via receptor-mediated or receptor-independent mechanisms. We aimed to find out whether melatonin can modify blood pressure (BP) and morphological parameters of heart and aorta in rats with metabolic syndrome.

Methods: Males, 6-week-old rats were divided into four groups: Wistar Kyoto rats; spontaneously hypertensive rats (SHR); rats with metabolic syndrome (SHR-cp); and SHR-cp treated with melatonin (10 mg/kg/day) for 3 weeks. BP was measured by telemetry. Tissue sections embedded in paraffin were stained with hematoxylin-eosin and picrosirius red. Heart and aorta collagen levels, cross section area and wall thickness of the aorta were analyzed. Melatonin MT(1) and MT(2) receptors were determined by immunohistochemical and Western blot methods.

Results: BP increase in SHR-cp was comparable to that of SHR. Melatonin treatment reduced BP increase of SHR-cp by 12%. Both heart and aorta collagen levels in SHR-cp were increased significantly comparing to SHR. Melatonin failed to affect fibrosis enlargement in the heart, while it reduced significantly fibrosis enlargement in the aorta. Comparing to SHR, expression of MT(1) receptors was elevated in SHR-cp with higher density in the aorta than in the heart. Thus, melatonin reduced fibrosis enlargement in the aorta – the tissue where expression of MT(1) receptors was shown to be higher.

In conclusion, decrease of fibrosis enlargement in the aorta after melatonin treatment may contribute to BP reduction in rats with metabolic syndrome. Moreover, contribution of MT(1) receptor – mediated effect of melatonin is suggested.

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B02-7

Protective role of melatonin against caspase depended apoptosis in thoracic aorta tissue of pinealectomised rat.

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The study was designed to investigate the protective role of melatonin on especially Caspase 8 and Caspase 3 depended apoptosis signalling pathway in thoracic aorta (TA) tissues. For this purpose, 18 Sprague Dawley male adult rats, kept under 12:00L-12:00D schedule at 22 °C, were randomly divided into three groups as following; SHAM operated control, pinealectomised groups and pinealectomised+melatonin administrated (s.c. 5 mg/kg/day) groups. Our results revealed that pinealectomy treatment causes traumatic conditions and DNA damage possibly involving an interaction between oxidative stress, disrupting equilibrium of essential elements, and triggers stress specific HSP70 (20.96 fold) expressions. Additionally proapoptotic genes TNFα, Caspase 8 and Caspase 3 significantly overexpressed. In the TA tissues, pinealectomy operation affected the

genomic band profiles and Genomic Template Stability that decreased to 80.22%. In this study, the GTS values were regulated by melatonin administration, and it was 100% in TA tissues. Melatonin plays protective roles on cell apoptosis through prevent pinealectomy-mediated DNA damage and stressful condition via elevated unfolded protein mechanisms that controlled HSPs family genes and inhibition oxidative damage. In addition, melatonin inhibits death mechanisms via suppressing TNF α , APAF1, Cyt-C gene expressions, significantly reducing caspase proteins and regulation the elements equilibrium. (This work is supported by TUBAP 2015/45).

B02-8

Acute exposure to hyperbaric oxygenation impairs endothelial nitric oxide production in Sprague-Dawley healthy male rats

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Question: Previously, study from our laboratory showed that acute exposure to hyperbaric oxygenation (HBO) increase oxidative stress production. This increased oxidative stress caused impaired endothelium-dependent vasodilation while superoxide scavenger TEMPOL *in vitro* restored that NO-dependent dilation. Present study aimed to determine the effects of acute HBO exposure on flow-induced endothelial NO production.

Methods: Healthy male 11 weeks old Sprague-Dawley rats were divided in: CTRL (control group, N=5) and A-HBO (acute single time exposure to HBO, N=5). Exposure to HBO was in hyperbaric oxygen chamber (100% oxygen; 2 bar/2 hours). Prior to decapitation rats were anesthetized with ketamin and midazolam. Middle cerebral arteries were isolated and cannulated and pressurized at $\Delta 80$ mmHg with flow on, on pressure myograph on, in the absence/presence of TEMPOL *in vitro*. NO production was determined by DAF-2DA to DAF-2T conversion fluorescence assay. All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals (directive 86/609) and were approved by institutional Ethical Committee.

Results: Flow-induced NO production was significantly lower in A-HBO group compared to flow-induced NO production in CTRL group ($p=0.020$). TEMPOL *in vitro* increased endothelial NO production in A-HBO group, compared to A-HBO in the absence of TEMPOL ($p=0.011$). NO production in control group was similar with/without TEMPOL *in vitro* ($p=0.62$) suggesting low level of oxidative stress in control rats.

Conclusion: Acute exposure to HBO increases oxidative stress. Superoxide scavenging by TEMPOL restores flow-induced NO production in A-HBO group.

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B02-9

Hydrogen sulfide restores redox status of heart tissues, diastolic heart function and endothelium dependent vasorelaxation in old animals

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The aim of the study was to investigate the effect of NaHS as exogenous H₂S donor on the heart redox status, cNOS coupling, cardiodynamics and vasorelaxation in old rats. The study was conducted on adult (6-8 months old) and old (22-24 months old) male Wistar rats. To evaluate the systolic and diastolic function of the heart we used pressure-volume (PV) conductance catheter system. Markers of oxidative and nitrosative stress determined by biochemical methods. It has been revealed that a combined oxidative and nitrosative stress develops in the heart of old rats, leading to cNOS uncoupling, which correlates with a decrease in diastolic function (dp/dtmin decreased by 33%,

end-diastolic pressure increased in 3 times, the time constant of left ventricular relaxation (Tau g) increased by 44%). At the same time acetylcholine induced vascular strips relaxation was significantly inhibited. Hydrogen sulfide donor (NaHS) increased H₂S pools in heart, suppressed oxidative stress ($\cdot\text{O}_2^-$ generation decreased in 7,4 times, hydrogen peroxide - 3,3 times, reactive hydroxyl radical ($\cdot\text{OH}$) reduced in 4,3 times). NaHS inhibited nitrosative stress: cNOS activity increased in 2,8 times; NO₂⁻ pools (constitutive NO synthesis marker) increased in 3,8 times that promoted to improvement of heart diastolic function and endothelium-dependent vasorelaxation in old rats. It was shown that dp/dt min increased by 20% ($P<0.05$), Tau decreased by 13% ($P<0.05$). NaHS also increased endothelium-dependent vasorelaxation. Thus, hydrogen sulfide inhibits oxidative and nitrosative stress, restores cNOS coupling and increases constitutive de novo synthesis of nitric oxide, improves diastolic heart function and endothelium-dependent vasorelaxation in old rats.

B02-10

The role of nitric oxide in endothelium-dependent control of murine basilar artery under conditions of acidosis

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Aim. Acidosis induces arterial relaxation, but its effects on endothelium are purely understood. We have shown earlier that NO exerts a powerful anticontractile effects and mediates a half of acetylcholine (ACh)-induced relaxation in murine basilar artery (BA). This study was aimed at the effects of acidosis on NO-ergic control of BA. **Methods.** BA segments from male C57BL/6 mice were mounted in wire myograph. NO effects were studied using L-NNA, given either alone or after blockade of other endothelial pathways by the combination of indomethacin, TRAM-34 and UCL-1684. Extracellular acidosis was induced by bubbling the solution with 10% CO₂ in O₂ (instead of 5% CO₂ under control conditions). **Results.** Contractile responses of BA to U46619 were suppressed by acidosis. However, under both control and acidic conditions L-NNA prominently increased maximum force and the sensitivity to U46619 (5-10-fold decrease of EC₅₀), while the combination of other blockers did not potentiate the contraction. Relaxation of BA to ACh (after U46619-precontraction) was slightly reduced by acidosis but demonstrated a paradoxical increase of NO-component along with strongly depressed contribution of other pathways. L-NNA halved the response under control conditions but reduced it by 90% in acidified BA; the combined effect of other blockers under acidosis was negligible. NO-sensitivity of BA (studied by relaxation to DEA-NO) was increased by acidosis as well. **Conclusions.** Acidosis did not affect anticontractile influence of NO. However, it strongly potentiated NO effects during activation of the endothelium, which may be important for vasomotor control under acidification of brain milieu. Supported by the Russian Science Foundation (grant N17-15-01433).

B02-11

Premature senescence of endothelial cells upon chronic exposure to TNF α can be prevented by N-acetyl cysteine and plumericin

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Cellular senescence is characterized by a permanent cell-cycle arrest and a pro-inflammatory secretory phenotype, and can be induced by a variety of stimuli, including ionizing radiation, oxidative stress, and inflammation. In endothelial cells, this phenomenon might contribute to vascular disease. Plasma levels of the inflammatory cytokine tumor necrosis factor alpha (TNF α) are increased in age-

related and chronic conditions such as atherosclerosis, rheumatoid arthritis, psoriasis, and Crohn's disease. Although TNF α is a known activator of the central inflammatory mediator NF- κ B, and can induce the intracellular generation of reactive oxygen species (ROS), the question whether TNF α can induce senescence has not been answered conclusively. Here, we investigated the effect of prolonged TNF α exposure on the fate of endothelial cells and found that such treatment induced premature senescence. Induction of endothelial senescence was prevented by the anti-oxidant N-acetyl cysteine, as well as by plumericin and PHA-408, inhibitors of the NF- κ B pathway. Our results indicated that prolonged TNF α exposure could have detrimental consequences to endothelial cells by causing senescence and, therefore, chronically increased TNF α levels might possibly contribute to the pathology of chronic inflammatory diseases by driving premature endothelial senescence.

B02-12

Obesity impairs vascular reactivity and Ca²⁺ homeostasis in *in situ* endothelial cells from rat aorta

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Despite the available evidences of a link between [Ca²⁺]_i and endothelial cell function, as well as, endothelial dysfunction and obesity, there are not studies correlating the vascular reactivity with Ca²⁺ signaling in *in situ* endothelial cells (IEC) from obese animals. **Question:** are vascular reactivity and IEC intracellular Ca²⁺, impairs in female Zucker Diabetic Fatty (ZDF) rat aorta?. **Methods:** Thoracic aorta was extracted, cut in 5mm rings. Vascular reactivity measurements were performed using isometric tension bath chamber. For Ca²⁺ signal recording, aortic rings were opened, loaded with Fura-2, and [Ca²⁺]_i measured by microfluorimetric techniques. **Results:** Female obese ZDF rats presented a significant increase in: body weight; abdominal circumference and periaabdominal adipose tissue compared with control rats. Non-significant differences were found in the glucose tolerant curve test between rat groups. Vascular reactivity in response to norepinephrine was increased by 201.5% in obese aortic ring with intact endothelium, and by 133% in obese aortic rings without endothelium. Vascular reactivity increase was not due to the nitric oxide bioavailability alterations because the relaxing effect of acetylcholine was similar in aortic rings obtained from obese and control rats. The application of adenosine triphosphate (ATP) 20 μ M to IEC evoked a Ca²⁺ signal consisting in a rapid increase in [Ca²⁺]_i followed by a slow decay to the baseline, however the peak amplitude was increased significantly in obese IEC. In addition, Ca²⁺ entry through store operated Ca²⁺ channels was increased in endothelium from obese rats. **Conclusion:** Obesity caused alterations in vascular contractility and endothelial Ca²⁺ homeostasis in rat aorta.

B03: Molecular & cellular physiology

B03-1

Radiofrequency Radiation Emitted from Cell Phone induces DNA Damage and Oxidative Stress in Rat Brain Tissue

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Questions: The brain tissue is one of the main tissues which are exposed to radiation emitting from cell phones. It is claimed that the radio frequency radiation (RFR) may cause deoxyribonucleic acid (DNA) damage in tissues. The aim of this study is to investigate the effects of radiations at different

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radio frequencies on brain tissues as DNA damage and oxidative stress. **Methods:** Male Sprague-Dawley rats were divided into four equal groups (n:7): sham-control, 900MHz, 1800MHz and 2100MHz. Experimental group of rats were exposed to RF radiation 2 hours/day for 6 months. Sham-control group rats were subjected to the same experimental procedure except of RF application. DNA damage were determined by employing the Comet assay technique to the brain tissue sections. Malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), total antioxidant status (TAS), total oxidant status (TOS) analyses were conducted in the brain tissue samples along with oxidative stress index (OSI) levels and serum nitric oxide (NO) levels. **Results :** It was determined that TAS levels decreased significantly in all experimental groups but a significant increase was found in TOS, OSI, MDA and 8-OHdG values compared to the sham group (p <0.01). While the increase of NO levels at 1800 MHz, 2100 MHz groups when compared to sham-control were significant (p<0.05), the increase was found insignificant at 900 MHz group (p>0.05). Regarding Comet assay, the increase of tail intensity in experimental groups was found to be significant in the 2100 MHz group (p <0.01). **Conclusions:** The exposure of rats to radiation emitting from cell phones may cause oxidative damage, induce an increase in lipid peroxidation and increase oxidative DNA damage formation in rat brain tissues. Furthermore, 2100 MHz RF radiation may cause formation of DNA single strand breaks. **Keywords:** Radio frequency radiation, Brain, Oxidative stress, DNA damage.

B03-2

Decreased inward rectifier potassium current IK1 in dystrophin-deficient ventricular cardiomyocytes

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Kir2.x channels in ventricular cardiomyocytes (most prominently Kir2.1) account for the inward rectifier potassium current IK1, which controls the resting membrane potential and the final phase of action potential repolarization. Recently it was hypothesized that the dystrophin-associated protein complex (DAPC) is important in the regulation of Kir2.x channels. To test this hypothesis, we investigated potential IK1 abnormalities in dystrophin-deficient ventricular cardiomyocytes derived from the hearts of Duchenne muscular dystrophy mouse models. We found that IK1 was substantially diminished in dystrophin-deficient cardiomyocytes when compared to wild type myocytes. This finding represents the first functional evidence for a significant role of the DAPC in the regulation of Kir2.x channels. This work was supported by the Austrian Science Fund (FWF) (P23060-B19 to K. Hilber).

B03-3

Effect of Glycine on Microglia during oxidative stress

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Microglia are antigen-presenting immune cells in the central nervous system and act as the first line of defence in the brain. Activated microglia release pro-inflammatory cytokines and can do an oxidative burst fighting against pathogens. In many neurodegenerative diseases, like Alzheimers or Parkinsons disease, inflammatory processes of microglia seem to play an important role.

To induce inflammation in cells lipopolysaccharide (LPS), an endotoxin of gram-negative bacteria, is used in many studies. In this study BV-2 microglia are stimulated with LPS combined with IFN- γ to induce cell stress. In addition, the cells are incubated with or without glycine for 24h. The amino acid glycine – besides its function as an inhibitory neurotransmitter – plays a major role in cellular homeostasis and anti-inflammation. Cells are analysed using Flow Cytometry technique. Annexin-V

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Poster Session B

and 7-AAD are used for apoptosis and necrosis detection and different surface markers are analysed for cell-status and -characterization using different fluorescence antibodies.

Glycine may be able to counteract the effect of LPS/ IFN- γ and leads to a decrease of apoptosis or necrosis. In higher concentrations glycine could have a positive effect on the immune reaction or even help cells getting a better tolerance against inflammatory factors and oxidative stress.

B03-4

OXIDATIVE STRESS IN THE LIVER NAD HEART INDUCED BY THIOACETAMIDE IN MALE AND FEMALE RATS. EFFECT ON HEART INNERVATION.

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Thioacetamide (TAA) is used experimentally for its specific hepatotoxic effect and the ability to produce liver damage with histological appearance similar to human hepatic fibrosis. Liver cirrhosis is associated with impairment of cardiovascular function including alterations of the heart innervation, humoral and nervous dysregulation, and electrophysiological abnormalities.

The aim of the study was (1) to evaluate the influence of gender on the oxidative stress in the liver and heart, and (2) to assess effect of TAA application on expression of enzymes for classical mediators, TH, DBH and CHAT in the heart.

Adult Wistar rats were treated with intraperitoneal injection of TAA for 12 weeks. After sacrifice, samples of heart, liver, and blood were taken. Levels of peroxidation were measured in the liver and heart. Relative expression of TH, DBH and CHAT were assessed in the left atrium of the heart.

Male Wistar rats showed higher susceptibility to the oxidative stress induced by subchronic administration of TAA than female rats. The dose of TAA that induced considerable increase in lipid peroxidation in the liver of male rats failed to produce such damage in female rats. Expression of mRNA for DBH and CHAT was decreased in TAA treated female rats but remained unchanged in male rats.

The response to TAA-induced oxidative tissue damage, as well as the effect of TAA treatment on intracardiac neurones is markedly influenced by gender. The influence of gender should be taken into consideration when TAA is used as a model substance for the evaluation of antioxidant properties of various therapeutic agents in rats.

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B03-5

Role of TASK-3 channels in the mitochondria of melanoma cells

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The expression of functional TASK-3 channels in the mitochondria of cultured melanoma cells was investigated using electrophysiology on isolated mitoplasts. We examined the effect of gene silencing on the metabolism and the expression of regulatory factors involved in this process. Our aim was also to prove the presence of these channels in the mitochondria of melanoma cells using electronmicroscopy.

Mitoplasts isolated from the mitochondrial fraction of non-transfected control, scrambled-shRNA transfected control and TASK-3 knockdown (KD) stable melanoma cultures exhibited functional TASK-3 channels. The open probability of these channels was significantly reduced under acidic condition in all of the examined colonies, while the average open probability was significantly lower in the gene silenced cells under normal pH condition.

Decreased expression of the TASK-3 channels in cultured melanoma cells markedly induced mitochondrial oxygen consumption via increasing glucose- and aminoacid oxidation, while the fatty acid metabolism was not effected. We could detect reduced ATP-release and superoxid production on the KD cultures, which denotes energy crisis and mitochondrial uncoupling following TASK-3 gene silencing.

Membrane structure labelled melanoma cultures investigated with transmission electronmicroscopy revealed significantly reduced mitochondria number in the TASK-3 KD cells, however the inner mitochondrial structure was not influenced.

All together we concluded that reducing TASK-3 channel expression in cultured melanoma cells significantly influences mitochondrial activity, which contributes to all changes observed in the cell viability, proliferation and tumorigenicity.

B03-6

Effects of Different Timing in Clamping of Umbilical Cord on Oxidative Markers

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Background: Timing of clamping procedure of umbilical cord varies depending to Clinique as early and late with offering advantages and disadvantages. Since leading to increased blood volume, Hct and Fe2+ of newborn, delayed clamping technique has being preferred. It is argued that birth may cause to increase of oxidative stress in both mother and newborn. In present study, possible oxidative stress differences originated from different clamping timings, MDA and GSH were measured.

Methods: Forty subjects were selected at Gynecology and Obstetrics Services Department, based on women who experienced a normal course of pregnancy and single delivery and caesarean section, Half of the subjects had deliveries with early-clamped newborn infants, and the other half had late-clamped deliveries. MDA and GSH measurements were done by manual method in venous blood samples

Results: Even though no statistically significant difference detected between early and delayed umbilical cord clamping in both normal and caesarean birth groups from plasma MDA and GSH levels point but possibly in erythrocyte. However, the oxidant and anti-oxidant parameters are not sufficient in off to explain the real situation if early or late clamping effects the system in plasma. We are still working on other different markers in both plasma and erythrocytes.

B03-7

PREVENTION of DOXORUBICIN-INDUCED CARDIOTOXICITY THROUGH ATP SENSITIVE POTASSIUM CHANNEL OPENING

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Doxorubicin (DOX) is a drug for solid tissue cancer treatment. However, it has some side effects on non-cancerous cells. The therapy for its undesirable cytotoxicity is still not found. DOX cytotoxicity might be related to reactive oxygen species (ROS) and to mitochondrial dysfunction. Protective effects of ATP-sensitive potassium channels (KATP) has been well documented against some pathological conditions, including ischemia-reperfusion. The aim of this study was to investigate whether the opening of KATP reverses the cardiotoxicity of DOX. Rat cardiomyocyte cell line (H9c2) was exposed for 24 hours to its medium served as control; Diazoxide (DIA), one of KATP opener; DOX; and DIA plus DOX. Distribution of actin filaments, mitochondrial membrane potential (MMP), and superoxide dismutase (SOD) enzyme's activity were analysed with proper tests and then statistical analysis was performed. Although DOX gave rise to decrease SOD enzyme activity, DIA co-treatment restored it. DOX destroyed cytoskeleton via actin distribution, but DIA ameliorated the distribution as well. Although DOX caused to elevate MMP, DIA reversed the DOX's effect on MMP. Cardiomyocytes loss by oxidative stress-mediated apoptosis is an important mechanism for DOX-induced cytotoxicity, and the alternations were attenuated with DIA co-treatment. Consequently, opening of KATP has protective effects on DOX-induced cardiotoxicity and DIA may be a candidate agent to protect the cell in DOX chemotherapy.

B03-8

EFFECTS of MELATONIN on ACUTE PANCREATITIS INDUCED by DOXORUBICIN in HUMAN PANCREATIC CELL LINES

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Doxorubicin (DOX) is an effective anticancer drug with some side effects on non-cancerous tissues, including heart, liver, and pancreas. Acute pancreatitis-induced by DOX has seldom been reported. So, the mechanism of acute pancreatitis-induced by DOX is not well known. Melatonin (MEL) with its anti-apoptotic and antioxidant properties has been suggested to have protective effects on many pathophysiological conditions. The aim of this study was to investigate whether MEL ameliorates the cytotoxicity of DOX on pancreatic beta cells through its anti-apoptotic function. Human pancreatic beta cell line (1.1B4) was used to conduct four groups: a control, MEL (1 mM), DOX (2.6 µM), and MEL-co-treatment with DOX (1 mM MEL+ 2.6 µM DOX). After 24 hours incubation, mitochondrial membrane potential (MMP) was analysed and apoptosis was determined by using TUNEL and IETD-FMK (FITC-IETD-FMK) methods. Actin filament distribution was also determined. Although DOX initiated the apoptotic pathway by increasing active caspase-8 levels and by MMP depolarization, MEL+DOX co-treatment ameliorated apoptotic beta cell loss by decreasing active caspase-8 levels via restoration of MMP. DOX treatment disrupted actin filaments while MEL reversed its effect on actin filaments. Consequently, MEL has a good candidate against DOX-induced acute pancreatitis for chemotherapy patients.

Keywords: Doxorubicin, Apoptosis, Melatonin

B03-9

Effect of Trans-3 Hydroxycinnamic Acid Against Liver Damage Induced-Methotrexate: An Experimental Study

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Purpose: Methotrexate (MTX) is usually used as a chemotherapeutic agent in the treatment of various types of cancer and some inflammatory diseases. The aim of this study was to determine the possible beneficial effects of trans-3 hydroxycinnamic acid (HC) as an antioxidant compound against oxidative liver damage induced by MTX. **Material and Methods:** In our study, we used 24 Wistar albino rats each weighing 200-220 g. The treatment was not applied in control group. Only a single dose 20 mg/kg of MTX was administered intraperitoneally to MTX group rats and sacrificed 5 days later. In HC group, we administered intraperitoneally 100 mg/kg of HC for 5 days. Intraperitoneally a single dose 20 mg/kg of MTX was given to MTX+HC rats on the first day of the experiment. Later, it was used 100 mg/kg dose of HC following 1 h after the MTX and HC was continued to be given for 5 days. Rats were sacrificed on the last day and the liver tissue was removed. Superoxide dismutase (SOD) activity and glutathione (GSH), malondialdehyde (MDA) levels were evaluated in tissue samples. **Results:** MDA levels increased in MTX group according to the biochemical results. But these levels decreased significantly in group treated with HC. Also, SOD activity decreased in MTX group. However, this enzyme activity increased depending on HC treatment. On the other hand, GSH level was significantly reduced in MTX group compared to controls in the rat's liver tissue. **Conclusion:** These results show that HC have a protective role against oxidative liver damage induced-MTX.

Keywords: Methotrexate, oxidative stress, liver damage, trans-3 hydroxycinnamic acid.

* This study has been generated from master thesis of Özge Arslan

B03-10

The effect of ferulic acid against myocardial damage induced by methotrexate

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Abstract

Aim: In our study, possible beneficial effect of ferulic acid (FA) on myocardial damage induced by methotrexate (MTX) has been investigated in rats. **Material and Methods:** Twenty-four Wistar type rats was equally and randomly divided into four group: group I (control group; no drug was administered), group II (only FA was applied in dose of 100mg/kg, i.p., b.w. for 1 week), group III (only MTX was given 20 mg/kg, i.p., b.w.), group IV (both FA+MTX was given in this group). Malondialdehyde (MDA) levels as oxidant parameter, superoxide dismutase (SOD) activity and glutathione (GSH) level were assessed in myocardial tissues. **Results:** MDA levels were increased in the group of MTX and while it decreased in the group of FA+MTX. SOD enzyme activity and GSH level were decreased in the group of MTX, however, these reduction in antioxidant enzyme activity was prevented the group receiving both FA and MTX. **Conclusion:** The results of our study have been demonstrated that FA has a significant role in the protection of heart against myocardial oxidant damage induced by MTX probably by preventing the decreases in various antioxidant enzymes.

Keywords: Ferulic acid, oxidative damage, methotrexate, heart.

B03-12

The role of p-Coumaric acid in methotrexate-induced neurotoxicity

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ABSTRACT

Aim: In this experimental study, it was aimed to assess the possible protective effect of p-coumaric acid on methotrexate induced neurotoxicity in rats. **Material and Methods:** This study was performed using twenty-four adult male rats. Groups were formed as control, methotrexate (20 mg/kg i.p. for single dose), p-coumaric acid (100 mg/kg i.p. for 1 week), and methotrexate +p-coumaric acid given groups. At the end of our experiments, brain tissues of rats were harvested under anesthesia. Malondialdehyde (MDA) level, a product of the lipid peroxidation, glutathione level and superoxide dismutase activity were assessed. **Results:** MDA level increased, whereas superoxide dismutase activity decreased in methotrexate group. However, in methotrexate+p-coumaric acid group, superoxide dismutase enzyme activity, glutathione level increased and MDA decreased. **Conclusion:** Our results showed that p-coumaric acid has protective effects against oxidatıf damage of brain tissue methotrexate-induced.

Keywords: Methotrexate , neurotoxicity, p-coumaric acid, rat.

B03-13

The effects of chronic intraperitoneally infusion of irisin on liver antioxidant balance in rats

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Irisin is a recently identified myokine. Irisin increases total energy consumption, increases food intake and reduces body weight. It has been shown that irisin levels are significantly lower in patients with nonalcoholic fatty liver. The aim of this study is to investigate the effect of irisin on the antioxidant balance in the rat liver.

In this study, 40 Sprague Dawley male rats were used, and were separated into four groups (n = 10). Sham and experiment groups received ceaselessly intraperitoneal infusion by means of osmotic mini pump filled with SF or irisin at concentrations of 10 and 100 nM (10ul / h) for fourteen days. Towards the end of infusion, the rats were sacrificed and their liver tissues were taken. The levels of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were determined in liver tissues spectrophotometrically. GSH-Px, SOD and CAT enzyme activations were significantly increased in the irisin treatment groups, on the other hand MDA levels were significantly decreased in the irisin groups (p <0.05).

This results suggests that irisin may be a potential therapeutic drug for metabolic diseases such as, liver nonalcoholic fatty liver disease.

B04: Endocrine, neuroendocrine and metabolism

B04-1

Energy homeostasis in a hypovitaminosis D-hypoirisinemic rat model

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Background: We created a rat model with hypovitaminosis D/hypoirisinemia and measured parameters of the energy homeostasis.

Methods: A total of 16 healthy weaned male albino rats were used. Rats were either fed on a normal balanced growth diet (Group A, n=8), or a normo-calcemic-vitamin D deficient diet with limited ultraviolet rays for 6 weeks (Group B, n=8). Then, a blood sample from the lateral tail vein were used to assess 25-hydroxy vitamin D3 (25-OHVD3) and irisin levels in both groups. Fasting rats were housed individually in Calo-cages of TSE PhenoMaster system for 24 h, where volumes of respiratory gases were measured by an open circuit indirect calorimetry. The respiratory quotient (RQ) & total energy expenditure (TEE) were calculated in addition to automatic food intake record. The measurements taken every 15 min and those of the first 6 h were deleted. Serum irisin, 25-OHVD3, calcium, glucose, and insulin were measured by ELISA.

Results: Irisin was found to show a positive correlation with 25-OHVD3 in both normal and deficient rats (n= 8, r=0.268, and 0.399 respectively, p<0.05). In hypoirisinemic-hypovitaminosis rats, a significant reduction in food intake, reduction of RQ (to the range of using the endogenous fat), with reduction of glucose and rise of insulin levels together with an insignificant increase of body weight or change of TEE were detected. Additionally, irisin was found to show a strong positive correlation with body weight in normal condition (r=0.773, p<0.05), and a moderate negative correlation in Hypoirisinemia (r=-496, p=0.211), while with TEE, irisin showed no correlation.

Conclusions: This study demonstrated the energy homeostasis and body weight changes during states of hypovitaminosis D/hypoirisinemia.

B04-2

Effects of oxidative stress and insulin on (pro)renin receptor expression in cultured human breast cancer cells

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Questions (Pro)renin receptor ((P)RR) is ubiquitously expressed throughout in the body. Plasma concentrations of soluble (P)RR, consisting of its extracellular domain, are elevated in patients with chronic kidney disease, diabetes mellitus, or obstructive sleep apnea syndrome. The aim of the present study is to clarify factors which regulate expression levels of soluble and full-length (P)RR.

Methods Effects of oxidative stress on expression levels of soluble and full-length (P)RR were studied using H₂O₂ in cultured T47D breast cancer cells. Effects of leptin and estradiol (E2) were also studied in T47D cells. Effects of insulin on (P)RR expression levels, Akt phosphorylation and cell proliferation were studied in cultured MCF7 breast cancer cells. (P)RR expression was analyzed by western blot analysis.

Results Treatment with H₂O₂ (10 - 1000 μM) for 48h resulted in a dose-dependent increase of soluble (P)RR expression, but not full-length (P)RR expression in T47D cells. E2 had no significant effects on H₂O₂-induced expression of soluble (P)RR. Treatment with a combination of H₂O₂ (1000 μM) and

leptin (10 ng/ml) enhanced H₂O₂-induced expression levels of soluble (P)RR protein greatly. Insulin (10 - 1000 ng/ml) increased cell proliferation, Akt phosphorylation and expression levels of full-length (P)RR, but not soluble (P)RR in MCF7 cells.

Conclusions Oxidative stress with leptin is a strong stimulator on soluble (P)RR expression, whereas insulin stimulates cell proliferation and expression of full-length (P)RR in cultured breast cancer cells.

B04-3

An Experimental Rat Model for the Effects of High Fat Diet-Induced Obesity on Spatial Learning

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Obesity is one of the most significant and important health concerns all over the world and identification of the obesity score is based on measurement of body mass index. There are many different health issues that related obesity. Either experimental or clinical researches show that, an important obesity-related health problem is cognitive deficits. The aim of this study is to investigate the effects of long term exposure to high fat diet to rats on the spatial learning via Morris Water Maze (MWM). Nine 8-week old male Wistar albino (WA) rats were housed with access standard rat food that contains approximately 65% carbohydrates, % 30 proteins and %5 lipids, and water ad libitum. Fourteen 8-week old male WA rats were housed with access fat-supplemented rat food that contains approximately %35 carbohydrates, 20% proteins and 45% lipids and water ad libitum. Rats were fed for 5 months and at the end of the research MWM was used to access spatial learning and memory. No physical exercise was applied until spatial test. During the feeding period, every week weight and height scores were monitored. Lee's body mass index (BMI) was used to determine the obesity of rats. All rats, fed with fat-supplemented diet, were become obese after the increasing of height was stopped approximately 3rd month of feeding. The rats fed with standard diet had normal BMI. Last five days of research spatial learning test was applied all rats. MWM results showed that, obese rats spent significantly longer time to find hidden platform than normal rats. Results of this research are showed that, long term high lipid consumption is a possible reason of spatial learning deficits and various experimental and clinical researches supported this probability.

Obesity, Spatial learning, Rat

B04-4

Traumatic brain injury induces plasma resistin levels in rat

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Questions: Apelin and resistin are adipocytokine and expressed peptides in the nervous system. Recent studies reports that these peptides may play an important role to protect the brain against traumatic brain injury (TBI). The aim of this study is to investigate change in plasma resistin and apelin level after TBI.

Methods: In our study, twenty eight Wistar Albino male rats (200-250 g) were used. Before TBI, cardiac blood samples were taken from the each rat. TBI was implemented to all animals under the deep anesthesia with weight-drop method. After TBI, the rats were grouped into 4 groups (each seven animals); in order to take cardiac blood samples in 1st, 3th, 5th and 7th hours. Apelin and resistin were measured by ELISA method in plasma samples. The results were evaluated with SPSS 20.0 software program by using Mann-Whitney U, Kruskal-Wallis and Wilcoxon tests.

Results: There was no significant difference between groups in term of apelin levels by hours. In addition, apelin level did not differ significantly between pre- and post-TBI in all groups. The resistin

level increased in groups after TBI vs before TBI. But this increase was found significant only in the first group after traumatic brain injury compared to before TBI (p<0.05).

Conclusions: According to our results that plasma apelin level does not show any change in traumatic brain injury in rats for 7 hours. The increase in post-injury resistin level may suggest that resistin may play a role in damage repair.

Keywords: apelin, resistin, traumatic brain injury

B04-5

Identification of potential biomarkers for autism spectrum disorders using urinary metabolomics

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Heterogeneity in etiology, phenotype and outcome of autism spectrum disorders (ASD) contribute to a clinical heterogeneity which manifests as diverse deficits or impairments in behavioral features and communicative functioning. Identification of specific biomarkers and the possibility of biological signatures contributing to the definition of subgroups of ASD move forward the quest for personalized medicine and treatment models in this highly heterogeneous population. Targeted metabolomics of urine samples of children with ASD and controls could bring new potential non-invasive biomarkers.

The first-morning urines of 35 children with ASD and 30 typically developing controls were analyzed by quantitative LC-MS/MS metabolomic analysis performed by combining direct injection mass spectrometry with a reverse-phase LC-MS/MS. Statistical analyses were performed in R using bootstrap two-sample t-test of mean difference (10000 bootstrap samples with replacement).

From 185 analytes, covering amino acid, glucose, fatty acid and lipid metabolism, 83 analytes were detected in more than 50% of samples. The difference between ASD and typically developing children was significant in three metabolites (p< 0.05). Pimelylcarnitine (C7-DC) and one glycerophospholipid (PC aa C34:1) were found significantly higher and nonylcarnitine (C9) was significantly lower in ASD children urine compared to the healthy controls.

Observed differences in metabolomic markers in urine samples may indicate an ASD-specific metabolic pattern. Diagnostic sensitivity and disease-specificity of the markers need to be investigated more detailed.

The study was supported by Slovak Research and Development Agency (APVV-15-0045 and APVV-15-0085).

B04-6

Ghrelin prevents skeletal muscle damage in septic rats

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Questions: Sepsis causes weakness and fatigue in muscle tissue due to increased loss of myofibrillary proteins. In septic conditions, exogenous ghrelin has been shown to decrease the inflammatory response, improve blood flow, and reduce tissue damage and mortality.

We investigated the effects of exogenous ghrelin on tissue histology in skeletal muscle during sepsis.

Methods: Male Wistar albino rats 200-250g were separated into four groups; Control, LPS (5 mg/kg), Ghrelin (10 nmol/kg i.v.), and LPS+Ghrelin. Muscle tissue was stained using modified Gomori trichrome (MGT), succinic dehydrogenase (SDH), and cytochrome oxidase (COX) and hematoxylin and eosin.

Results and Conclusions: Using hematoxylin and eosin (H&E) staining, weak fiber boundaries and irregular-shaped nuclei were seen in the LPS group. In the ghrelin-treated septic groups, the histologic appearance of the muscle fibers was normal as with the controls. Animals that were treated with LPS showed myopathic changes, with rounding of muscle fibers and fiber size variation. SDH and COX activities were the same as the control group. In the ghrelin and ghrelin+LPS group there were no myopathic changes, and SDH, COX results were same as the control group. As a result, ghrelin improved myopathic damage in skeletal muscle during sepsis.

Acknowledgment: Our study was granted from Istanbul University Research Projects (Project No: 42363)

B04-7 EFFECTS OF INTRACEREBROVENTRICULAR FGF21 INFUSION ON THE ENERGY METABOLISM

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Aim: Fibroblast growth factor 21 (FGF21), playing an important role in the regulation of metabolism, is a member of the endocrine FGF subfamily. Thyroid hormones also regulate FGF21 gene expression in liver and adipose tissues and serum FGF21 levels are positively associated with circulating T3 and T4 levels. Thyroid hormones have important roles in energy homeostasis and thermogenesis and are required uncoupling protein 1 (UCP1) expression in white adipose tissue (WAT) and brown adipose tissue (BAT). Therefore, the aim of this study is to determine the effects of intracerebroventricular (icv) FGF21 administration on energy metabolism in adipose tissues.

Methods: In the study, 30 male *Wistar albino* rats randomly divided into three groups: control, sham and FGF21 (n=10). FGF21 was intracerebroventricularly infused to experimental group (0.72 µg/day) and vehicle (artificial Cerebrospinal Fluid) was infused to sham group via osmotic mini pumps for seven days. After seven days, all animals were sacrificed and serum T3 and T4 levels were analyzed by ELISA and UCP1 gene expression levels in WAT and BAT were determined by Real-Time PCR.

Results: The serum T3 (p<0.05) and T4 (p<0.001) levels statistically increased after icv FGF21 infusion compared to sham and control groups. UCP1 gene expression levels in WAT were found significantly higher than control and sham groups (p<0.05), but UCP1 gene expression levels in BAT were not statistically different.

Conclusion: Our results suggest that central FGF21 infusion can have some roles on regulation of thermogenesis and energy expenditure by increasing UCP1 gene expression.

This study was supported by the Inonu University-BAP (Project no:2014/16).

B04-8 EFFECTS OF SHORT-TERM AND LONG-TERM OF OBESITY ON RETN, IAPP, and DRD5 MRNA LEVELS

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Objective: Adipocyte gene expression is altered in subjects with obesity through multiple metabolic and biochemical pathways. Our study aimed to examine the gene expressions of the Resistin (RETN), Amylin (IAPP), and (D1-like dopamine receptor) (DRD5) genes previously suggested to contribute to the pathogenesis of obesity, albeit with controversial missing points, in the pathogenesis of obesity. We also aimed to determine the effects of short and long-term of obesity on mRNA levels of these genes in C57BL/6J mice.

Methods: Two obesity models were formed in our study, with the first in T1 group (20 mice) fed with HFD (a high-fat diet- 60% fat) for 3 months and the other in T2 group (20 mice) fed with HFD (60% fat) for 6 months. The T0 (control- 20 mice) group fed with a diet with a 10% kcal fat supplement for 6 months. At the end of the experiment adipose tissues were dissected surgically. Tissue samples of each group were pooled to isolate RNA and cDNA synthesis was carried out. mRNA levels were examined with the qRT-PCR.

Result: The mRNA expression of RETN showed a moderate upregulation (Fold change: 5.69) in adipose tissue in T2. IAPP expression level was slightly upregulated (Fold change: 2.49) in T1 but it was significantly upregulated in T2 (Fold Change: 16.23). 5.12 fold upregulation for DRD5 is observed in adipose tissue in T2.

Conclusion: Our study demonstrated that the mRNA levels of the genetic markers considered to play a role in adipogenesis were different in short-term and long-term obesity models formed by HFD in C57BL/6J mice.

B04-9 Comparison of Methods for Alpha-Amylase Measurement in Saliva

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Aim: Studies related stress physiology requires non-invasive objective evaluation of symphato-adrenal axis. Salivary alpha-amylase is one of these parameters but the methods available for its determination have not been compared for saliva. The aim of current study was to evaluate two available methods for alpha-amylase measurements in saliva.

Material and Method: Starch-iodine method, substrate (CNPG3) method and dinitrosalisilic acid method were studied and their advantages and disadvantages were established. For that purpose, standard curves were established by using varying doses of alpha-amylase ranging from 0.06 to 30 IU/ml. For starch-iodine test, saliva samples were added on starch solution and incubated for 30 min at 50 degree Celcius. Afterwards, iodine solution was added and color formed was read in a plate reader spectrophotometer at 580 nm. For CNPG3 test, saliva samples were added on PBS solution and incubated for 1 hour at 37 degree Celcius. Following addition of CNPG3 solution, the color formed was read 405 nm.

Results: Standard curves for starch-iodine method and CNPG3 tests were successfully established and were linear. Sensitivity and dynamic range of the methods were between 0,06-1.0 IU/ml and

0.002-0.02 IU/ml, respectively for CNPG3 and starch-iodine tests. Samples needed to be diluted before test at 4000x and 5x, respectively for starch-iodine method and CNPG3 method.

Conclusion: It has been determined that starch-iodine and CNPG3 methods are cheap, easily applicable, relatively shorter than other tests and suitable for stress physiology studies. Starch-iodine test was cheaper than the CNPG3 but the latter was more practical as it involved less stages and dilutions.

This study was supported by Inonu University BAP:2015/82.

B04-10

Late-night eating increased cortisol awakening response but did not affect heart rate variability in the next morning

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QUESTIONS: Effect of eating late in the night on the hypothalamo-pituitary-adrenal axis (HPA) and autonomous nervous system (ANS) activity in the next morning is not known. Aim of the current study was, therefore, to measure effects of late-night eating on sleep quality, cortisol awakening response (CAR) as an indicator of HPA and heart rate variability (HRV) as an indicator of ANS activity.

METHODS: Medical students (n=17, 20-26 year-old) were followed for three consecutive days: a control day, morning after sweet food (within 1 hour before sleeping), fatty and protein meals (10:00 p.m before sleeping). In each day, sleep dairies were filled; salivary samples were taken at 0, 15, 30 and 60 min post-awakening for measurement of CAR; and electrocardiogram was recorded for 5 min for determination of HRV. The data were not distributed normally and Friedman test was used to determine the statistical differences between the groups.

RESULTS: Late-night eating increased CAR (area under the curve) and disturbed sleep (p<0.05) but did not affect time- and frequency- domain parameters of HRV (p>0.05)

CONCLUSIONS: The results suggest that late- night eating is associated with increased CAR rather than changes in HRV and, therefore, it might be concluded that late- night eating affects HPA activity rather than ANS activity in the next morning.

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B04-11

Afamin, a potential marker of metabolic syndrome associated with lipid accumulation in liver, is not affected by 3-months exercise intervention

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Epidemiological evidence indicates that levels of afamin predict the risk of metabolic syndrome. **Aim** of our study was to determine associations of metabolic, biochemical, histological and behavioral patients' characteristics with circulating afamin.

Methods: Insulin sensitivity (EHC), glucose tolerance (OGTT), abdominal fat (MRI), hepatic lipids (1H-MRS), daily physical activity (accelerometer), circulating afamin (ELISA), lysophosphatidylcholine 18:2 (LPC 18:2) and adipocyte size were determined in healthy middle aged men (BMI: 22.3±0.5 kg.m-2, n=19), in men with obesity (BMI: 31.5±0.6 kg.m-2, n=20), prediabetes (BMI: 32.6±0.6 kg.m-2, n=16) & type 2 diabetes (T2D, 31.2±1.0 kg.m-2, n=16).

Results: Circulating afamin was increased in patients with obesity (23%, p<0.05), prediabetes (38%, p<0.001) and T2D (36%, p<0.001). It was positively associated with circulating triglycerides (r=0.47, p<0.001), insulin (r=0.68, p<0.001), C-peptide (r=0.61, p<0.001) and with hepatic lipids (r=0.72, p<0.001). Moreover, afamin negatively correlated with the whole-body (r=-0.60, p<0.001) and the adipocyte tissue-specific insulin sensitivity (r=-0.31, p<0.01), physical activity (r=-0.29, p<0.05), adiponectinemia (r=-0.48, p<0.001) and positively with BMI (r=0.57, p<0.001), adipocyte size (r=0.52, p<0.001) and body fat (r=0.52, p<0.001). Higher levels of afamin were in lean healthy individuals associated with family history of obesity and 3-month exercise training did not affect the serum afamin.

Conclusion: Progression of obesity-related metabolic disease, especially hepatic lipid accumulation was paralleled by increase in circulating afamin. Afamin, however, was not affected by the 3-months exercise training.

B04-12

Effects of Zinc and Melatonin Supplements on Immunity Parameters of Rats with Breast Cancer

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The aim of the study was to determine the effects of zinc and melatonin supplements on the immunity parameters of female rats with induced breast cancer. Group 1; Control, Group 2; 7,12-Dimethylbenz[*a*]anthracene (DMBA), Group 3;DMBA+Zinc, Group 4;DMBA+ Melatonin, Group 5; DMBA+Zinc+Melatonin. The rats' breast cancer was induced by DMBA 80 mg/kg. Groups 3,4,5 received daily 5 mg/kg doses of zinc, melatonin, and zinc+melatonin respectively. Lymphocyte rates, T-lymphocyte subgroups, B-lymphocyte and natural killer cells NK and NKT were evaluated. It was found that a notable increase occurred in the cell types related to the immunity parameters in the supplemented groups; especially compared to the Group 2. The most significant increase in lymphocyte, T-lymphocyte and CD4 lymphocyte rates was found in Group 5. The highest NKT cell rates were found in Group 3. Findings show that zinc and melatonin supplements have led to an increase in the immunity parameters of rats with breast cancer. The most significant increase in immunity parameters have occurred in group 5.

B05: Sports & exercise physiology

B05-1

Concurrent exercise training improves anthropometric measures in schizophrenic individuals by engaging epigenetic mechanism and inflammatory modulation

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Poster Session B

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Poster Session B

Among possible factors, an imbalance on epigenetic machinery and inflammatory markers have been recognized in schizophrenia (SZ) physiopathogenesis and course. The patients with SZ usually adopts a sedentary lifestyle, which has been partially associated with the increase in obesity and diseases. Exercise emerged as an important additional therapeutic option for this population, promoting benefits to physical and mental health. Few studies pointed out that the positive effects of exercise in different populations engage the modulation of epigenetic and inflammatory markers. However, data reporting interaction in SZ patients are lack. Furthermore, these studies generally use aerobic and/or resistance programs, while less attention have been devoted to concurrent protocols. Therefore, we aimed to evaluate the effect of a concurrent exercise protocol (CEP) on anthropometric parameters, global histone H4 acetylation levels and inflammatory markers (IL-4, IL-6 and IFN- γ) in peripheral blood of SZ patients. The participants (n=15) were submitted to the CEP during 90 days, 3 times a week/60 minutes-session. Blood samples were collected pre, 30, 60 and 90 days after the intervention began. The CEP significantly reduced body mass index and body mass and induced a remarkable histone H4 hypoacetylation status in all times evaluated when compared to the baseline period. A reduction in IL-6 levels during the 60 and 90 days compared to the baseline period was observed and diminished IFN- γ levels were found in the 90 days period compared to the baseline and 30 days after periods. The improvement in anthropometric measures following CEP might be associated with the reduction on histone H4 acetylation and anti-inflammatory cytokines levels.

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B05-2

How the walking dynamics of obese individuals change by low vs fast walking speed with respect to the normal-weight counterparts?

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Obesity is an excess fat accumulation situation caused by imbalance between energy intake and consumption. The walking is typically advised activity to obese. We aimed to compare the energy expenditure of obese and normal weight, and to interpret data in terms of walking parameters.

Our study consists 14 normal weight and 13 obese subjects, and energy expenditures were measured by indirect calorimetry during resting and walking in 3 speeds (preferred walking speed (PWS), 30 % less and more than PWS) on treadmill. During walking, the temporospatial parameters and mediolateral and vertical displacements of center of body mass (COM) were recorded.

The resting oxygen consumption (VO₂) was higher in obese (p<0,001). The significance was disappeared when resting VO₂ normalized to corrected fat-free mass (cFFM) (p=0,552). VO₂ was significantly different between two groups in all 3 speeds (p<0,05). There is no significant difference in PWS, stride and stride length (p>0,05). However, step width of obese was significantly higher in all 3 speeds (p<0,001). COM mediolateral displacement was significantly higher in obese in all 3 speeds (p<0,001). However, vertical displacement of COM did not change significantly between two groups (p>0,05). While mediolateral displacement of COM was decreased, vertical displacement was increased significantly with each speed increment (p<0,05).

In order to compare the REE of obese with other body mass index groups, the normalization of REE to the cFFM can be preferred. Obese individuals may adapt their walking pattern to the walking speed increment as consuming more energy, increasing the step width, and restraining vertical displacement of COM.

Keywords: Obesity, energy expenditure, gait analysis

B05-3

No hemodynamic effects after one-month ischemic training during the muscle metaboreflex activation

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Introduction

Ischemic training (IT) has been proposed as a tool to induce muscle hypertrophy, but its safety remains controversial as it may cause mean blood pressure (MBP) increments due to the activation of the muscle metaboreflex (MM). However, IT also causes metabolites accumulation that in turn may desensitise type III and IV nerve endings, which are thought to trigger the MM1. Then, we hypothesised that a period of IT would result in a blunted hemodynamic activation during the MM.

Methods

17 young male healthy (age 18-25 yrs) took part in this study. Hemodynamics during the MM was obtained by the method of the post-exercise muscle ischemia₂ at baseline (T₀) and after one month (T₁) of dynamic IT (handgrip), conducted at 50% of maximum voluntary contraction in the dominant arm with circulatory occlusion, which was obtained with a pressure of 50 mmHg above systolic blood pressure. IT was applied for 3 days/week.

Results

The main results were that none of the studied parameters changed in response to MM after IT. In detail, MBP response was +4.09±3.87 vs. +2.23±4.65 mmHg at T₀ and T₁ respectively (p>0.05). Similarly, there was no difference in heart rate (-3.47±9.26 vs -0.08±9.96 bpm), cardiac output (+349±1693.4 vs. +57.2±801.9 ml·l⁻¹·min⁻¹), and systemic vascular resistance (-14.6±201.6 vs. +5.59±104.9 dynes·s·cm⁻⁵).

Discussion

Contrary to initial hypothesis, this investigation provides evidence that a period of IT is not able to change the hemodynamic response to metaboreflex activation in young healthy male subjects. Thus, the IT protocol employed in the present investigation was not able to desensitise type III and IV nerve endings.

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B05-4

INFLUENCE OF EXERCISE ON AGING PROCESS

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Introduction. Exercise is known to be beneficial to aging process, improving health and preventing chronic disease appearance. The aim of the study was to investigate oxidative stress (OS) markers related to exercise.

Methods. A number of 38 male and 32 female were examined and divided in 3 age groups: I group to 30 years old; II group 31-50 years; and III group above 51 years. Each group was divided to subgroups of sedentary subjects (SS) and subjects who exercise (SE). Lipid peroxidation (LP) as a fluorimetric method with thiobarbituric acid was used to estimate OS. Antioxidative status was determined by cell antioxidants such as enzymes - superoxide dismutase (SOD), glutathione peroxidase (Gpx and glucose 6 phosphate (G-6-PD) and by extra cell antioxidants such as glutathione reductase (GR), nitric oxide (NO) and total antioxidant capacity (TAC).

Results. Increased values of LP were noticed along the aging process ($p<0.05$), but no statistical significance was found between male and female subjects. Statistical significance for OS was not found between SS and SE in I group as it was found in II group ($p<0.05$) and in III group ($p<0.01$). For cell antioxidants no statistical difference was found, but NO and TAC showed lower values in SS compared to SE in II group ($p<0.05$) and in III group ($p<0.05$).

Conclusion. Aging process per se showed increase OS probably by impaired function of free radical scavengers. Well balanced exercise might keep the integrity of blood vessel endothelium which slows down the aging process. Due to obtained results we may conclude that OS is diminished in subjects who performed exercise.

Key words: exercise; aging process; oxidative stress.

B05-5

Time Course of Hemorheological Alterations Following an Acute Bout of Isokinetic Exercise in Active Male Subjects

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Question: Isokinetic exercise is used to reduce strength imbalance and to enhance performance. Hemorheological parameters (Erythrocyte deformability, aggregation) play critical role in exercise influencing oxygenation. Although previous studies presented hemorheological alterations induced by different types of exercise, changes in red blood cell (RBC) deformability and aggregation following isokinetic exercise remain unknown. Present study was designed to explore the time course of hemorheological alterations after an acute bout of isokinetic exercise.

Methods: 11 healthy, male, active subjects (mean age 19.45 ± 0.31 , BMI 22.05 ± 0.51) were enrolled. They performed single, unilateral eccentric contractions of knee flexors and extensors with dominant leg on a dynamometer. Isokinetic hamstring, quadriceps strength were recorded at eccentric (30 , 120°s^{-1}) angular velocities. Blood was obtained before, after, 2-3 days following the exercise. Hemorheological parameters were measured by an ektacytometer. Friedman test was used for statistical analysis.

Results: Isokinetic exercise didn't change deformability but, increased RBC aggregation index (AI, $p=0.037$). AI returned to pre-exercise levels within 2 days. RBC deformability measured at 0.30 Pa was decreased 2 days ($p=0.040$), at 1.69 , 9.49 - 30.00 Pa was impaired 3 days ($p<0.05$) after the exercise compared to post-exercise values.

Conclusion: Our results demonstrating enhanced RBC aggregation until a recovery period of 2 days and decreased deformability 2-3 days following the exercise session suggest that, an acute bout of isokinetic exercise doesn't seem beneficial for tissue perfusion in hemorheological view.

Keywords: isokinetic exercise, Hemorheology

B05-6

A 12-week vigorous exercise protocol in a healthy group of persons over 65: Study of physical function by means of the Senior Fitness Test

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Questions: The aim of this study was to assess the effects of vigorous exercise on functional abilities by means of a Senior Fitness Test (SFT) in a group of elderly adults. **Methods:** Twenty healthy and inactive people performed vigorous exercise (VE: 12 men and 8 women, aged 69.6 ± 3.9 years). At the beginning of the study (T0) and after 3 months (T1) each subject's functional ability was tested for muscular strength, agility, cardiovascular fitness, flexibility and balance. The VE was designed with continuous and interval exercise involving large muscle activities. Functional exercises were performed with a platform step in accordance with ACSM guidelines between 60% and 84% of heart rate reserve (HRR) for duration of 65 minutes. **Results:** Five out of the 6 SFTs performed were found significantly improved: Chair Stand (T0 12.4 ± 2.4 ; T1 13.5 ± 2.6 ; $p<0.01$), Arm Curl (T0 14.2 ± 3.6 ; T1 16.6 ± 3.6 , $p<0.01$), 2 min step (T0 98.2 ± 15.7 ; T1 108.9 ± 16.2 , $p<0.01$), Chair sit-and-reach (T0 -9.9 ± 7.7 cm; T1 1.7 ± 6.3 cm, $p<0.01$), Back Scratch (T0 -15.8 ± 10.9 cm; T1 -8.4 ± 13.1 cm, $p<0.01$). **Conclusions:** Our results suggest that a high intensity protocol and functional exercises can improve functional mobility and muscle endurance in those over 65 years of age. Senior fitness tests are an effective method for assessing improvements in the functional capacity of elderly adults.

B05-7

Hemorheological Alterations Following an Acute Bout of Nordic Hamstring Exercise in Active Male Subjects

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Question: The Nordic hamstring exercise (NHE) is a bodyweight movement commonly prescribed to increase eccentric hamstring strength and reduce the incidence of strain injury in sport. Hamstring injuries present a great impact on sport practice. Hemorheological parameters (Erythrocyte deformability and aggregation) play a critical role in exercise influencing oxygenation. Although previous studies presented hemorheological alterations induced by different types of exercise, changes in red blood cell (RBC) deformability and aggregation following NHE remain unknown. Present study was designed to explore hemorheological alterations after an acute bout of NHE in order to provide new insights into the exercise physiology field.

Methods: 10 healthy, male, active subjects (mean age 19.9 ± 0.23 , BMI: 21.56 ± 0.54) participated to the study. They performed a single session of seven-repetitions of NHE followed by a familiarisation period. Blood samples were obtained before and immediately after the exercise from the antecubital vein. Hemorheological parameters were measured by an ektacytometer. Paired-t-test was used for statistical analysis.

Results: NHE didn't change deformability but, increased RBC aggregation index (AI, $p=0.011$) and decreased RBC aggregation half time ($t_{1/2}$, $p=0.009$). The increment observed in AI is concordant with decrement of $t_{1/2}$ and indicate augmentation of RBC aggregation.

Conclusion: Our results demonstrating enhanced RBC aggregation following the exercise session suggest that, an acute bout of NHE doesn't seem beneficial for tissue perfusion in hemorheological point of view.

Keywords: nordic hamstring exercise, RBC deformability, erythrocyte aggregation

B05-8

Comparatively Determination of Ventilatory Efficiency from Constant Load and Incremental Exercise Tests

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Background: The analysis of the relationships between minute ventilation (VE) to CO₂ output (VCO₂), termed as ventilatory efficiency, in response to the incremental exercise test has shown as an useful index for assessing the presence and severity of cardiopulmonary and metabolic diseases.

Objective: The effects of constant load exercise test performed at the work intensity associated with the anaerobic threshold (AT) and respiratory compensation point (RCP), on accurate measurements of ventilatory efficiency are not well known. The aim of this present study was to investigate the reliability of VE/VCO₂ ratio obtained from the constant load exercise tests performed with two important metabolic rates (AT and RCP) and compared those of incremental exercise test.

Methods: Total of 20 young male (20.8±0.4 yr) subjects initially performed an incremental and then two constant load exercise tests, on different days. The study protocol was approved by the Local Ethics Committee. Respiratory and pulmonary gas exchange variables were measured breath-by-breath and used to estimate AT and RCP. A paired t-test was used to analyse data.

Results: AT and RCP occurred the at 60% and at 71% of peak O₂ uptake, respectively. The lowest VE/VCO₂ ratio that occurred within first 2 minutes of constant load exercise tests with work load AT (26.4±0.3) and RCP (26.7±0.5) were not statistically different those obtained from incremental exercise test (26.0±0.7).

Conclusion: Despite to the different metabolic rates, VE increases closely with CO₂ production, reflecting optimal ventilation and perfusion ratio. The clinicians should be consider the constant load exercise test work load associated with AT and RCP which provide a meaningful lowest value for the ventilatory efficiency.

B05-9

Cardiopulmonary test parameters in patients with coronary artery disease

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Introduction. It is well known that cardiovascular diseases compromise exercise capacity. Cardiopulmonary exercise testing (CPET) is an established tool to evaluate patients' physical capacity. The aim of the present investigation was to compare the response in CPET parameters of coronary artery disease (CAD) patients with responses obtained in a control healthy population (CTL). Our purpose was to find out which of the CPET parameters showed the most striking differences between the two population.

Methods. 11 CAD patients (age 57.54 ± 6.6) and 11 CTL subject (age 48.2 ± 5.6) were enrolled and underwent CPET during an incremental exercise test on a electromagnetically braked cycle-ergometer (protocol 10watt/min up to exhaustion). Heart rate (HR) was assessed by electrocardiographic monitoring. Oxygen uptake (VO₂), carbon dioxide production (VCO₂), and pulmonary ventilation (VE) were measured by means of portable gas analyser (VO2000). Moreover, oxygen pulse (OP, defined as VO₂/HR) and ventilator-carbon dioxide linear regression slope (VE/VCO₂ slope) were obtained from CPET parameters. Differences between groups were find out at REST, at anaerobic threshold (AT) and at maximum (MAX) of exercise by means of two-way ANOVA.

Results. Statistics did not found any difference between groups at REST. However, CAD patients showed significant lower values of HR, VO₂, VCO₂, VE, OP and VE/VCO₂ slope both at SA and at MAX in comparison with CTL.

Conclusions. This study demonstrates that CPET is able to discover differences between CAD and CTL during exercise. Both at AT and at MAX significant differences were detected in all parameters taken into consideration. Thus, CPET should be recommended in the physical evaluation of CAD patients.

B05-10

Cardioprotective Effects of Exercise on the Experimental Type 1 Diabetes Mellitus; Investigating the Oxidative and Antioxidative Status

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Exercise plays a key role in prevention and the treatment of obesity, metabolic syndrome and diabetes. There are many factors in the development of diabetic cardiomyopathy (DKMP), which is an important complication of diabetes. Oxidative stress causes cell damage by the increase of reactive oxygen species which known to have a role in the pathogenesis of DKMP. Type 1 diabetes mellitus which accounts for about 10% of total diabetics is more likely to develop acute complications such as hypoglycemia and diabetic ketoacidosis so that studies investigating the effect of exercise on type 1 diabetes mellitus are relatively few. The aim of the study was to investigate the effects of exercise on oxidative-antioxidative status in type 1 diabetic hearts with two different training protocols.

12 weeks old male Wistar albino rats were divided into two main groups as non diabetic and diabetic, each group including sedentary, moderate and high-intense training subgroups (n=5). Exercises were performed for 6 weeks at increasing speed and inclination in the training groups. Heart tissues were harvested 24 hours after the last exercise. Total oxidant and antioxidant status (TOS,TAS) levels were determined by ELISA from homogenates of left ventricles. Oxidative stress indexes (OSI) were evaluated.

There was not a statistically significant difference among the groups by means of TOS and OSI. Sedentary group has the lowest while sedentary DM group has the highest OSI, increase in the exercise intense decreased the OSI in diabetics but there was not a statistically significant difference among the groups.

Decrease of the OSI in exercised diabetic promotes the benefits of exercise. In order to reveal the exercise effects n numbers will be increased and further experiments will be performed.

B05-11

Effect of Progressive Resistance Exercise, Targeting Muscles with High Type 1 Fiber, on Aerobic Capacity of Young Sedentary Individuals

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Aerobic capacity and related parameters are best indicators of physical fitness and working capacity of individuals. The aim of this study was to investigate possible effects of progressive resistance exercises (PRE) specifically targeting the muscles, known to have high percentage of type 1 fibers, on aerobic capacity of sedentary subjects.

Sedentary volunteers (university students, confirmed to be sedentary by self rating of global physical activity questionnaire) were recruited after informed consent. Analysis involved somatotype calculations (body mass index, fat-free mass) with cardiopulmonary exercise testing for basal values.

The first group performed PRE for 6-weeks specifically targeting five muscles (namely soleus, tibialis anterior, biceps femoris, vastus medialis, adductor magnus) which predominantly have type 1 fibers while the individuals in the other group performed only walking exercises for 6 weeks, with same exercise durations (25 minutes per session). Statistical analysis were performed using the Wilcoxon matched-pairs test.

Mean value of maximum oxygen uptake (VO₂ max) for the progressive resistance exercise group was 31.12±6.08 ml/kg/min initially and significantly increased up to 38.71±8.45 ml/kg/min (p=0.018, n=7) after 6-weeks of PRE. The walking groups' basal levels of VO₂ max was 32.94±6.84 and this value did not change significantly (33.88±7.12) after 6 weeks study period (p=0.344, n=7).

Results of this study indicates that 6-weeks of progressive resistance training targeting the muscles with high percentage of type 1 fibers provides significant improvement in aerobic capacity of sedentary subjects. The results of this study might be of importance for training and rehabilitation.

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B05-12

Influence of Rhodiola Rosea product and physical training, on acute physical stress

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Questions. Rhodiola Rosea (RR) it is a well known adaptogen. The objective was to highlight an RR product (RRP) and the physical training (PT) action on blood sugar (BS) and anxiety (A), in acute physical stress.

Methods. Sedentary subjects (n=24 males) were organized into 3 groups: 1) control (C=8) no RRP, no PT; 2) with RRP, no PT (RR=8); 3) with RRP and PT (RRPT=8). Study steps: a) RRP (P1) for RR and RRPT: 21days, at physical rest; b) PT (P2) for RRPT: pedaling on a cycloergometer, a week, 12 min/day; c) physical stress (P3) for all groups: running on a treadmill Excite+Run MD. BS and A measurings: T1 = before P1; T2, T3, T4 = at the end of P1, P2, P3 respectively. Assessments: BS with a portable glucometer; A with Beck inventory. Statistical evaluation was based on Student test.

Results. T4 comparison: a) at RR versus GC: BS decreased moderate significantly(p=0.05); A decreased significantly (p=0.005); b) BS and A decreased at RRPT: intensive significantly versus C (BS,p=0.003; A,p=0.001); moderate significantly versus RR (BS,p=0.05; A,p=0.03).

Conclusions. 1) RR acted more intense on A than BS. 2) RRPT proved comparable stress protection, for BS and A. 3) RRPT combination acted more efficient on BS and A, comparing with the use of only RR under physical stress. 4) RR and especially RRPT may be useful to modulate BS and A on acute physical stress, in sedentary persons.

Key words: Rhodiola Rosea, glycemia, anxiety, physical stress

B05-13

The Impact of Physical Exercise Performed at Different Times of Day on Serum Nesfatin-1 and Irisin Levels in Trained and Untrained Young Male Subjects

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Objectives: This study aimed to investigate the potential beneficial effects of acute exercise performed at different times of day on both nesfatin-1 and irisin levels in relation to the subjects training statuses.

Methods: This study's protocol was approved by the Local Ethics. Both trained (n=14, 18.3±0.1 yr, 61.4±2.3 kg) and untrained (n=14, 18.6±0.1 yr, 63.2±2.4 kg) male subjects performed in soccer matches at three different times of day: morning, afternoon and night. All matches were performed on the same field (lasted 60 min each; three days between each match). Pre- and post-match venous blood samples were taken, and levels of both nesfatin-1 and irisin were analyzed using the ELISA method. The Wilcoxon signed-rank test and Mann-Whitney U test were used to analyze the significance of data.

Results: The baseline nesfatin-1 levels were significantly higher in the untrained subjects (p<0.05), and baseline irisin levels were significantly higher in the trained subjects (p<0.001). Following all matches, the subjects irisin levels increased significantly in both groups (p<0.0001). Nesfatin-1 levels were also increased after the workouts; however, the increase was statistically significant only for night-time exercise in both groups (28.3% trained and 20.9% untrained, p<0.05).

Conclusions: The different workout times have different effects on both irisin and nesfatin-1 levels, irrespective of training status. Although the reason for the increases seen during this experiment are unclear, night-time seems to be the preferable exercise time for the improvement of metabolic system regulation via nesfatin-1 and irisin hormones, which may have important implications for both weight management and impaired energy metabolism.

B09: Neurobiology

B09-1

The relationship between global acetylation histone H4 levels and spinal cord injury: an experimental study

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Emerging evidences have been pointed out that the imbalance on epigenetic machinery exert a pivotal role in the physiopathology of several neurological, neurodegenerative and neuropsychiatric conditions. However, this relationship in spinal cord injury (SCI) have been poorly investigated. Therefore, this study aimed to evaluate the modulation of global histone H4 acetylation levels, an important epigenetic mark, after a thoracic SCI model in rats. Male Wistar rats aged 3 months were

submitted to a thoracic SCI model and global histone H4 acetylation levels were measured at different time-points: 6h, 24h, 48h, 72h and 7days after. The global histone H4 acetylation levels were determined using the Global Histone H4 Acetylation Assay Kit (Colorimetric Detection, EpiQuik USA) according to the manufacturer's instructions. The Animal Bioethics Committee of both Federal University of Rio Grande do Sul (number 26116) and Pontifical Catholic University of Rio Grande do Sul (number 15/00492) approved the study protocol. It was observed that global histone H4 acetylation levels changed at the evaluated time-groups ($P=0.0001$). Post hoc tests showed the 72h post-SCI group was significantly increased from all the other groups ($P\leq 0.03$). Moreover, there was an additional difference between the 24h and 7 day post-SCI groups ($P=0.01$). Taken together, our findings suggest histone H4 acetylation levels as novel possible biomarker in SCI. We also showed that this modulation in the perilesional tissue are time-dependent after SCI.

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B09-2

The role of P2X7 receptors in penicillin-induced epileptiform activity*

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Questions: P2X receptors are an ATP-gated ion channels that expresses in neurones and glia in the brain. Inhibition of P2X7R has been proposed as a potential role in wide range of neurological diseases including epilepsy. The present study was designed to determine the role of P2X7R in the experimental epilepsy model of penicillin.

Methods: A total 98 male Wistar rats, weighing 180-240 g were used in this study. Animals were placed in rat stereotaxic apparatus under anaesthesia. Bipolar two electrodes were placed over somatomotor cortex. Intracerebroventricular injections of P2X7R agonist Bz-ATP, at doses of 25, 50, 100, 200 µg and antagonist A-438079, at doses of 5, 10, 20, 40 µg, were administered into the left lateral ventricle of each rat after induction of epilepsy by penicillin.

Results: P2X7R antagonist A-438079, at a dose of 5 µg, did not affect either frequency or amplitude of epileptiform activity. All other doses (10, 20, 40 µg) decreased the frequency of epileptiform activity without changing amplitude. A-438079, at doses of 20 and 40 µg, was the most effective in decreasing the frequency of penicillin-induced epileptiform activity. P2X7R agonist Bz-ATP, at a dose of 25 µg, did not change the frequency and amplitude of epileptiform activity. All other doses of Bz-ATP increased the frequency of epileptiform activity without changing amplitude. Bz-ATP, at doses of 100 and 200 µg, was the most effective in increasing the frequency of epileptiform activity.

Conclusions: P2X7R antagonist (A-438079) reduced penicillin-induced epileptiform activity, whereas agonist (Bz-ATP) enhanced the frequency of epileptiform activity in rat. Inhibition of P2X7R seems a good therapeutic strategy to suppress acute seizure development in epilepsy.

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B09-3

The effect of hemopressin on ECoG activity of absence epilepsy model in WAG/Rij rats*

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Poster Session B

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Questions: Cannabinoid systems contribute to regulate seizure activity in the brain through the activation of cannabinoid CB1 receptors. Hemopressin originates from the alpha chain of haemoglobin and binds to the CB1 receptors. However it is not clear that hemopressin is a typically CB1 receptor agonist or antagonist yet. For the first time, the aim of present study was evaluate effect of hemopressin on ECoG activity of absence epilepsy model in WAG/Rij rats.

Methods: A total 28 male WAG/Rij rats, weighing 200-240 g were used in this study. Animals were placed in rat stereotaxic apparatus under anaesthesia. Tripolar electrodes were placed on skulls and control ECoG activities were recorded. Experimental groups received 0.015 µg, 0.03 µg ve 0.6 µg intracerebroventricular injections and ECoG recordings were repeated. The number of spike wave discharges (SWDs) and their durations were calculated.

Results: Hemopressin, at doses of 0.03 and 0.6 µg, reduced the total number of SWDs in 30 and 20 minutes after hemopressin injection, respectively while hemopressin, at a dose of 0.015 µg, did not affect it. All doses of hemopressin did not change the amplitude of SWDs in all groups. The effective doses of hemopressin (0.03 and 0.6 µg) also reduced the duration of SWDs. The most effective dose in decreasing the frequency and duration of SWDs was 0.6 µg hemopressin in this study.

Conclusions: The intracerebroventricular administration of hemopressin attenuated the number and duration of SWDs seen in the ECoG recordings of genetic absence epilepsy seizures in WAG/Rij rats. Therefore it can be concluded that hemopressin behaves like a CB1 receptor agonist, at least in the absence epilepsy model in WAG/Rij rats.

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B09-4

The difference of gastrointestinal microbiota of children with and without autism in Slovakia.

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Autism is a neurodevelopmental disorder, presented as social and communication abnormalities associated with stereotyped repetitive behaviors and often accompanied by gastrointestinal (GI) disorders. Autism development is based on the genetic predispositions triggered by environmental factors, which importance is accumulating evidences. In our study we were looking at the difference of fecal microbiota (an environmental factor) in children with and without autism in Slovakia using real-time PCR. After we found that the gut microflora assessed from stool samples in children with autism differs from the one in children without autism on the level of phylum as well as on the level of species, we attempted to look for its possible role in the development of GI disorders and/or other manifestations of autism. We established that the *Bacteroidetes/Firmicutes* ratio was decreased and the *Lactobacillus* abundance was elevated in stool samples of children with autism. We also observed trends for elevated *Clostridia* cluster I and *Desulfovibrio* incidences in children with autism. Our results showed a correlation of the amount of *Desulfovibrio* with the severity of autism. Among the participants of our study the autism severity (ADI) correlated with the severity of GI dysfunction. We also measured three pro-inflammatory markers (TNFα, DHEA-S, calprotectin) which had different levels in children with autism and at the present moment we are looking at their correlation with the autism severity. To expand our knowledge about the role of GI microbiota in manifestations of autism, we are going to investigate it in larger groups of participants.

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Poster Session B

B09-5

THE EFFECT OF MELATONIN ON THE EXPERIMENTALLY PRODUCED ALZHEIMER IN RATS AND RELATIONSHIP WITH FEZ1 GENE EXPRESSION

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Aim: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by memory dysfunction and loss of cognitive functions. Immunohistochemical studies have shown that many neurologic diseases may be related with the FEZ1 (Fasciculation and elongation protein zeta-1) expressed in neocortex. The present study was designed to determine the effect of melatonin on the Alzheimer and relation between the FEZ1.

Methods: 30 male rats weighing 220-280 gr were used in the study. The rats were divided into three groups (n=10), sham, streptozotocin (STZ), melatonin+STZ. The intracerebroventricular STZ injections were applied to the rats on the 1st and 3rd days as 3 mg/kg. Melatonin applications (intraperitoneally 10 mg/kg/day) were started one hour before the first dose of STZ, and were continued for 14 days. The rats were killed and hippocampus tissues were removed. The FEZ1 gene expressions were determined with qRT-PCR and protein levels of FEZ1 were measured by using Western Blot.

Results: In the icv STZ group, the protein levels of FEZ1 were found higher than sham group (p<0.05). While the protein levels of FEZ1 in the icv STZ+melatonin group were similar to sham groups, it was statistically lower than STZ injected group (p<0.05).

Conclusions: Our study results demonstrated that FEZ1 levels were high in the rat models of Alzheimer's Disease and these increases in FEZ1 levels were turned back by melatonin.

B09-6

Role of alpha-adrenoceptor agonists in meningeal nociception

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Questions: Sympathetic activity is involved in peripheral processes of pain generation upon activation of alpha-adrenoceptors expressed in nociceptive afferents under pathophysiological conditions like nerve lesions. We aimed to clarify the role of alpha-receptor agonists in meningeal nociception using neuropeptide release as readout.

Methods: In perfusion-fixed rodent dura mater and trigeminal ganglia polyclonal antibodies recognizing calcitonin gene-related peptide (CGRP) or alpha-adrenoceptors were used to identify possible receptor sites by confocal imaging. CGRP release from fresh rat dura mater in the hemisected rat head and from isolated mouse trigeminal ganglia was quantified by an ELISA.

Results: Immunoreactivity for alpha-adrenoceptors was localized in 12.5% of small to medium sized mouse trigeminal ganglion neurons but not co-localized with CGRP immunoreactivity. In the dura mater alpha-receptor immunoreactivity was not found associated with nerve fibers. Agonists at the alpha-receptor (phenylephrine and norepinephrine > 10 mM) caused CGRP release from the rat dura mater. Blocking TRPA1 receptor channels or alpha-receptors did not interact with the phenylephrine effect, whereas blocking TRPV1 receptor channels abolished the phenylephrine effect. Phenylephrine caused also CGRP release from trigeminal ganglia of wildtype mice but not from mice lacking TRPV1 receptors.

Conclusions: CGRP release from the rodent dura mater and trigeminal ganglia induced by alpha-adrenoceptor agonists at high doses can occur partly via opening of TRPV1 receptor channels but these results do not support the assumption that alpha-adrenoceptor activation is involved in meningeal nociception and the generation of headaches.

B09-7

The Evidences of Electrophysiological Symptoms of Acute Toxoplasmosis in Rats

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Zoonotic, neurotrophic, intracellular parasite, toxoplasma gondii causes many neuropsychiatric diseases mainly seizure like discharges depending on the region in which the brain is located. It is reported that 1/3 of the world population is infected with this parasite. It is known that some parasites are responsible for the etiology of idiopathic and cryptogenic epilepsies. Some viral, bacterial and parasite infections, such as neurosysticosis, toxoplasmosis, neuroborreliosis, prion diseases, JCV, HSV have been shown to cause epilepsy. The first objective of this study was to determine whether the experimentally induced toxoplasma gondii infection caused epileptic seizures. Secondly, if seizures occur, it is necessary to determine whether these epileptic seizures have different characteristics in acute phase of the infection. In this study, 2-4 month old wistar albino rats were used. All groups received permanent electrodes under ketamine/xylazine (10/90 mg /kg) anesthesia. Animals were divided into two groups: (G1) Positive control (n = 4) and (G2) experimental group (n=4). The control group received 40 mg / kg PTZ and epileptic seizure was induced and 1 hour EEG recording was obtained. Animals in the experimental group were injected with 1x10⁴/ml of tachyzoite via IP. On the 8th, 15th, and 30th days of infection from the same animals in the study group, the 1-hour EEG recordings were taken awake and the number of seizures was assessed with seizure duration and DDD (spike wave discharges) one way ANOVA(post hoc TUKEY).According to this result, the efficacy of EEG on waves of toxoplasma gondii infection is similar that of epileptic PTZ model. It may cause similar to the PTZ model. There was also no significant difference between records taken during the infectious process. This study is supported by The Scientific And Technological Research Council Of Turkey (Tubitak) given project number as 115S223.

B09-8

Dynamics of changes in heart rate variability after prolonged exposure to dark

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Introduction: Aim of this study was to evaluate the influence of long exposure of dark on heart rate variability (HRV) in young people. It's an experimental method currently used in psychology. Existing results of "Chamber-Rest therapy" point out an improvement of mental health in people under constant stress. So far, no evidence of changes in autonomic nervous system function and its influence on cardiovascular system were measured after a long term stay in the dark.

Methods: 14 students (19 to 26 years) were placed into a room with maximal darkness for 96 hours. The room met the requirements for a comfortable stay, located in a quiet, socially isolated place. The participants received food and drinks as requested without using of any device emitting light or showing the actual time. Orthostatic test was used for measuring power LF, HF and LF/HF ratio. The first measurement was performed the day before starting the therapy, next measurement was taken 30 minutes after completing the therapeutic session, followed by two more measurements in the fourth and the seventh day after exiting the dark chamber.

Results: The power HF showed a significant change between the first and the second measurement (increasing activity of HF) in the horizontal position ($p \leq 0,05$), with a similar trend observed during consecutive measurements. The LF/HF ratio pointed non-significantly to a modulatory influence of sympathetic and parasympathetic nervous system during the orthostatic test.

Conclusion: 96 hours of darkness therapy and subsequent time disorientation likely influenced the parasympathetic nervous system regulation after completing the therapy. This discovery was further supported by a lowered heart rate which can also affect cardiovascular system.

B09-9

Muscarinic acetylcholine receptors activation enhances neurite outgrowth in cultured hippocampal neurons and exerts anxiolytic-like effects by modulating BDNF and FGF2 in the rat hippocampus

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Background and Aim – Muscarinic acetylcholine receptors (mAChRs) are a family of G protein-coupled receptors involved in the regulation of synaptic plasticity, neurogenesis, neuronal differentiation and neuroprotection. Recently, it has been demonstrated that mAChRs can rapidly transactivate tyrosine kinase receptors in absence of their specific ligands. In this work, we aimed to explore: a) whether mAChRs activation by oxotremorine, a nonselective mAChR agonist, may transactivate FGFR1 and exert trophic effects in cultured hippocampal neurons; b) the anxiolytic-like effect of oxotremorine on anxiety-like behavior induced by chronic restraint stress.

Methods – Oxotremorine-induced FGFR1 transactivation and related trophic effect was tested in primary hippocampal neurons. Oxotremorine anxiolytic-like effect was evaluated using four different paradigms: forced swim test, novelty suppressed feeding test, elevated plus maze and dark/light. Western blotting was used to detect BDNF and FGF2 levels.

Results – Treatment with oxotremorine was able to transactivate FGFR1 in primary hippocampal neurons, producing a significant increase in the primary neurite outgrowth. Oxotremorine treatment was effective in alleviating the anxiety in rats. Chronic restraint stress was associated with the reduction of BDNF and FGF2 levels in the hippocampus, and oxotremorine administration was able to recovery both neurotrophic factors levels.

Conclusions – The present findings, by showing a functional mAChR/FGFR1 interaction in the hippocampus and the oxotremorine anxiolytic-like effects, mediated by neurotrophic factors modulation, will contribute to advance the understanding of cholinergic drugs mechanisms involved in neuronal plasticity.

B09-10

Neurospecific proteins determination in plasma of newborns with perinatal hypoxic lesion of the central nervous system

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Immunochemical determination of neuronal specific proteins (such as brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF) and neuron specific enolase (NSE)) in blood regarded as a promising approach for early diagnosis of cerebral lesions. The aim of the investigation is to estimate the informative value of BDNF, GDNF and NSE determination in the plasma of newborns with perinatal hypoxic damage of the central nervous system (CNS).

BDNF, GDNF and NSE concentrations were determined in plasma of newborns with 1) signs of intrauterine hypoxia (score by Apgar test <7); 2) convulsive states and 3) with severe perinatal CNS damage, by using ELISA kits (R&D Systems, USA and Vector Best, Russia).

Infants from group 1 showed a significantly low content of BDNF, from group 2 a significant increase of NSE concentration was shown, from group 3 a low GDNF content was found.

The obtained data showed that the determination of neurospecific proteins level in plasma suggests its application in clinical neonatology as an informative test for predicting the severity of CNS damage.

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B09-11

Do the activities of redox regulating enzymes decline during ageing and in the brains of Parkinsons disease patients?

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The pathological hallmarks of Parkinsons disease (PD) are the loss of dopaminergic neurons of the substantia nigra pars compacta (SNpc), and the deposition of aggregated α -synuclein in Lewy bodies. Age is the principal risk factor for neurodegenerative diseases such as PD. Cellular oxidative damage is one of the molecular mechanisms that has been proposed to drive normal ageing and also brain pathological damage. To examine the influence of human ageing on brain redox regulation, we have quantified the level and activity of the redox stress regulating enzymes, superoxide dismutase 1 (SOD1) and catalase, in the prefrontal cortex of *post-mortem* human brain tissue over the age range of 21-84 years. 6 human control subjects were assessed for each decade between the second and ninth decades. Furthermore, to examine regional vulnerability of the SNpc region to loss of redox regulation and accumulation of redox stress, SOD1 levels and activity were quantified in *post-mortem* tissue from the prefrontal cortex, caudate nucleus, cerebellum, hippocampus, corpus callosum, thalamus, and SNpc in 10 PD patients and 10 age and sex matched control subjects. Variation of the levels and activity of SOD1 and catalase during normal ageing and in different brain regions of control and PD patients will be discussed.

B09-12

Chemogenetics modulation of kisspeptin neuron activity and its role in anxiety behavior in mice

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Kisspeptin is known for its key role in reproduction. However, a large fraction of kisspeptin expressing neurons also make projections to various brain regions. The role of these connections are not well understood. We used chemogenetic neuronal activity manipulator, hM3D, to selectively stimulate

kisspeptinergic neurons and investigate its effects in a number of behavioral assays related to anxiety behavior, such as elevated plus maze and open field tasks in mice. Obtained preliminary findings suggest that active Kiss1 neurons may be related to anxiety behavior.

Key words: Kisspeptin, hM3D, chemogenetics, Brain, Anxiety, Behavior

B12: Sensory and motor neurophysiology

B12-1

Intrinsic discharge patterns of floccular Purkinje cells in rats

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The main question in the field of learning and memory is how the brain encodes the pattern of experience into the molecular and cellular level. To answer this question, eye movement modulated by the cerebellum is one of the most used tools due to its well-defined and simple circuitry. However, in spite of extensive researches on the modulation of eye movement by the cerebellum during the last few decades, the intrinsic cellular properties of the floccular Purkinje cells (PCs) in the cerebellum, which is thought to be an important site for modulating eye movement, have not yet been defined. Therefore, we investigated the passive and active properties of the floccular PCs in slices prepared from rats (P21-23) by using whole-cell patch clamp technique. Four types of firing patterns were identified in response to depolarizing current injections in floccular PCs; tonic-firing showing continuous Na⁺ spikes throughout the current pulse (53%), initial-bursting showing short firing of few action potentials (29%), complex-bursting showing both Na⁺ spikes and Ca²⁺-Na⁺ bursts (14%), and gap-firing showing a pause of firing between the first and second spike (4%). The passive membrane properties were not significantly different over the firing patterns. As for the active membrane properties, initial bursting neurons had a tendency to exhibit lower excitability than other firing pattern neurons. Our result is the first that discusses about the intrinsic excitability of the floccular PCs in vitro. These electrophysiological properties may provide useful details for understanding how the floccular PCs integrate input signals to produce output signals, which modulate eye movement.

B12-2

The intra-limb anticipatory postural adjustments and their role in movement performance

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Anticipatory Postural Adjustments (APAs) are commonly described as unconscious muscular activities aimed to counterbalance the perturbation caused by the primary movement, so as to ensure the whole-body balance. These activities usually create one or more fixation chains which spread over several muscles of different limbs, and may be thus called *inter-limb* APAs. However, we reported that APAs also precede voluntary movements involving tiny masses, like a flexion/extension of the wrist or even a brisk flexion of the index-finger. In particular, such movements are preceded by an *intra-limb* APA chain, that involves muscles acting on the proximal joints. Considering the small mass of the moving segments, it is unlikely that the ensuing perturbation could threaten the whole-body balance, so that it is interesting to enquire the physiological role of *intra-limb* APAs and their organization and control compared to *inter-limb* APAs.

Since several years, our research is focused on *intra-limb* APAs and highlighted a strict correspondence in their behaviour and temporal/spatial organization with respect to *inter-limb* APAs. Hence we suggested that both are manifestations of the same phenomenon. Particular emphasis has

been given to *intra-limb* APAs preceding index-finger flexion, because their relatively simple biomechanics and the fact that muscular actions were limited to a single arm allowed peculiar investigations, leading to important conclusions. Indeed, such paradigm provided evidence that APAs and prime mover activation are driven by a shared motor command, and also that by granting a proper fixation of those body segments proximal to the moving one, APAs are involved in refining movement precision.

B12-3

Impact of photoreceptor failure on inner retinal function

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Although retina is just two-three hundred microns thick, it is able to convert and to elaborate images of the outside world in neural code. The complexity and intensity of this process are intrinsically susceptible to a genetic defect or oxidative stress and, considering the high energy at play, the more susceptible element of this chain is photoreceptor. In this talk, I would like to highlight the strict relationship between photoreceptor failure and inner retina malfunctioning. I will show the long-term consequences that absence of light experience, during the critical period, has on retinal ganglion cells ability to filter and decode space and time. A similar paradigm will be then transposed in an animal model for Retinitis Pigmentosa: the Royal College of Surgeon rat. Finally, I will introduce a new technological platform that allows the simultaneous recording of light-evoked responses from thousands of retinal ganglion cells in the ex-vivo retina of mouse, rat, and monkey, thus opening new perspectives in this field of research.

B12-4

Dynamic weight bearing test for assessing effects of acute intramuscular administration of botulinum neurotoxin type A1 in the rat

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Questions

The Dynamic Weight Bearing (DWB) test in the rat measures distribution of body weight across paws in a freely-moving animal. We investigated whether DWB test is sensitive to muscle-relaxant effects of botulinum neurotoxin type A1 (BoNT/A1). Effects in the DWB test were compared to those assessed by the compound muscle action potential (CMAP) and digit abduction score (DAS) tests in the same animals. We also investigated whether injections given as 1 vs 2 volumes affected the results.

Methods

Female Sprague-Dawley rats received intramuscular injection of BoNT/A1 (0.1, 1, 10 pg/rat) into the right gastrocnemius muscle, while the left received vehicle. Control animals received vehicle in both muscles. Injections were made using 1x30 µL or 2x15 µL. Rats were tested for DWB and CMAP 1 and 2 days post-injection, respectively, and each test was preceded by a DAS test. Differences assessed by 1 way ANOVA followed by Dunnett's test.

Results

BoNT/A1 dose-dependently reduced DWB in toxin- vs vehicle-injected limbs, causing >50% reduction at 10 pg/rat dose (p<0.001). Dose-dependent reductions were also observed in the CMAP amplitude in the toxin-injected muscles, with greater than 60% reductions at 1 pg/animal (p<0.01) and full suppression at 10 pg/rat (p<0.01). Overall, the two injections were associated with greater efficacy of the intermediate dose in DWB and CMAP. However, modest increases in DAS scores were only observed in a limited number of animals.

Conclusions

The DWB test is sensitive to muscle-relaxant effects of BoNTs following acute intramuscular administration. It has a greater sensitivity than the DAS, but lesser one than the CMAP. Thus, it can be used as a non-invasive measure of the biological properties of neurotoxins in vivo.

B12-5

Are psychogenic startles anxiety-enhanced physiological startles? A latencies-based answer

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Questions: Startles are physiological responses to an unexpected, possibly threatening sensory stimulation. They can be enhanced by anxiety, through an amygdala-based modulation. Under psychogenic disorders, a pathological startle (easily triggered, unexhausted, exaggerated) is observed. Distinguishing between an anxiety-enhanced startle and a "pseudo" startle is not always clinically easy. This study aims at determining psychogenic startles latency occurrence variance in 5 patients.

Methods: We retrospectively examined a number of patients with psychogenic startles (clinical diagnosis). Scrutinizing EEG-EMG video recordings, we noted for each patient the latency between the triggering factor and muscle contraction onset.

Results: Latency mean duration of muscle contraction persisted and varied in the 5 patients (614 +/- 275ms). These values are higher than the mean reaction time obtained in 10 subjects (447 +/- 55ms) and above the physiological startles reaction time; all muscular responses normally occur within the first 100 milliseconds after stimulation.

Conclusions: The study of muscular latencies responding to a psychogenic startle stimulation shows that latencies exceed a normal reaction time and that they are much superior to a physiological startles contraction latency. A psychogenic burst is not therefore an anxiety-enhanced physiological startle, but a behavioral response to an unexpected stimulation, which is not a startle in the first place. Contrary to conventional wisdom, this is not an elimination diagnosis, but a true positive diagnosis based on a range of clinical and neurophysiological arguments: long stimulus-response latency (> 100 ms), variable, persistent muscular contraction, unusual and variable pattern.

B12-6

Shared neural input between muscles activated during shoulder abduction and adduction

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Shoulder muscle synergies have been identified during isometric abduction/extension and adduction/flexion, and during point-to-point reaching tasks to study activation of temporal synergies. These studies applied a non-negative matrix factorization analysis which does not reveal the neural drive to the muscles. Most shoulder muscles serve multiple functions so details of muscles sharing common neural input are probably complex. Interactions between muscle pairs at the shoulder or between shoulder and arm are rarely studied, leading to gaps in our understanding of these common pathways. Cross-correlation and frequency coherence analysis have previously been used to identify common neural input to muscle pairs. Here, sEMG recordings were made from shoulder and arm muscles primarily activated during shoulder abduction (deltoids, trapezius, triceps) and adduction (pec. major, serratus anterior, latissimus dorsi). We investigated muscle interactions during three tasks: an isometric maximum voluntary contraction in the direction of either (1) abduction or (2) adduction, and (3) a novel maximum voluntary effort task. Cross-correlation and frequency coherence analysis were applied between muscle pairs during different tasks and shoulder angles. We expect the

muscle interactions to be altered, possibly due to adjustments in biomechanical as well as central or peripheral neural drive.

B12-7

On genito-urological pathophysiology I

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Introduction: Guinea pig detrusor (D) of urinary bladder, vas deferens (VD) & ureter (U) are model preparations in biological & medical physiology.

METHOD: Effects of cypermethrin (CY) & deltamethrin (DE) 0.1-100µM on spontaneous (phasic) (SPC, 1-5/min) and contractions to electrical neurogenic (TTX-blockade) stimulation with 10&100Hz, 3ms, 3s (CES10/100) were recorded.

RESULTS: Present experiments demonstrate a negative chrono- and inotropic pyrethroid action on SPC of D, but only a strong neg. inotropic of U. Important differences in degree of inhibitory effects between CY/DE on CES of D & VD are evident, with the exception: DE 1-10µM had an augmentory effect of CES10 in VD (113.0±5.7%). Statistical analysis and ref. see part II.

CONCLUSION: Pathophysiological motor reactions are a sensitive indicator for functional disturbances in the urogenital system after toxicants (eg human D reacted to Hg at 0.1-1 nM). The different effects of CY & DE on CES10/100 support results about the existence of two sets of postganglionic neuro-vegetative effector regulation of VD (incl. human, with low & high pyrethroid- & thermosensitivity, excitable at 10&100 Hz resp.).

Ref.: Neu, Michailov et al.: DPG-Congr 2017 Greifswald, Acta Physiol. 291/S71, p. 97-98. EurJPhysiol 2002-Tübingen 443/S 1997-Rostock 433/6:R65 1992-Düsseldorf 420/1:R99. Urol. Res. 8, 238, 1980; 5, 36, 1977. Urol. int. 38, 234-242, 1983; 36, 225-234, 1981.

B12-8

Changes in static perimetry during chamber-rest: a pilot study

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Introduction. The aim of this study is to evaluate the effect of CHAMBER REST on visual field in young people. The results of this experimental therapeutic method, which is based on staying in complete darkness to improve health of people living under constant stress, suggest that it may positively influence the sensitivity of the retina as well as its regeneration.

Methods. We evaluated 14 students (19 to 26 years), who were placed into a special room with maximal darkness for 96 hours. The participants did not use any device emitting light or showing the time. The first measurement was performed the day before starting the therapy. The next measurement was taken 30 minutes after completing the therapeutic session, followed by two measurements in the 4th and the 7th day after exiting the darkness. In total, 4 measurements were obtained for both left and right eye by using the OCULUS Centerfield 2, in cooperation with the MaculaThreshold software for testing static perimetry. The MeanDefect (MD) visual field index, which

is the most important index describing the mean loss of sensitivity and the reduction of the visual field, was used for the evaluation.

Results. In this study, the results of the MD index (measured 1 day before starting the therapy and 7 days after finishing it) were compared. 71 % of students showed an improvement of the MD index in both eyes, 21 % improved only in one eye and no improvement was found in 7 % of the students. A statistical analysis proved these results to be statistically significant ($p = 0,0167$).

Conclusions. Results demonstrate the positive effect of long exposure of darkness on the retina and the visual field in young people. It is necessary to perform more research and confirm this hypothesis on a larger group of participants.

B17: Stem cells

B17-1

Development of a multi-layer scaffold for artificial tissue with mesenchymal stem cells

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Commonly used scaffolds with the varying size of pores formed by disorderly arranged fibrin and collagen fibers have limited applications for tissue engineering. There is a risk that cells could escape to the neighboring environment from such implanted scaffold. Development of a multi-layer scaffold with an impermeable outer layer could be a proper solution to overcome the latter disadvantages. For that purpose we tested hMSCs behaviour on micromachined polyimide film and poly(lactic acid) electrospun fiber scaffolds. There were no changes in migration, proliferation and monolayer formation properties. Optimal spacing between the micro-holes for cell adhesion was found to be 45 μm . Metabolic and genetic coupling was enabled between 2 sides of PI film through 3.1 \pm 0.5 μm diameter of micro-hole. We applied combined electrospinning and laser micro-machining techniques to develop multi-layer scaffold. An inner layer of the scaffold, providing proper environment for cell inhabitation, could be produced by electrospun mats. Our results suggest that the intercellular communication between two sides of PI film could be established through 3.83 \pm 0.45 μm diameter holes by tunnelling tubes. PI could be suitable as cell migration from scaffold barrier.

B17-2

Synergistic effects of TGF- β and IGF-1 on chondrogenic potential of adipose tissue derived stem cells

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Objectives

Adipose tissue derived stem cells (ADSCs) can proliferate extensively and offers a promising cell source for regenerative medicine. In the present study, we aimed to examine the effects of transforming growth factor- β (TGF- β) and insulin like growth factor-1 (IGF-1) on the chondrogenic potentials of ADSCs.

Methods

ADSCs were isolated from the visceral adipose tissue of the adult Sprague Dawley rats. The effects of TGF- β and IGF-1 on chondrogenic differentiation were assessed by using three-dimensional pellet culture system. Pellet cultures (1×10^6 cells) were incubated in a chondrogenic basal media consisting of high glucose-DMEM supplemented by TGF- β (1, 5 and 10 ng/ml) and/or IGF-1 (10, 50 and 100 ng/ml) for 21 days. Finally, the pellets were harvested for histological (toluidine blue staining) and biochemical (proteoglycan levels) analysis. All quantitative data were analyzed by using Kruskal-Wallis and Mann-Whitney U tests. Statistical significance was set at $p < 0.05$.

Results

TGF- β and IGF-1 treated pellets were larger than control pellets while TGF- β +IGF-1 showed the biggest size of pellet. Histologically, TGF- β +IGF-1 treated pellets clearly showed cartilage-like extracellular matrix particularly. Also, the quantitative analysis of proteoglycans demonstrated a dose and time-dependent increase in proteoglycan content in TGF- β and IGF-1 treated cultures. Induction of pellets with both 10 ng/ml TGF- β and 100 ng/ml IGF-1 resulted a significant increase in proteoglycan production compared to study groups ($p < 0.05$).

Conclusions

In conclusion, our results suggested that TGF- β and IGF-1 could exert synergistic effect on chondrogenic differentiation of ADSCs.

POSTER SESSION C

C01: Cardiac physiology

C01-1

The cardioprotective remote ischemic preconditioning in SHR rats : role of age and activation of RISK signaling pathway.

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Remote ischemic preconditioning (RIP) represents a novel form of innate cardioprotection conferred by short episodes of ischemia applied in a distant organ/tissue. RIP has been shown to exert its cardioprotective effect by activating intrinsic pro-survival signaling cascades such as reperfusion injury salvage kinase (RISK) pathway in healthy animals, however, there is no evidence on this effect of RIP in hearts from SHR animals. The aim of this study was to investigate the role of RISK pathway in effect of RIP on cardiac tolerance to I/R in SHR rats of different ages.

Rats of age three, five and eight months (3/5/8m) were anesthetized and RIP was performed on the right hind limb. Its protocol consisted of three cycles of 5min non-invasive limb occlusion followed by 5min reperfusion. Subsequently, hearts were excised, Langendorff-perfused and exposed to 30min global I and 2h R for the evaluation of reperfusion-induced ventricular arrhythmias, infarct size and recovery of contractile function.

Enhanced resistance to myocardial infarction after RIP was observed in all experimental groups. Moreover, in 3m and 5m animals RIP exhibited antiarrhythmic effect, while in 8m SHR rats its effect was either proarrhythmic. Protective effect of RIP was accompanied with increased Akt and GSK-3 β activation as well as with decreased proapoptotic signaling only in hearts from 3m and 5m animals, while in 8m rats the Akt and GSK-3 β activity and apoptotic signaling were not changed after RIP.

Cardioprotective effects of RIP in SHR rats show partial age-dependency, since in older adult animals, RIP decreased size of lethal injury but worsened arrhythmogenesis compared to younger individuals. These effects of RIP may be attributed to differences in activation of RISK pathway.

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C01-2

Remote ischemic preconditioning: protection of myocardial energetics

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The effect of noninvasive remote ischemic preconditioning (RIP) on the functional remodeling of heart mitochondrial membrane and its cardioprotective contribution to ischemic-reperfusion injury was observed.

Methods: RIP was induced by short-term occlusion of the artery supplying the lower limb. Heart mitochondria were isolated and forwarded to biochemical and biophysical investigation performed after 15 minute stabilised perfusion, 30 minute ischemia and 40 minute reperfusion. Activity of mitochondrial Mg²⁺-ATPase was determined spectrophotometrically as the concentration of anorganic phosphate formed by ATP splitting. Mitochondrial membrane fluidity was determined by fluorescence anisotropy. Content of oxidised isoforms of coenzyme Q (CoQ9ox a CoQ10ox) was measured by HPLC method.

Results: We noticed the significant ($p < 0.05$) 5.05% increase in mitochondrial membrane fluidity of RIP group in comparison with the control group after reperfusion. RIP caused 6.95% increase in total mitochondrial Mg²⁺-ATPase activity after reperfusion compared to the control group. The nonsignificant increase in oxidised isoforms of coenzyme Q (CoQ9ox, CoQ10ox) during stabilisation induced by RIP reflects the moderate increase of free radicals having just a signal character and initiates the protective mechanisms. In RIP group after ischemic-reperfusion load, the content of oxidized isoforms CoQ9ox was nonsignificantly reduced by 5.62% compared to control group after reperfusion phase of myocard.

Conclusions: Functional remodeling of mitochondrial membrane triggered by RIP effectively contributed to improved recovery of myocard after ischemic-reperfusion injury. Grants: APVV-15-0119; VEGA 2/0133/15, 2/0201/15.

C01-3

Hypertension and oxidant stress: Effects of angiotensin II receptor antagonists and calcium-channel antagonists on oxidant status in Algerian hypertensive men.

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Questions: Oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and their detoxification by antioxidants, is involved in atherosclerosis and HTA. ROS are responsible for membrane lipid peroxidation, nitrosation / nitro-sylation of proteins, and increased intracellular calcium, impairing endothelial cell function. The effects of calcium antagonists (amlodipine) and angiotensin II receptor antagonists (telmisartan) on oxidative markers were investigated in Algerian hypertensive patients.

Methods: In this study, we included adult men patients with essential HTA. This study was a stratified, randomized, investigator-blinded trial that evaluated the effects of telmisartan monotherapy or amlodipine monotherapy in hypertensive adults was treated for the period of 1 year. At the beginning and after 1 year of antihypertensive therapy, adult patients with essential HTA were followed and oxidative markers (nitric oxide, superoxide anion, malondialdehyde and carbonyl proteins) were determined.

Results: The results of this study indicate that telmisartan and amlodipine are effective antihypertensive agents in the treatment of hypertension because a significant reduction in systolic and diastolic blood pressure was observed in all hypertensive patients after 1 year of treatment. Our results show also that telmisartan and amlodipine treatments counteracted hypertension-dependent and oxidative stress. All hypertensive patients present high levels of pro-oxidant markers.

Conclusion: It seems reasonable to consider therapeutic agents with beneficial effects on blood oxidative stress markers, such as telmisartan and amlodipine. In addition, telmisartan, which reverses all redox changes associated with HTA, should be prescribed, especially in hypertensive patients with severe oxidative stress and its damages.

C01-4

Role of altered Ca^{2+} homeostasis during adverse cardiac remodeling after ischemia and reperfusion

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Acute myocardial infarction (AMI) due to coronary artery occlusion represents a major cause of morbidity and mortality in humans. Increasing evidences demonstrated that despite successful reperfusion therapies, heart failure (HF) appears in ~ 10% of patients due to adverse ventricular remodeling. HF is characterized by dysfunction and abnormalities of intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) handling with altered disturbed excitation-contraction coupling (EC-coupling). $[\text{Ca}^{2+}]_i$ alteration is also involved in activation of Ca^{2+} -dependent transcription factors related to adverse cardiac remodeling.

Methods: $[\text{Ca}^{2+}]_i$ handling was studied in rat model of I/R subjected to transient (40 minutes) ligation of left descending coronary artery. Changes in cytosolic ($[\text{Ca}^{2+}]_{\text{cyto}}$) and intranuclear ($[\text{Ca}^{2+}]_{\text{nuc}}$) were studied in a cardiac myocyte isolated from remote and infarcted zone 1 week after surgery.

Results: Using echography and nuclear magnetic resonance we observed that rat undergoing I/R protocols have depressed cardiac contractile capacity as soon as one week after surgery. I/R treatment produces a decreased cytosolic ($[\text{Ca}^{2+}]_{\text{cyto}}$) and intranuclear ($[\text{Ca}^{2+}]_{\text{nuc}}$) transients in adult cardiomyocytes, not only in the risk zone but also in the remote zone. I/R treatment also induces significant reduction in sarcoplasmic reticulum Ca^{2+} content in both. These alterations were associated with changes in the expression of several ion channels both in remote and ischemic zones.

Conclusion: The calcium homeostasis undergoes significant changes during I/R, not only in the ischemic area, but also occurs in the remote area. These calcium changes may contribute to the development of adverse cardiac remodeling and further heart failure.

C01-5

Fluoxetine Attenuates Remote Myocardial Ischemia Reperfusion Injury

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Questions: Aortic ischemia reperfusion is an important factor in development of postoperative acute cardiac injury following abdominal aortic surgery. Reactive oxygen species has been implicated as a corner stone of reperfusion injury. The aim of the study is to answer the questions; what are the antioxidant effects of fluoxetine (Flx) in the context of ischemia – reperfusion (IR) injury and what are its effects on cardiac function and cellular integrity?

Methods: Male Wistar rats were divided into 3 groups (n=7 per group): 1) control; 2) IR by occlusion of infrarenal abdominal aorta (60-min ischemia and 120-min reperfusion); 3) Flx+IR (20 mg/kg/d, i.p. for 3 d before surgery). The serum creatine kinase (CK) and creatine kinase-MB (CK-MB) levels were considered as cardiac function markers. Lipid hydroperoxide (LOOH), malondialdehyde (MDA), superoxide dismutase activity (Cu,Zn-SOD), glutathione peroxidase (GSH), pro-oxidant antioxidant

balance (PAB) and ferric reducing/antioxidant power (FRAP) levels were determined. Tissue leucocytes infiltration and cellular integrity were assessed histologically.

Results: IR led to a significant increase in CK and CK-MB, LOOH, PAB, MDA levels ($p<0.01$) and a decrease in FRAP, GSH, SOD levels ($p<0.01$). Flx was able to restore these parameters significantly. CK, CK-MB and MDA levels were decreased ($p<0.05$), along with LOOH and PAB levels ($p<0.01$) while FRAP, GSH, SOD levels were found increased compared to IR ($p<0.01$, $p<0.01$, $p<0.001$). Flx attenuated the disruption in cellular integrity induced by IR.

Conclusions: Our study clearly demonstrates that fluoxetine confers protection against aortic IR-induced cardiac injury, tissue leucocyte infiltration and cellular integrity.

C01-6

Beneficial effect of molecular hydrogen and hypoxic postconditioning on ischemia reperfusion injury of isolated rat hearts

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Molecular hydrogen (H_2) is considered as a selective antioxidant able to react with strong oxidants and preserve cell signaling mediated by NO and superoxide radicals. This study aimed to verify whether H_2 can potentiate protective effect of hypoxic postconditioning (HpostC) against ischemia-reperfusion (I/R) injury. Isolated rat hearts perfused with Krebs-Henseleit buffer (KHB) were exposed to 30-min global ischemia/120-min reperfusion. HpostC was induced by 4 cycles of 1-min perfusion with oxygen-free KHB intercepted by 1-min perfusion with normal KHB, while in H_2 +HpostC group, oxygen-free KHB was enriched with H_2 . Severity of I/R injury was evaluated by measurement of infarct size (IS) within the area at risk (AR) (IS/AR, TTC staining) and recovery of function. IS/AR was markedly reduced in HpostC group to $24.6 \pm 0.9\%$ compared with $38.7 \pm 1.4\%$ in non-conditioned controls, and even more significantly in H_2 +HpostC group ($16.6 \pm 0.8\%$; $P<0.05$ vs. both, controls and HpostC). Post-I/R recovery of systolic function (LVDP) was improved in H_2 +HpostC group: $53 \pm 11\%$ to the levels of statistical significance vs. $23 \pm 1.6\%$ in controls. End-diastolic pressure (LVEDP) was decreased in both conditioned groups to a similar level (HpostC: 22.1 ± 5.9 mmHg, H_2 +HpostC: 28.6 ± 5.6 , both $P<0.05$ vs. 55.2 ± 6.9 mmHg in controls). Application of H_2 potentiated the beneficial effect of HpostC. Grants: VEGA SR 2/0201/15, 2/0021/15, APVV-0102-11, APVV-0241-11, APVV-15-0376.

C01-7

THE EFFECTS OF ZOFENOPRIL ON CARDIAC FUNCTION AND PRO-OXIDATIVE PARAMETERS IN THE STREPTOZOTOCIN-INDUCED DIABETIC RAT HEART

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Questions: Renin–angiotensin–aldosterone system is one of the main modulators of chronic hyperglycaemia while hyperglycaemia-induced oxidative stress is an important factor in diabetic cardiomyopathy. The present study was designed to assess heart performance in the early stage of diabetic cardiomyopathy development after 4 weeks of hyperglycemia, in the stage known as increased tissue RAAS activity.

Methods: Investigation was carried out on 24 adult male Wistar albino rats whose hearts were perfused according to Langendorff technique. We evaluated the influence of acute administration of zofenopril on myocardial function from rats with streptozotocin-induced diabetes mellitus (STZ-DM),

with a special emphasis on cardiodynamic and oxidative stress parameters in diabetic rat hearts. Rats were divided randomly into two groups (12 animals per group): control nondiabetic animals (C) were healthy rats perfused with 1.5 μ M of zofenopril, and STZ-treated diabetic animals were diabetic animals perfused with 1.5 μ M of zofenopril 4 weeks after the induction of diabetes.

Results: STZ-induced diabetic rats are characterized by a depressed cardiac performance and that these changes seems to not be mediated by via in oxidative stress. However, acute application of zofenopril failed to improve these hyperglycemia-induced changes of cardiac function.

Conslusions: Long-term follow-up intervention trials are necessary to fully demonstrate the benefit of zofenopril in this context.

Key words: zofenopril, cardiac function, diabetic rat heart

C01-8

THE LONG-TERM EFFECTS OF ATORVASTATIN ON OXIDANT/ANTIOXIDANT STATUS OF HYPEHOMOCYSTEINEMIC RATS

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Questions

The objective of our study was to evaluate the association between atorvastatin administration and body weight, food intake, plasma total homocysteine (tHcy), cholesterol (tCHOL), Low-density lipoprotein (LDL), High-density lipoproteins (HDL), triglycerides (TRY) levels, as well as pro-oxidative (superoxide anion radical, hydrogen peroxide, index of lipif peroxidation) and antioxidative markers (reduced glutathione, catalase and superoxide dismutase) in Wistar albino rats.

Methods

Study was conducted on adult male *Wistar albino* rats (n=30; 4 weeks old; 100±15g body mass) in which HHcy was achieved by dietary manipulation. For 4 weeks, the animals were fed with one of the following diets: standard rodent chow (n = 10) (control fed); diet enriched in methionine with no deficient in B vitamins (folic acid, B6 and B12) (n = 10); diet enriched in methionine and deficient in B vitamins (folic acid, B6 and B12) (n = 10). Atorvastatin was administrated daily for 4 weeks, 3 mg/kg i.p.

Results

After 4-wk feeding with purified diets, blood concentrations of the antioxidant GSH in blood were significantly affected, as well as CAT activity and parameters of lipid status (p<0.05). We found significant differences between the body weights and food intakes among all groups (p<0.05) and strong positive correlation between Hcy levels, prooxidative and lipid parameters, and negative correlation with antioxidant parameters in blood after administration of atorvastatin (p<0.05).

Conclusions

Atorvastatin could inhibit progression at any stage of oxidative stress and should therefore be proactively administered to the patient with dyslipidemia and hyperhomocysteinemia, regardless of disease severity.

Key words: HMG-CoA reductase inhibitors, homocysteine, oxidative stress

C01-9

THE EFFECTS OF CHRONIC ADMINISTRATION OF CISPLATIN ON OXIDATIVE STRESS IN ISOLATED RAT HEART

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Questions

Taken into consideration that molecular and cellular mechanisms involved in cardiotoxicity are still not clear, the aim of this study was to compare the production of oxidative stress parameters in the isolated rat heart between animals chronically treated with cisplatin and saline.

Methods

The hearts of male Wistar albino rats (n = 24, 12 per group, age 8 weeks, body mass 250±50 g) were excised and perfused according to the Langendorff technique at gradually increased coronary perfusion pressures (40-120 cmH₂O). Over the entire CPP range, we measured levels of superoxide anion radicals, hydrogen peroxide, nitrites and index of lipid peroxidation in order to determine if oxidative stress is involved in coronary endothelium response in conditions of hypoxia (lower than 60 cm H₂O) and hyperoxia (higher than 80 cm H₂O).

Results

Levels of superoxide anion radicals, hydrogen peroxide, nitrites and index of lipid peroxidation were significantly altered (p<0.05). Higher levels of CPP increased the values of oxidative stress.

Conclusion

We can conclude that damaged endothelium of cisplatin-treated animals had weaker response to hyperoxia and also lower antioxidant capacity. This increment is more prominent in control group as a result of preserved endothelium and its more powerful response to hyperoxia.

Keywords: cisplatin, isolated rat heart, oxidative stress

C01-10

THE EFFECTS OF MODULATION OF N-METHYL-D-ASPARTATE RECEPTORS ON OXIDATIVE STATUS IN ISOLATED RAT HEART

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The role of N-methyl-D-aspartate receptor (NMDA-R) in cardiovascular system is not fully understood jet. The aim of the present study was to examine the effects of MK-801, as a NMDA-R blocker, alone and its combination with glycine and/or glutamate on oxidative status in isolated rat heart. The hearts of male Wistar albino rats were excised and perfused according to Langendorff technique and in samples of coronary venous effluent were spectrophotometrically determined values of biomarkers of oxidative stress – index of lipid peroxidation measured as TBARS, nitrites (NO₂⁻), superoxide anion radical (O₂⁻) and hydrogen peroxide (H₂O₂). Only in group treated with MK-801, glutamate and glycine there was an increase in value O₂⁻, while in all other groups all other measured biomarkers of oxidative stress were decreased or remained unaltered. Based on the obtained results it can be concluded that NMDA-R activation allows the entry of certain quantities of calcium and thus influence the redox balance in myocardium.

C01-11

EFFECT OF MATURATION ON RESISTANCE OF RAT HEARTS TO ISCHEMIA AND EFFECTS OF CLASSICAL AND REMOTE ISCHEMIC PRECONDITIONING. STUDY OF POTENTIAL MOLECULAR MECHANISMS

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Questions: Aging affects tolerance to ischemia/reperfusion (IR), however, its onset and cellular mechanisms behind are less known. Blunting of ischemic preconditioning (IPC) and defects in protective signaling are suggested. Although remote IPC (RIPC) protects young and aged human hearts, its age-dependency in animals is less explored.

Methods: We studied response to IR, effects of IPC and RIPC in isolated hearts of juvenile, younger and mature adult (1.5-, 3-, and 6-month-old) rats exposed to 30-min I/120-min R, and proteins of "pro-survival" pathways. IPC was induced by 1 cycle of IR, 5 min each. RIPC was evoked by pressure cuff inflation (200 mmHg)/deflation (3 cycles, 5 min each) on hind limb. We measured infarct size (IS), arrhythmias and contractile recovery (LVDP), levels of Akt, phosphorylated Akt (p-Akt), endothelial NO synthase (eNOS) and protein kinase C ϵ (PKC ϵ) (WB).

Results: Maturation impaired response to lethal injury and promoted arrhythmogenesis. IPC reduced arrhythmias occurrence, IS and improved LVDP recovery in younger animals, while its effect was attenuated in mature ones. Loss of protection was associated with age-dependent decrease in p-Akt, eNOS and PKC ϵ in the hearts of mature animals, and with a failure of IPC to upregulate these proteins. RIPC also reduced severity of arrhythmias, IS and improved LVDP recovery in younger rats. However, protection was preserved even in the mature adults coupled with upregulation of all selected proteins.

Conclusions: Maturation starts to impair the resistance of rat hearts against IR injury and causes gradual loss in IPC efficiency, while RIPC appears more effective and easily performed clinically relevant intervention.

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C01-12

EMAP II provides restoration of heart function in Langendorff ischemia-reperfusion model.

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Endothelial monocyte-activating polypeptide (EMAP) II is a proinflammatory cytokine that is released from apoptotic and hypoxic cells. EMAP II negatively modulates lung neovascularization. Others data suggest that EMAP II stimulates vasodilatation via iNOS activation. However, the role of EMAP II in ischemia-reperfusion is not highlighted. The aim of our study was to examine the effect of EMAP II at heart function recovery in ischemia-reperfusion model. We used male Wistar rats aged 6 month. Recombinant human protein EMAP II in dose of 30 mg/kg was injected in tail vein. After 30 min rats were sacrificed and hearts were perfused by Langendorff preparation. We registered contractile activity, coronary flow and oxygen consumption. Hearts were subjected to 20 min ischemia followed by 40 min of reperfusion. EMAP II prevented myocardial contracture during ischemic period and strongly supported restoration of left ventricular pressure that averaged 90% during all the reperfusion vs 30% in control rats. Notably, there was 25% increase of coronary flow right after reperfusion: we observed reaction of reactive hyperemia after perfusion renovation. As a result oxygen cost of myocardial work did not changed significantly comparing to control where it was 4 time increase

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indicating non-effective oxygen utilization and ROS formation. Thus, EMAP II seems to be perspective tool for development of anti-ischemic approach against contracture and non-effective oxygen utilization by myocardium.

C01-13

Oxidative stress and deficient of nitric oxide synthesis as possible reasons of impaired Frank-Starling low in rat heart due to prolonged lighting

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Prolonged lighting (PL) as a result of sleep deprivation is known to decrease melatonin synthesis which contributes to the cardiovascular control. We hypothesized that PL induce disturbances of oxidative metabolism and NO production at mitochondrial level.

Wistar male rats were exposed to 24h-lighting for 1 and 3 weeks. Hearts were perfused by Langendorff preparation. We studied dependence of left ventricular pressure from volume (PV, Frank-Starling low). Activities of NO synthases as well as generation rate of reactive oxygen species in cardiac mitochondria were measured. PCR analysis for UCP3 expression was used.

PL for 1 week resulted in a pronounced impairment of heart function. The contractile activity (dP/dtmax) as well as coronary flow was decreased. Lowering of dP/dtmin indicated the impairment of diastolic function. Negative impact of PL was aggravated after 3 weeks. The coronary flow was reduced by 43%; the heart rate was slowed by 21%. 1 week of PL for did not affect the shape of PV curve. However, disturbances of heterometric regulation were significant after 3 weeks. The functional changes were accompanied with increased O₂- and *OH (by 4.4- and 4-times respectively) in cardiac mitochondria. The activity of constitutive NO synthase was 3-times decreased. As a result, the level of NO₂- was decreased by 34%. The 5-times increase of inducible NO synthase activity was accompanied with increase in NO₃- content by 19%. Notable downregulation of cardiac UCP3 gene expression (P<0.01) was observed right after 1 week as well as after 3 weeks.

Deficient of NO synthesis and increased reactive oxygen species in cardiac mitochondria might underlie PL-induced heart function disturbances and decreased adaptive abilities of myocardium.

C02: Vascular physiology

C02-1

Impaired expression of voltage-gated K⁺ channel during early phase of diabetes in the rat mesenteric arterial smooth muscle

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This study investigated the alteration of voltage-dependent K⁺ (Kv) channels in mesenteric arterial smooth muscle cells from control (LETO) and diabetic (OLETF) rats during the early and chronic phases of diabetes. In the early phase of diabetes, the amplitude of mesenteric Kv currents induced by depolarizing pulses was greater in OLETF rats than in LETO rats. The contractile response of the mesenteric artery induced by the Kv inhibitor, 4-aminopyridine (4-AP), was also greater in OLETF rats. The expression levels of most Kv subtypes were increased in mesenteric arterial smooth muscle from OLETF rats compared with LETO rats. However, in the chronic phase of diabetes, the Kv current amplitude did not differ between LETO and OLETF rats. In addition, the 4-AP-induced contractile response of the mesenteric artery and the expression of Kv subtypes did not differ between the two groups. In summary, the increased Kv current amplitude and Kv channel-related contractile response

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were attributable to the increase in Kv channel expression during the early phase of diabetes. The increased Kv current amplitude and Kv channel-related contractile response were reversed during the chronic phase of diabetes.

C02-2

The vasodilatory effect of repaglinide, a member of meglitinide class anti-diabetic drugs, via activation of PKG and PKA in aortic smooth muscle

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We investigated the vasorelaxant effect of repaglinide and its related signaling pathways using phenylephrine (Phe)-induced pre-contracted aortic rings. Repaglinide induced vasorelaxation in a concentration-dependent manner. The repaglinide-induced vasorelaxation was not affected by removal of endothelium. Pre-treatment with adenylyl cyclase inhibitor or the PKA inhibitor effectively reduced repaglinide-induced vasorelaxation. Also, pretreatment with guanylyl cyclase inhibitor or the PKG inhibitor effectively inhibited repaglinide-induced vasorelaxation. However, pretreatment with voltage-dependent K⁺ channel inhibitor (4-AP), ATP-sensitive K⁺ channel inhibitor (glibenclamide), big-conductance Ca²⁺-activated K⁺ channel inhibitor (paxilline), and the inwardly rectifying K⁺ channel inhibitor (Ba²⁺) did not affect the vasorelaxant effect of repaglinide. Furthermore, pretreatment with Ca²⁺ inhibitor (nifedipine) and SERCA inhibitor (thapsigargin) also did not affect the vasorelaxant effect of repaglinide. From these results, we concluded that repaglinide induced vasorelaxation by activation of adenylyl cyclase/PKA and guanylyl cyclase/PKG signaling pathway independently of endothelium, K⁺ channels, Ca²⁺ channel and intracellular Ca²⁺ ([Ca²⁺]_i).

C02-3

Inhibitory effect of nortriptyline, a tricyclic antidepressant, on voltage-dependent K⁺ channels in coronary arterial smooth muscle cells

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We demonstrated the effect of nortriptyline, a tricyclic antidepressant drug and serotonin reuptake inhibitor, on voltage-dependent K⁺ (Kv) channels in freshly isolated rabbit coronary arterial smooth muscle cells using a whole-cell patch clamp technique. Nortriptyline inhibited Kv currents in a concentration-dependent manner, with an apparent IC₅₀ value of $2.86 \pm 0.52 \mu\text{M}$ and a Hill coefficient of 0.77 ± 0.1 . Although application of nortriptyline did not change the activation curve, nortriptyline shifted the inactivation current toward a more negative potential. Application of train pulses (1 or 2 Hz) did not change the nortriptyline-induced Kv channel inhibition, suggesting that the effects of nortriptyline were not use-dependent. Preincubation with the Kv1.5 and Kv2.1/2.2 inhibitors, DPO-1 and guangxitoxin did not affect nortriptyline inhibition of Kv channels. From these results, we concluded that nortriptyline inhibited Kv channels in a concentration-dependent and state-independent manner by changing the steady-state inactivation curves independently of serotonin reuptake.

C02-4

The vasorelaxant effect of nateglinide, a member of meglitinide class of anti-diabetic drugs, via activation of voltage-gated K⁺ channels in aortic smooth muscle

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We investigated the vasorelaxant effect of nateglinide using phenylephrine-induced pre-contracted aortic rings. The application of nateglinide induced vasorelaxation in a concentration-dependent manner. Pretreatment with the BKCa channel inhibitor paxilline, Kir channel inhibitor Ba²⁺, and KATP channel inhibitor glibenclamide, did not affect the vasorelaxant effect of nateglinide. However, pretreatment with the Kv channel inhibitor 4-AP, effectively reduced the vasorelaxant effect of nateglinide. Pretreatment with the Ca²⁺ inhibitor nifedipine and the SERCA inhibitor thapsigargin did not change the vasorelaxant effect of nateglinide. Additionally, the vasorelaxant effect of nateglinide was not altered in the presence of an adenylyl cyclase, a protein kinase A, a guanylyl cyclase, or a protein kinase G inhibitor. The vasorelaxant effect of nateglinide was not affected by the elimination of the endothelium. In addition, pretreatment with a nitric oxide synthase inhibitor, L-NAME, and a SKCa channel inhibitor, apamin did not change the vasorelaxant effect of nateglinide. From these results, we concluded that nateglinide induced vasorelaxation via the activation of the Kv channel independent of other K⁺ channels, Ca²⁺ channels, intracellular Ca²⁺ ([Ca²⁺]_i), and the endothelium.

C02-5

The inhibitory effect of dapoxetine, a selective serotonin reuptake inhibitor on voltage-gated K⁺ channels in rabbit coronary arterial smooth muscle cells

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We investigated the inhibitory effect of dapoxetine, a selective serotonin reuptake inhibitor (SSRI), on voltage-dependent K⁺ (Kv) channels using native smooth muscle cells from rabbit coronary arteries. Dapoxetine inhibited Kv channel currents in a concentration-dependent manner, with an IC₅₀ value of $2.68 \pm 0.94 \text{ mM}$ and a slope value (Hill coefficient) of 0.63 ± 0.11 . Application of 10 mM dapoxetine accelerated the rate of inactivation of Kv currents. Although dapoxetine did not modify current activation kinetics, it caused a significant negative shift in the inactivation curves. Application of train step (1 or 2 Hz) progressively increased the inhibitory effect of dapoxetine on Kv channels. In addition, the recovery time constant was extended in its presence, suggesting that the longer recovery time constant from inactivation underlies a use-dependent inhibition of the channel. From these results, we conclude that dapoxetine inhibits Kv channels in a dose-, time-, use-, and state (open)-dependent manner, independent of serotonin reuptake inhibition.

C02-6

Direct inhibition of the class III anti-arrhythmic agent, amiodarone on voltage-dependent K⁺ channels in coronary arterial smooth muscle cells from rabbit

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We examined the inhibitory effect of amiodarone, a class III anti-arrhythmic agent, on voltage-dependent K⁺ (Kv) currents in freshly isolated rabbit coronary arterial smooth muscle cells, using a whole-cell patch clamp technique. Amiodarone inhibited Kv currents in a concentration-dependent manner, with a half-maximal inhibitory concentration (IC₅₀) value of $3.9 \pm 1.44 \mu\text{M}$ and a Hill coefficient of 0.45 ± 0.14 . Amiodarone did not have a significant effect on the steady-state activation of Kv channels, but shifted the inactivation current toward a more negative potential. Application of consecutive pulses progressively augmented the amiodarone-induced Kv channel inhibition. Another class III anti-arrhythmic agent, dofetilide, did not inhibit the Kv current or change the inhibitory effect of amiodarone on Kv channels. Therefore, these results strongly suggest that amiodarone inhibits Kv currents in a concentration- and state-dependent manner.

C02-7

Cav1.2 L-type Ca²⁺ channel form a signal complex with Orai1 and TRPC1 in vascular smooth muscle cells: Role in vascular tone regulation

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Rationale: Voltage-dependent Cav1.2 L-type Ca²⁺ channels (LTCC) are considered the main route for calcium entry in vascular smooth muscle cells (VSMCs). However, independent studies have determined the relevant role of store-operated Ca²⁺ channels (SOCC), formed by Orai1 and TRPC1, in vascular tone regulation.

Objective: We aimed to characterize the crosstalk between Orai1- and TRPC1- dependent SOCC and Cav1.2 LTCC in VSMCs isolated from mice aorta and rat coronary artery.

Methods and results: Serotonin (5-HT) and endothelin-1 (ET-1) evoked significant vasoconstriction and intracellular Ca²⁺ increase in aorta and coronary artery isolated from mice and rat respectively. The induced vasoconstriction was sensitive to the widely used inhibitors of LTCC and SOCC. Immunofluorescence experiments using proximity ligation assay (PLA) determined that both Orai1 and TRPC1 share the same subcellular microdomains and interact with Cav1.2 both in aortic and coronary VSMCs. Interestingly, Orai1 and TRPC1 enhanced their interaction with Cav1.2 upon VSMCs with agonists or upon store depletion with thapsigargin.

Conclusions: Our data suggest that vasoactive agonists promote vessel contraction by co-activation of Cav1.2-dependent LTCC and SOCC channels formed by Orai1 and TRPC1.

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Keywords: Cav1.2; Orai1; TRPC1, Store depletion; Vascular tone regulation.

C02-8

Effects of PCSK9 inhibitor in obese Zucker (fa/fa) rats.

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Proprotein convertase subtilisin/kexin type (PCSK9) is an enzyme that binds to the LDL receptors. If PCSK9 is blocked, more LDLRs are recycled and are presented on the cell surface to remove LDL-particles from the extracellular fluid. Therefore, blocking PCSK9 can lower blood LDL-particle concentrations.

Male obese Zucker (fa/fa) rats and Zucker lean (lean) rats, aged 12 weeks were divided into three groups: Zucker (lean) - control, Zucker (fa/fa) – obese control, Zucker fa/fa – treated with inhibitor of PCSK9 (iPCSK9), n=6 in each group. Inhibitor of PCSK9 was administrated intraperitoneally three times during six weeks (10 mg/kg per one application). Blood pressure was measured by the tail-cuff-plethysmography. Lipid profile was analysed in the plasma and concentration of conjugated dienes (CD, marker of lipid peroxidation) was measured in the kidney and liver. Total nitric oxide synthase (NOS) activity was examined by measuring the rate of conversion from [3H]L-arginine to [3H]L-citrulline in the heart, aorta and kidney. Protein expression of NOS isoforms were determined by Western blot analysis in the same tissues. Administration of iPCSK9 decreased LDL-cholesterol in obese Zucker (fa/fa) rats without affecting other components of lipid profile.

Moreover, iPCSK9 was able to reduce CD concentration in the kidney and liver and increase NOS activity in the aorta, however, without affecting blood pressure yet. In conclusion, the increase of NOS activity, in addition to reducing LDL-cholesterol and lipid peroxidation, may contribute to the beneficial effects of iPCSK9 during hypercholesterolemic conditions.

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C02-9

Protective effects of nanoparticle-loaded renin inhibitor in experimental hypertension

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Introduction: Despite beneficial effects, clinical use of renin inhibitor - aliskiren is limited by short lifetime of this drug. We aimed to determine the effects of nanoparticle-loaded aliskiren, with gradually realized drug, on blood pressure (BP), nitric oxide synthase (NOS) activity, and structural alterations developed due to hypertension.

Materials and methods: 12-week-old male SHR were divided to the untreated group, group treated with powdered aliskiren, or nanoparticle-loaded aliskiren (25mg/kg per day), and nanoparticles only for 3 weeks by gavage. NOS activity including isoforms expressions, and collagen and elastin contents were determined in both heart and aorta. Wall thickness (WT), inner diameter (ID) and cross sectional area (CSA) were determined in the aorta.

Results: At the end of experiment, BP was lower in both powdered aliskiren and nanoparticle-loaded aliskiren groups with more pronounced effect in the second one. Moreover, nanoparticle-loaded aliskiren was able to decrease collagen content (by 11%) and CSA (by 25%) in the aorta in comparison to the powdered aliskiren group, while it had no significant effect on the similar parameters in the heart. There were no significant changes in the elastin content, WT and ID among aliskiren groups and control group. Only nanoparticle-loaded aliskiren increased the activity of NOS in the heart (7.4±0.4 pkat/g) and aorta (9.8±0.5 pkat/g) in comparison to the untreated SHR (5.1±0.3 pkat/g and 7.0±0.5 pkat/g, respectively).

In conclusion, nanoparticle-loaded aliskiren seems to be promising drug in blood vessel protection during hypertensive conditions.

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C02-10

Ranolazine improves vascular sensitivity to insulin in rabbit femoral arteries.

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Questions: Insulin resistance impairs vascular function through an imbalance between vasoconstrictor and vasodilator pathways, and by increasing reactive oxygen species production. Ranolazine, a late Na⁺ current (I_{NaL}) blocker, improves glycemic control and reduces HbA1c in type II diabetic patients. Thus, the purpose of the present study was to evaluate if three different I_{NaL} blockers (GS967, GS6615 and ranolazine) enhance vascular sensitivity to insulin.

Methods: Rabbit femoral artery rings were mounted for isometric tension recording in organ baths. In rings pre-contracted with noradrenaline (10–6 M), cumulative concentration curves of insulin (10–13 to

10–7 M) were constructed in the absence and presence of ranolazine (10-6M), GS967 (3x10-7M) and GS6615 (3x10-7M).

Results: Insulin induced a concentration-dependent relaxant response in rings pre-contracted with noradrenaline (Emax = 43.5 ± 8.3). Vascular relaxation to insulin was blocked by GS967 (Emax = 14.8 ± 16.9) but not by GS6615 (Emax = 50.3 ± 3.9). However, ranolazine enhanced vascular response to insulin (Emax = 64.9 ± 5.6).

Conclusions: Ranolazine enhances vascular relaxant effects induced by insulin in rabbit femoral arteries and this effect seems to be independent of I NaL blockade.

C02-11

Renal vascular Kv7.1 channels – potential targets for renoprotection

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Question: Vascular smooth muscle Kv7 channels, mainly Kv7.4 and Kv7.5, have been shown to contribute to vasoconstriction and vasodilation. However, Kv7.1 channel function is largely unexplored. Thus, this study addressed the hypothesis that Kv7.1 channels contribute to blood flow regulation in the renal vasculature, a vascular bed with high expression of Kv7.1 channels.

Methods: Wistar rat renal segmental arteries and intact kidneys were studied using real-time qPCR, isometric vessel myography and constant-flow organ perfusion.

Results: In renal arteries, Kv7.1 channel mRNA expression was at a similar level compared to Kv7.4 and Kv7.5 channels. The Kv7.1 channel opener R-L3 reduced methoxamine (MX)-induced contraction of isolated vessels. This effect was inhibited by the pan-Kv7 channel blocker XE991 and by HMR1556, a selective Kv7.1 channel blocker. HMR1556 alone was without effect on MX-induced contraction. The Kv7.2-7.5 channel opener retigabine reduced MX-induced contractions. This effect was abolished by XE991 but was not affected by HMR1556, pointing to the absence of Kv7.1-Kv7.x heteromultimeric channels. Neither HMR1556 nor XE991 affected the anti-contraction effect of the cGMP-coupled vasodilator ANP or the cAMP-coupled vasodilator urocortin. In intact kidneys, R-L3 reduced MX-induced increases in perfusion pressure. This effect was inhibited by XE991 and HMR1556. HMR1556 alone was without effect on MX-induced increases in perfusion pressure.

Conclusion: The results show that opening of renal vascular Kv7.1 channels facilitates kidney blood flow without altering vasoconstrictor- and vasodilator-induced blood flow adaptation suggesting that these channels may serve as targets for renoprotection.

C02-12

The Effects of Nifedipine in Heart Injury Induced by Renal Ischemia Reperfusion

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Aim: It has been shown that acute renal injury may lead to dysfunction in far organs like the heart and liver. Ischemia Reperfusion (I/R) borne injury might occur due to the increase and activation of leucocytes, release of reactive oxygen types like hydrogen peroxide (H2O2), and intracellular calcium (Ca2+) increase. In our study, we examined the effects of nifedipine, which is a nonspecific calcium channel antagonist, in heart injury induced by Renal I/R by determining some oxidative stress markers

and the CD38 and cyclic adenosine diphosphate ribose (cADPR) levels that have roles in intracellular calcium regulation.

Methods: 24 Wistar Albino male rats weighing 240-260g were used in our study. 4 groups were formed each of which had 6 animals. The 1st Group was the Control Group (C). In the 2nd Group, the Sham (S) Group; right kidney was dissected. In the 3rd Group (I/R), 1hour ischemia 24hour reperfusion were applied to the left kidney after the right kidney was dissected. In the 4th Group (N), the same surgical procedures were applied as in the 3rd Group, and 4mg/kg nifedipine was administered intraperitoneally before the reperfusion started. The statistical analyses and the results are given as mean±SD. The differences were compared with the Tukey Post Hoc Analysis following the One-Way ANOVA test.

Results: It was observed that applying nifedipine in heart injury occurring due to renal I/R decreased the MDA, SOD, MPO and H2O2 levels in the group which received nifedipine, when compared with the I/R Group, and increased the GSH, Cat, CD38 and cADPR levels; however, these changes are not significant. In the histological examinations; the renal injury increasing with I/R, Caspase-3 expression have decreased with the application of calcium canal antagonists.

Key words: Ischemia reperfusion, Nifedipine, Oxidative Stress, Calcium

This study was supported by Atatürk University SRP (Project no: 2014/146).

C03: Molecular & cellular physiology

C03-1

Iron oxide nanoparticles increase nuclear textural entropy in buccal epithelial cells

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Questions: Although it is known that iron oxide nanoparticles (IONPs) have certain toxic potential in cells and tissues, many issues regarding their interaction with cell nucleus remain unclear. In this study, we demonstrate that certain parameters of nuclear texture of buccal epithelial cells (BECs) change after exposure to IONPs in *in vitro* conditions.

Methods: Human BECs were kept in RPMI-1640 medium at 37°C, with the addition of L-glutamine. The cells were put in special chamber/slides for tissue culture (Lab-Tek, IL, USA) and treated with magnetite, Fe3O4 nanoparticles (spherical shape, diameter 80-100 nanometers, 120 mg/L). Digital micrographs of the cell nuclei (50 nuclei of treated, and 50 of untreated, control cells) were made with Pro-DEM 200 High-Speed color CMOS Chip (Oplenic Optonics, Hangzhou, CN) mounted on optical microscope. Textural analysis was done using Grey level co-occurrence matrix algorithm. For each nucleus, average values of entropy, as well as angular second moment (ASM) and inverse difference moment (IDM), were calculated.

Results: Nuclear textural entropy of BECs significantly increased (p<0.05) after the treatment with iron oxide nanoparticles. Values of angular second moment, on the other hand, did not significantly change. Similarly, no significant change in average values of nuclear inverse difference moment was detected after the treatment (p>0.05).

Conclusions: Our study shows that iron oxide nanoparticles may, in some circumstances, increase the level of textural chaos and disorder of cell nucleus. This is the first study to demonstrate this phenomenon in buccal epithelial cells.

Keywords: Nucleus, Nanomaterial, Texture

C03-2

Gender-dependent expression of miRNA in human colorectal cancer and adjacent colonic tissues

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Key words: miR-21-5p, miR-21-3p, miR-16-5p

Questions: miRNAs are short regulatory non-coding RNA involved in post-transcriptional down-regulation of genes. Mature miRNA consists from a leading and a passenger strand. Co-existence and functionality of both miRNA strands have been reported recently. Deregulated levels of miRNAs were found in a variety of diseases including cancer. We focused on evaluation of the expression of miRNA in tumor and its comparison to the adjacent tissues and plasma levels.

Methods: The tissue and plasma samples from the patients with colorectal cancer were used. The tissue samples were taken from the tumor and proximal (min. 10cm above the tumor) and distal parts (2cm under the tumor) of resected colon. Expression of miR-21-5p, miR-21-3p and miR-16-5p was measured by Real Time PCR. miRNA expression profiling in the plasma, tumor and adjacent tissues was performed to identify changes in miRNA expression.

Results: We observed up-regulation of miR-21-5p, miR-21-3p and miR-16 in the tumor tissue compared to adjacent tissues. Tumors and adjacent tissues showed higher expression of miR-21-5p than miR-21-3p and positive correlation between them. The expression pattern exhibited gender-dependent differences in miRNA levels. miRNAs identified by profiling that showed different expression in the adjacent and cancer tissue were correlated with miRNA plasma levels.

Conclusions: Our findings indicate a gender-dependent expression of miRNA which should be considered as an important factor in generating new prognostic or diagnostic biomarkers.

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C03-3

Nanoparticles at the neurovascular unit: in vitro and in vivo studies to assess the blood-brain barrier permeability and function

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The brain is always confronted with the dilemma of the protection from noxious substances from the blood and the delivery of vital metabolites. Endothelial cells, forming together with other cells the blood-brain barrier (BBB), are known as the "gatekeepers" of this trafficking. It is known that many common drugs cannot cross the BBB in appreciable concentrations, thus decreasing the rate of possible available treatments for many central nervous system (CNS) diseases. In the last decades, nanomedicine has increase its role in developing strategies to deliver drugs to the CNS. In our previous studies we administrated liposomes functionalized with phosphatidic acid and an ApoE-

derived peptide as a potential treatment for Alzheimer's disease (AD): their administration reduced brain beta-amyloid burden and ameliorated impaired memory in AD mice. Furthermore, we evaluated the adaptability of warm microemulsion process for ligand surface modification of solid lipid nanoparticles with ApoE to target the BBB and we investigated how the different administration routes affect their brain bioavailability. The aim of this study is to evaluate the interaction of lipid based nanoparticles (NPs) at the neurovascular unit. In light of our previous results we here assess the NPs interaction with human cerebral microvascular cells (hCMEC/D3) as in vitro BBB model and mice brain neuronal slices by means of patch clamp recordings and simultaneously calcium imaging measurements to follow calcium dynamics transients. Our studies of the NPs impact to the main neurophysiological functions should encourage further applications of NPs based drug delivery strategies for future clinical treatments of CNS diseases.

C03-4

In Vitro Cell Death Discrimination and Screening Method by Simple and Cost-Effective Viability Analysis.

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Questions:

There are two major different kinds of cell death: apoptosis and necrosis. Discrimination is essential for *in vitro* testing of potential drugs or signal transduction modifiers. Viability analysis performed at two different time points post treatment can provide valuable information after death induction because metabolic activity of apoptotic and necrotic cells is different. In this study this was verified by the use of specific caspase and membrane integrity tests.

Methods:

A431 (epidermoid carcinoma) cells were treated with 3 different established chemical apoptosis inducers (actinomycin-D, TBB, RO 31-8220), H₂O₂ and photodynamic treatment (PDT). Viability was measured 2 and 24 hours post treatment using the resazurin assay. Additionally, Caspase-Glo® 3/7 - and membrane integrity assays were conducted to verify apoptosis and necrosis and results of at least three independent experiments were plotted.

Results:

A difference curve between 2 and 24 hours of the resazurin measurements were calculated – the main features of the difference curve are: a positive difference signal indicates apoptosis while an early reduction of the viability signal indicates necrosis. This was confirmed by the results of the caspase and membrane integrity assays.

Conclusion:

Viability analysis at two different time points can provide clear and valuable information with minimal effort of time and financial resources about the concentration or dose ranges of a cytotoxic reagent where apoptotic or necrotic cell death appears.

C03-5

Progesterone and selective membrane progesterone receptor ligands as immunomodulators in human T-lymphocytes

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Progesterone (P4) ensures pregnancy preservation and prevents allogeneic fetal rejection. The mechanism of P4 action on immune cells is not well understood. The effects of progestins are mediated both by nuclear (nPRs) and membrane receptors (mPRs) of the progestin and adipoQ receptor family. The mPR α and mPR β are expressed in T-lymphocytes, whereas the nPRs expression is not detected. Among the synthesized compounds, we identified two selective ligands of mPRs that do not interact with nPRs: 19-hydroxypregn-4-en-20-one (I) and 19-hydroxypregn-3-en-20-one (II). We assessed the effects of these compounds and P4 on the levels of cytokines (IL-2, IL-10, TGF beta and TNF alpha) mRNA in Jurkat cells by means of qRT-PCR. Cells were stimulated with phorbol esters and incubated with hormones (1 to 50 μ M) for 48 hours. 1-10 μ M of any steroid did not significantly influence the cytokines mRNAs levels. 20 μ M P4 and both selective ligands significantly reduced the TNF-alpha mRNA level (by about 30% compared to the control), 50 μ M P4 reduced it even more, whereas I and II little changed their effects. The IL-2 mRNA level declined significantly after exposure to P4 and compound I at both concentrations, but not after the treatment with II. The IL-10 mRNA level significantly increased under the action of 50 μ M P4 and compound II. None of the three steroids caused changes in the TGF-beta mRNA level. Therefore, progestins suppress the levels of pro-inflammatory TNF-alpha and IL-2 mRNA and augment the IL-10 mRNA level through mPRs in T-cells. The differences in effects of compounds I and II may be due to their different affinity for the mPR α and β subtypes, whereas P4 binds to both mPRs.

C03-6

Tolfenamic Acid Induces Apoptosis by Increasing TNF-alpha Gene Expression in rat hepatocellular carcinoma cells

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Question:

Tolfenamic acid (TA) is a non-steroidal anti-inflammatory drug that has shown to have apoptotic effect on many cancer cell lines. The aim of this study is to investigate the effect of TA on mRNA abundance of caspase3, IL-1 β , Nf κ B and TNF-alpha on rat hepatocellular carcinoma (H4IIE) cells.

Method:

We treated H4IIE cells with 10 and 50 μ M dose of TA for 48 hours. After treatment, we collected the cells and just after total RNA were isolated using High Pure RNA Isolation Kit (Roche, Germany). cDNA was synthesized by using the reverse transcriptase cDNA synthesis kit (Roche Nano Lightcycler Roche Diagnostics, Mannheim, Germany). The abundance of caspase-3, IL-1 β , Nf κ B and TNF-alpha mRNA were analyzed using the beta-actin as a reference gene. Measurements were performed using a Roche Nano Lightcycler (Roche Diagnostics, Mannheim, Germany). Data were analyzed by relative quantification method, 2- $\Delta\Delta$ Ct calculation and statistical analyses were made by using one-way ANOVA and posthoc test TUKEY. Differences with P values <0,5 were considered significant.

Results

Caspase3, IL-1 β and Nf κ B mRNA abundance did not change significantly between the groups. However TNF-alpha mRNA abundance increased significantly in the 50 μ M TA group when compared to control.

Conclusions :

The apoptotic effect of TA on cancer cell lines may be related to its transcriptive effect on TNF-alpha.

C03-7

The apoptotic effect of quercetin in human hepatoma cell line HEP3B that Nf-KB pathway suppressed by CAPE

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The present study was designed to investigate the antiproliferative efficiency and genetic mechanisms of the anticancer properties of caffeic acid phenethyl ester (CAPE) and Quercetin, single and combined treated to human hepatocellular carcinoma, Hep3B cell lines. After single and combined treatments with the CAPE and Quercetin, cell viability was monitored using the MTT assay, necroptosis-apoptosis was observed following cell membrane staining by annexinV/propidium-iodide using a TALI cytometer. The gene expression studies belong to NF- κ B and mitochondrial apoptosis pathways were carried out using a qRT-PCR assay. Our results indicated that individually treated both CAPE and Quercetin dramatically reduces cell viability in dose and time depended manner and selectively induces caspase-dependent apoptosis via both CASP8 and CASP3 in Hep3B. NF- κ B inhibition was observed significantly CAPE treated experimental groups. Combined application decreased death cell population and apoptosis rate in all experimental groups and IC50 dose of Quercetin 88 and 34 fold increased in CAPE IC50 and CAPE 2IC50 treated groups, respectively. The result of the present study emphasize that CAPE and Quercetin combination cause antagonistic effect in spite of individually treated CAPE and Quercetin are potential anticancer agent with sufficient antiproliferative effect and spesific apoptotic potential. (This work was supported by Research Fund of the Trakya University. Project Number: 2016/212)

C03-8

Trancriptional regulation of metabolic reactions in breast cancer cells

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We found that the proliferation rate of cancer cell lines from the NCI-60 collection corellated with the expression of all the genes in the human metabolic network (Feizi and Bordel, 2013). The metabolic pathways showing highest correlation with cell proliferation resulted to be both lipid synthesis and degradation. Even if it was previously believed that these processes cannot coexist in the same cells, we hypothesized that this phenomenon could be involved in a shuttle of redox potential from the cytoplasm to the mitochondrion, in which reductive power from cytosolic NADPH is transferred to mitochondrial NADH. By comparing gene expression of cancer cell lines (in the NCI-60 collection) with 8 types of healthy stem cells. We observed that 5 enzymes involved in the degradation of valine, leucine and isoleucine were highly over-expressed. Using public data (Jain *et al.*, 2012) about uptake and secretion rates and a genome-scale human metabolic model we estimated the contribution of these 3 amino-acids to the total cellular ATP supply. We observed that this contribution is as important as the lactic fermentation. We silenced 3 genes (BCAT2, ECHS1, FASN) coding for metabolic enzymes involved in alternative supply of reductive potential for cancer cells. The silencing of these genes decreased significantly the proliferation of breast cancer cell lines (MDA-MB-231, MCF7 and BCC). We found that proliferation of cancer cells is impaired by the transcriptional suppression of enzymes involved in alternative pathways to supply reductive potential to the mitochondrion. This is in agreement with our initial hypothesis and reveals new potential anti-cancer targets.

C03-9

Synthesis of New 1,1,3,3-Tetra(4'-oxy-3-substituted-chalcone)-5,5-diphenylcyclotriphosphazene Derivatives and Investigation of Their Anti-Cancer Activities

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The compounds, so called phosphazene, contain phosphorous-nitrogen double bond. Phosphazenes are the largest class of inorganic macromolecules that cover small molecules through polymers depending on number of the repeating unit, N=PX₂-group, in their structure. In the present study, 1,1,3,3-tetra(4'-oxy-3-fluorochalcone)-5,5-diphenylcyclotriphosphazene (**1a**), 1,1,3,3-tetra(4'-oxy-3-chlorochalcone)-5,5-diphenylcyclotriphosphazene (**1b**) and 1,1,3,3-tetra(4'-oxy-3-bromochalcone)-5,5-diphenylcyclotriphosphazene (**1c**) compounds were obtained from the reactions of 1,1,3,3-tetrachloro-5,5-diphenylcyclotriphosphazene[4] with 4-hydroxy-3-fluorochalcone, 4-hydroxy-3-chlorochalcone and 4-hydroxy-3-bromochalcone respectively. The cytotoxicity effects of compounds **1a-c** against A2780 cancer cell lines at 1, 5, 25, 50 and 100 µM concentrations were determined with using MTT assay method. The anti-cancer properties of 1,1,3,3-Tetra(4'-oxy-3-substituted-chalcone)-5,5-diphenylcyclotriphosphazene derivatives were assessed *in vitro* using A2780 cell line at 1, 5, 25, 50 and 100 µM doses. All the compounds (**1a-c**) were reduced % cell-viability as dose-dependent (p<0.05) towards A2780 cell lines (p<0.05). When the structure activities of the compounds (**1a-c**) were investigated, the -Cl substituted compound (**1b**) against A2780 cell lines were observed more active than the others. In summary, cyclotriphosphazene compounds bearing phenyl and substituted chalcone compounds containing fluoro (**1a**), chloro (**1b**) and bromo (**1c**) groups at meta position were conducted to investigate the effects on A2780 cell line. The results displayed that cyclotriphosphazene derivatives bearing phenyl and substituted chalcone compounds have anticancer activity against A2780 cancer cell lines.

C03-10

Effects of N-(p-aminylcinnaomyl) anthranilic acid (ACA) on various human cancer cell lines

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Cancer is one of the most public health problem in the world. There is currently no therapy to cure of cancer, and hence studies aiming to cancer treatment are ongoing. It has been shown that N-(p-aminylcinnaomyl) anthranilic acid (ACA) inhibit transient receptor potential melastatin-2 (TRPM2). TRPM2 isoforms were shown to be overexpressed in several cancers, including melanoma, breast, and lung cancer. Inhibition or RNA silencing of TRPM2 in prostate cancer cells led to decreased proliferation. This study is done to prove that TRPM2 inhibitor ACA have anticancer activity against human prostate (PC3), over (A2780) and breast cancer (MCF-7) cell lines.

We investigated of ACA in terms of antitumor properties were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on these cancer cell lines (PC-3, A2780 and MCF-7). Different concentrations (1, 5, 25, 50 and 100 µM) of ACA was treated with PC-3, A2780 and MCF-7 cell lines for 24 h. Additionally, we calculated LogIC₅₀ concentration of ACA for PC-3, A2780 and MCF-7 cells, by using a Graphpad prism 6 programs on a computer.

ACA reduced cell viability of PC-3, A2780 and MCF-7 cells (p <0.05). We conclude that TRPM2 is essential for prostate, over and breast cancer cell a proliferation and may be a potential target for the treatment of these cancers. TRPM2 channels pharmacologic inhibition can potentially provide an innovative strategy to eradicate the tumors associated with many types of cancers.

C03-11

Effects of saxagliptin on human prostate and breast cancer: An in vitro study

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Dipeptidyl peptidase (DPP- 4) inhibitors are class of oral antidiabetic drugs. They are used for the treatment of Type 2 Diabetes mellitus. DPP-4 is an enzyme which puts down the action of hormone, incretin. Incretins belong to the group of hypoglycaemic gastrointestinal hormones. Some studies show that DPP-4 inhibitors causes cancer and some study show that they have anticancer property. This study is done to prove that DPP-4 inhibitor (Saxagliptin) have anticancer activity against human prostate (LNCaP) and breast cancer (MCF-7) cell line. We investigated of *saxagliptin* in terms of anticancer properties were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on LNCaP and MCF-7 cells. 1, 5, 25, 50 and 100 µg of concentration of *saxagliptine* was treated with human prostate and breast cancer cell lines for 24 h. Additionally, we calculated LogIC₅₀ concentration of Saxagliptin for LNCaP and MCF-7 cells, by using a Graphpad prism 6 programs on a computer. We observed saxagliptin were reduced % cell-viability as dose-dependent (Expect 1 µg) on LNCaP and A2780 cell lines (p<0.05). This significant anticancer activity of DPP-4 inhibitor Saxagliptine could play a role as a cytotoxic agent in many tumour conditions.

C03-12

The influence of enzyme matrix metalloproteinase-9 and innate immune cells in the pathogenesis of tumor response

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Introduction: Matrix metalloproteinase-9 (MMP-9) or gelatinase B belongs to the family of enzymes that commonly called matrix metalloproteinases. Gelatinase B is synthesized in many cell types, such as: keratinocytes, monocytes, tissue macrophages, polymorphonuclear leukocytes and many types of tumor cells. The intensity of release of active enzyme is dependent on the amount of the enzymes stored in granules of these cells. Statistically significant expression of matrix metalloproteinase-9 is demonstrated in various cases of lung cancer and in inflammatory conditions, where is involved in many processes of proliferation, differentiation and migration of mast cells.

Patients and methods: we hypothesized that circulating levels of MMP-9 were abnormal in patients with colorectal cancer and these levels were compared with those in matched controls. The method of enzyme immunoassay (ELISA) was used to determine enzyme expression of matrix metalloproteinase-9 (MMP-9).

Results: our results showed a large increase in the enzyme MMP-9 in the urine and the percentage of the cells of innate immunity (NKT cells and regulatory T cells) in peripheral blood of patients with colorectal cancer with significant correlation of these values. The increased levels of these cells, as well as, the concentration of MMP 9 correlate with the stage of tumor.

New possibilities for better monitoring the disease are very important. We verified the activity of MMPs in the urine of patients with diagnosed colorectal cancer in different stages of disease.

Acknowledgement: This work was supported by grants from University of Rijeka (13.06.1.1.14 and 13.06.1.1.15).

C03-13

Investigation of the effects of a sulfite molecule on human neuroblastoma cells via a novel oncogene URG4/URGCP

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Aim: The aim of this study is to determine the anticancer effect of sulfite on SH-SY5Y neuroblastoma cells in vitro conditions and elucidate underlying molecular mechanism of sulfite and explore its therapeutic activity.

Main methods: In this study, cytotoxic effects of sulfite in SH-SY5Y cells were detected over time in a dose dependent manner with the IC50 doses ranging from 0.5 to 10 mM. Genotoxic effect of sulfite was shown by comet assay. IC50 doses in the SH-SY5Y cells were detected as 5 mM. Expression profiles of the target genes related to apoptosis and cell cycle control were determined by quantitative RT-PCR. Protein changes were determined by western blot analysis.

Key findings: URG4/URGCP, CCND1, CCND2, CDK4, CDK6, E2F4 and BCL-2 gene expression levels were significantly reduced and RB1, TP53, BAX, BID, CASP2, CASP3, CASP9 and DIABLO gene expressions were significantly increased in dose group cells. The mechanism of this result may be related to sulfite dependent inhibition of cell cycle at the G1 phase by down-regulating URG4/URGCP or CCND1, CDK4, CDK6 gene expression and stimulating apoptosis via the intrinsic pathway. Sulfite suppressed invasion and colony formation in SH-SY5Y cell line using Matrigel invasion chamber and colony formation assay, respectively.

Significance: It is thought that sulfite demonstrates anticarcinogenesis activity by affecting cell cycle arrest, apoptosis, invasion, and colony formation on SH-SY5Y cells. Sulfite may be an effective agent for treatment of neuroblastoma as a single agent or in combination with other agents.

C04: Endocrine, neuroendocrine and metabolism

C04-1

The Effects of Thyroid Dysfunction on Nesfatin-1 Levels in Rats

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Nesfatin-1 was recently discovered anorexigenic peptide in the brain and it has inhibitor effects on food intake as dose-dependently. The aim of this study was to determine the effects of thyroid dysfunction on nesfatin-1 levels in rat. The study was performed on the 40 male Sprague-Dawley rats. Experiment groups were designed as follows.

1. Control group (n=8): Animals in this group were killed without any application and blood samples were collected for hormone analysis.

2. Hypothyroidism group (n=8): To induce hypothyroidism PTU was applied by intraperitoneal as 10 mg/kg/day for 3 weeks.

3. Hypothyroidism + Thyroxine group (n=8): Previously animals were made with hypothyroidism by 2 weeks PTU application and following 1 week L-thyroxine was given by intraperitoneal as 1.5 mg/kg/day.
4. Hyperthyroidism group (n=8): Rats were made with hyperthyroidism by 3 weeks L-thyroxine supplementation (0.3 mg/kg/day).
5. Hyperthyroidism +PTU group (n=8): Animals were made hyperthyroidism by L-thyroxine as groups 4, then 1 week PTU was applied to correct hyperthyroidism.

The end of supplementaion animals were sacrificed and blood samples were collected for FT3, FT4 and nesfatin-1 analysis. FT3 ve FT4 levels were reduced significantly in hypothyroidism while increased in hyperthyroidism (P<0.001). Hypothyroidism caused to increase in nesfatin-1 (P<0.001). In experimental hyperthyroidism nesfatin-1 levels increased significantly (P<0.001). Although hypothyroidism increased nesfatin 1 level (P<0.001), hyperthyroidism increased nesfatin 1 levels much more than hypothyroidism (P<0.001).

The results of study show that experimental hypothyroidism and hyperthyroidism lead to significantly change to nesfatin-1 levels. However, changed hormone levels were normalised after correction of thyroid function.

C04-2

Experimental Hypothyroidism and Hyperthyroidism Have Similar Effects on Cardiac Irisin Levels in Rats

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Irisin is a newly discovered myokine and adipokine that increases total body energy expenditure. This effect is considered to be achieved by converting the white fat tissue to brown fat tissue. The purpose of this study was to determine the effect of experimental hypothyroidism and hyperthyroidism on the levels of irisin in heart tissue in rats. The study was performed on the 40 male Sprague-Dawley rats. Experimental groups were designed as; Control, Hypothyroidism, Hypothyroidism+Thyroxine, Hyperthyroidism and Hyperthyroidism +PTU. Following 3 weeks experimental period, irisin levels were determined in heart tissues. Irisin levels in the experimental groups were respectively, 32.50 ± 6.55 ng / g tissue; 40.53 ± 4.69 ng / g tissue; 33.31 ± 6.33 ng / g tissue; 47.52 ± 11.70 ng / g tissue; 34.13 ± 08.07 ng / g tissue. Hypothyroidism group values of irisin are higher than control group but lower than hyperthyroidism group. The hyperthyroidism group has the highest levels of cardiac irisin.

The results of the study show that the experimental hypo and hyperthyroidism increase the heart irisin levels but the increase in the hyperthyroidism group is much higher than hypothyroidism group.

C04-3

EFFECT OF BISPHENOL A AND DIETHYLHEXYL PHTHALATE ON PROGESTERONE SECRETION BY LUTEAL CELLS

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Questions: This study investigates the effects of bisphenol A (BPA) and diethylhexyl phthalate (DEHP) as endocrine disrupting compounds (EDCs), on progesterone secretion by bovine luteal cells.

Methods: Luteal cells were isolated from the midluteal ovaries of healthy cows and distributed in 6 well plate wells as 3x10⁴ cells/2 mL culture medium. Cells were incubated for 24 hours to adhere to the bottom of the plate. Then, the incubation was continued by replacing the media with different

concentrations of BPA (1, 3, 10 and 30 µM) and DEHP (1, 3, 10 and 30 µM). Media collected at hour 96 and hour 120 were stored at -20 °C until the progesterone measurement.

Results: At hour 96 of incubation, it was observed that all doses of BPA and 3 and 30 µM doses of DEHP significantly reduced ($p < 0.05$) the progesterone level as compared to the control. Also, progesterone synthesis was decreased ($p < 0.05$) in 3, 10 and 30 µM doses of BPA and in all doses of DEHP as compared to the control at hour 120 of incubation. Progesterone levels decreased ($p < 0.05$) in control and the highest dose of BPA (30 µM) and in all doses of DEHP including control depending on the length of the incubation.

Conclusions: The results of this study showed that BPA and DEHP disrupted luteal steroidogenesis by suppressing progesterone synthesis depending on the dosage and incubation time. It is thought that this effect can cause infertility problems in cows by disturbing the hormonal balance of the ovary. It should be necessary to restrict the use of these chemicals and spread in nature.

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Key words: BPA, DEHP, Luteal cell

C04-4 c-AMP DURING OESTRUS CYCLE IN RATS

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Introduction. The mammalian pineal gland is under adrenergic control. The physiological oscillations of gonadal steroids could strongly affect the melatonin synthesis and secretion by acting on the pre- and postsynaptic levels and by modulation of the target cells replay. The aim of this study was to determine the basal levels of cAMP in the pineal gland during the various phases of oestrus cycle in normothensive (NTR), Wistar rats and spontaneously hypertensive (SHR) Okamoto and Aoki rats and to describe the histological finding of the pineal gland tissues.

Methods. Two hundred female mature rats (100NTR and 100SHR) were investigated. They were divided in 4 groups according to the phases of the oestrus cycle (diestrus, proestrus, estrus and metaestrus). The phase of oestrus cycle has been determined by microscopic analysis of the vaginal smears. The level of cAMP (RIA) in the pineal gland was the parameter of its intracellular activity. The pineal gland tissues were stained on HaEo.

Results. In SHR there is a slight shortening of the oestrus cycle. In NTR there was an increase of the cAMP level from proestrus to metaestrus, contrary to the dramatic decrease in SHR. Histological findings of pineal glands showed the presence of many changed pinealocytes with picnotic nucleuses, while the neuroepithelial cells, in the upper parts of the glands, were separated in gland-like islets. There was a normal pineal histology in NTR.

Conclusion. This study indicated significant neurohormonal differences between NTR and SHR. The changed adrenal activity in SHR correlated with histological findings in the pineal gland.

Key words: c-AMP; oestrus cycle; rats

C04-5

Effect of Zinc and Melatonin on Oxidative Stress and Serum Inhibin-B Levels in a Rat Testicular Torsion-Detorsion Model

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The present study was aimed to examine the effects of 3-week zinc and melatonin administration on testicular tissue injury caused by unilateral testicular torsion-detorsion in rats and their serum Inhibin-B levels.

The study was performed on 60 Wistar Albino type adult male rats. The animals were allocated to 6 groups. 1. Control; 2. Sham; 3. Ischemia-Reperfusion; 4. Zinc + Ischemia-Reperfusion; 5. Melatonin + Ischemia-Reperfusion; 6. Zinc + Melatonin + Ischemia-Reperfusion. Zinc and melatonin were administered before ischemia-reperfusion at doses of 5 and 3 mg/kg respectively through the intraperitoneal route for a period of 3 weeks. Blood and testicular tissue samples were collected to analyze erythrocyte and tissue GSH and plasma and tissue MDA, Inhibin-B levels.

The highest erythrocyte and testis GSH values were found in zinc, melatonin, and zinc + melatonin. Torsion-detorsion group had significantly lower erythrocyte GSH and higher MDA values. Serum inhibin-B and spermatogenic activity levels in the torsion-detorsion group were also significantly lower than those in the other groups. However, zinc, melatonin and melatonin + zinc supplemented groups have higher inhibin-B and spermatogenic activity.

The results of the study show that zinc, melatonin and melatonin + zinc administration partially restores the increased oxidative stress, as well as the reduced inhibin-B and spermatogenic activity levels in testis ischemia-reperfusion in rats.

Suppressed inhibin-B levels in the testicular tissue may be a marker of oxidative stress.

C04-6

Combined Effects of Flavonoid Fisetin and Endocrine Disruptor Bisphenol A on Progesterone Production by Granulosa Cells

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Proper function of the ovaries is essential for maintaining female reproductive health. Currently, many industrial agents termed endocrine disruptors (EDs) are linked to the increased fertility disorders. In contrast, health protective effects of phytochemicals, e.g. flavonoids, are assumed. The possibility to address ED-involved reproductive dysfunctions by natural compounds would be desirable; yet, the data on such mutual effects are limited. We examined the ability of the flavonoid fisetin (Fis) to modulate effects of a ubiquitous ED Bisphenol A (BPA), on the function of ovarian granulosa cells (GCs). Porcine GCs were treated with different concentrations of BPA, Fis, or their combinations. Progesterone (P4) production by GCs was determined by radioimmunoanalysis, viability of GCs was assessed by MTT assay, expression of relevant genes was determined by real-time PCR. BPA inhibited P4 production by GCs at the highest concentration. Fis reduced P4 production dose-dependently, and in this manner, Fis further altered P4 production when added to BPA-treated GCs. This effect could partly result from the decreased viability of GCs via up-regulation of *CASP3*. Nevertheless, the combined action of Fis and BPA significantly down-regulated steroidogenesis-related enzymes (*STAR*, *CYP11A1*, *HSD3B*) what seems to contribute to P4 synthesis inhibition most. Our results suggest that Fis might interfere with ovarian steroidogenesis, and has no beneficial effects in terms of restoring P4 synthesis altered by BPA. Considering the constant human exposure to myriad of environmental and dietary chemicals, physiological effects of such mixtures need to be investigated. *Acknowledgements: The work was supported by the VEGA project 2/0198/15.*

C04-7

Determining the Correlation between Thyroid Hormone and Adropine Hormone in Rats which received Cold Restraint Stress

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Aim: The Hypothalamic hypophyseal thyroid axis has various roles in regulation of the body temperature, protection of metabolic speed and many other physiological processes. It is already known that stress is related with neurochemical and hormonal changes including the changes in the thyroid hormone levels. In this study, we investigated the correlation between the adropine hormone whose expression is defined in central neural system and encoded by the gene that is related with energy homeostasis and the thyroid hormones in rats which received Cold Restraint Stress (CRS).

Method: 16 Wistar Albino male rats were used in this study. Two groups were formed in the study as Control and CRS Groups (n=8). No applications were made to the rats in Control Group. CRS application was made as follows: The rats were placed in a restraining chamber. The tails of the rats were fixed to the edge of the chamber. Sufficient respiration was ensured with big holes. The rats in the CRS group were subjected to CRS in groups of 4 at 4°C for 4 hours. The animals were sacrificed at the end of the study and their blood was collected. TSH, T3, T4 and adropine hormone levels in the plasma samples were determined with ELISA Method. The Spearman Correlation Analysis was used for statistical analysis.

Results: There were no correlations between the hormones in the Control group. In the groups which received CRS, no correlations were determined between the TSH and T4 hormones of the adropine hormone, and a negative correlation was detected with T3 hormone [$r(8)=-0,922$; $p<0.01$].

Conclusion: As a result of the CRS application, we showed that T3 level decreased and adropine level increased.

This study was supported by Atatürk University SRP (Project No: 2015/281, 2015/39)

C04-8

Thyroid axis functioning is associated with health status and shorter survival of brain tumor patients

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QUESTIONS. To investigate if thyroid hormone levels are associated with health status and prognosis of brain tumor patients.

METHODS: Two-hundred and thirty brain tumor patients (70% women) before surgery were evaluated for cognitive (Mini mental State Examination; MMSE) and functional (Barthel index; BI) status, and thyroid function profile. The Low tri-iodothyronine (T3) syndrome was defined as T3 concentration below the reference range. Unfavorable hospital discharge outcomes were determined as Glasgow outcome scale score of ≤ 3 . Follow-up continued until November, 2015.

RESULTS. Seventy-four percent of patients had Low T3 syndrome. Lower total T3 concentrations were associated with lower MMSE ($p=.013$) and BI ($p=.023$) scores independent of age, gender and histological diagnosis. Preoperative Low T3 syndrome increased risk for unfavorable discharge outcomes adjusting for age, gender and histological diagnosis ($OR=2.944$, 95%CI [1.314-6.597], $p=.009$). In all patients, lower total ($p=.038$) and free ($p=.014$) T3 concentrations were associated with greater mortality adjusting for age, gender, extent of resection, adjuvant treatment and histological diagnosis. The Low T3 syndrome was associated with greater 5-year mortality for glioma patients

($HR=2.197$; 95%CI [1.160-4.163], $p=.016$) and with shorter survival (249 [260] vs. 352 [399] days; $p=.029$) of high grade glioma patients independent of age, gender, extent of resection and adjuvant treatment.

CONCLUSIONS. Reduction of T3 concentrations is common in brain tumor patients and is associated with worse health status and worse discharge outcomes.

C04-9

Pregnancy induced changes in innate immunity during autoimmune thireoid disease

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Aim: Autoimmune thyroid dysfunction (ATD), which comprises two main clinical entities: Graves' disease and Hashimoto thyroiditis, often affect women of reproductive age. In a healthy pregnancy predominant is Th2 over Th1 immunity, which explains the improvement of autoimmune disease during pregnancy, while after birth due to changes in Th1/Th2 ratios often leads to deterioration of ATD. NKT and Tregs seem to play an important part in mediating maternal tolerance to fetus. Although many researches have been done in the field of thyroid autoimmunity, very few studies investigated the role of innate immunity in AITD during human pregnancy and in the postpartal period

Results: We investigated the presence of ATD in pregnant and postpartum period in women with hormonal status determination, the titer of thyroid antibodies and auto antibodies and compared them with healthy pregnant women and subjects postpartum and not pregnant women. After intracellular and surface staining using flow cytometry, we analyzed the phenotype and cytolytic potential of isolated peripheral blood mononuclear cells of pregnant women and postpartum women, and not pregnant women.

Conclusion: pregnancy and the postpartum period influence the function of the thyroid gland. In the presence of thyroid autoimmunity changes are more pronounced, especially postpartum. Apart from pregnancy and postpartum period influence the course of ATD and thyroid autoimmunity affects thyroid function in pregnancy and the postpartum period.

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C04-10

Comparison of extraction methods for measurement of hair cortisol

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Introduction: Hair cortisol measurements provide an important tool for the assessment of long-term stress in humans. However, extraction methods differ between the studies. Therefore, the aim of the current study was to compare the effect of different extraction methods on hair cortisol concentration.

Materials and methods: Washed or unwashed male hair samples were cut into small pieces by a scissor or ground by using liquid nitrogen. Afterwards, one set of samples were incubated for 16 or 36 h at 52 degrees Celcius. Another set was sonicated 30 min, 1.0 h or 2 h at 35 degrees Celcius. A different set was both sonicated and incubated at 52 degrees for 16 h. For control comparisons, one set of samples were kept under room temperature for 90 h without using ultrasound. Following these

extraction protocols, all samples were centrifuged 10000 rpm for 30 min and the supernatant was used for cortisol analyses. All supernatants were evaporated, re-suspended in phosphate buffered saline, vortexed and analyzed by a validated ELISA method.

Result: Variations were observed between the hair cortisol concentrations of different individuals. All extraction protocols resulted in cortisol concentrations that were within pg/mg range and readable with the ELISA test used.

Conclusions: Extraction methods appear to affect hair cortisol concentration. However, as all methods used resulted in levels within acceptable range, it might be recommended to use any of the extraction methods used in the current study.

C04-11

Lengths of the menstrual cycle and menstruation are positively correlated with general tiredness in long-term entrained students

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Introduction: Long-term participation in sports might affect lengths of the menstrual cycle and menstruation in young females. Therefore, the aim of the current study was to find out the correlations between parameters of stress and lengths of the menstrual cycles and menstruation in female students actively participating in sports.

Materials and methods: Female students (n=193) actively participating in sports as a part of their education were studied in the current study. Lengths of menstrual cycle and menstruation in the last three cycles were recorded together with 40-item state and trait anxiety scales. Statistical analyses were carried out by using Pearson's correlations.

Results: Lengths of the menstrual cycle and menstruation were 29.3±0.3 and 5.6±0.1 days, respectively. Length of the menstrual cycle was positively correlated with the length of menstruation (R-sq=0.905; P<0.001). There were positive linear correlations between the scores of general tiredness and that of length of the menstruation (R-sq=0.213; P=0.003) and length of the menstruation (R-sq=0.172; P=0.017).

Conclusions: The results of the current study suggest that (1) the lengths of the menstrual cycles and menstruation were within normal range in long-term entrained female student and that (2) increased menstruation increases level of tiredness. The latter might be associated with increased iron loss by prolonged menstruation.

C05: Sports & exercise physiology

C05-1

The Effect of Resveratrol Supplementation on Element Metabolism in Bone Tissue of Rats with Acute Swimming Exercise

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The aim of the present study was to investigate how affect resveratrol supplementation element metabolism in bone tissue of rats with acute swimming exercise

Animals were divided to 4 groups. 1.Control, 2.Swimming; Rats were fed by standart rat food and exposed to 30 minutes swimming exercise at the end of study. 3.Resveratrol: Animals were fed by

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Poster Session C

standard rat food plus resveratrol for 4 weeks (10 mg/kg/day) by drinking water. 4.Resveratrol + Swimming: Animals were fed by standard rat food plus resveratrol (10 mg/kg/day) by drinking water for 4 weeks and exposed to swimming exercise for 30 minutes at the end of study.

The end of 4 weeks study, bone tissue samples analyzed at the Atomic Emission (mg/L).

The findings of the study show that resveratrol supplementation increased zinc, calcium, phosphorus, magnesium and boron levels in bone tissue independently from exercise.

One of the main findings of study was that resveratrol supplementation has protective and/or regulator activity in bone tissue indepedently from exercise and may be consider.

C05-2

Cardiorespiratory fitness effect on cerebral oxygenation in chronic obstructive pulmonary patients

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Introduction:

Low cerebral oxygenation is associated with cognitive decline and may lead to higher risk of neurodegenerative disease. However, positive impact of physical activity on brain health is recognized (Dupuy et al, 2015). Chronic obstructive pulmonary disease (COPD) is often associated with brain functioning deregulation and lower cerebral oxygenation than healthy during exercise (Vogziatis et al, 2014). The aim of this study was to assess the influence of cardiorespiratory fitness on cerebral oxygenation during exercise in COPD patients.

Material and Method:

Forty-one COPD patients (64.6 ± 9.8 years), classified GOLD 2-3, VEMS (%pred) 57.3 ± 14.0 were included in the study. All performed a maximal incremental test on ergocycle (10W/min). During the test, cerebral oxygenation (NIRS sytem, Artinis MS NL) and pulmonary gaz exchanges (Ergocard, Medisoft, Dinant, B) were recorded. The NIRS optode was put on the left frontal lobe. Tissue Saturation Index, total haemoglobin, deoxyhaemoglobin and oxyhaemoglobin (TSI, tHb, HHb and HbO2 respectively) were measured by a NIRS system. Correlations were performed using Pearson tests.

Results:

Mean VO2peak were 16.2 ± 4.5ml/min/Kg and power peak were 77.0 ± 19.8W. Two positive correlations were found: 1) VO2peak vs tHbpeak (r=0.40, p<0.05) and 2) VO2peak vs HbO2peak (r=0.42, p<0.05). Neither HHbpeak nor TSI were correlated with VO2peak.

Discussion Conclusion:

This study confirms the link, in COPD patients, between cerebral oxygenation and cardiorespiratory fitness. The patients who presented a higher VO2peak also had a higher cerebral oxygenation. As cerebral oxygenation is a major feature of brain functioning and health, COPD patients should be encouraged to be active.

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Poster Session C

C05-3

Effects of Acute Exhaustive Exercise on Oxidant and Antioxidant System Parameters in Rats with Streptozotocin Induced Diabetes Mellitus

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Questions: Oxidative stress (OS) is responsible for both the development and complications of diabetes mellitus (DM). Acute exercises are a well known source of OS. DM patients may experience strenuous physical activity conditions in daily life. Therefore we investigated how oxidant-antioxidant system responds to acute exhaustive exercise (AEE) in an experimental DM model.

Materials and Methods: 16 Sprague-Dawley rats were randomly divided into two groups: control (n = 8) and DM group (n = 8). Streptozotocin (STZ) (65 mg/kg intraperitoneal injection) was administered to DM group rats. Three days after the administration, blood glucose levels were evaluated and rats with levels above 200 mg/dL was considered as DM. Serum was separated from blood samples immediately after AEE. 8-OH-deoxyguanosine, 3-nitrotyrosine, lipid hydroperoxide, protein carbonyl, CuZnSOD, glutathione and glutathione peroxidase assays were performed by ELISA method.

Results: 3-nitrotyrosine (p = 0.001) and protein carbonyl (p = 0.013) were significantly higher and 8-OH-deoxyguanosine was significantly lower in the DM group compared to control group (p = 0.001). There was no significant difference in lipid hydroperoxide levels between the groups. When antioxidant parameters compared, there was no significant difference in Cu-Zn-SOD but glutathione (p = 0.013) and glutathione peroxidase (p = 0.001) levels were significantly higher in the DM group.

Conclusion: Antioxidant system showed an increase in response to AEE induced OS in DM group. Although this increase may protect against DNA damage, it could not prevent protein oxidation.

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C05-4

Diving response after a one-week diet and overnight fasting

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Questions: We hypothesized that overnight fasting after a short dietary period could allow performing breath-hold diving with no restraint for diaphragm excursion and blood shift and without any increase of metabolism, and in turn improve the diving response. **Methods:** During two separate sessions, 8 divers carried out two trials: (A) a 30-metre depth dive, three hours after a normal breakfast and (B) a dive to the same depth, but after following a diet and fasting overnight. Each test consisted of 3 apnea phases: descent, static and ascent. An impedance cardiograph, housed in an underwater torch, provided data on trans-thoracic fluid index (TFI), stroke volume (SV), heart rate (HR) and cardiac output (CO). Mean blood pressure (MBP), arterial O₂ saturation (SaO₂), blood glucose (Glu) and blood lactate (BLa) were also collected. **Results:** In condition B, duration of the static phase of the dive was longer than A (37.8±7.4 vs. 27.3±8.4 s respectively, P<0.05). In static phases, mean Δ SV value (difference between basal and nadir values) during fasting was lower than breakfast one (-2.6±5.1 vs. 5.7±7.6 ml, P<0.05). Since mean Δ HR values were equally decreased in both metabolic conditions, mean Δ CO value during static after fasting was lower than the same phase after breakfast (-0.4±0.5 vs. 0.4±0.5 L·min⁻¹ respectively, P<0.05). At emersion, despite the greater duration of dives during fasting, SaO₂ was higher than A (92.0±2.7 vs. 89.4±2.9 % respectively, P<0.05) and BLa was lower in the same comparison (4.2±0.7 vs. 5.3±1.1 mmol·L⁻¹, P<0.05). **Conclusions:** An adequate balance between metabolic and splanchnic status may improve the diving response during a dive at a depth of 30m, in safe conditions for the athletes health.

C05-5

Relationship between regular exercise-induced cardiac hypertrophy and microRNA

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Questions: Exercise-induced cardiac hypertrophy (CH) is a type of physiologic CH. MicroRNAs (miRNAs) are involved in cardiac development, hypertrophy and angiogenesis. We investigated the role of miRNAs in regular exercise-induced cardiac hypertrophy.

Material & Methods: Male Sprague Dawley rats were divided into Exercise-group (EG, n=9) and Control-group (CG, n=6). Swimming sessions began with 60 min/5 days/8 weeks and continued with on the 9th week 2x/day, and on the 10th week 3x/day. Dimensions of the left ventricle and myocardial wall thickness were measured by transthoracic echocardiography (TTE). miRNAs were assessed by miRNA microarray and confirmed by real time PCR. Apoptosis, necrosis, and cell proliferation were evaluated histologically.

Results: In TTE left ventricular mass, end-diastolic diameter of the left ventricle and end-systolic diameter of the left ventricle, the thickness of the posterior wall and interventricular septum thickness were found to be increased significantly in EG. Genetic analysis showed upregulation of the expression of miR-132-3p and miR-194-5p and downregulation of the expression of miR-290 in EG. In histological analysis although there was necrosis in cardiac tissue, there were no cell proliferation and apoptosis in TG.

Conclusions: We suggest that in exercise-induced CH, heart may be protected from fibrosis due to changes in the expression of the genes miR-132-3p and miR-290. Increase in expression of miR-132-3p in blood may be a predictor of fibrosis. Also an increase in the expression of miR-194-5p may be an indicator of exercise induced CH. However these findings should be validated with further research.

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C05-6

Prognostic Value of 6-Minute Walk Test in children with congenital anemia

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Introduction: Anemia is the main cause of dyspnea, muscle deconditioning and than exercise intolerance. The 6-min walk Test (6MWT) is a simple and safe test that usually used to evaluate global response to submaximal exercise and which have reliable prognostic value.

Aim: The aim of this study was to evaluate the relationship between 6-Minute Walk Test (6MWT) distance, and respectively Muscle Mass (MM) and hemoglobin levels in a group of children with beta thalassemia or Sickle Cell Disease (SCD).

Methods: Our study included 24 children who regularly followed up in a pediatric consultation. This population is composed by 11 beta-thalassemia and 13 SCD patients with sex ratio equal to 0.41. We

performed for each patient a blood sampling test for hemoglobin measurement, bio-electrical impedance for MM measurement and 6MWT for distance walked measurement.

Results: The averages of age, hemoglobin level and MM were respectively 12 ± 3.4 years, 7.9 ± 0.7 g/dl and 49.5 ± 8.2 %. Contrasting with normal MM, data revealed a severe reduction of average walking distance expressed as a percentage of the theoretical value calculated according to the Troosters equation ($41 \pm 13.6\%$). The, 6MWT distance was strongly correlated with Hemoglobin levels ($p < 0.05$) but no significant correlation between MM and anemia was found.

Conclusion: This study highlights an important limitation of 6MWT distance which correlated to anemia severity and reflected poor prognosis in patients with congenital anemia. These alarming data could be seriously taken into consideration by health authorities to better management of anemia.

C05-7

Case Study of a Male Ocean Racer: body composition and nutritional intake during world solo sailing record attempt

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The Italian Sailor Gaetano Mura tried to beat the world record of non-stop solo globe circumnavigation in Class 40 (record held by the Chinese sailor Guo Chuan) in October 2016, without stopovers or assistance, a physically demanding challenge for which appropriate nutrition should be crucial to maintain energy balance, ensure optimum performance and to maintain optimal body composition. His daily recommended nutritional intake (NI) during the voyage, detected with sensewear armband during preparation, that had to be about 130 days, was 3000 Kcal/day with carbohydrate and protein intake goals of 335 g/day and 100g/day, respectively. Unfortunately he had to stop in Australia for a technical failure after 70 days of navigation and did not continue the challenge. Fat mass (FM) and fat-free mass (FFM) were assessed, by means of plicometry, during his preparation (4 months before the race-T0) pre- (15 days-T1) and postrace (10 days-T2), and body mass was also measured. Measurements enlightened that during the voyage the racer did not lost body mass ($\Delta T0-T1$ 2.1% $\Delta T0-T2$ 2.1%) and his body composition remained similar pre and after the race (FFM $\Delta T0-T1$ 1.8% $\Delta T0-T2$ 2.2%; FM $\Delta T0-T1$ 5.3% $\Delta T0-T2$ 1.8%), moreover, he reported good sensations about his nutrition on board. This intervention demonstrates that racers' nutrition strategy can be improved to facilitate meeting more optimal NI goals for performance and health. And shows that further studies can provide important information for optimizing nutritional strategies for ocean racing.

C05-8

VITAMIN C SUPPLEMENTATION MITIGATES DIVING-INDUCED CHANGES IN CEREBRAL CIRCULATION

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SCUBA related decrements may be associated with impairment in the cerebral circulation and we investigated if it could be prevented by oral antioxidant supplementation. Fourteen divers performed a single SCUBA dive and participated in a follow-up study involving 60% oxygen breathing at ambient pressure. Prior to both studies, participants ingested ascorbic acid (2g) and two weeks later placebo daily for six days. After two weeks of study interruption subjects switched groups and received the opposite pre-treatment. Transcranial Doppler ultrasound was used to measure cerebral blood velocities (CBV) for 10 minutes pre-dive and through 90 minutes post-dive. CBV measures were analyzed by two-way repeated measure ANOVA for the two studies (time – pre/30/60/90min, trial –

placebo/VitC). Velocity in the middle cerebral artery (MCAv) increased 30 minutes post-dive from 60.08 ± 8.99 cm/s to 63.14 ± 10.01 cm/s and in the posterior cerebral artery PCAv from 40.05 ± 6.14 cm/s to 43.94 ± 5.79 cm/s, respectively ($p < 0.05$). Thirty minutes post-dive MCAv and PCAv were significantly higher in the placebo trial compared to the Vitamin C trial ($p < 0.05$). There were no main effects of time or trial in the oxygen breathing study. Transient elevations of CBV were present only 30 minutes post-dive and were mitigated by vitamin C; but hyperoxia as a diving related stress factor showed no independent influence on CBV and did not explain diving related changes in the cerebral vasculature.

C05-9

The Investigation of the Effects of Mask and Mouthpiece Types with Different Dead Space Volumes on the Energy Expenditure Measurements

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The indirect calorimetry is widely used technique for evaluating the energy expenditure (EE) of the subjects. During respiration, gases can be collected by using different types of devices. We aimed to investigate the effect of the miscalculation of dead space volume (DSV) of two masks and mouthpiece (actual, 25% less and 25% more of DSV) during resting and walking EE measurements, and also to compare comfortableness of these apparatuses.

There was no significant agreement among the masks and mouthpiece in terms of resting EE data (ICC= 0.65). Although ICC for the actual, 25% less and 25% more of the DSV of 1st mask was moderate, the resting EE data for the three DSVs of 2nd mask were not significantly agreed (ICC=0.68). There was an excellent agreement among the resting EE measurements of the three DSVs of the mouthpiece (ICC=0.91). Among two masks and mouthpiece used for walking EE measurement, ICC was moderate. Although ICC for 1st mask was good, ICC for 2nd mask was excellent for all the three DSVs during walking. There was a moderate agreement among the measurements with mouthpiece (ICC=0.81).

We suggested that same apparatus should be used for whole study for the resting EE measurement. Although the 25% error in DSV for the 1st mask and mouthpiece may not have a significant effect on the resting EE data, the DSV of the 2nd mask needs to be correctly entered to the program, which was the most comfortable one. The 25% error in DSV for the both masks and mouthpiece also had no significant effect on the walking EE. In addition, three apparatuses can be used instead of each other in the walking EE measurement.

C05-10

The Contraction-Induced Hypertrophic Response of Myostatin Suppression Is Intrinsically Impaired in Myotubes from Obese Individuals.

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Introduction

Loss of skeletal muscle mass and function with age is a key contributor to frailty and the incidence of chronic disease. Importantly, such loss of muscle mass and quality is associated with increased adiposity. However, the intrinsic molecular mechanisms that underpin the relationship between adiposity and loss of muscle mass are poorly understood. This study aimed to characterise the hypertrophic response of primary human myotubes from lean and obese individuals in response to muscle contraction *in vitro*.

Methods

Skeletal muscle (gluteus maximus) was obtained from lean and obese patients undergoing elective total hip replacement surgery (NRES 14ES1044). Myostatin mRNA expression in skeletal muscle, cultured myotubes and myotubes subject to electrical pulse stimulation (EPS) was quantified by qRT-PCR. EPS was performed using an Ion Optix C-Pace EP for 24 h (1Hz, 2ms and 11V). All data are presented as mean \pm SEM. Data was analysed by paired and unpaired t-tests as appropriate.

Results

Myostatin expression was significantly greater in skeletal muscle of obese (n=6), compared to lean subjects (n=6) ($p<0.01$). Myostatin expression was also significantly greater in myotubes cultured from obese subjects (n=5), compared to lean (n=4) ($p<0.01$). EPS for 24h reduced myostatin expression (2-fold) in myotubes from lean subjects (n=4) ($p<0.01$). No effect of EPS on myostatin expression was observed in myotubes cultured from obese subjects (n=5).

Discussion

These data suggest that skeletal muscle myotubes from obese individuals are intrinsically altered, resulting in an impaired hypertrophic response to exercise stimulated downregulation of myostatin.

C05-11

The Effects of Voluntary Physical Activity in Female Rats Fed with Fructose Rich Diet

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The aim of this study was to investigate the effects of voluntary physical activity on body weight, blood pressure, serum lipid and glucose levels in rats that were fed with fructose rich diet during six weeks.

Sprague-Dawley female rats were separated as control (C; n=7), voluntary physical activity (A; n=7), fructose (F; n=7) and fructose active group (FA; n=7). Fructose groups were fed 20% fructose in drinking water for six weeks. The rats were kept in cages with running wheel during six weeks. Lee Index (body weight^{1/3}/naso-anal length) was used in order to determine obesity. Blood pressure was measured with the tail-cuff method at the last day of feeding period. Serum triglyceride, total cholesterol, HDL, LDL and glucose levels were determined by using enzymatic method, insulin level measured by using the ELISA method. Two-way ANOVA and Student's t-Test were used for statistical comparisons.

Fructose intake increased systolic blood pressure ($p=0.001$), diastolic blood pressure ($p=0.002$), liver weight ($p=0.035$), glucose ($p=0.041$), insulin ($p=0.001$), cholesterol ($p=0.001$) and trygliseride ($p=0.001$) levels. Physical activity decreased heart rate and Lee index (respectively $p=0.016$; $p=0.018$). No significant interaction was observed between fructose intake and voluntary physical activity in groups. There was no significant difference of daily walking distance between FA and A groups.

Our findings considered that voluntary physical activity decreases obesity and heart rate but may not be effective on increased blood pressure, blood glucose and lipid levels in female rats fed with high fructose diet. This study has been supported by TUBAP (2016/84).

Key Words: voluntary physical activity, fructose rich diet, exercise

C05-12

Effects of Exercise on ADAMTS-4 and ADAMTS-5 Levels in Sport Horses

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The wellness and early diagnosis of the diseases in the locomotor system of sport horses are important. A disintegrin-like and metalloproteinase with thrombospondin motifs (ADAMTS) proteinase family play an important role in many physiological and physiopathological processes. In this study, we aimed to determine the changes of ADAMTS-4 and ADAMTS-5 levels on sport horses before and after exercise. The Oldenburg and Selle Français horse-breed types which are healthy, 6-15 years old, around 650-750 kg, and distinct genders were used (n=10). Following the physical examinations, the horses were subjected to 50 minutes regular exercise program. Blood samples were collected into anticoagulant-free tubes which were centrifugated as earliest as possible for 10 minutes at 3000 rpm in order to determine ADAMTS-4 and ADAMTS-5 levels before and after exercise. Horse specific ELISA kits (Sunred Bio, China) were used and results were evaluated by GraphPad Prism 5.0 software. Interestingly, although no differences were observed with at the level of ADAMTS-4 ($p=0.39$), ADAMTS-5 level significantly increased 1.2 fold ($p=0.0032$). In conclusion, ADAMTS-4 and ADAMTS-5, known as the potential therapeutic targets and responsible for the enzymatic cleavage of the major component of the cartilage tissue aggrecan proteoglycan and contribution to the restructuring of cartilage, play an important role in the early diagnosis and treatment of articular cartilage injuries and diseases observed in humans and various animals. In this terms, the increase in the serum ADAMTS-5 levels may be one of the potential biomarkers of these disorders and it is necessary to investigate more extensively to clarify its action with clinical evidence.

Acknowledgment: This study was supported by Cukurova University Scientific Research Projects (BAP), Project No: 9288

C05-13

Eight-weeks of treadmill exercise ameliorates neuropathic pain in diabetic rats

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The aim of this study was to investigate effects of exercise on diabetes-induced neuropathic pain and possible role of endogenous irisin.

Adult male Sprague-Dawley rats were kept under standart conditions with free acces to water food. Animals were habituated to both treadmil exercise and pain threshold measurement set up before being divided into control (normoglycemic) and diabetic groups. Diabetes (serum glucose ≥ 300 mg/dL) was induced by i.p. injection of streptozotocin. Diabetes was confirmed by glucose measurement from blood of fasting animals collected from the tail vein, 48 hours after STZ injection.

Animals in the diabetes group was further divided into diabetes only, diabetes + low intensity exercise, diabetes + high intensity exercise groups. The low intensity exercise protocol was 30 min/day by running at 0.5 km/h for 5 days/week and animals on high intensity exercise group performed 60 min/day by running at 1 km/h for 5 days/week, for 8 weeks.

Pain threshold, paw withdrawal in response to radiant heat, measurements were performed at baseline and at 4, 6 and 8th weeks after STZ by heat-induced plantar test. Data are compared using Dunnet test.

At the beginning of the experiment, the pain threshold values were not statistically different among the groups. After induction of diabetes, the pain threshold values were significantly increased. Exercise,

both low and high-intensity exercise, attenuated the diabetes-induced increase in pain threshold, only being significant at 8th weeks of exercise.

Results from this study indicates that chronic exercise provides beneficial effect on diabetes-induced neuropathic pain.

Acknowledgements: This study was supported by Karadeniz Technical university Scientific Research Unit (TDK-2016-5352)

C07: Gastrointestinal physiology

C07-1

Effect of Pinealectomy and Melatonin Supplementation on Metallothionein, Zinc Transport Protein Levels in the Small Intestine Sections of the Rat

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The objective of the present study is to explore the relationship between levels of metallothionein, zinc transport protein levels, which comprise a basic mechanism in the absorption of zinc, in the parts of the small intestines, of rats whose pineal glands were removed, which were supplemented with melatonin after pinealectomy, and which were supplemented with melatonin without touching the pineal gland.

The study was carried out at the Wistar type adult male rats.

Group 1, Control, Group 2, Pinealectomy, Group 3, Pinealectomy + Melatonin, Group 4, Melatonin.

The percentages of ZnT2, ZIP2, ZIP4 and metallothionein were determined using the immunohistochemical method.

The results of the study indicate that reduced levels of ZnT2, ZIP-2, ZIP-4, and metallothionein, especially in the duodenum after pinealectomy are almost restored to control values after melatonin supplementation.

C07-2

Comparative study between esophageal hypomotility and inefficient esophagus about 420 cases

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Questions: Esophageal hypomotility is defined by an average pressure of contractions following a deglutition at the esophagus <50mmHg. It is fairly frequent pathology (31%) with a new character described these last years as an inefficient esophagus defined by an average pressure at the esophagus <30mmHg.

Aims:

* To study epidemiological and manometric data of a population of patients with moderate esophageal hypomotility and a population of patients with inefficient esophageal motility.

* To compare the collected data

Methods: A retrospective study of all esophageal manometers collected by the digestive functional exploration unit of the Gastro-enterology department during five years.

Results: We examined 420 patients: 223 patients with moderate esophageal hypomotility and 197 patients with inefficient esophageal motility. Comparing the two hypomotility groups, we found the following: our population is quite young whatever the intensity of the esophageal hypomotility. The two groups include a majority of females. The main esophageal manometric indicators (dysphagia, scleroderma and a reflux pre-intervention medical checkup) are quite similar. However, indicators distribution is different across groups. The two groups had a hypotonia at the lower sphincter of the esophagus, with a slightly higher frequency observed for the moderate hypomotility group. A statistically significant difference between the two groups (p=0, 02) is found at the motor disorder as highlighted by the manometer.

Conclusions: In this study, we found evidence pointing to some differences between moderate esophageal hypomotility and inefficient esophageal motility. Mainly, we found a higher frequency for achalasia and contamination of the scleroderma of the esophagus in the serious hypomotility group while the reflex and the diffused spasms disease are often associated with moderate hypomotility.

C07-3

Investigation of anticancer mechanism of isoorientin isolated from eremurus spectabilis leaves in HT-29 human colorectal adenocarcinoma cells.

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Question: Isoorientin is a flavonoid compound that can be extracted from plant species some of them are *Phyllostachys pubescens*, *Patrinia*, and *Drosophyllum lusitanicum*. The main aim of this study is to investigate the potential anti-proliferative effects of isoorientin in HT-29 human colorectal adenocarcinoma cell line in vitro, specifically on cell viability, apoptosis, and cell cycle pathways.

Method: The cytotoxic effect of ISO isolated from *E. Spectabilis* was measured by XTT method in HT-29 cell lines. Total RNA was isolated with Tri-Reagent protocol. Effects of ISO on apoptosis related gene were determined by using RT-PCR. The analyses of findings were made by using $\Delta\Delta CT$ method and quantitated with a computer programme. The comparison of groups was done with "VolcanoPlot" analysis, from "RT² . ProfilesTM PCR Array Data Analysis", which assessed statistically using the Student t-test.

Result: In our study, IC₅₀ (inhibitory concentration where 50% of the cells die) of ISO was detected as 125 μM at the 48th hours in HT-29 cells by XTT assay. Real-time PCR analysis in HT-29 cells showed that CCND1, CDK6, casp-3, casp-8, Bax, Bcl-2, CHEK1, CHEK2 and ERCC1 expressions were reduced in ISO treated group of cells compared with the control group of cells. P53, p21, caspase-9 and ATR expressions were increased in ISO treated group of cells compared with the control group of cells (p<0.05).

Conclusion: The effects of isoorientin were given in this study. ISO effected cell proliferation of colorectal cancer cells via cell cycle pathways. It also altered apoptosis gene expression. These results demonstrated that ISO can be therapeutic agent for colorectal cancer treatment, however, further studies are needed to clarify the mechanism of actions of ISO.

C07-4

Association between chromatin fractal lacunarity and nuclear envelope circularity in mice hepatocytes

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Questions: Relationship between chromatin structural properties and nuclear shape remains poorly understood. In our study, we tested the existence and strength of correlation between nuclear envelope circularity as the main parameter of nuclear shape, and chromatin fractal lacunarity in mice hepatocytes.

Methods: A total of 100 nuclear structures from 10 healthy male mice were evaluated using National Institutes of Health (Bethesda, MD) software and its subprogram / mathematical algorithm for fractal analysis. Chromatin was stained using DNA/RNA - specific toluidine blue method. Circularity of nuclear envelope was calculated based on nuclear area and perimeter. Chromatin fractal lacunarity was determined using box-counting algorithm.

Results: There was a statistically highly significant negative correlation ($p < 0.01$) between the chromatin fractal lacunarity and nuclear envelope circularity. Circularity decreased as the lacunarity increased and vice versa. No such correlation was evident between nuclear perimeter and lacunarity, nor between nuclear area and lacunarity.

Conclusions: The results are in accordance to previously published research indicating that fractal organization of chromatin architecture is related to nuclear shape. The study presents a basis for further research in the field of cell physiology, molecular biology and biophysics.

Keywords: Chromatin; Lacunarity; Shape; Nucleus

C07-5

VX-809 restores the alcohol-induced expression defect of cystic fibrosis transmembrane conductance regulator in Capan-1 cells

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Introduction: Heavy alcohol intake is one of the most common causes of acute pancreatitis (AP). Our group previously showed that ethanol and fatty acids cause severe functional defect and impaired expression of the cystic fibrosis transmembrane conductance regulator (CFTR), which increases the severity of acute ethanol-induced pancreatitis. New compounds, (such as ivacaftor-VX-707 and lumacaftor-VX-809), are available that correct the impaired CFTR function and expression in cystic fibrosis patients with specific mutations, which might be utilized in the treatment of alcohol-induced AP.

Aims: Our aim was to test the effect of VX-809 treatment on the CFTR expression during ethanol exposure.

Materials & methods: CFTR expression was evaluated by immunofluorescent staining in Capan-1 cells and isolated guinea pig pancreatic ducts. Images were captured by confocal microscopy.

Results: Exposure of Capan-1 cells and guinea pig pancreatic ductal cells to 100mM ethanol for 12 hours significantly decreased the plasma membrane expression of CFTR. In parallel the cytoplasmic CFTR expression was increased. 10 μ M VX-809 alone had no effect on the CFTR expression. Whereas the application of 10 μ M VX-809 in pretreatment (treatment started 6 h prior to ethanol exposure), or post-treatment (treatment started 6 h after to ethanol exposure) significantly improved the plasma membrane expression of CFTR in Capan-1 cells.

Conclusion: These preliminary findings suggest that VX-809 might be able to restore the CFTR expression defect caused by alcohol. Further extended in vitro and in vivo studies need to clarify the effect of VX-809 on alcohol-induced pancreatic injury.

C07-6

THE CYTOTOXIC AND GENOTOXIC EFFECTS OF DAIDZEIN IN MIA PACA-2 HUMAN PANCREATIC CARCINOMA CELLS

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Question: Pancreatic cancer is one of the most fatal malign diseases, with a worse survival prognosis, rapid growth and metastatic distribution. Daidzein, a flavonoid compound extracted from soybeans, has anticancer activity. The results of the *genotoxicity tests* play a significant role in the assessment of heritable and carcinogenic risks. The main object of the study was to investigate cytotoxic and genotoxic effects of daidzein in MIA PaCa-2 human pancreatic carcinoma cells.

Method: The cytotoxic effect of daidzein in MIA PaCa-2 cell line was measured by XTT method according to time and dose dependent manner within the range of 25-1000 μ M. In addition, its genotoxic effects were also investigated with Comet Assay. Data were analyzed by using student t-test in SPSS 20.

Result: In this study, the IC50 (inhibitory concentration where 50% of the cells die) of daidzein was found as 200 μ M in Mia Paca-2 cells at the 48th hour by XTT assay. Comet assay analysis in Mia Paca-2 cells showed that Head Length and Head Intensity were reduced in the experimental cell groups treated with daidzein compared with the control group. Tail Length, Tail Intensity, Tail moment and Tail migration were increased in the cell groups treated with daidzein compared with the control group ($p < 0.01$).

Conclusion: This study displayed that daidzein has cytotoxic and genotoxic effects in MIA PaCa-2 human pancreatic carcinoma cells. These results suggest that daidzein may be used as a therapeutic agent for the treatment of pancreatic carcinoma alone or in combination with other drugs. However, further studies are needed to clarify the mechanism/s of cytotoxic and genotoxic action of daidzein.

C07-7

Mechanism of glutamate secretion on the pancreatic juice by acinar cells

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The pancreas efficiently absorbs amino acids for the synthesis of enzymes, but also secretes free amino acids in the pancreatic juice (PJ). From the 20 proteinogenic amino acids analyzed, glutamate (Glu) is the most concentrated. Under protein restriction, the PJ enzymes are decreased, but free Glu secretion is maintained. The aim of this study is to investigate the mechanism of Glu concentration in acinar cells and its mechanism of secretion.

Using mouse pancreata we analyzed the expression of possible carriers for Glu secretion. Freshly isolated acini were used for measuring Glu secretion in the presence of enzyme and channel inhibitors.

Our results showed that acinar cells accumulated Glu mainly via the metabolism of glutamine (Gln). The inhibition of the enzyme glutaminase (DON) reduced Glu accumulation in the cells and its secretion. The efflux mechanism of Glu in secretory cells is unknown, but recently several anion channels were shown to be able to efflux Glu and we analyzed their expression in pancreas. We observed that acinar cells express the calcium activated chloride channel ANO1/TMEM16, all the subunits forming the volume regulated anion channel LRR8CA-E/VRAC, and as previously showed the connexin 26 (CX26). TMEM16A expression was unchanged, but the VRAC isoform LRR8CA and CX26 increased and LRR8CB expression decreased in the pancreas of mice under protein restriction, suggesting that they may be involved in Glu secretion and or cell volume regulation. We are currently testing the effect of anion channel inhibitors in acinar Glu secretion.

Our results suggested that Glu is mainly synthesized from Gln in acinar cells. Our ongoing experiments will clarify the role of anion channels in the secretory mechanism of Glu by acinar cells.

C07-8

Investigation of the pancreatic ductal ion secretion in pancreatic ductal organoid cultures

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Intoruction: Pancreatic ductal fluid and HCO_3^- secretion are crucially important in the physiology and pathophysiology of the exocrine pancreas. However the study of human pancreatic secretory processes is great challenge due to the limited access to human pancreatic ductal cells. The recently developed three-dimensional pancreatic organoid cultures (OC) may help to overcome this limitation. However the ion secretory processes in pancreatic OC is not known.

Aims: Our aim was to characterize the ion transport processes in mouse pancreatic OCs.

Materials and Methods: Mouse pancreatic ductal fragments were isolated by enzymatic digestion. The isolated ducts were grown in Matrigel on 37°C for a week in OC media. Changes of the intracellular pH was measured to characterize the ion transporter activities of the epithelial cells in OC.

Results: Basolateral administration of 20mM NH_4Cl in standard HEPES or $\text{CO}_2/\text{HCO}_3^-$ buffered solution resulted in rapid intracellular alkalization, which was followed by a recovery phase. Removal of NH_4Cl induced rapid acidification followed by regeneration to the resting pH levels. The regeneration phase was inhibited by the removal of extracellular Na^+ . The administration of 10 μM CFTR_{inh}172, a selective inhibitor of cystic fibrosis transmembrane conductance regulator decreased the regeneration from alkali load. Basolateral administration of 20mM amiloride and 20mM H_2DIDS decreased the intracellular pH suggesting the activity of Na^+/H^+ exchanger and $\text{Na}^+/\text{HCO}_3^-$ cotransporter on the basolateral membrane.

Conclusion: The ion transport activities in mouse OC are similar to those observed in freshly isolated primary tissue. This suggest that OC will be suitable to study human ductal epithelial ion transport.

C07-9

Role Of Vagal Afferents On High Fat Diet Induced Alterations in Rat Behaviour And Gut Motility

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Poster Session C

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Questions: If is there any role of vagal afferent nerves on high fat diet (HFD) induced alterations in cognitive functions and gut motility?

Method: Ten-week old male Sprague-Dawley rats (n=38) were treated with either perivagal 1% capsaicin (n=19) or vehicle (10% Tween 80 in oil) (n=19). After 3 weeks of recovery, rats were pair-fed with chow (fat 2-7%) (n=18) or HFD (% 45 fat) (n=20) for 5 weeks until decapitation. In between 4th and 5th weeks of HFD rats were subjected to elevated plus maze and open field tests (anxiety and depression-like behaviour); novel object recognition, passive avoidance tests (memory). Food and water intake were measured after 16 hrs. of food and 24 hrs. of water deprivation. Weight of faeces for 16 hrs. and transit time with charcoal were measured. Data was expressed as mean standard deviation, comparison between groups were done with two- way ANOVA.

Results: VAD increased body weight significantly (p <0,05) during feeding period irrespective of fat content of the diet. Fat content and VAD had no effect on 1 hr food intake after food deprivation. HFD decreased water intake (p<0,0075), VAD blunted this effect (p<0,05). Both HFD and VAD decreased faeces weight significantly (p <0,0001 and p<0,05 respectively) but there was not any change in intestinal transit. HFD impaired short -term memory (p<0,02), whereas VAD compromised spatial learning (p<0,04). HFD rats were more anxious in OFT (p <0,01) and in EPM (p<0,03).

Conclusion: HFD induced alterations in memory and anxiety were not affected by VAD but VAD blunted effect of HFD on water intake and faeces weight, suggesting that their operating mechanisms are different. VAD by itself impaired spatial memory that requires further investigation.

C07-10

FLUID AND HCO_3^- SECRETION AND CFTR ACTIVITY ARE INHIBITED BY CIGARETTE SMOKE EXTRACT IN GUINEA PIG PANCREATIC DUCTAL CELLS

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Background: Smoking represents an independent risk factor for the development of chronic pancreatitis (CP). It is well documented that secretion of pancreatic ductal alkaline fluid (which is regulated mostly by the anion exchanger and CFTR) is diminished in CP. Aim: In this study we would like to understand whether smoking has any effects on pancreatic ductal fluid and HCO_3^- secretion.

Materials & methods: Guinea pigs were exposed to cigarette smoke four times a day for 30 min for 6 weeks. The CFTR expression was analysed by immunohistochemistry. Pancreatic ducts were isolated from guinea pig pancreas. Cigarette smoke extract (CSE) was prepared by smoking of 15 cigarettes into 10 ml distilled water by a smoking machine. Intracellular Ca^{2+} concentration and pH were evaluated by microfluorometry. Fluid secretion was measured by video microscopy. CFTR currents were detected by whole cell configuration of patch clamp technique.

Results: Cigarette smoking significantly diminished the expression of CFTR and the fluid and HCO_3^- secretion in guinea pig pancreas. CSE dose dependently decreased fluid and HCO_3^- secretion in guinea pig pancreatic ducts via inhibition of anion exchanger, Na^+/H^+ exchanger and $\text{Na}^+/\text{HCO}_3^-$ cotransporter and also forskolin-stimulated Cl^- current of CFTR Cl^- channel. CSE incubation altered

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the pattern of carbachol-induced Ca²⁺ signal in pancreatic ducts suggesting that some of the inhibitory effects may be regulated by calcium signalling.

Conclusion: Cigarette smoking and CSE inhibits pancreatic ductal fluid and HCO₃⁻ secretion and the activity of the CFTR which may play role in the smoke-induced pancreatic damage. This study was supported by OTKA, MTA, SZTA and ÚNKP.

C14: Ion channels

C14-1

Different modulation of the excitability of hippocampal and cerebellar neurons by a fibrotic scar model

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Multiple functional and morphological changes accompany a traumatic brain injury. Little is known about alteration of single neuron excitability at, or close to, the site of injury. We have used a fibrotic scar model developed by Kimura-Kuroda and coauthors to compare changes in excitability of rat hippocampal neurons (HN) and/or cerebellar granule cells (CGC) under the conditions mimicking those in injured brain.

When HN from newborn rats were cultured on a fibrotic scar model, they started to fire action potential series already at the Day 3 *in vitro* (DIV3). Control HN fired at the DIV3 single action potential only. Further, the density of voltage activated sodium, and potassium, but not calcium currents was significantly increased. Chondroitin sulfate proteoglycans played substantial role in these effects, as they were fully reversed by Chondroitinase ABC.

CGCs from 6 day old rats generated single action potential only when they were cultured for 3 days either on a fibrotic scar or in a control conditions. In a sharp contrast to HN, both sodium and potassium currents were significantly inhibited in CGCs cultured on a fibrotic scar at the DIV3. In line with our observation on HN, calcium currents were not altered. Again, observed effects were fully reversed by Chondroitinase ABC.

In conclusion, environment modeling the conditions of traumatic brain injury may have strikingly different effects on neurons in different parts of the brain. The hippocampal excitability was significantly enhanced and such enhancement may facilitate rise of epilepsy, which usually follows after brain injury. In contrast, excitability of CGCs was attenuated under these conditions.

C14-2

Glycine Uptake via Sodium/Neutral Amino Acid Transporters Activates a Swelling-Dependent Anion Conductance in Microglial Cells

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Questions: In microglial cells formation of engulfment pseudopodia and particle uptake is associated with activation of a swelling-dependent Cl⁻ current (ICl_{swell}) and blockers of ICl_{swell} inhibit phagocytosis. Likewise, an increase in extracellular glycine stimulates phagocytosis, causes cell swelling and

depolarizes the cell membrane potential (V_{mem}) due to glycine uptake via Na⁺/neutral amino acid transporters (SNATs). Here we investigated if cell swelling under glycine induces ICl_{swell}, ICl_{swell} activation affects V_{mem} and glycine influences cell migration. **Methods:** Flow cytometric mean cell volume (MCV) measurements, whole-cell patch clamp and trans-well migration assays were used on murine BV-2 cells. **Results:** Glycine (5 mM) caused an increase in MCV under isotonic conditions by ~8% within 15 min. This was paralleled by the activation of a Cl⁻ conductance with biophysical and pharmacological characteristics of ICl_{swell}. Glycine uptake via SNATs induced a rapid, stable depolarization, which was enhanced by additional hypotonic stimulation of ICl_{swell}, but also under isotonic conditions upon long-term (>20 min) exposure to glycine. Cell migration was stimulated by glycine (0.6–5 mM). The ICl_{swell} inhibitor DCPIB (10 μM) completely counteracted both hypotonicity- and glycine-induced depolarizations, inhibited glycine-stimulated migration and augmented glycine-induced cell swelling. **Conclusions:** The findings indicate an interplay between cell volume regulatory processes and glycine-stimulated phagocytosis/migration in microglial cells – a mechanism which might be particularly relevant in case of brain trauma or ischemia, where high interstitial glycine concentrations occur due to cell damage.

C14-3

Noradrenaline Suppresses a Cl⁻ Current as well as Phagocytosis in Murine Microglia

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Questions: In the central nervous system (CNS), neurodegenerative diseases are associated with a decrease in noradrenaline (NA). As microglial cells, macrophage-derived immune cells of the CNS, express adrenergic receptors (AR), their response to catecholamines is of interest. We found that in microglia a cell volume-regulatory Cl⁻ current (ICl_{swell}) is involved in volume-related functions like migration and phagocytosis. Since NA has been shown to suppress phagocytosis in microglial cells and fMLP-induced migration in neutrophils, we investigated if NA affects ICl_{swell} in microglial cells. **Methods:** Whole-cell Cl⁻ currents were recorded in murine BV-2 microglial cells using perforated patch clamp leaving the cytosolic milieu intact. Phagocytosis was quantified by exposing BV-2 cells or primary murine microglia to polystyrene microspheres for 15 min and counting the number of cells containing at least one microsphere using scanning electron microscopy. **Results:** Hypotonic cell swelling induced an outwardly rectifying Cl⁻ current (ICl_{swell}), which was reduced by addition of NA (1 nM or 1 μM). Similarly, ICl_{swell} was suppressed by the β-AR agonist isoproterenol, the Epac-specific analog 8-pCPT-2'-O-Me-cAMP and the PKA inhibitor H89. NA in the pM and nM range suppressed phagocytosis and the α₂-AR antagonist yohimbine enhanced the suppressing effect of NA. **Conclusions:** We show that AR stimulation suppresses ICl_{swell} in microglial cells, probably via altered cAMP levels. Given the role of ICl_{swell} in cell volume regulation and cell volume-related processes like formation of lamellipodia/engulfment pseudopodia and cell migration, its inhibition might underlie the observed suppression of phagocytosis upon AR stimulation.

C14-4

Cloxyquin is a selective and state-dependent activator of TWIK-related spinal cord K⁺ channel (TRESK)

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Questions:

Cloxyquin (5-chloroquinolin-8-ol) has been previously identified as an activator of TRESK background K⁺ channel (K_{2P}18.1, TWIK-related spinal cord K⁺ channel). We have examined the specificity of the drug by testing several K_{2P} channels. We also investigated the mechanism of cloxyquin-mediated TRESK activation, with emphasis on the differences between the physiologically relevant regulatory states of the channel.

Methods:

Potassium currents were measured by two-electrode voltage clamp in *Xenopus* oocytes and by whole-cell patch clamp in mouse dorsal root ganglion (DRG) neurons.

Results:

Cloxyquin (100 μ M) activated both mouse (4.4 \pm 0.3-fold, EC₅₀=26.4 μ M) and human TRESK (3.9 \pm 0.3-fold, EC₅₀=43.9 μ M). TRESK was potently activated by cloxyquin in the resting state. The activation was not mediated by cytosolic [Ca²⁺] (it was maintained in EGTA-injected oocytes) or activation of calcineurin (verified using calcineurin inhibitors and mutant channels with abolished calcineurin binding). The compound did not influence mouse TRESK and only slightly affected the human channel after activation via calcium signal evoked by the stimulation of Gq-coupled receptors. Constitutively active mutants could not be further stimulated by cloxyquin. The drug selectively targeted TRESK in the K_{2P} channel family. In a subpopulation of isolated DRG neurons cloxyquin application activated the background K⁺ current.

Conclusions:

Cloxyquin activates TRESK by a Ca²⁺/calcineurin-independent mechanism. The drug is specific for TRESK within the K_{2P} channel family and useful for studying TRESK currents in native cells. Cloxyquin may be a useful parent compound for the development of selective TRESK modulators.

C14-5

Ion channels in anticancer drugs painful side effects

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Platin-based drugs and taxane are used in the treatment of breast, ovary, testes, kidney, or head and neck solid tumors. Platin-based drugs cause cell death through the formation of DNA adducts while taxane class of drugs are mitotic inhibitors. Interestingly in patients, these anticancer drugs with two completely different mechanisms of action exhibit a range of similar side effects that occur shortly after treatment and can last for years. Those include modification of touch perception, allodynia, cold hypersensitivity, imbalance, and tinnitus.

As cisplatin had been shown to modify membrane properties in different cell systems, we first had investigated its effects on mechanosensitive channels and found some interesting candidates for its action (Milosavljevic N. Cancer research – 2010). In second part, we investigated if platinum-drugs and taxanes can modulate gene expression of several channels involved in touch and pain perception. We also studied the expression of transcription factors modulate by xenobiotics and carrying a site to bind the promotor of our identified targets.

Strikingly, both platinum-based drugs and taxanes at doses used in chemotherapy, reveal a common profile of modified gene expression for two ion channels among those tested, which is correlated with

a modification of their protein activity. Interestingly, we identified a transcription factor specifically modulates as its two targets. Moreover, we observed a reversion of these effects by using drug acting on this transcription factor in parallel of the anticancer drugs treatment. Taken together, we hope that these results will provide us with new clues on possible common denominators to previously-unrelated side effects of these drugs.

C15: Other

C15-1

EVALUATION OF ESTRADIOL LEVEL AND SERUM LIPIDS IN WHITE WISTAR RATS OF FEMALE GENDER DURING THEIR GENERATIVE LIFE

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Objectives. Clinical and experimental data underscore the cardioprotective effects of female sex hormones, particularly estrogens. 50% of the antiatherogenic effects of estrogens are attributable to effects on lipoprotein metabolism. The values of estradiol and serum lipids were examined in white Wistar rats of female gender during their generative life.

Material and methods. The study included a total of 40 white Wistar rats of female gender divided into two groups according to their age (sex maturity): control group of 22 mature rats, with regular estrus cycle and experimental group of 18 rats in the period of reproductive involution at the age of eighteen months. Estradiol level was determined with standardized tests based on the radioimmunological method and serum lipid concentration was determined with the method of fractionation sedimentation according to the specific weight.

Results. The investigation has shown that there was a significant reduction of the estradiol level in experimental group (12.4 \pm 3.8 pg / ml) in comparison to control group (23.9 \pm 1.5 pg / ml), (p< 0.05) and a significant increase of the level of LDL-CH in experimental group (2.6 \pm 1.3) in comparison to control group of female rats (1.1 \pm 0.6) (p< 0.05). Nevertheless, there were no significant differences in the level of HDL-CH, total cholesterol, and triglycerides in two groups.

Conclusions. We can conclude that there is a severe impairment of lipid profile (increase of LDL cholesterol), during the involution period of female white Wistar rats, in comparison with the reproductive period of life.

C15-2

Discovery of a new voltage-gated proton channel

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A new H_v1 gene was discovered in *Nicoletia phytophila*, an insect species from the Zygentoma order, one of the first terrestrial animals on Earth. We have called the protein NpH_v1 following the common nomenclature used for proton channels. Interestingly, NpH_v1 is genetically closer to its human homolog (33 % of identity) than to other species studied. NpH_v1 was successfully expressed in human cells presenting proton currents higher than 400 pA, suitable for electrophysiological studies. The detailed electrophysiological characterization has proved that NpH_v1 is highly proton selective, and shows other hallmarks of H_v1 as voltage-dependent gating and pH-dependent gating. Curiously, NpH_v1 has demonstrated to have an enhanced pH-dependence of gating when comparing with the human one (hH_v1). This pH-dependent gating is a unique characteristic for H_v1 which allows their main physiological role, cell's pH regulation. However, how the channel sense and adjusts its gating

depending on the pH across the cell membrane is still unknown. Here, further studies focused in differences on the amino acids sequence between NpH_v1 and hH_v1 could elucidate the intrinsic mechanism. Finally, substitutions of Asp66 by non acidic amino acids have shown anion permeation meanwhile those done with acidic amino acids showed conserved proton selectivity. Therefore, we probed that Asp66 (Asp112 for humans) is the selectivity filter for NpH_v1, confirming it as the general selectivity mechanism for all known H_v1.

Conclusions

- This work is the first description of a proton channel in insects.
- NpH_v1 presents the same biophysical characteristics for all known proton channels.
- NpH_v1 shows an enhanced pH-dependent gating in comparison with the human protein.
- Asp66 is the selectivity filter for NpH_v1 and confirms the general selectivity mechanism for all the proton channels.

C15-3

The determination of interaction between naringin and different chemotherapy agents in neuroblastoma and astrocyte cell lines

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Neuroblastoma is a cancer type seen in children under five years old. Chemotherapy (doxorubicin, cisplatin and etoposide) use for the treatment in addition to surgery, radiation and stem cell transplantation. Because of the side effects of chemotherapeutic agents, some plant-derived components are used for protecting healthy cells. Naringin is a citrus flavonoid have antioxidant, apoptotic, antiinflammatory properties. In this study, we aimed that the determination of single and combine effects of naringin and chemotherapy agents (doxorubicin and cisplatin) in neuroblastoma N1E-115 (ATCC® CRL-2263™) and astrocyte C8-D1A [Astrocyte type I clone] (ATCC® CRL-2541™) cell lines. With this aim, the effects of the combinations following exposure to the sequentially and simultaneously on apoptosis analyzed by image-based cytometer and gene expressions of apoptosis pathway. According to results of the study, naringin induced intrinsic apoptosis pathway as evidenced by the induction of p53, Bax, Cyt-c and caspase-3 in neuroblastoma cells. In addition, pre- or post treatment of naringin with chemotherapy agent caused different apoptotic effects. In conclusion, naringin treatment before cisplatin and after doxorubicin caused more apoptosis in neuroblastoma cells. Furthermore pretreatment of naringin showed protective effect against cisplatin toxicity in astrocyte cell lines. This study was supported by Trakya University Research Project Foundation (Project Number: TÜBAP-2016-231), Edirne/Turkey

C15-4

Critical analysis of dietary habits in people with type 2 diabetes

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Diabetes is a formidable disease for the complications it causes (infarction, renal insufficiency, blindness, ...). Thus, it is better to diagnose this disease early to learn more about its different forms, its screening and its treatment. Thus, in this work, we proposed to evaluate and criticize the quantitative and qualitative aspects of the spontaneous feeding of a group of patients with type 2 diabetes, and to highlight the different diet gaps for a good catch in charge. The objectives of this study:

To highlight the different dietary habits of a group of patients with type 2 diabetes, Assess the quantitative and qualitative aspects of the spontaneous feeding of this group, Criticize the main regime differences.

This was a prospective study involving 70 patients with type 2 diabetes, who were recruited from the outpatient department of the National Institute of Nutrition and Food Technology in Tunis over a period of one month. We were interested in the various anthropometric parameters, as well as a food survey and a questionnaire on personal data of the patients.

From our results, it emerges: A caloric normo diet A hyper-carbohydrate, normo-protein and normo-lipid ration. Atherothrombogenic diet (AGPI / AGMI)> 1

These results show that there is an imbalance in the dietary intake of diabetics studied which contributes to diabetes imbalance especially with excessive carbohydrate intake. Our results can be explained by high consumption of cereals and moderate consumption of other foods.

The situation is alarming for both young people and adults. However, they continue to neglect preventive measures by adapting unbalanced eating behaviors. Critical analysis of dietary habits in people with type 2 diabetes.

C16: Neuro-immunology

C16-1

Association of TNFAIP3 and TRAF1 polymorphisms with susceptibility to systemic lupus erythematosus and rheumatoid arthritis in Egyptian Population.

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Background:

Recent genome-wide association studies demonstrated association of single nucleotide polymorphisms (SNP) in the TNFAIP3 and TRAF1 with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in European populations.

Aim of study:

To determine whether the Tumor necrosis factor, alpha-induced protein 3 (TNFAIP3) polymorphism (rs2230926) and tumor necrosis factor (TNF)-receptor associated factor 1 (TRAF 1) polymorphism (rs10818488) confer susceptibility to systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in Egyptian population.

Materials and Methods:

This was a case-control study in which 90 individual with SLE and 105 individual with RA and 75 healthy controls were included. Genotyping was performed using TaqMan genotyping assay for two single nucleotide polymorphisms (SNPs) that showed the best evidence of association in the previous Caucasian studies.

Results:

We detected significant differences in allele frequency of rs2230926 G allele with SLE (OR: 3.13, CI: 1.37-7.12; P=0.006) and RA (OR: 2.9; CI: 1.31-6.65; P =0.008). A allele of TRAF1 was significantly increased in RA compared to control (50% versus 40.6%). Carriers of the A allele were significantly more likely to develop RA (OR: 1.45; 95% CI: 0.95-2.22; P=0.008), while TRAF 1 polymorphism did not exhibited any statistical significant difference in the frequencies of genotypes or alleles in SLE and controls (OR: 0.67; 95% CI: 0.43 -1.06; P=0.03).

Conclusion:

These results indicated that TNFAIP3 is a susceptibility gene to SLE and RA in the Egyptian population. Also Association of TRAF1 locus with RA susceptibility was detected in the Egyptian population, while no significant association was observed for SLE.

Keyword: TNFAIP3; TRAF1; polymorphisms; systemic lupus erythematosus; rheumatoid arthritis; Egyptian.

C16-2

Antibodies against vimentin -An early biomarker of ischemia?

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Although anti-vimentin (cytoskeletal protein) autoantibodies (AVA) are not associated with a specific autoimmune disease, they are also a patency that can be seen in diseases such as Rheumatoid Arthritis, anti-phospholipid syndrome, SLE and some infections. The clinical provision is not yet fully understood. In this study, we aimed to investigate the clinical features of AVA positive patients who were followed up in our clinic. The patients who came up with different diagnostic diseases such as vasculitis, dysmyelinating disease, multiple sclerosis, anti-phospholipid antibody syndrome, ischemic stroke were demended from Neurology Department to be tested anti-nuclear antibodies (ANA). These tests were conducted Department of Medical microbiology and Immunology laboratory at the desired and Indirect Fluorescent antibody test 10 cases were studied retrospectively. According to the manufacturers recommendations $\geq 1/100$ dilution of serum titres are considered positive. Three of the female cases had ischemic cerebrovascular disease. One of these patients, had an APS, one had actinic keratosis and the other had FMF. A 53 year old patient had coronary artery disease. A 68-year-old patient had CAD and additionally hashimoto thyroiditis. A 33 year old patient was diagnosed with MS. A 3 year old dysmyelinating patient and her investigations were still continuing. Two patients aged 34 and 39 had RA diagnosis. A 32-year-old male patient was diagnosed with MS and vasculitis. AVA positivity in patients with ischemic processes is at the forefront, this in addition to autoimmune patients and additional diseases in character. In patients with rheumatic disease in particular autoimmune character AVA is positive in terms of the early biomarker of ischemia caused by more extensive studies are needed.

C18: Teaching & e-learning

C18-1

Near-Peer Teaching Program in Medical Physiology at Comenius University

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Traditional curriculum in Human Physiology at Faculty of Medicine, Comenius University in Bratislava involves lectures for 300 students and direct teaching in small group labs, all taught by Faculty. Recently, near-peer(NP) teaching pilot program has been added as a novel method of teaching in English program.

The aim of our study was to analyze if adding NP teaching model would increase understanding and motivation among our students. Students who finished Physiology curriculum were selected as NP teachers based on academic performance, leadership skills, motivation, and willingness to teach. Preclinical students participated in 6 structured, three hour-long tutorials for each module. 2 sessions were held on each topic by 2-3 tutors, using different modes of teaching (manikin simulators, OSCE, PBL, hands-on experiments, power-point presentations, NP teachers also provided self-made online videos and handouts).

Total of 17 NP teachers (N=17, 9 female and 8 male) participated in the study. 100% of them considered teaching beneficial for their knowledge, teaching skills, and would consider to do it again, if asked.

35 (92,1%) anonymous self-reported detailed Likert-style questionnaires were collected from students (n=35, 20 female and 15 male). 90% of them reported that NP program increased their knowledge and

improved final test results. 85% of them mentioned that they would like to participate again, if asked. For 85% of them, program enhanced their inner motivation towards studying Physiology.

NP program was found to be beneficial for both students and NP teachers, as valuable addition to Physiology traditional classes. In the future, we plan on expanding tutorials to give equal opportunities for all students.

C18-2

Team-Based Learning in Medical Physiology

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Team-based learning (TBL) is a teaching concept which allows performing small group teaching in a lecture hall setting. We introduced TBL for Physiology teaching for 740 students/semester. TBL consists of a preparatory phase, where the students acquire all knowledge necessary for the actual TBL. In our setting the preparatory phase (4 weeks) includes plenary lectures, seminars, practical courses, and self-studying of various physiological contents. For the actual TBL (4 x 2 hours distributed over 1 week) students are randomly assigned to a team consisting of 5-7 persons; we teach 10 teams per lecture hall. At the beginning of each of these 4 courses students have to individually complete a readiness assurance test consisting of 5 to 8 multiple choice questions presented as PowerPoint slides. Students write down their answers, and immediately after this individual test, they take the same test as a team. After a discussion within the team, the teams have to decide for one answer and display their answer per audience response system. Teachers and students see the answers of the different teams, and teams have to defend their answers against those of other teams. Teachers facilitate the discussion between teams, ask questions to explore the topic, and give explanations if necessary. The most important task for teachers is to prepare multiple choice questions that connect many different fields of Physiology and that stimulate discussion. Teachers have to be open for surprising questions and answers from the students, and should not be afraid of noise in the class room. In TBL students are motivated to reflect what they have learned in the preparatory phase and make the experience that they usually perform better as a team than as an individual.

POSTER SESSION D

D01: Cardiac physiology

D01-1

Serotonergic 5-HT2B receptors in mitral valvulopathy: bone marrow mobilization of endothelial progenitors

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Valvular heart disease is highly prevalent in industrialized countries. Chronic use of anorexigens, amphetamine or ergot derivatives targeting the serotonin system has been associated with valvular heart disease.

Here, we investigated the contribution of serotonin receptors in a mouse model of valve degeneration induced by nordexfenfluramine the main metabolite of the anorexigens dexfenfluramine and benfluorex.

Chronically activated 5-HT2B receptors by nordexfenfluramine in mice mimicked early steps of mitral valve remodelling attested by increased valve thickness, and cell density in a thick extracellular matrix. Lesions were totally prevented by inhibition of both 5-HT2A and 5-HT2B receptors by antagonists, in transgenic Htr2B^{-/-}, or Htr2A/2B^{-/-} mice. Surprisingly, we found that valve lesions are mainly formed by numerous non-proliferative CD34⁺ endothelial progenitors. We show that these progenitors originate from bone marrow as revealed by bone marrow transplantation. Initial steps of mitral valve remodelling involve bone-marrow derived CD34⁺CD31⁺ cells mobilization by 5-HT2B receptor stimulation. Moreover, the analysis of human mitral valve prolapse, showing spontaneous

degenerative lesions highlights the presence of non-proliferating CD34⁺/CD309⁺/NOS3⁺ endothelial progenitors expressing 5-HT2B receptor. This work reveals a crucial contribution of bone-marrow derived endothelial progenitor cells in valve tissue remodelling and highlights the contribution of this new mechanism involved in human valvular heart disease.

D01-2

Cardioprotective effect of Aqueous Viscum album extract on isoproterenol induced myocardial infarction in rats .

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Viscum album has prosperous cardiovascular effects such as antihypertensive and vasodilator activity increased by NO synthase and antioxidant. However, little is known about its effects on myocardial injury. The current study was designed to investigate the cardioprotective effect of *Viscum album* L.aqueous extract (AVa) against ISO induced acute myocardial injury in male rats by demonstrating the changes in hemodynamics, biochemical parameters and histopathological architecture. Sprague Dawley rats were randomly divided 6 groups(n=8); control, ISO, Sham, AVa (0.1,1,10 mg/kg). AVa was administered intraperitoneally at a dose 0.1,1,10 mg/kg for 10 days. Acute myocardial infarction was induced by subcutaneous injection of 150 mg/kg-1 of ISO at an interval of 24 h to the groups on 9 th and 10th day.Blood and tissue samples were taken for chemical and histopathological evaluations

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Poster Session D

following electrocardiography recording on day 11th.ISO caused ST segment elevation (0.348 ± 0.03 mV compared control P < 0.01) and increase heart rate 351±11.6 bmp compared control P< 0.01. Tissue MDA significantly decreased in all pretreatment with AVa groups compared ISO induced group (7.69±1.25, p<0.01).Viscum album significantly increased in endogenous antioxidant level by alone (1.23±0.4, p < 0.05) compared to the other groups.Plasma nitrate level significantly increased in highest dose of AVa (10mg/kg) compared ISO and control groups (126.1±42.2, p< 0.05) ISO induced hearts revealed significant extensive myonecrosis, myocellular edema and inflammation. Pretreatment with Va (0.1 and 10 mg/kg) significantly eliminated (p < 0.01) ISO induced histopathological changes and decreased the myocardial necrosis to a greater extent. Present study provides first scientific report on protective effect of aqueous Viscum album crude extract given intraperitoneally against ISO induced myocardial damage in rats.

D01-3

EFFECTS OF THE NITRIC OXIDE DONOR S-NITROSOGLUTATHIONE AND ACUTE LOCAL VENTRICULAR STRETCH ON ISOLATED RABBIT HEART.

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Questions

Myocardial stretch is a proarrhythmic factor. Little is known of the effects of nitric oxide (NO) upon mechanoelectric feedback. We used isolated and perfused rabbit hearts to study the changes in mechanoelectric feedback produced by the NO donor S-Nitrosoglutathione (GSNO).

Methods

32 WNZ male rabbits were used, and anaesthetized to euthanasia, to extract the hearts. Epicardial multielectrodes were used to record myocardial activation at baseline, during and after stretching of the left ventricular free wall produced by an intraventricular device. Series: 1) control (n=10); 2) GSNO 10 µM (n=11) and 3) GSNO 50 µM (n=11). The changes in ventricular fibrillation (VF) pattern caused by stretching were analyzed.

Results

GSNO 10 µM did not modify VF at baseline but attenuated acceleration of the arrhythmia (dominant frequency was 15.6±1.7 vs 21.3±3.8Hz) and reduced the percentile 5 (P5) of the activation intervals (42±3 vs 38±4ms) caused by stretching, although it did not prevent from achieving complexity indexes of VF similar to controls. In contrast, GSNO at 50 µM concentration shortened P5 at baseline (40±7 vs 52±10ms) and increased the complexity index (1.77±0.18 vs 1.27±0.13). During stretching we obtained the lowest P5 values (34±3ms) and the highest levels of complexity (1.84 ± 0.17). A correlation between complexity index and P5 was found (r=-0.592). The stepwise regression model only admitted P5 (constant=2.4, slope=-0.02, R=0.6).

Conclusions

S-nitrosoglutathione 10 µM attenuates the effects of mechanoelectrical coupling, while at 50 µM the drug alters the baseline VF pattern and accentuates the increase in complexity of the arrhythmia induced by myocardial stretch.

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Poster Session D

D01-4

Extracellular diadenosine tetraphosphate affects contractility and cytoplasm calcium level via protein kinase C pathway

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Diadenosine polyphosphates (Ap(n)A) are recently considered as endogenous signaling compounds which are present in numerous mammalian tissues, including heart. Extracellular Ap(n)A cause suppression of working myocardium contractility, induce inhibitory effects in cardiac pacemaker and alter bioelectrical activity via P1- or P2-purine receptors. However, the particular intracellular mechanisms which underlie Ap(n)A cardiac effects remain unknown. In the present study we show the role of P2Y-associated regulatory pathway in mediation of Ap4A effects in the rat heart.

Effects of Ap4A on myocardial contractility were estimated in isolated Langendorff-perfused paced (4 Hz) hearts of male Wistar rats. Action potentials (APs) were recorded with sharp microelectrodes in isolated multicellular preparations. Alteration of cytosolic calcium ([Ca²⁺]_i) transients was measured using Fluo-4 fluorescent dye in enzymatically isolated rat ventricular myocytes.

Diadenosine tetraphosphate (10 μM, n=7) induced significant decrease in left ventricular developed pressure, maximal rate of contraction and relaxation in isolated rat heart. Inhibitory effects of Ap4A were significantly suppressed by protein kinase C (PKC) blocker chelerythrine (5 μM, n=7). Also, Ap4A (10 μM, n=12) produced AP shortening in both atrial and ventricle myocardial preparations. Chelerythrine (5 μM, n=7) application significantly reduced effect of Ap4A on AP duration. In addition, substantial suppression of cytoplasm [Ca²⁺]_i transients was observed in the presence of Ap4A (10 μM, n=6). PKC inhibitor (5 μM) significantly restored the amplitude of [Ca²⁺]_i transients (n=7).

Thus, we suggest that negative effects of Ap4A in the rat heart are mediated by PKC dependent pathways.

This study was supported by Russian Science Foundation [grant no. 14-15-00268].

D01-5

Effect of anti-HMGB1 protein in experimental myocardial infarction

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Introduction: High mobility group box 1 (HMGB1) is a DNA-binding protein associated with various pathological conditions such as cardiovascular disease, cancer, and ischemia/reperfusion injury. The aim of this study was to evaluate the effects of HMGB1 protein on biochemical and morphological parameters after experimental myocardial infarction (MI).

Methods: 12-week-old WKY male rats used for the study were divided into following groups: sham operated WKY without MI, WKY with MI, WKY + IM+ anti-HMGB1 protein (10 in each group). In vivo model of experimental MI was induced by ligation of the left descending coronary artery and lasted 20 min. Before reperfusion anti HMGB1 protein was administrated i.v. Animals survived 7 days after MI. NOS activity was determined by conversion of 3[H] Arginine to 3[H] Citrulline in the aorta and ischemic, border and non-ischemic region of the heart. For morphological parameters, the hearts were excised and used for TTC-staining procedure. Cytokine levels were investigated using the Bio-Plex Pro Cytokine kit in the plasma. Concentration of CD was measured spectrophotometrically.

Results: Anti-HMGB1 protein increased NOS activity in both ischemic and border part of the heart, as well as in the aorta. It significantly decreased TNF-alpha and IL-6 level in plasma. Simultaneously, anti HMGB1 protein decreased MI part as well as border region of the heart.

Discussion: Considering the results, HMGB1 protein is a promising molecule for reduction the negative effects of the myocardium infarction, as well as a promising agent for the treatment of cardiovascular diseases.

Supported by: VEGA 2/0170/17, 2/0144/14, APVV-14-0932

Keywords: myocardial infarction, HMGB1 protein

D01-6

IMPACT OF SIMVASTATIN ON LIPID AND NON-LIPID BIOCHEMICAL RISK FACTORS IN DIET-INDUCED HYPERHOMOCISTEINEMIA IN WISTAR ALBINO RATS

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Questions

The objective of our study was to evaluate the association between long-term administration of simvastatin and body weight, food intake, plasma total homocysteine (tHcy), cholesterol (tCHOL), low-density lipoprotein (LDL), high-density lipoproteins (HDL), triglycerides (TRY) levels, as well as pro-oxidative (superoxid anion radical, hydrogen peroxide, index of lipid peroxidation) and antioxidative markers (reduced glutathione, catalase and superoxide dismutase) in Wistar albino rats.

Methods

Study was conducted on adult male *Wistar albino* rats (n=30; 4 weeks old; 100±15g body mass) in which HHcy was achieved by dietary manipulation. For 4 weeks, the animals were fed with one of the following diets: standard rodent chow (n = 10) (control fed); diet enriched in methionine with no deficient in B vitamins (folic acid, B6 and B12) (n = 10); diet enriched in methionine and deficient in B vitamins (folic acid, B6 and B12) (n = 10). Simvastatin was administrated daily for 4 weeks, 5mg/kg i.p.

Results

We found significant differences between the body weights and food intakes among all groups (p<0.05) and significant strong positive correlation between Hcy levels, prooxidative and lipid parameters, and negative correlation with antioxidant parameters in blood after administration of simvastatin (p<0.05). Also, blood concentrations of the antioxidant SOD in blood were significantly affected, as well as CAT activity and all lipoproteins (p<0.05).

Conclusions

These data support the association between higher methionine intake and increasing of lipid and non-lipid biochemical risk factors for cardiovascular disease, with antioxidative protective role of HMG-CoA reductase inhibitors (simvastatin) in these cascade reactions.

Key words: HMG-CoA reductase inhibitors, homocysteine, rat.

D01-7

Investigation of the Effects of Some Calcium Channel Blockers on in vivo, in vitro and ischemia / Reperfusion Injured Rat Heart Acetylcholinesterase Enzyme

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AIM: Some calcium channel blockers were tested on lacetylcholinesterase (AChE) enzyme in vitro. Nifedipine was found to be the most effective one among the drugs tested. In order to investigate the effect of nifedipine drug on heart tissue AChE enzyme in Ischemia/reperfusion (I/R) and I/R injured rats.

METHODS: Control, sham, I / R and nifedipine + I / R are as follows: A total of 24 male Wistar albino rats weighing 250300 g were equally and randomly divided into four groups. In the last group, nifedipine was administered at 4 mg/kg dose before intraperitoneal ischemia period. Heart tissues were removed after the bilateral I/R process. Enzymatic activity was measured by using the spectrophotometric method of Ellman. The reaction mixture include TrisHCl buffer (1 M, pH 8.0), 5,5'dithiobisbis nitrobenzoic acid (DTNB, 0.5 mM), acetylthiocholine iodide (ATChI, 10 mM) and AChE (0.28 units/mL).

RESULTS: Different calcium channel blockers were tested in vitro on AChE enzyme and nifedipin was observed to be the most effective one among the tested drugs. Specific activity values were determined for AChE enzyme at four different experimental groups. Groups were determined as follows: Control group 0.239 ± 0.012 EU/mg protein, sham group 0.162 ± 0.005 EU/mg protein, I/R group 0.134 ± 0.004 EU/mg protein, and nifedipine + I/R group 0.128 ± 0.004 EU/mg protein.

CONCLUSION: As a result, the activity of the AChE enzyme was determined to exist mostly in ischemia/reperfusion+nifedipine group. This study has been supported by Ataturk University Scientific research project unit (2014/146).

D01-8

Association of α -adrenoceptor Polymorphisms with Cardiac Autonomic Control

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As important components of the sympathetic nervous system, α -adrenergic receptors (α -AR) modulate cardiovascular (CV) function and the changes in their function may play a role in the initiation and progression of CV diseases.

The aim of present study was to evaluate the relation between α -AR genetic polymorphisms and autonomic nervous system (ANS) function at rest or during orthostatic stress in young healthy adolescents. Subjects were genotyped for α 1A-AR (rs1048101) and for α 2A-AR (rs1800544) polymorphisms and underwent evaluation of ANS function using basic cardiovascular and hemodynamics measures: R-R interval, systolic, diastolic and mean blood pressure (SBP, DBP, MBP, respectively), stroke volume, cardiac output, total peripheral vascular resistance, pre-ejection period and left ventricular ejection time. All measures were recorded noninvasively, continuously and simultaneously at each heart beat in supine rest, head-up tilt and supine recovery. Reactivity was

evaluated by subtracting the mean value for pre-stress resting period from the mean for head-up tilt period.

Results revealed significant associations between α 1A-AR and α 2A-AR polymorphisms and several basic CV measures. Reactivity of SBP, DBP and MBP to head-up tilt were found to be associated with α 1A-AR polymorphism.

Our findings demonstrate that genetic variations in the alpha-adrenergic receptors are associated with the alterations in sympathetic cardiac control indicating their potential role in the association between AR genetic variations and CV diseases initiation and progression.

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D01-9

Influence of thioacetamide administration on autonomic control of the heart atria in rats

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Cardiac dysfunction is one of the complications of liver cirrhosis. During chronic heart failure persistent sympathetic hyperactivity was observed. Its effect is supported by sympathetic cotransmitters, e.g. neuropeptide Y (NPY) and its Y1receptor (Y1R). The calcitonin receptor-like receptor (CRLR) forms with RAMP molecules specific receptor to bind other heart neuropeptides. Release of additional neurotransmitters can have deleterious consequences for cardiac function.

Adult female Wistar rats (n=7) received thioacetamid (TAA, 200 mg/kg i.p), three times a week for 12 weeks. The rats were decapitated in 4 weeks after the end of TAA application. Blood, heart and liver samples were taken and compared with controls (n=7). In the serum enzymes were estimated, such as alanine transaminase (ALT), aspartate transaminase (AST) and glutamate dehydrogenase (GLDH) to evaluate the hepatic injury. Lipid peroxidation (LP), reduced glutathione (GSH) and activities of glutathione peroxidase (CPx), glutathione reductase (GR) and catalase (CAT) were determined in liver and kidney tissue homogenates. Relative expression of NPY, Y1R and CRLR were measured in the left and right atrium.

Administration of TAA significantly increased liver enzymes activity. The markers of oxidative damage were significantly decreased in liver. Significant increase of LP was found in kidneys. Expression of NPY and Y1R remained unchanged. In TAA induced hepatic fibrosis the expression of CRLR in the left atrium was upregulated, which could be one of cofactors contributing to autonomic dysregulation.

This study was supported by the National Sustainability Program I (No. LO1503) provided by the Ministry of Education, Youth and Sports of the Czech Republic.

D01-10

Effects of Sertraline in Healthy and Damaged Rat Aorta

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AIM: Selective serotonin reuptake inhibitor group anti-depressant drugs are commonly used in patients with cardiovascular disorders. However, their effects on rat aorta are not investigated particularly experimentally. We aimed to investigate the effect both on healthy and damaged rat aorta.

MATERIAL AND METHODS: 24 Wistar albino rats in 2 groups (n=24). Group 1 aorta-intact endothelium (n=12), group 2 aorta-damaged endothelium (n=12). Descending thoracic aorta was isolated after cervical dislocation. Aorta tissues were cleaned sectioned into 3-4 mm long rings. Rings were placed in organ baths containing Krebs solution, thermoregulated at 37.0°C and aerated (95 % O₂ and 5% CO₂). Changes in isometric tension of aorta rings were recorded using a four channel force displacement transducer. Phenylephrine (PE 10-6M) was applied contractions were recorded in both groups. Then sertraline (SE 50 mg) was given cumulatively (10-9 -10-4 M) to group 1. In group 2 aorta damage was achieved by tearing endothelium with needle. After controlling endothelial damage by applying acetylcholine (Ach 10 -6 M), damaged strips were washed for one hour and second dose of PE was administered, then SE was given cumulatively to group 2, contractions were recorded.

RESULTS: After SE was given cumulatively to group 1, significant inhibition of spontaneous contractions was noticed in SE 10-9, 10-8, 10-7 doses (p<0.05), inhibition of contractions kept on in the rest of SE doses. SE 10-6 - -4 made significant inhibition in contractions according to 10-9 -8 -7 . Group 2 inhibition of second PE contractions continued after SE doses, but it was less significant when compared with group 1 (p<0.05).

CONCLUSION: Sertraline may be safely used in patients with aorta disease.

D01-11

Possible Effects of Sertraline on Human Heart Muscle Contractility: An in vitro experimental study

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AIM: Patients who underwent cardiac surgery may have depression in the early postoperative period and they may need treatment with anti-depressant drugs. In this study we aimed to investigate the in vitro effects of sertraline on human atrium muscle contractility.

METHODS: Human atrium tissues (n=28) are taken from the cardiac surgery performed cases for coronary bypass surgery during the venous cannulation through right atrium appendage before the initiation of cardiopulmonary bypass. The patients age were between 47 to 72. All atrium tissues are placed into isolated organ bath and washed for 3 hours in order to diminish the effects of anaesthetic agents. Adrenaline 10-1 was administered in tissue cabs for producing isometric contractions. Contraction width measurements were used as contraction parameters. Cumulative sertraline (10-9 to 10-4) doses were added in organ baths. The contractions were recorded accordingly. Friedman and Kruskal Wallis tests were used for statistical evaluation.

RESULTS: Inhibition of contraction was statistically significant at the 10-7 M, 10-6 M, 10-5 M, 10-4 M doses of sertraline following the initial administration of adrenaline. Also statistically significant inhibition of contraction occurred at 10-6 , 10-5 , 10-4 M doses when compared with 10-9, 10-8, 10-7 M doses of sertraline.

CONCLUSIONS: Results of this study showed that sertraline which is a selective serotonin re-uptake inhibitor agent, may cause negative inotropic effects on human atrium muscle. This negative effect is markedly seen when sertraline doses are augmented. Therefore, sertraline may be carefully used in cardiac surgery performed patients , especially in the early postoperative period.

D01-12

Cerebral oxygenation in Metabolic Syndrome patients during mental task and muscles metaboreflex activation: a preliminary study

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Introduction.

Muscles metaboreflex activation increases sympathetic tone and it is a well established method to study cardiovascular adjustments to exercise as well as to reveal cardiovascular diseases^{1,2}. Our hypothesis was that, in patients suffering from Metabolic Syndrome (MS), cerebral oxygenation decreased during the metaboreflex elicited by post-exercise muscle ischemia (PEMI) method, ought to an exaggerated increase in sympathetic tone in these patients³. Furthermore, we hypothesized that, in MS patients, during the metaboreflex cerebral oxygenation was further impaired by superimposing a mental task (MT).

Methods.

15 subjects (7 females, age 33-58 yrs.) suffering from MS took part in this investigation. They were free from any known sympathetic dysfunction. Patients underwent 5 different tests each lasting 12 min: PEMI, control exercise recovery (CER), PEMI+MT, CER+MT, and MT alone. During each session, cerebral oxygenation was detected by Near Infrared Spectroscopy (NIRS) by applying sensors to the skin of the forehead.

Results.

Data analysis showed that during MT test, cerebral oxygenation was significantly reduced with respect to the other tests. No reduction was found in cerebral oxygenation during PEMI. The MT test added to the PEMI test did not induce any significant decrease in cerebral oxygenation.

Conclusions.

From our results it can be concluded that in patients with MS adding MT to PEMI does not impair cerebral oxygenation. It remains to ascertain whether the response to this tests is similar in healthy subjects.

¹Roberto et al. J Appl Physiol 2012 113: 1323-31.

²Roberto et al. J Appl Physiol 2017 122: 376-385.

³Crisafulli and Roberto Curr Diabetes Rev 2017 (at press)

D01-13

A method for isolation of functional human ventricular myocytes from fresh epicardial biopsies

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Most of basic research findings in the field of cardiac cell physiology rely on data gathered in cells obtained from various animal models or tissues other than human ventricle (atrial myocardium or derived from iPS cells). Although these approaches offer possibilities of mechanistic investigations, they still have many limitations leading to difficult translatability to real-life human and clinical situations. We developed a protocol for isolation of functional cardiac myocytes from small epicardial biopsy samples of human left ventricle obtained during open-chest coronary artery-bypass grafting surgery.

Biopsy was performed in patients undergoing such surgery who gave their informed consent. Biopsy samples weighing 5-10 mg were immersed in ice-cold BDM-supplemented cardioplegia solution, cut to 350 mm pieces and incubated in Joklik medium containing trypsin and Liberase TM enzymes, and gently shaken for approximately 60 min at 37 °C. Viable rod-shaped cardiomyocytes appear after 45 min of digestion. Afterwards, 1 ml of cell suspension is transferred to enzyme-blocking solution and settled for 10 min. This step is repeated every 5 min until tissue pieces become completely dissolved. Joklik medium is then replaced with Tyrode solution and calcium is gradually added to 1.2 mM. In the end, calcium-tolerant cardiomyocytes were loaded with Fura-2AM and were able to contract upon electrostimulation and exhibit stable calcium transients.

Cardiomyocytes obtained by the described procedure could be used for other purposes, such as immunocytochemistry, patch clamping, contractility assessment, etc. The procedure of sample collection is straightforward, safe for patients and is not limited to specific type of cardiac surgery.

D02: Vascular physiology

D02-1

Crowding stress results in long-term vascular and behavioral alterations of in prehypertensive rats

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This study determined long-term influence of social stress on vascular function and behavior in young male borderline hypertensive rats (BHR) and Wistar-Kyoto rats (WKY). Five-week rats were exposed to crowding stress for two weeks and then returned to control conditions for two weeks. Systolic blood pressure (BP) of five-week BHR was significantly increased vs. WKY (127±3 vs. 104±3 mmHg, $p<0.01$). Crowding significantly accelerated BP increase and elevated plasma corticosterone vs. controls only in BHR, which persisted two weeks post stress. Maximal acetylcholine-induced relaxation of the femoral artery was unchanged by stress yet a significantly reduced nitric oxide (NO)-dependent component of relaxation was detected in BHR two weeks post stress in agreement with reduced aortic NO synthase activity. Stress reduced NO production in the cerebellum brain stem and hypothalamus in both rat strains, which persisted during post stress period. In stressed BHR, open arm distance traveled in elevated plus maze was increased vs. age-matched controls without changes in total distances traveled, which was not seen in WKY. In conclusion, data showed that exposure to social stress in peripubertal period led to long-term elevation of plasma corticosterone, reduced NO production, acceleration of the early development of hypertension and delayed behavioral alterations only in rats with borderline elevated blood pressure. Study was supported by grants Nos. APVV-0523-10 and VEGA 2/0160/17.

D02-2

Enhanced inhibition of endothelial cell proliferation and migration by multikinase inhibitor and blocking of metabolism

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Angiogenesis contributes to pathophysiological processes such as inflammation and cancer and therefore several antiangiogenic therapies have been established. Blocking of angiogenesis signals has become an attractive strategy. Metabolism of endothelial cells is an important regulator of angiogenesis and therefore glycolysis represents another promising target. We investigated the efficiency of combined approach for inhibition of glucose metabolism together with blocking of angiogenesis by multikinase inhibitor. Effects were evaluated on the basis of proliferation and migration of endothelial cells and postreceptor signaling pathways underlying both processes.

Human umbilical vein endothelial cells (HUVEC) were treated with multikinase inhibitor (sunitinib) and inhibitor of glucose metabolism 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) alone or in combination. Inhibition of HUVECs proliferation was tested by MTT assay and migration was evaluated by wound healing assay. Signaling pathways were analyzed by western blot analysis.

3PO had an inhibitory effect on cell migration and proliferation and sunitinib suppressed both processes in a dose dependent manner. Combined treatment with sunitinib and 3PO had additive suppressive effect on HUVECs migration and proliferation as compared to cells treated with individual inhibitors.

We showed stronger effects of simultaneous inhibition of glucose metabolism together with multikinase inhibitor treatment on reduction of migration and proliferation of endothelial cells *in vitro*. Results provide a basis for identification of new pathways, which are promising for effective inhibition of angiogenesis.

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D02-3

CHOLINESTERASES IN RAT AORTA

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QUESTIONS. The endothelium-dependent vasodilatory effect of acetylcholine (ACh) is well described, but only little is known about ACh-hydrolyzing cholinesterases (ChE) in blood vessels. Therefore, the aim of our project was to examine the presence of ChE in rat aorta, and to describe their possible physiological functions. **METHODS.** Thoracic aortas isolated from 12 weeks old male Wistar rats were used. Modified Karnovsky and Roots method was used for staining of ChE in the present of selective inhibitors for acetylcholinesterase (AChE), BW284C51 or butyrylcholinesterase (BChE), iso-OMPA. Immunohistological experiments were performed with polyclonal anti-AChE and monoclonal anti-BChE antibodies prepared in our laboratory. Commercially available specific markers of smooth muscle (anti-MLCK) and endothelium (anti-e-NOS) were used. Expression of AChE and BChE were analyzed using qRT-PCR. Effect of BChE on the ACh – induced vasodilatation in isolated aorta was observed by adding iso-OMPA. **RESULTS.** Activity staining, immunohistochemistry and qRT-PCR methods confirmed presence of AChE and BChE in rat thoracic aorta, with precise localization in the smooth muscle. Interestingly, BChE was clearly predominant in rat aorta. Selective inhibition of BChE by iso-OMPA lead to decrease of ACh-induced vascular relaxation in comparison to control aorta. **CONCLUSION.** We conclude that BChE is the dominant ChE in rat aorta and plays an important role in the regulation of the vascular tone.

The project was supported by grants: VEGA 1/0855/15, UK 415/2015, ITMS 26240120031.

D02-4

Levamisole, a cocaine adulterant, impairs acetylcholine dependent relaxation in the rabbit renal artery.

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Questions: Cocaine induces endothelial dysfunction mainly by reducing nitric oxide (NO) production. Levamisole, increasingly used as cocaine adulterant, produces vasculitis, but its effects on vascular tone are not fully investigated.

Methods: Rabbit renal rings were mounted for isometric tension recording in organ baths. Concentration-response curves to acetylcholine (ACh) were obtained in the absence and presence of levamisole, cocaine and the combination of both drugs. Furthermore, ACh curves were performed in the presence of L-NG-Nitroarginine methyl ester (L-NAME, 10^{-4} M) and superoxide dismutase (SOD, 200 U/L) to study NO-dependent vasorelaxation and ROS production.

Results: Relaxant response to ACh was reduced by both cocaine (10^{-5} and 10^{-4} M) and levamisole (10^{-3} M). Levamisole blockade was higher than that induced by cocaine (pD2 6.6 ± 0.1 for levamisole 10^{-3} M vs 7.5 ± 0.2 for cocaine 10^{-4} M, $p < 0.05$). Cocaine plus levamisole did not potentiate the response of levamisole. Reduced ACh response in the presence of L-NAME was not modified by cocaine whereas levamisole further potentiated the blockade. SOD completely prevented the effects induced by cocaine but partially those induced by levamisole.

Conclusions: Cocaine impairs endothelium-dependent relaxation by reducing NO bioavailability that could be related to increased ROS production. Decreased ACh-response to levamisole is independent of NO and could be in part mediated by oxidative stress.

D02-5

Acute adrenergic effects of levamisole, a cocaine adulterant, in rabbit carotid artery.

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Questions: Cocaine effects on vascular tone are primarily mediated by an enhancement of the sympathetic activity. Levamisole, a cocaine adulterant, blocks the reuptake of noradrenaline in cell cultures, potentiating the actions of cocaine. However, the synergistic effects of cocaine and levamisole in vessels have not yet been evaluated.

Methods: Rabbit carotid rings were mounted for isometric tension recording in organ baths. Concentration-response curves to phenylephrine (Phe) (10^{-9} - 3×10^{-5} M) were obtained in the absence and presence of levamisole (10^{-5} - 10^{-3} M), cocaine (10^{-5} - 10^{-4} M) and the combination of both drugs. Electrical field stimulation response was obtained at 8 Hz in the absence and presence of levamisole (10^{-6} - 10^{-3} M), cocaine (10^{-5} - 10^{-4} M) and the combination of cocaine (10^{-4} M) plus levamisole (10^{-6} - 10^{-3} M).

Results: Cocaine 10^{-4} M potentiated the contractile response to Phe (pD2= 5.7 ± 0.1 vs 6.0 ± 0.1 , control vs cocaine $p < 0.05$). Levamisole 10^{-3} M reduced the contractile response to Phe (pD2= 5.7 ± 0.1 vs 5.1 ± 0.1 , control vs levamisole $p < 0.05$) that was not modified in the presence of cocaine. Both cocaine (10^{-5} - 10^{-4} M) and levamisole (10^{-6} - 10^{-4} M) produced a concentration-dependent potentiation of EFS ($26 \pm 2\%$ for control, $43 \pm 3\%$ for cocaine (10^{-4} M) and $52 \pm 5\%$ for levamisole (10^{-4} M). This effect was further increased by combination of cocaine 10^{-4} M plus levamisole 10^{-4} M ($60 \pm 3\%$, $p < 0.05$). Levamisole 10^{-3} M abolished the contractile response to EFS either alone or in the presence of cocaine.

Conclusions: Levamisole potentiates the cocaine sympathetic response. However, at higher concentrations, levamisole acts as an α 1-adrenergic antagonist and abolishes adrenergic neurotransmission, pointing to a toxic effect.

D02-6

Protein expression of HIF-1 alpha, VEGF and cyclooxygenases in cerebral blood vessels of Sprague-Dawley rats on a short-term high salt diet

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Question: Our previous study showed increased oxidative stress and impaired vascular reactivity in cerebral blood vessels of rats on high salt diet (HSD). Increased oxidative stress can affect HIF-1 α expression and its downstream proteins, such as COX-2. Purpose of this experiment was to determine effect of HS diet on protein expression of HIF-1 α , its target protein VEGF and COX-1 and COX-2 in cerebral blood vessels.

Methods: 11 week old Sprague-Dawley rats (N=8 per group) were divided in three groups: CTRL (control group of rat); HSD (rats on HS diet for 7 days, 4% NaCl) and HSD+TEMPOL (rats on HS diet and 1mM TEMPOL in drinking water for 7 days). Prior to decapitation and surface cerebral blood vessels collection, rats were anesthetized with ketamin-chlorid (75 mg/kg) and midazolam (0.5 mg/kg). Protein levels of HIF-1 α , VEGF and COX-1 and COX-2 were assessed. One-way ANOVA or student t-test was used when appropriate (SigmaPlot v11.2, Systat Software, Chicago, USA), $p < 0.05$ was considered significant. All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals (directive 86/609) and were approved by the local and national Ethical Committee.

Results: Results show significantly increased expression of HIF-1 α transcriptional factor and VEGF in HSD groups compared to CTRL and HSD+TEMPOL group. There were no differences in COX-1 expression. Expression of COX-2 was increased in HSD group compared to HSD+TEMPOL group.

Conclusion: Increased protein levels of HIF-1 α and COX-2 in brain blood vessels may be related to increased oxidative stress caused by high salt diet and reversed by TEMPOL in vivo.

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D02-7

REMODELING OF CORONARY ARTERY NETWORK DURING QUERCETIN SUPPLEMENTATION

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Objective: Polyphenols, including quercetin, present in human diet, have various physiological effects. Short term vasodilatory actions on coronary arterioles have been demonstrated by us, but no information is available concerning their long term effects on microvascular networks.

Methods: Male Wistar rats were divided into two groups. In group Q, animals were treated with 30 mg/kg quercetin per os (n=9), while group C was kept in parallel (n=10) for 8 weeks. Animals were sacrificed, and left descending coronary artery network was prepared down to 80 μ m inner diameter. Orifice was cannulated, and continuous saline flow and input pressure were maintained during recording. Pictures of the network were analyzed digitally.

Results: Quercetin treatment reduced hemodynamically disadvantageous components in the network: branching angles under 45° or over 105° were less frequent in group Q (9% vs. 23%, $p < 0.05$). Segmental tortuosity decreased ($11.0 \pm 0.1\%$ vs. $9.4 \pm 0.1\%$, $p < 0.01$), similar to the frequency of broken courses (13.5 ± 0.6 vs. 1.0 ± 0.5 pcs, $p < 0.01$). At bifurcations, asymmetry of daughter branches decreased (2.3 ± 0.2 vs. 1.7 ± 0.1 , $p < 0.03$). Vessel wall thickened beyond 400 μ m arterioles ($p < 0.05$). Network tortuosity, an indicator of order in coronary networks, was decreased as an effect of quercetin treatment ($11.5 \pm 0.2\%$ vs. $9.7 \pm 0.1\%$, $p < 0.01$).

Conclusions: Chronic administration of quercetin resulted in reduced number of hemodynamically disadvantageous sections of the coronary networks. It is assumed that quercetin can delay non-beneficial vascular remodeling caused by e.g. long term hemodynamic stress, or ageing.

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D02-8

Age-related changes in endothelial function of pulmonary arteries in an experimental model of essential hypertension

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Questions: The spontaneously hypertensive rat (SHR) is the commonly used model of essential hypertension (EH). Hypertension in SHR may progress to heart failure (HF). Hypertrophy of the right heart ventricle (RHV) is a reliable marker of the HF. The failing SHR become less active. SHR can also serve as a model of pulmonary hypertension due to left ventricular dysfunction, but a detailed functional study of the pulmonary arteries (PA) has not been carried out. The aim of this study was therefore to analyze the endothelium-dependent relaxation (EDR) of the PA in the young adult (20-week-old) and old (52-week-old) male SHR compared with WKY rats.

Methods: Blood pressure (BP) was determined by tail-cuff and locomotor activity using the open-field. EDR of large PA and small mesenteric arteries (SMA) was investigated in the presence and absence of nitric oxide (NO) synthase inhibitor.

Results: In both SHR group, BP and locomotor activity was increased. Biometric analyses indicated left ventricular hypertrophy, but not hypertrophy of the RHV in both SHR groups. EDR was significantly reduced in SMA of adult SHR vs. WKY, which was not observed in the PA. We observed NO-related endothelial dysfunction (ED) in the PA in old SHR.

Conclusions: Results demonstrated ED in the PA of the old SHR, however without signs of HF. Thus, ED in the PA may not be associated with RHV hypertrophy and/or HF in SHR.

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D02-9

Tissue oxygenation modulates muscle compression-induced hyperaemia

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The characteristic of skeletal muscles to develop a rapid hyperemia in response to muscle compression was recently pointed out by several studies. Controversial are the hemodynamic effects of repeated compressions, reported in the literature. The issue is relevant for medical treatments aimed at improving local circulation by repetitive tissue compression, e.g., intermittent pneumatic compressions (IPC).

The present study aims at investigating changes of compression-induced hyperemia upon repetitive stimulation. Leg mechanical compressions were delivered at different inter-stimulus intervals (ISI, ranging from 20 to 160 s) through a customized IPC device based on five pneumatic cuffs wrapped around the lower part of right limb of ten healthy subjects. A comprehensive approach was employed, including continuous monitoring of tissue oxygenation and blood volume in calf muscles by near infrared spectroscopy (NIRS), as well as simultaneous eco-Doppler monitoring of femoral artery inflow and vein outflow.

The results indicates that: i) a long lasting increase in local tissue oxygenation follows the compression-induced hyperemia; ii) the amplitude of the hyperemia is not associated with the amount of blood volume displaced by the compression iii) the hyperemia in response to the repetitive stimulation exhibits different patterns of attenuation depending on the ISI; iv) the extent of the attenuation of the hyperemic response to each single compression correlates with the extent of oxygenation already present in the tissue ($r = -0.78$, $P < 0.05$).

Conclusions: i) increased tissue oxygenation limits the hyperemia in response to repetitive compressions; ii) NIRS is a useful tool for monitoring the outcome of IPC treatments.

D02-10

Different structural alterations in conduit arteries of spontaneously hypertensive rats compared to Wistar rats from the prehypertensive period to late adulthood

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Systolic blood pressure (BP) and structure of thoracic aorta, carotid and iliac artery of spontaneously hypertensive rats (SHRs) were assessed during ontogeny. Wistar rats and SHRs aged 3, 17, and 52 weeks were used. BP was measured by plethysmography weekly. The vascular system was perfused with a fixative under the pressure of 90 mm Hg in 3 and 120 mm Hg in 17- and 52-week-old rats. The middle parts of the arteries were processed for electron microscopy. The wall thickness (intima+media) (WT), cross-sectional area (intima+media) (CSA), inner diameter (ID), and WT/ID were measured in light microscopy. The volume densities of endothelial cells (ECs), muscle cells (SMCs), and extracellular matrix (ECM) were measured in aorta using electron microscopy, and their CSAs were calculated. In 3-week-old SHR compared to Wistar rats, the BP did not differ; in the thoracic aorta, all of the parameters (WT, CSA, ID, WT/ID, CSA of SMCs, CSA of ECs, and CSA of ECM) were decreased; in the carotid artery, WT and CSA did not differ, ID was decreased, and WT/ID was increased; in the iliac artery, WT, CSA, WT/ID were increased, ID was decreased. In 17- and 52-week-old SHRs, the sBP and all of the above parameters in all arteries were increased, only ID in iliac artery in 52-week-old SHRs and CSA of ECs in the aorta in 17-week-old SHRs did not change. The study revealed that in some conduit arteries of SHRs, (i) morphological changes are present in the prehypertensive period, and (ii) there is a positive correlation but quantitative disproportional relationship between BP increase and structural alterations during ontogeny. Different modes of

alteration can reflect the flexibility of the arterial tree to the different needs of supplied areas. Supported by VEGA 2/0048/17, APVV-15-0565.

D02-11

Quercetin supplementation moderates hypertension induced remodeling of coronary artery network

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Aims: Previously, we demonstrated beneficial acute and chronic effects of quercetin on coronary network properties and function. In this work, it was studied if chronic administration of quercetin could influence network remodeling of coronary vessels induced by experimental hypertension.

Methods: Male Wistar rats were divided into two groups. HQ group was treated with quercetin (30 mg/kg), while HC group was kept as parallel control. After 28 days, osmotic minipumps were implanted to infuse angiotensin II (200 ng/min/kg) for 28 days. At the end of treatment, animals were sacrificed, left descending coronary with its ramifications were prepared down to 80 µm and perfused. Using computerized videomicroscopy, network properties were analyzed offline.

Results: Quercetin treatment resulted in decreased blood pressure (161±7/122±6 vs. 137±10/110±10 mmHg, p<0.05). Segmental tortuosity of coronaries decreased in HQ group (1.3±0.19 vs. 0.4±0.06%, p<0.01), vessel wall slightly thickened over 200 µm inner diameter (p<0.01). Proximally, extremely widened (600-800 µm) artery segments appear in HC group, while distally more resistance size vessels were found (100-300 µm) in HQ group (p<0.01). Hemodynamically disadvantageous branching angles were less frequent in HQ group (24 vs. 16 % of total). Network tortuosity, an indicator of disorganization, decreased in HQ group (14±1 vs. 10±0.1%, p<0.01). Number of vascular anomalies decreased in HQ group (6.8±0.7 vs. 3.6±0.7 pcs, p<0.01).

Conclusion: Chronic quercetin treatment moderates angiotensin induced hypertension and disadvantageous remodeling of coronary artery network.

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D02-12

Exploring the murine microvascular response variability to hyperoxia with the wavelet transform

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Oxygen inhalation is widely used to dynamically assess microcirculation in vivo, and it is known to produce variable responses. Laser Doppler flowmetry (LDF) is a reference technique to quantify in vivo microcirculation, providing an oscillatory signal with several frequency components, whose components are still, however, poorly described in murine. Our aim was to describe the components of the murine cutaneous microcirculation using the wavelet transform (WT). 16 male C57/BL6 mice (18 ± 10 weeks old) were subjected to a 100% oxygen breathing protocol under ketamine-xylazine anesthesia, consisting in three phases – 10 min room atmosphere breathing, 10 min 100% oxygen breathing, and 10 min recovery. LDF signals were recorded on both hind paws. All procedures involving animal experimentation were ethically supervised. Two different vascular responses to

hyperoxia were observed – perfusion decrease (PD) in 20/32 paws and perfusion increase (PI) in 12/32 paws. WT allowed the identification of several LDF frequency ranges, compatible with the cardiac (5.3-4.6 Hz), respiratory (3.8-3.2 Hz), myogenic (0.17-0.059 Hz), sympathetic (0.052-0.020 Hz), endothelial NO dependent (0.017-0.0094 Hz) and endothelial NO independent (0.0084-0.0042 Hz). The animals that responded with PD showed a significant increase in the cardiac and myogenic activities and a significant decrease in the NO-dependent activity. The animals that responded with PI showed a significant increase in the cardiac and myogenic activities, with no significant changes in the endothelial components. These results show the murine LDF frequency ranges, and also highlight the usefulness of the wavelet transform for the characterization of microvascular reactivity to hyperoxia.

D03: Molecular & cellular physiology

D03-1

The Role of Palmitoylation in Glutamate-Mediated Excitotoxicity in Neurodegenerative Diseases.

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Chronic neurodegenerative disorders such as Alzheimer's and Huntington's disease are associated with neuronal degeneration due to glutamate-mediated excitotoxicity as a result of dysfunctional proteins in the glutamatergic tripartite synapse. Palmitoylation controls several synaptic proteins in physiological glutamate neurotransmission but the role of palmitoylation in neurodegenerative diseases is unknown.

An integrated approach was taken to review the published literature and to make novel predictions to determine whether synaptic protein palmitoylation was disrupted and contributed to excitotoxicity in neurodegenerative diseases, with a focus on proteins already implicated in these disorders.

The major conclusion drawn is that disrupted palmitoylation to proteins throughout the tripartite synapse contributes to the mechanisms controlling excitotoxicity in Huntington's disease. The majority of synaptic proteins implicated in disease are predicted to be palmitoylated. Therefore, aberrant palmitoylation in each component of the tripartite synapse is predicted to affect homeostatic glutamate neurotransmission.

This research develops our understanding of the role of palmitoylation in neurodegenerative disease. Palmitoylation is disrupted in Huntington's disease which highlights a novel therapeutic target in the treatment of this disorder. But further research is necessary to establish whether aberrant palmitoylation is a common pathological mechanism in other life-limiting, neurodegenerative diseases.

D03-2

Cellular Calcium Balance in Chronic Kidney Disease

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Questions: Calcium cations (Ca²⁺) are an important ubiquitous messenger, controlling a broad range of cellular processes. Disturbances in cellular calcium homeostasis in patients with chronic kidney disease (CKD) represent a complex process which aggravates with CKD progression. We aimed to

investigate the state of cellular Ca^{2+} , Ca^{2+} entry via Ca^{2+} /cation channels and Ca^{2+} removal through plasma membrane Ca^{2+} -ATPases (PMCA) in the blood cells of CKD patients.

Methods: The study involved 22 healthy volunteers and 22 CKD stage 2-3 patients. Cytosolic Ca^{2+} measurements were performed by Fluo-3 fluorimetry. To examine the Ca^{2+} entry via calcium release activated calcium (CRAC) channels, an inhibitor of these channels, was applied. To determine the function of P2X_7 receptors, the antagonist and agonist of these receptors were used. The activity of PMCA was determined by UV-VIS spectrofluorimetry.

Results: Cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and Ca^{2+} concentration of intracellular stores of CKD patients was higher in comparison with healthy subjects. The Ca^{2+} entry via CRAC channels were increased in CKD patients. Activation of P2X_7 receptors caused an increase in $[\text{Ca}^{2+}]_i$ in both groups, but the effect was significantly smaller in CKD patients, likely by reason of higher $[\text{Ca}^{2+}]_i$ under basal conditions. Inhibition of P2X_7 receptors reduced $[\text{Ca}^{2+}]_i$ in CKD, but had no effect in healthy subjects. The activity of PMCA, was found to be decreased by 25 % when compared to healthy subjects.

Conclusions: Our results demonstrate that all these alterations in Ca^{2+} signaling are contributing to the elevated $[\text{Ca}^{2+}]_i$ from early stages of CKD.

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D03-3

An Investigation into the Effects of Extracellular Acidification on Mouse Uterine Contractions; Are ASICs Involved?

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Adequate uterine contraction is crucial to safe delivery of the foetus. Acidity can play an important role in affecting myometrial contraction but the effects, especially concerning external acidification, are unclear. This study was designed to determine the effects of acidic pH on myometrium and any gestational differences, and to examine ASICs as possible mediators to the effects. **Methods:** Mouse uterus was used either on day 14 or 19 (term) of gestation. Changes in pH to 6.9 were made for 10 minutes, by adding HCl to physiological saline. Spontaneous contractions were measured and data analysed. Statistical differences were tested with Student's t test and taken at $P < 0.05$. Western Blotting was done following the protocol used previously in our lab. All assays. Mouse brain and urinary bladder were used as a control for ASICs expression. **Results:** At 14 days, extracellular acidification to pH 6.9 significantly increased AUC, mainly due to an increased frequency and amplitude. At 19 days, extracellular acidification to 6.9 also significantly increased AUC, frequency and amplitude (P value < 0.05). Application of repetitive 10 min episodes of acidification (pHo 6.9) caused sequential increase in the amplitude of the force on term myometrium, but not in 14 days pregnant mouse. Western blotting showed ASICs 2a and 3 proteins were expressed in the mouse myometrium. **Discussion:** In mouse, a decrease of pHo stimulated pregnant uterine contraction. Extracellular acidification significantly increased the amplitude in term pregnant mouse more than mid-pregnant uterus which suggests a possible role of external acidification in labour. ASICs have been reported, and thus may contribute to increases in force via depolarization and increased Ca entry

D03-4

The effects of the luteal cells cocultured with islet cells on cell viability and functionality in rats*

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Questions: It was aimed to evaluate the islet cell viability and functionality by coculturing the islet cells with luteal cells.

Methods: After isolation of luteal cells from the superovulated corpus luteum, they seeded to wells to attach the bottom of the wells and incubated for 24 hours. Islet cells were isolated at 24th hours of incubation of luteal cells. Then luteal cells and islet cells cultured separately or together by coculturing for 96 hours. Islets viability and glucose stimulation tests performed and stimulation index (SI) was calculated and total insulin secretion was determined in the islet medium and coculture medium at 48th and 96th hours.

Results: It was observed that islets viability increased significantly ($p < 0.05$) at 48th h of the incubation in the cocultured group as compared to the only islet group. Islets viability was also increased at the 96th h in the cocultured group, but this increase did not found to be significantly. Islet functionality and insulin secretion were increased ($P < 0.05$) in the cocultured group as compared to the only islet groups both 48th and 96th hours.

Conclusions: Although islet cells were incubated up to 48 h to transplant in routine procedure; in this study, viability and functionality of islet cells were maintained up to 96 hours, by coculturing with luteal cells and increased insulin secretion. These results suggest that the coculturing of islets cells with luteal cells may help the induction of insulin secretion after transplantation, by maintaining the viability and functionalities of the islets and it is extremely important for transplantation success.

Keywords: coculture, islet cell, luteal cell

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D03-5

Role and transcription profiles of $\alpha 1$ and $\beta 2$ adrenergic receptors in tissues of yellow and silver European eels

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Adrenergic receptors (AR) belong to the wide family of G-protein coupled receptors (GPCR) and specifically bind catecholamines, i.e. epinephrine and norepinephrine. According to mammalian classification, there are two main groups of AR, α and β , with several subtypes: $\alpha 1$ (a Gq coupled receptor) and $\alpha 2$ (a Gi coupled receptor); $\beta 1$, $\beta 2$ and $\beta 3$ (all coupled to Gs proteins). Pharmacological ligands were of limited help to demonstrate the presence and role of α and β subtypes in tissues from teleost fish, while further insights have recently been provided thanks to molecular approaches. In the present study, we evaluated the glucose output from isolated hepatocytes of yellow (feeding phase) and silver (sexually developing and migrating phase) European eels (*Anguilla anguilla*), treated with epinephrine. The hormone induced a time and dose-dependent glucose output, which appeared to be slightly different between yellow and silver eels. Major differences were instead observed in AR transcription profiles. The expression of $\alpha 1$ and $\beta 2$ AR was evaluated by qPCR in different tissues of eels. Expression $\alpha 1$ -AR mRNA was in the range of tens copies/ng RNA in liver, brain, gills and muscle of yellow eels, with values about 10-fold higher in the liver of silver eels. Expression of $\beta 2$ -AR mRNA in liver, brain, gills and muscle of yellow eels was in the range of hundreds of copies/ng RNA, with

values about 5-fold higher in the liver of silver eels. Differently, expression of $\alpha 1$ - and $\beta 2$ -AR mRNA in the heart was about 8-fold lower and about 2-fold higher in silver versus yellow eels, respectively.

D03-6

A new animal model for epithelial ion transport modeling (focusing on CFTR) – wild type ferrets

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Cystic fibrosis (CF) is a lethal genetic disease affecting several organs, including the pancreas. Several animal models are available to study the CF related tissue damage but they have clear limitations. Recently a cystic fibrosis transmembrane regulator (CFTR) knock out ferret model was generated, they born with a normal pancreas, but, within a short period they develop a CF related pancreatic damage. This model would be the first available one to study pharmacological prevention of the disease development.

Intra/interlobular pancreatic ducts were isolated from the WT ferret pancreas. Expression of CFTR was detected by immunohistochemistry. Resting pH, buffer capacity and Cl⁻/HCO₃⁻—exchange activity were evaluated by microfluorometry. Buffer capacity was calculated by measuring Δ pHi in response to different concentrations of NH₄Cl/HCO₃⁻—pulses in Na⁺-free solutions. Fluid secretion was examined by video microscopy.

CFTR was expressed on the luminal membrane of ferret pancreatic ducts. The resting intracellular pH of pancreatic epithelial cells is lower (7.17 \pm 0.08) in ferrets compared to mice (7.31) or to guinea pigs (7.36). Concerning the bicarbonate influx mechanisms, functionally active sodium/hydrogen exchanger and sodium/bicarbonate cotransporter were detected. Anion exchanger activity measured by NH₄Cl-technique, Cl⁻ removal and inhibitory stop methods indicated that ferret pancreatic ducts secrete similar amount of bicarbonate as mice and guinea pigs. Video microscopy revealed a significant increase in fluid secretion to HCO₃⁻— and to 5 μ M forskolin stimulation.

Major epithelial ion transporters are expressed in the ferret pancreatic ductal epithelial cells. Our results indicate that ferret could be a suitable model organism to study the CF style pancreatic damage.

D03-7

Interactions of cyclic adenosine monophosphate production and store operated Ca²⁺ entry

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Introduction. The cyclic adenosine monophosphate (cAMP) and Ca²⁺ signaling play central role in the regulation of the secretory functions of epithelial cells. The two signaling system were reported to localized to specific intracellular microdomains and have multiple synergistic interactions helping to optimize the cellular response to stimulation. One of the recently described interactions suggest connection between the store operated Ca²⁺ entry with adenylyl cyclase 8 (AC8) that increase cAMP production, however the molecular mechanism of this process is not known.

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Poster Session D

Aim. We aimed to characterize the interactions of SOCE with cAMP.

Methods. Human embrional kidney (HEK) cells were transfected with plasmids encoding the proteins of interest. Cellular cAMP production was measured by fluorescence resonance energy transfer (FRET) using the cAMP reporter Epac1.

Results. The stimulation of the cells with 5 μ M forskolin and 100 μ M 3-isobutyl-1-methylxanthine (IBMX) resulted in reversible elevation in cAMP production. The expression of AC8 significantly elevated the cAMP response. The overexpression of the endoplasmic reticulum (ER) Ca²⁺ sensor Stim1, or the Ca²⁺ channel Orai1 (components of SOCE) increased the cAMP production upon stimulation. The effect of Stim1 and Orai1 was not depending on the ER Ca²⁺ content, or extracellular Ca²⁺ influx. Extended synaptotagmin 1 (E-Syt1), a recently described ER-plasma membrane tethering protein, increased the cAMP response, similarly to Stim1-Orai1.

Conclusions. Our results showed the interaction of SOCE and cAMP production that might play an important role in the regulation of cAMP production. However further studies are required to clarify the mechanisms of the interaction.

D03-8

INVESTIGATION OF PROTECTIVE EFFECT OF PARIETIN AGAINST GLUTAMATE EXCITOTOXICITY IN PRIMARY CORTICAL NEURON CULTURE

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QUESTION: Glutamate is one of the neurotransmitters in the central nervous system. Glutamate accumulation can excessively activate the N-methyl-D-aspartate receptors and cause excitotoxicity. In this study, the neuroprotective activity of parietin in primary cortical neuron cultures against glutamate excitotoxicity was evaluated.

METHOD: Primary cortical neuron cultures prepared from cerebral cortices of newborn rats. Cultures were exposed to 10⁻⁵ M glutamate to induce glutamate excitotoxicity. Then, different concentrations of (final concentrations in the well to be 2.5-500 μ M) parietin were added into the medium. Cell survival in different parietin groups were measured by MTT assay. TAS-TOS analyses were used to evaluate reactive oxygen species generation. Obtained data were evaluated by using One-way anova with post-hoc LSD test using SPSS 20.0 software.

RESULT: In our study, IC₅₀ (inhibitory concentration where 50% of the cells die) of parietin was determined to be 10 μ M at the 24th hour cortical neuron culteres by MTT assay. TAS assay results demonstrated that 10 μ M of parietin increased the antioxidant level in cells, which might help to protect neurons against glutamate induced exitotoxicity. TOS assay result exhibited that 10⁻⁵ M glutamate and higher doses of parietin increased oxidant level in cells inducing stress to neurons.

CONCLUSION: In this study; Parietin showed high neuroprotective effect in primary rat cortical neuron culteres against glutamate excitotoxicity. These results suggest that parietin can be used as a therapeutic agent for glutamate excitotoxicity, however, further studies are needed to clarify the mechanism/s of action of parietin.

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Poster Session D

D03-9

Association analysis between A163G and T245G gene polymorphisms of osteoprotegerin and bone mineral density in Turkish postmenopausal women

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Purpose: One of the most significant nominee genes for osteoporosis predisposition is Osteoprotegerin (OPG). The objective of this study was to evaluate the relationship between the A163G and T245G polymorphism in the OPG promoter with bone mineral density (BMD) of postmenopausal women.

Method and Materials: We found 194 voluntary subjects containing 109 with primary postmenopausal osteoporosis and 85 healthy people as controls. The BMD of L1, L4, total lumbar spine (L1-4), neck hip and total hip were evaluated by dual-energy X-ray absorptiometry (DEXA). The A163G and T245G polymorphisms in the OPG promoter were determined by using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Result and Discussion: The frequencies of genotypes were AG+GG (46.8%), AA (53.2%) for A163G and GG+TG (36.7%), TT (63.3%) for T245G polymorphisms in postmenopausal women. When compared with healthy women, remarkably more women with osteoporosis were found to have AG+GG ($p=0.005$) and GG+TG ($p=0.049$). An important association of genotypes with BMD at the lumbar spine ($p=0.027$, $p=0.019$, $p=0.024$, and $p=0.003$, respectively) was observed for T245G and A163G polymorphisms in postmenopausal women and controls, respectively. Genotypes GG+TG were related with lower BMD as compared with TT genotype for T245G polymorphisms. Similarly A163G polymorphisms, genotypes AG+GG were also related with lower BMD as compared with AA genotype.

Conclusion: Our results suggest that T245G and A163G polymorphisms in the OPG promoter might make a contribution to the genetic regulation of BMD. These results will be beneficial to examine the role of OPG gene in osteoporosis in studies to be carried out in the future.

Key words osteoprotegerin, osteoporosis, gene polymorphism, bone mineral density, polymerase chain reaction.

D03-10

Cell penetrating protein C inhibitor (PCI): Internalization, nuclear translocation, and potential intracellular targets

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PCI is a secreted serine protease inhibitor (serpin) with very broad protease reactivity and tissue distribution. Binding of glycosaminoglycans and phospholipids modulates its inhibitory activity and target enzyme specificity. We have shown that PCI can be internalized by cells most likely by directly crossing the phospholipid bilayer. Inside the cell it translocates to the nucleus. Currently we are analyzing the exact mechanism of PCI internalization and its intracellular role. N-terminally cleaved PCI as well as N-terminally truncated, functionally active PCI-mutants are not internalized by cells. Testisin, a glycosylphosphatidylinositol-anchored serine protease, which co-localizes with PCI in the testis, cleaves PCI not only at its reactive site but also at a site close to its N-terminus. This N-terminal cleavage releases a peptide rich in basic amino acids which was recognized as cell-penetrating

peptide. Local proteases might therefore regulate the cellular uptake of PCI. With cultured cells we observed uptake of PCI by only 10-15% of cells. Therefore there must be also hitherto unidentified cellular mechanisms regulating its internalization.

We have shown that PCI contains a functional nuclear localization signal in its H-helix. Analysis of subcellular fractions revealed that PCI is mainly found in the nuclear envelope fraction, where it interacts with cathepsin L. Enrichment of cathepsin L in the nucleus has been observed in cancer cells and in some transformed cell lines. Several substrates of nuclear cathepsin L have recently been identified, such as histone H3, 53BP1, and the transcription factor CUX1. Internalized, nuclear PCI may therefore be involved in the regulation of epigenetic modifications and/or in cell cycle progression.

D03-11

Significance of co-expression of transient receptor potential vanilloid 4 and aquaporin5 in pregnant uterine contractility in rats

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Questions: The expression of aquaporin5 (AQP5) is under multifactorial control (e.g. steroids and oxytocin). AQP5 has a crucial role in cellular osmotic changes. The increased AQP5 expression is followed by reduced uterine contractility. The transient receptor potential vanilloid 4 (TRPV4) is a Ca2+-permeable cation channel activated by osmotic changes. Our aim was to investigate the co-expression and cooperation of AQP5 and TRPV4 in pregnant uterine contractions in rat.

Methods: Reverse-transcriptase PCR and Western blot techniques were used to detect the changes in AQP5 and TRPV4 expression in pregnant rat uteri. The uterine contractions were measured in an isolated organ bath system with selective TRPV4 agonist (RN1747). Immunohistochemical method was used to determine the localization of AQP5 and TRPV4.

Results: The TRPV4 expression continuously increased from day 5 to the last day of pregnancy. The maximum of AQP5 expression was detected at day 18 of pregnancy, but it was practically disappeared at day 22. The TRPV4 and AQP5 co-expressed on pregnancy days 18 and 20. On day 22, however, TRPV4 expression was proved, only. The TRPV4 agonist did not influence the uterine contractions on pregnancy day 18, but it increased the activity at day 22.

Conclusion: Presumably AQP5 causes hypertonic state in the cells that inhibits the TRPV4 function. The lack of AQP5 expression at the end of pregnancy triggers a hypotonic stress which activates the TRPV4 and uterine contraction.

Key words: AQP5, TRPV4, pregnancy

D03-12

Sex, age and weight as determinants of plasma DNA: a cross-sectional study

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Questions: Extracellular DNA (ecDNA) is studied in many diseases as a possible marker but also as a trigger of an immune response important for the pathogenesis, both, in clinical studies and in animal experiments. The aim of our study was to describe the interindividual variability of ecDNA in plasma of healthy mice and to analyze potential determinants of the variability including sex, age and bodyweight.

Methods: For this experiment 78 adult CD1 mice (41 female and 37 female) of a variable age (126–493 days) and bodyweight (22–55 g) were used. DNA was isolated from plasma. Total ecDNA concentration in plasma was measured fluorometrically. Nuclear ecDNA and mitochondrial ecDNA were quantified using real time PCR. Deoxyribonuclease activity was measured using SRED method. A simple correlation analysis was conducted with age and bodyweight.

Results: No gender differences were found in plasma ecDNA and its subcellular origin (ecDNA average values 51,68 ng/ml of plasma for male and 52,67 ng/ml of plasma for female) or in DNase activity (0,0398 KU/μl for male and 0,0391 KU/μl for female). Correlations were found for total, nuclear and mitochondrial ecDNA neither with bodyweight, nor with age of the mice.

Conclusions: The results suggest that the interindividual variability of ecDNA in laboratory mice is with a coefficient of variability of 138% high. Sex, age and bodyweight seem not to affect plasma ecDNA in mice. The major contributors to the variability are yet to be identified in future studies taking into account the endogenous deoxyribonuclease activity.

D03-13

The role of aquaporin-4 e isoform in the regulation of rapid cell volume changes in astrocytes

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Water channel aquaporin 4 (AQP4) plays a key role in the regulation of water homeostasis in the brain. It is predominantly expressed in astrocytes at blood-brain and blood-liquor interfaces. In the mammalian brain several AQP4 isoforms have been identified. Two of them (AQP4a (M1) and AQP4c (M23)) have been confirmed to cluster into plasma membrane supramolecular structures, termed orthogonal arrays of particles (OAPs) and to enhance water transport through the plasma membrane. However, the role of the newly described water conductive mammalian isoform AQP4e is unknown. The focus of our study was to learn the dynamics of AQP4e aggregation into OAPs and its role in the regulation of astrocyte water homeostasis. With super-resolution structured illumination microscopy we determined, that AQP4e isoform is co-localized in OAPs in rat astrocytes. We observed that in hypotonic conditions which elicit cell edema, OAP formation is considerably enhanced by overexpressed AQP4e. This suggests that AQP4e may be involved in regulatory volume changes in astrocytes. We tested this hypothesis by using atomic force and confocal microscopies. Our results revealed that AQP4e is key in regulating rapid changes of volume in astrocytes.

D06: Respiratory physiology

D06-1

Exogenous surfactant enriched with anti-IL-8 antibody additionally improved lung functions in experimental meconium-induced lung injury

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Introduction

Meconium aspiration syndrome (MAS) is associated with massive neutrophils influx into the lungs and resulting inflammation and oxidation which could inactivate surfactant and induce respiratory failure. We hypothesized that addition of anti-IL-8 antibody to therapeutic surfactant could suppress neutrophil influx and inflammation what can contribute to improved lung functions and enhanced surfactant therapy in MAS.

Methods

New Zealand rabbits with meconium-induced respiratory failure were divided to: non-treated (M), exogenous surfactant treated (M+S) and treated with combined surfactant and anti-IL-8 antibody (M+S+anti-IL-8) groups. Blood gases and ventilation parameters, i.e. PaO₂/FiO₂, oxygenation index (OI), ventilation efficiency index (VEI), mean airway pressure (MAP), dynamic lung compliance (C_{dyn}), alveolar-arterial gradient (AaG) and O₂ saturation (SatO₂), were observed before and 30 min after meconium instillation and at 30 min, 1, 2, 3, 4, 5 h of the therapy (Th). Total and differential leukocyte counts were determined in bronchoalveolar lavage fluid (BAL).

Results

Combined M+S+anti-IL-8 therapy significantly improved PaO₂/FiO₂, OI, VEI at 2h and 5h Th compared to surfactant (p<0.05 vs. M+S) and reduced count of neutrophils and monocytes in BAL (p<0.01 and p<0.05 vs. M). Other lung function parameters showed comparable effects for both therapies M+S and M+S+anti-IL-8 vs. M.

Conclusions

Addition of anti-IL-8 antibody to surfactant therapy improved lung functions and reduced neutrophils and monocytes in BAL. Targeted inhibition of neutrophil influx and neutrophil-induced inflammation/oxidation by anti-IL-8 antibody likely stabilized surfactant and enhanced therapy of MAS.

Support: VEGA 1/0305/14; VEGA 1/0469/16; APVV-0435-11; APVV-15-0075

D06-2

Effects of inhaled roflumilast and tadalafil on airway reactivity and inflammation in ovalbumin-sensitized guinea pigs

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Question: Phosphodiesterases (PDEs) are enzymes involved in degradation of cAMP and cGMP. The clinical effectiveness of selective PDE4 inhibitors has been confirmed previously in chronic obstructive pulmonary disease associated with neutrophil inflammation. The aim of this study was to evaluate effects of roflumilast (selective PDE4 inhibitor) and tadalafil (selective PDE5 inhibitor) on *in vivo* and *in vitro* airway smooth muscle reactivity to histamine, changes in blood cells count in blood and bronchoalveolar lavage fluid, and some inflammatory mediators (IL-4 and IL-5) in ovalbumin sensitized guinea pigs with experimentally induced eosinophil inflammation.

Methods: Sensitized animals were exposed to aerosol of roflumilast, tadalafil, or both for 3 minutes once daily for 7 days.

Results: Sensitization with ovalbumin led to a significant increase in *in vivo* and *in vitro* airway reactivity. Similarly, increased plasmatic levels of IL-4, and IL-5 in blood were observed, with significant increase in differential counts of eosinophils both in blood and BAL fluid.

Conclusions: The administration of roflumilast and tadalafil suppressed significantly *in vivo* airway reactivity, *in vitro* airway reactivity in tracheal tissue strips, and IL-4 and IL-5 levels, suggesting that PDE4 and PDE5 inhibition has potent anti-inflammatory effects in guinea pigs with ovalbumin induced eosinophil inflammation.

Keywords: phosphodiesterase, inhibitors, airway reactivity, interleukin, inflammation

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D06-3

Comparison of three types of lung-protective ventilation in an experimental model of meconium aspiration syndrome

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Questions: Ventilation support in severe meconium aspiration syndrome (MAS) is realized with lung-protective techniques: conventional mechanical ventilation (CMV), high-frequency oscillatory ventilation (HFOV), or high-frequency jet ventilation (HFJV). This experimental study compared effects of three ventilation modes on the lung functions and inflammation in a model of MAS to estimate their efficacy.

Methods: Model of MAS was induced in young rabbits by intratracheal instillation of meconium suspension (25 mg/ml, 4 ml/kg). After induction of respiratory distress, meconium-instilled animals were ventilated either with CMV, HFOV, or HFJV (n=7 in each group) for additional 4 h, n=6 of healthy non-ventilated animals served as controls. Parameters of ventilation and gas exchange were measured regularly. At the end of experiment, lung edema formation was determined from wet-dry lung weight ratio. Concentrations of proinflammatory cytokines (TNF α , IL-1 β , IL-8) were measured in the plasma and lung tissue homogenates by ELISA methods.

Results: Meconium instillation decreased lung compliance, deteriorated gas exchange, and triggered inflammation and edema formation. All ventilations supplied sufficient gas exchange without clear differences in the lung functions, edema formation, or cytokine concentrations between the groups, with slightly better results observed in HFOV group.

Conclusions: All tested lung-protective ventilations exerted effective gas exchange and nearly comparable impact on the lung tissue, with a slight preference for HFOV.

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D06-4

Oxidative and inflammatory modifications in the extra-pulmonary organs associated with primary acute lung injury

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Questions: Acute lung injury (ALI) is characterized by diffuse lung damage, inflammation, edema, and surfactant dysfunction leading to hypoxemia. Severe ALI pathomechanisms could also accelerate progression of extra-pulmonary organs injury, but it's poorly known. Therefore this study evaluated oxidative and inflammatory modifications of heart and liver.

Methods: ALI was induced in rabbits by 1) intratracheal instillation of meconium (Mec-ALI), 2) repetitive saline lung lavage (Lav-ALI) and compared with healthy controls (C), all ventilated for 5 hours. Concentrations of markers of inflammation (TNF α ; galectin-3, Gal-3), oxidative damage to lipids (thiobarbituric acid reactive substances, TBARS) and proteins (3-nitrotyrosine, 3NT), vascular damage (receptor for advanced glycation end products, RAGE) in heart and liver homogenates were determined.

Results: In ALI groups, significantly increased levels of the mentioned markers were observed compared to controls, i.e. in the heart: TBARS ($p<0.05$ Mec-ALI); 3-NT ($p<0.05$ Mec-ALI, $p<0.02$ Lav-ALI); TNF α ($p<0.04$ Mec-ALI, $p<0.004$ Lav-ALI); Gal-3 ($p<0.03$ Mec-ALI); RAGE ($p<0.01$ Mec-ALI, $p<0.04$ Lav-ALI); in the liver: TBARS ($p<0.04$ Mec-ALI); 3-NT ($p<0.04$ Lav-ALI); TNF α ($p<0.03$ Mec-ALI, $p<0.01$ Lav-ALI); Gal-3 ($p<0.04$ Mec-ALI, $p<0.03$ Lav-ALI).

Conclusions: In both ALI models, elevated markers of inflammation, oxidative and vascular damage in heart and liver were observed compared to controls. Severe ALI showed direct effect on extra-pulmonary organs.

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D06-5

Exogenous superoxide dismutase in the surfactant treatment of experimental meconium aspiration syndrome

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Production of reactive oxygen species is important pathway in meconium aspiration syndrome (MAS) pathogenesis. Oxidative stress causes bronchoconstriction, impairs endogenous and exogenous surfactant and destructs pneumocytes. Intratracheal administration of superoxide dismutase (SOD) has proved anti-inflammatory effects in MAS. However, coaction of SOD and surfactant in the treatment of MAS has not been studied yet. Thus, we wanted to verify if SOD addition would improve surfactant therapy of MAS.

MAS had been induced in young New Zealand rabbits of 2.36 ± 0.12 kg of body weight by intratracheal meconium application. One group was left without the treatment (Mec; n=8), the treated groups were administered an exogenous surfactant (Curosurf® – Surf; n=8) or surfactant combined with SOD (Surf+SOD; n=8). Animals were ventilated for 5 hours by FiO₂ 1.0. Respiratory parameters were recorded, and *post mortem* IL-1 β , IL-6 and TBARS in lung tissue homogenate and total and differential leukocyte count in bronchoalveolar lavage fluid (BALF) was evaluated.

SOD addition into surfactant preparation led to strong, but transient betterment in all measured respiratory parameters (PaO₂/FiO₂, OI, VEI, PaCO₂, AaG), with significant improvement compared to surfactant monotherapy seen in some of them. After combined therapy, IL production was decreased compared to solely surfactant, while no effect was seen in total and differential leukocytes in BALF.

Although SOD therapy of MAS had strong effect in respiratory parameters when added to surfactant, it seems that the effect is just transient and SOD is being inactivated by meconium presence.

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D06-6

Effects a phosphodiesterase-4 inhibitor on the inflammation and oxidative stress in an experimental model of acute lung injury

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Questions: Acute lung injury (ALI) is characterized by loss of alveolar–capillary membrane integrity, migration and activation of inflammatory cells into the lungs, and release of pro-inflammatory mediators. Phosphodiesterase (PDE)-4 is mainly expressed in inflammatory cells and its inhibition reduces action of inflammatory cells. The main goal of our study was to evaluate if systemic administration of selective PDE-4 inhibitor roflumilast influences inflammation and oxidative damage in saline lavage-induced model of acute lung injury.

Methods: ALI was induced by repetitive saline lung lavage (30 ml/kg) until arterial PO₂ reaches values <26.7 kPa in oxygen ventilation. Animals were divided into 3 groups: animals with ALI model without therapy (ALI), animals with ALI treated with intravenous roflumilast (1 mg/kg; ALI+Rof), and healthy non-ventilated controls (Control). After 4 hours of ventilation, total and differential counts of cells in bronchoalveolar lavage fluid (BAL) were measured. Lung edema was expressed as wet/dry weight ratio. Concentrations of markers of oxidative stress (TBARS, 3NT), inflammation (TNF α , IL-6 and -8) were analysed in the lung tissue and plasma.

Results: Roflumilast therapy reduced leak of cells into the lung (P<0.05), mainly neutrophils (P<0.05) and monocytes/macrophages (P<0.01), lung edema formation (P<0.05), concentrations of markers of inflammation (TNF α , IL-6, and -8, P<0.05) and oxidative damage of lipids (TBARS, P<0.01).

Conclusion: Therapy with selective PDE-4 inhibitor roflumilast positively affected the inflammation and oxidative stress in lung tissue and plasma.

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D06-7

Exogenous surfactant reduces endotoxin-induced inflammation and oxidative stress in rat lungs

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Questions: Pulmonary surfactant is a lipoprotein complex situated at the air-liquid interface that may interfere with many substances. This causes decrease of its activity, with functional effects on the respiratory system. Lipopolysaccharide (LPS) is a major component of outer membrane of Gram-negative bacteria that interacts with structures of the lungs. The aim of the study was to investigate the influence of exogenous surfactant on acute lung injury induced by intratracheal instillation of LPS.

Methods: In adult rats (Wistar, 340 \pm 30g), lung injury was induced by intratracheal instillation of LPS (100, 500 or 1000 μ g/kg of b.w.). Treated group received exogenous surfactant (Curosurf[®]) at dose 50 mg PL/kg and control received saline (for all 2.2 ml/kg b.w.). After 5 hours of artificial ventilation IL-1 β , MCP-1, ANGPT2, surfactant protein A and oxidative stress (TBARS) were evaluated in homogenized lung (HL) tissue and bronchoalveolar lavage fluid (BALF). Lung edema was expressed as wet/dry weight ratio.

Results: Instillation of LPS at dose 500 and 1000 μ g/kg caused formation of lung edema (p<0.01), increased levels of IL-1 β (p<0.01), ANGPT2 (p<0.05) in HL and BALF and oxidative stress in HL (TBARS, p<0.05). The administration of surfactant decreased ANGPT2 in BALF (p<0.05 vs. LPS 500). Decrease of IL-1 β , MCP-1 in BALF was not significant (p>0.05). Surfactant therapy also reduced the lung edema (p<0.05 vs. LPS).

Conclusions: Intratracheal administration of LPS leads to changes reminiscent of bacterial infection. Administration of exogenous surfactant reduces inflammation, edema formation and oxidative stress.

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D06-8

The effects of Simvastatin on Galectin-3 and TBARS in Lung Tissue during Endotoxemia

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Questions: Galectins constitute a soluble mammalian β -galactoside binding lectin family which play homeostatic roles in regulation of cell cycle and apoptosis, as well as display inflammatory and immune modulatory activities in various pathological conditions. Previous studies have implicated galectin-3 in regulation of various inflammatory conditions including endotoxemia and airway inflammation. Statins, a key precursor inhibitors of HMG-CoA reductase, may prevent development and progression of inflammation when prior statin treatment in sepsis. Endotoxemia causes formation of Thiobarbituric acid reactive substances (TBARS), the consequence of lipid peroxidation reactions by damaging of the cell membrane, and oxidation of proteins, carbohydrates, and DNA molecules. In this study, we aimed to show the effects of Simvastatin on Galectin-3 and TBARS in lung tissue of rats treated with Lipopolysaccharides (LPS) during the early phase of sepsis.

Methods: Rats were divided into four groups: control, LPS (20 mg/kg, i.p.), Simvastatin (20 mg/kg, p.o.), and Simvastatin+LPS group. Galectin (gal-3) expression in formalin fixed and paraffin embedded lung tissue sections showed by immunohistochemistry. The levels of TBARS were determined using thiobarbituric acid (TBA) assay and measured spectrophotometrically 532 nm.

Results and Conclusions: There were decreased densities and the number of the gal-3 immunoreactivities in Simvastatin+LPS group compared to LPS group in pneumocytes and bronchial epithelium of lung tissue. However TBARS levels were significantly decreased in LPS+Simvastatin group according to LPS group (p<0.001). In conclusion, simvastatin protective effect on lung tissue during endotoxemia.

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D06-9

The Effect of Splenectomy on Lung Inflammation in Rats: The Protective Role of Curcumin

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Spleen derived leukocytes and their mediators may have important antiinflammatory functions. In this case splenectomy may cause lung inflammation. NF- κ B plays a critical role in inflammatory response. Curcumin is suggested to be a inhibitor of NF- κ B. Thus we have investigated whether splenectomy causes lung inflammation and the protective effect of curcumin. In study female Wistar Albino rats was divided into four groups containing 8 in each; splenectomy, splenectomy+curcumin, splenectomy+corn oil and control. Curcumin was given intragastrically with corn oil for 28days after splenectomy. At the

end, anesthetized rats were sacrificed and their lungs were removed. NF- κ B, VEGF, PDGF, MDA; Cu-ZnSOD activity and GPx levels are determined in samples. Also samples were examined histopathologically. Non-parametric Mann-Whitney U test was used for statistical evaluation. In splenectomy group, NF- κ B, VEGF, PDGF and MDA increased significantly ($p < 0.01$) compared to control group and SOD and GPX decreased significantly ($p < 0.05$). A significant decrease in inflammatory parameters and a significant increase in antioxidant parameters were detected in splenectomy+curcumin group. Histopathologic findings suggest that splenectomy induces acute and chronic inflammation, however curcumin alleviates acute inflammation and reduces the formation of tertiary lymphoid follicles. Splenectomy may cause NF- κ B-mediated inflammation. Curcumin may exhibit antiinflammatory properties with inhibition of NF- κ B.

D06-10

Effect of lipopolysaccharide on alveolar epithelial type II cells

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Introduction: Alveolar epithelial type II (ATII) cells have a critical role in the production of pulmonary surfactant which is required for a lung homeostasis and normal breathing. ATII cells can be damaged during lung inflammation caused by bacterial lipopolysaccharide (LPS). Exposure of ATII cells to this endotoxin could impair cell viability and modulate their ability to produce surfactant. However, impact of LPS on ATII cells is still not well known. Therefore this study evaluates the effect of LPS on ATII cells viability and function.

Methods: Human ATII cells were cultivated under standard conditions (Cell Biologics, USA). Different concentrations of LPS (E. coli 055:B5, Santa Cruz Biotechnology) 1, 5, 10, 15 and 20 μ g/ml were added to ATII cells and incubated for 1, 4 and 24 hours. The survival of ATII cells was determined microscopically and by the biochemical method (MTT assay).

Results and conclusions: Our results indicate that cell viability was not affected with LPS in time and dose-dependent manners. Even so, the surfactant production still remain to be determined to prove the LPS effect on ATII cells function.

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D08: Behavioral and cognitive neuroscience

D08-1

How the Emotional Status of the Emoji Characters Affect the Process of Response Activation?

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Introduction: Emoji characters allow people to express their concomitant feelings or answers quickly to other side through the digital communication. It makes us to wonder about whether the emotional content of the emoji characters has an impact on event-related potentials (ERP). We aimed to evaluate the effects of emoji characters on the process of response activation by using a visual go-nogo task.

Methods: ERPs were recorded with 30 electrodes using a visual go-nogo task from 18 male healthy volunteers. Go and nogo stimuli were randomly presented with 1.5 s inter-stimulus intervals (ISI) and probabilities of 0.6 and 0.4, respectively. We designed a go-nogo paradigm including three types of go stimuli as positive, negative, and neutral emoji characters presented in equal percentages. A yellow circle which has exactly the same radius with go stimuli is set as nogo stimuli. The amplitudes and latencies of ERPs to each go stimuli groups were analyzed by repeated measure of ANOVA.

Results: The reaction time of the positive emoji icons was found to be significantly lower than both the negative and neutral emoji icons ($p = 0.001$). The amplitude of the P3 potential in response to the positive emoji icons was significantly higher than the other two go stimuli groups ($p = 0.001$).

Conclusions: Our results suggest that the response activation to the positive emoji icons is higher than both the negative and neutral emoji icons. During the evaluation processes of the positive emoji icons, more brain resources were used than the other two groups. Since the amplitude of the P3 potential reflects both the amount of the attentional resources used and the information which can be gathered from the stimuli.

Keywords: Emoji characters, go-nogo task, response activation

D08-2

The Effects of Preceding Context on the Processes of Response Inhibition in Healthy Adults

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Introduction: The evaluation of event related potentials (ERPs) is one of the electrophysiological assessment methods can provide information of neural networks with a high temporal resolution. Both the response activation (go condition) and inhibition processes (nogo condition) in the brain can be analyzed with the go-nogo task. In this study, we aimed to investigate the effects of preceding context on response inhibition process by a visual go-nogo paradigm.

Methods: ERP data were recorded from 17 healthy volunteers (19.94 ± 0.83 yrs) via 30 scalp electrodes in an electrically shielded, sound attenuated room. Go and nogo stimuli were pseudo-randomly presented with 0.3 s stimulus duration and the probabilities with 0.6 and 0.4, respectively. According to stimulus type (nogo or go with three different difficulty levels) of preceding nogo stimuli, they were divided into four groups. The averaged responses to nogo stimuli were analyzed by repeated measures of ANOVA for each nogo stimulus groups.

Results: It is observed that subjects made more commission errors to nogo stimuli if the preceding stimulus is a nogo stimuli ($p = 0.001$). The amplitudes of P3 potentials of nogo responses were lower if the preceding stimuli are nogo stimuli ($p = 0.003$). The amplitude of P3 potentials of nogo stimuli were higher if the preceding go stimuli are easier ($p = 0.049$). The latency of P3 potentials of nogo stimuli were longer if the preceding go stimuli are more difficult ($p = 0.001$).

Conclusion: Our results suggest that type and the difficulty level of preceding stimuli affected the amplitudes and latencies of nogo P3 potentials by modulating the response inhibition.

This research was supported by the Research Fund of Mersin University (project 2017-1-TP2-2191).

D08-3

THE EFFICACY OF AUTISM-RISK SCREENING OF YOUNG CHILDREN IN SLOVAK POPULATION SAMPLE

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Questions: Autism Spectrum Disorders (ASD) are neurodevelopmental disorders characterized by deficits in social communication and restrictive, repetitive behavior. The aim of our study was to assess efficacy of screening for ASD using *M-CHAT questionnaire* in a sample of Slovak high-risk population and compare it with the objective diagnostic tools such as *Autism Diagnostic Observation Schedule - second revision (ADOS-2)* and *Autism Diagnostic Interview - Revised (ADI-R)*.

Methods: Study population included 92 children, age 1,7 - 6, who were signed up by their parents for ASD testing at our institution during years 2013 to 2016. Children were screened for ASD by M-CHAT questionnaire filled by their parent, and then examined using ADI-R and ADOS-2.

Results: Significant difference of summary score of M-CHAT between group of children with ASD and without ASD, diagnosed using ADOS and ADI-R was observed ($p < 0.001$). Two out of three diagnostic domains of ADI-R: scores of Abnormalities in Reciprocal Social Interaction and Abnormalities in Communication significantly correlated with total M-CHAT score ($p < 0.01$). Total M-CHAT score correlated with overall rate of symptoms in ADOS ($p < 0.05$) and as well as with Social Affect score ($p < 0.01$) and Restricted and Repetitive Behavior scores ($p < 0.01$).

Conclusions: Our results in the sample of high-risk children demonstrated that the M-CHAT score can be used to differentiate between children with and without ASD risk and that it correlates with objective measures for diagnosis of ASD, i.e. with ADOS and ADI-R. Further research is needed to determine the sensitivity and specificity of M-CHAT in ASD screening in general population of children in Slovakia.

Supported by grants: APVV-15-0045, APVV-15-0085.

D08-4

Levels of Faecal Calprotectin Correlate With Behavioural Markers in a Sample of Individuals with Autism Spectrum Disorders from Slovakia

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Questions: Autism Spectrum Disorders (ASD) are neurodevelopmental disorders which affect social interaction, communication and behaviour. There is an evidence that intestinal inflammation is involved in aetiology of ASD, and elevated levels of inflammatory markers are associated with more aberrant behaviours. Levels of faecal calprotectin (FC) reflect local inflammation of the digestive tract. The aim of the study was to assess concentrations of FC and their correlations with behavioural markers in a sample of subjects with ASD.

Methods: Concentration of FC was determined by ELISA method in 87 individuals with ASD, and 51 controls (29 siblings and 22 non-related controls).

Results: In non-related controls significantly lower values of FC were observed than in both subjects with ASD and their siblings. In the group with ASD, significant correlations of FC with all three main domains of the diagnostic tool ADI-R (Autism Diagnostic Interview-Revised) were found: qualitative abnormalities in social interaction and communication, restrictive and repetitive patterns of behaviour. Results indicate that higher levels of FC correlate with more serious behavioural impairments.

Conclusions: Our results suggest that intestinal inflammation may be one of the factors implicated in the pathophysiology of ASD. More studies are needed to validate the utilization of faecal calprotectin as a diagnostic marker for ASD.

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D08-5

Study of Acylcarnitine Profile in Dry Blood Sample of Children with Autism Spectrum Disorders

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Questions. Autism spectrum disorders (ASD) are disorders of neurodevelopment characterized by deficits in social communication and interactions, and restricted pattern of behaviours. The specific aetiology of ASD is only partially explained, and it was proposed that ASD can be associated with mitochondrial and fatty acid oxidation disorders. Acylcarnitines (ACC) can be diagnostic for a number of β -oxidation defects. The aim of this pilot study was to investigate the levels of acylcarnitines in dry blood samples of children with and without ASD.

Methods. A total of 59 subjects with ASD and 96 controls without ASD aged 2-17 years were included in the study. Dry blood sample was taken during office visit. Commercial kit Chromsystems® was used for determination of ACC levels by the method of tandem mass spectrometry. Groups were compared using analysis of variance.

Results. All patients except one showed normal ACC profiles, or ACC profiles nonspecific for a certain disorder. However, ACC species C0, C2, C8, C10, C10:1, C12, C14:1, as well as several of their ratios were scored as statistically significantly higher, and C5 significantly lower in children with ASD in comparison with children without ASD.

Conclusions. Significant differences in dry blood ACC of children with and without ASD were found that provide a basis for their further investigation as potential markers for an early diagnosis of ASD.

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D08-6

LOW DOSE CAFFEINE PROTECTS FROM PSYCHOLOGICAL STRESS AND IMPROVES COGNITIVE FUNCTION

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INTRODUCTION

Caffeine is an adrenergic antagonist that enhances neuronal activity. Psychological stress depresses cognitive function.

AIM

To investigate the effects of acute and chronic low dose caffeine on anxiety-like behavior and cognitive functions of acute or chronic psychological stressed rats.

METHODS

Acute or chronic caffeine (3mg/kg) was administered to male Sprague Dawley rats (n=42) before acute (cat odor) and chronic variable psychological stress (restraint overcrowding stress, elevated plus maze, cat odor, forced swimming) induction. Anxiety and cognitive functions were evaluated by hole-board and object recognition tests. The brain glutathione and malondialdehyde (MDA) assays, myeloperoxidase (MPO), nitric oxide (NO), superoxide dismutase (SOD), luminol and lucigenin activity and histological examination were done. ANOVA and Student's t-test were used for statistical analysis.

RESULTS

The depressed cognitive function with chronic stress exposure and the increased anxiety-like behavior with both stress inductions were improved via both caffeine applications ($p<0.05-0.001$). Both caffeine pretreatments in chronic stressed rats, and chronic caffeine in acute stressed ones reduced the elevated MPO activities ($p<0.05-0.01$). The increased MDA, lucigenin and NO levels with acute stress were inhibited with chronic caffeine ($p<0.05-0.01$), MDA and NO levels were declined by acute caffeine ($p<0.001$). The induced histological damage with both stress exposures was ameliorated with chronic caffeine.

CONCLUSION

The increased anxiety-like behavior and depleted cognitive functions under stress conditions were improved with both caffeine pretreatments by decreasing oxidative damage parameters.

D08-7

Long shift hours was associated with increased attention performance in pediatric registrars

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Introduction: Long shift hours are generally thought to negatively affect working performance. Pediatric registrars normally have long continuing shifts lasting and this thought to affect their attention. Therefore, the aim of the current study was to assess the effects of long shifts on their performance in attention tests.

Materials and methods: Pediatric registrars who had 32 hours continuous shifts (n=9) were compared to nurses who had normal work hours (0800 to 16:00 h; n=10) in terms of attention tests. The study started at 08:00 h on Day 1 and continued until 17:00 h on Day 2. On both days of the study, an attention test was filled at 08:00 and 16:00 h. The test consisted of sequential numbers dispersed on an A4 paper and participants were asked to follow these numbers as quickly as they can by starting from number 1 to number 25. The time was recorded by using a chronometer. The data was analyzed by using t tests.

Results: Mean time for completing attention test was 22 ± 1.5 seconds for pediatric registrars and 30 ± 2.7 seconds for nurses who had normal day time work hours ($P=0.023$). Additionally, performance of both groups were increased by time (28.7 ± 2.5 and 24.6 ± 1.9 seconds for Day 1 morning and Day 2 evening, respectively, $P=0.045$).

Conclusions: Unexpected better performance in pediatric registrars might be due to a work-oriented attention, which may be associated with vital interventions in a susceptible pediatric population.

D08-8

Influence of Green tea extract and Passiflora, on heart rate and fatigue sensation, in intense mental stress

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Questions. Green tea (GT) and Passiflora (PS) are known for their anti-stress and adaptogen effects. The aim was assessing the influence of a GT extract product (GTP) and PS product (PSP), in intense mental stress, by measuring heart rate (HR) and fatigue sensation (FS).

Methods. The chosen subjects (n = 24 males) were organized into 4 groups: 1) control (C=6) no GTP, no PSP; 2) only with GTP (GT=6); 3) only with PSP (PS=6); 4) with both GTP and PSP (GTPS=6). Stress was represented by a verbal arithmetic mental effort. The analyzed indicators were HR and FS. Statistical evaluation was done on the basis of Student test.

Results. Comparing GTP and PSP separated administration, the two parameters were significantly reduced, post mental stress: HR, more intense after GTP ($p=0.002$), FS more intense after PSP ($p=0.003$). Compared to GTP and PSP, GTPS reduced more, post mental stress, both HR (GT-GTPS, $p=0.03$; PS-GTPS, $p=0.01$) and FS (GT-GTPS, $p=0.02$; PS-GTPS, $p=0.04$).

Conclusions. 1) Under the GTP respectively PSP influence, HR and the FS were significantly reduced. 2) There are differences between the GTP and PSP treated groups, both for the HR and FS evolution. 3) It has been proven that the GTPS effect is significantly higher than the GTP and PSP effects, on HR and FS. 4) We suggest the using of GTP and PSP combination, in mental stress modulation.

Key words: green tea, passiflora, stress, mental stress, heart rate, fatigue sensation

D08-9

Evaluation of the influence of Romanian product "Emotional comfort" on facial expressions impact, in acute physical stress

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Questions. Facial expression of the public can influence mood and functional parameters of an athlete. The objective of this study was to evaluate the influence of "Emotional comfort" product (ECP), on the facial expressions (FE) impact in acute physical stress.

Methods. Untrained volunteers (n=12 men) were divided in group: a) without ECP (C=6); b) with ECP (EC=6). Chosen FE: neutral (N), disappointment (D) and encouragement (E), were played in three short films, while the subject was running on treadmill. Stress was represented by an intense short term physical effort on a treadmill Excite+Run MD, in three successive physical sequences (PS), in PS+N/ PS+D/ PS+E. Evaluated parameters: heart rate (HR); three positive emotions (energized EG, relaxed RL, in control IC). *Parameters assessments:* 24h (T1) and 15 min (T2) before PS+N; 15 min after PS+N (T3), PS+D (T4) and PS+E (T5); 24h after PS+E (T6). Statistical evaluation was made on the basis of Student test.

Results. At EC, compared to C group, HR values were significantly reduced at: T2 ($p=0.005$), T3 ($p=0.02$), T4 ($p=0.04$) and T5 ($p=0.004$). At EC - compared to T1- EG, RL and IC were: a) almost unchanged at T2 and T3; b) moderately significantly reduced, at T4 (EG, $p=0.05$; RL, $p=0.04$; IC, $p=0.04$); c) significantly increased at T5 (EG, $p=0.02$; RL, $p=0.001$; IC, $p=0.01$).

Conclusions. 1) Compared to C, influence of ECP+N/D/E was significantly intense in EC, both for HR and positive emotions. 2) Compared to C, ECP: decreased the effect of FE-D, and increased the effect of FE-E. 3) ECP influence on EG, RL and IC was almost similar. 4) ECP could be used as a modulator of FE impact on acute physical stress.

Key words: phytotherapeutic product, facial expression, heart rate, positive emotions

D11: Blood

D11-1

The effects of long-term and short-term water and food deprivation on blood antioxidant defense system

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Malnutrition and temporal dehydration can lead to the intensification of free-radicals oxidation that contributes to the progression of physiological and metabolic dysfunctions in response to stress. The aim of this study was to investigate the effects of long-term and short-term water and food deprivation on blood antioxidant defense system. Adult male Wistar rats were divided into four experimental (48- and 96-hours water and food deprivation) and one control groups. Measurements of blood hemoglobin level, the concentration of protein in blood plasma, general antioxidant activity, catalase activity in blood and activity of extracellular (SOD3) and cytoplasmic (SOD1) forms of superoxide dismutase as well as the levels of non-enzymatic antioxidants (non-protein thiols and ceruloplasmin) and thiobarbiturate acid-active products in blood were performed to identify the changes in blood antioxidant defense system. Short-term 48-hours food deprivation led to an increase in activity of SOD3. Water deprivation of the same duration demonstrated more profound effect on blood antioxidant defense system via an increase in hemoglobin level and general antioxidant activity along with the decrease in the level of nonprotein thiols in the blood. Long-term 96-hours food deprivation resulted in an increase of hemoglobin level whereas general antioxidant activity and activity of SOD1 decreased. Dehydration caused by long-term 96-hours water deprivation led to an increase in the hemoglobin level, concentration of ceruloplasmin, general antioxidant activity and catalase activity in blood. Water deprivation in comparison to food deprivation caused more significant changes in blood antioxidant defense system with more profound effects of long-term water deprivation. Short-term food deprivation during 48 hours resulted in smaller changes in the response of blood antioxidant defense system.

D11-3

Investigation of the effects of major autohemotherapy ozone application on erythrocyte deformability and aggregation

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Question: Currently, with reappraisal of ozone therapy especially for the protection of health, it has been utilized worldwide in research. Hemorheological conditions (*ie.* Erythrocyte deformability and aggregation) basically influence tissue perfusion, oxygen and nutrient supply. Limited number of studies exploring effect of ozone on red blood cell (RBC) deformability and aggregation reported conflicting results depending on the application way of ozone, dose and the species the experiments were carried out. The effect of ozone therapy on hemorheological parameters of healthy humans was not demonstrated before. Thus, the results of the current study are expected to provide new insights into the ozon therapy field.

Methods: 10 and 50 µg/ml doses ozone were applied for 20 minute to 15ml venous blood samples obtained from 10 healthy male volunteers (age 20-25). Erythrocyte aggregation, and deformability at 9

shear stresses were measured by an ektacytometer. Shapiro–Wilk's test was used for determination of normal distribution. Continuous variables were defined by mean ± standard deviation. Repeated Measures ANOVA, Friedman Tests were used for comparing three dependent groups. For post hoc analysis Bonferroni method was used.

Results: Ozone application of 10 and 50 µg/ml doses did not alter erythrocyte aggregation. On the other hand, 50 µg/ml ozone increased RBC deformability measured at 0,53 Pa ($p=0.045$).

Conclusion: Our results demonstrate that although 10 µg/ml concentration of ozone has no effect on hemorheology, ozone therapy at 50 µg/ml concentration may have beneficial effects on circulation through reducing RBC deformability.

Keywords: ozone therapy, RBC deformability, erythrocyte aggregation

D11-4

Enhancement of erythrocyte deformability after dark chocolate ingestion in healthy humans.

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The deformability of erythrocytes is important property of erythrocytes that considerably affects blood flow and hemodynamics in general. The high content of polyphenols present in dark chocolate has been reported to play a protective role in functionality of erythrocytes. We hypothesized that chocolate might influence erythrocytes not only after repeated chronic intake, but also immediately after its ingestion. Thus, we determined the acute effect of dark chocolate and milk (with lower content of biologically active substances) chocolate intake on erythrocyte deformability. We were also focused on selected factors that may affect erythrocyte deformability, specifically nitric oxide (NO) production in erythrocytes and total antioxidant capacity of plasma. We determined post-treatment changes in mentioned parameters 2 hours after consumption of chocolate compared with their levels before consumption of chocolate. In contrary to milk chocolate intake, the dark chocolate led to significantly higher increase in erythrocyte deformability. NO production in erythrocytes was not changed after dark chocolate intake, but significantly decreased after milk chocolate. The plasma total antioxidant capacity remained unaffected after ingestion of both chocolates. We may conclude that our hypothesis was confirmed. Single ingestion of dark chocolate improved erythrocyte deformability despite unchanged NO production and antioxidant capacity of plasma. Increased deformability of erythrocytes may considerably improve rheological properties of blood and thus hemodynamics in humans, resulting in better tissue oxygenation.

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D11-5

The effects of hyperbaric oxygen therapy on the erythrocyte osmotic deformability (Osmoscan) parameters in patients with various disorders.

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Poster Session D

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Poster Session D

Aim: Hyperbaric oxygen therapy (HBOT) is applied using 100% oxygen under high pressure condition, which increases the amount of dissolved oxygen in plasma. Previously, it was shown that high dissolved oxygen level damaged the erythrocytes. However recently we have shown that HBOT did not affect any hemorheological parameters. Osmotic gradient ektacytometry is a very sensitive method for detecting early erythrocyte morphology, structure and cell surface/volume ratio changes. Therefore, we aimed to measure osmotic deformability parameters in patients undergoing HBOT.

Method: After the approval of local ethical committee, the venous blood samples were collected from 29 patients (19 M/10 F) before the first and after 20th HBOT at 2.4 ATA. Erythrocyte deformability in osmotic gradient conditions was measured using osmotic gradient ektacytometry (Osmoscan). Minimal elongation index (EI) at low-osmotic environment (Elmin), maximal EI (Elmax), half of the maximal EI at high-osmotic environment (Elhyper), osmolality at EI min (Omin), osmolality at Elmax (Omax), osmolality at Elhyper (Ohyper) and the area under the individual EI (Area) were measured.

Results: Elmax and Elhyper decreased after treatment ($p < 0.004$ and $p < 0.01$ respectively), whereas the differences in other parameters were not significant.

Our findings pointed out that the HBOT affects erythrocytes and they become less deformable at the high osmotic environments.

D11-6

***In Vitro* Effects of Some Pesticides on Some Human Carbonic Anhydrases**

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AIM: Some pesticides (Carbofuran, propoxur, simazine, and atrazine) were tested on human carbonic anhydrase (CA) I and II enzymes *in vitro*. A better inhibitory activity has been observed with atrazine investigated here for the inhibition of the rapid cytosolic isozyme hCA II.

METHODS: Human hCA I and hCA II isoenzyme were isolated by means of affinity chromatography. CA activity was determined by the esterase method which follows the formation of 4-nitrophenylacetate to 4-nitrophenol at 348 nm. Activit%-[Inhibitor] graphs were drawn and IC50 values were calculated. KI values were calculated by using the Cheng-Prusoff equation.

RESULTS: Carbofuran, propoxur, simazine, and atrazine exhibited important inhibitory features with KI values in the range of 4.95 to 16.125 nM for CA I and II. Atrazine was observed to be the most effective one among the tested compounds.

CONCLUSION: As a result, activity of the CA enzyme was determined to be mostly inhibited in atrazine among all tested pesticides.

D11-7

Investigation of the Effects of Gossypin on *in vivo*, *in vitro* and Ischemia / Reperfusion Injured Rat Erythrocytes Carbonic Anhydrase Enzyme

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AIM: Some phenolic compounds (Resorcinol, catechol, curcumin, and gossypin) were tested on carbonic anhydrase (CA) I and II enzymes *in vitro*. Gossypin was found to be the most effective one

among the phenols tested. Is to investigate the effect of gossypin on erythrocytes CA enzyme in Ischemia/reperfusion (I/R) and I/R injured rats.

METHODS: Control, sham, I / R, and gossypin + I / R are as follows: A total of 32 male Wistar albino rats weighing 256 ± 5.3 g were equally and randomly divided into four groups. In the last group, gossypin was administered at 400 µg/kg dose before intraperitoneal ischemia period. Enzymatic activity was measured by using the spectrophotometric method of Verpoorte.

RESULTS: Resorcinol, catechol, curcumin, and gossypin exhibited important inhibitory features with KI values in the range of 6.08 to 4014 µM for CA I and II. Gossypin was observed to be the most effective one among the tested compounds. Specific activity values were determined for total erythrocytes CA enzyme at four different experimental groups. Gossypin + I / R group was found to be the most effective one among the tested group. Groups was determined as 400 µg/kg dose gossypin + I/R group 0.149 ± 0.005 EU/mg protein.

CONCLUSION: As a result, activity of the CA enzyme was determined to be mostly inhibited in gossypin + I/R group among all applications.

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D11-8

Investigation of the Effect of Carbonic Anhydrase of Ischemia/Reperfusion Injured Rat

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AIM: The purpose of this study is to investigate the effect of erythrocytes carbonic anhydrase (CA) enzyme in ischemia / reperfusion (I / R) and I / R injured rats.

METHODS: Control, sham, I / R, are as follows: A total of 24 male Wistar albino rats weighing 256±5.3 g were equally and randomly divided into three groups. Erythrocyte levels of enzyme activity of carbonic anhydrase (CA) were measured. Enzymatic activity was measured by using Verpoortes method by using a spectrophotometer. The assay system contained 0.05 M Tris-SO4 buffer pH: 7.4, including 3 mM 4-nitrophenylacetate.

RESULTS: Specific activity values were determined for CA enzyme at three different experimental groups. Groups were determined as follows: Control group 0.194 ± 0.008 EU/mg protein, sham group 0.180 ± 0.005 EU/mg protein, I/R group 0.246 ± 0.011 EU/mg protein.

CONCLUSION: As a result, the activity of the CA enzyme was determined to be mostly inhibited in sham group among all applications. Activity value of sham group decreased by about 8% compared to controls was observed that $p \leq 0.05$ is as meaningful.

D11-9

Nurses have higher blood leucocyte counts following night-shift works

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Aims: Shift works place a great pressure on metabolism and this, in turn, may affect body's defense and immune system. Whole blood counts provide information about the level of immune system activation. Therefore, the aim of the current study was to evaluate whole blood counts in nurses following night-shift works.

Materials and methods: Nurses having night-shift works (n=13) were compared to age-matched post-graduate students who did not have night shifts (control, n=10). They were followed for three days and blood samples were collected on the last day in the morning immediately after the commencement of the normal workday. Blood samples were collected into vacutainer tubes with EDTA as anticoagulant. As soon as taking the blood samples, they were analyzed by automated whole blood counter. The data was analyzed by GLM models of MINITAB statistical package.

Results: White blood cell counts were higher in nurses having night-shift works than the control group (P=0.053) but red blood cell counts, sedimentation rate, platelet counts, hemoglobin concentration and hematocrit values did not differ between the groups. In general, differential leucocyte counts were also similar except basophils which were higher in nurses which had night-shift works (P=0.036).

Conclusion: The results suggest that immune system is activated in night-shift nurses and this might be due to increased workload, perturbed sleep or increased exposure to microorganisms or immune activating substances.

D11-10

Evaluation of effects of hyperthermic intraperitoneal chemotherapy treatment on erythrocyte deformability

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Aim: Cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (HIPEC) is a major surgical procedure that is being used increasingly frequently in therapeutic option for selected patients with peritoneal surface malignancies. Our purpose in this experimental study is to evaluate effects of intraperitoneal chemotherapy on erythrocyte deformability.

Materials and Methods: 24 New Zealand rabbits included and divided four groups. Proper laryngeal mask was placed and they were connected to mechanical ventilation. Two catheters were placed in to abdominal cavity one to upper quadrant another to lower quadrant. Perfusion was applied as closed abdomen technique. Perfusate was given intraperitoneal cavity following heated up to 42 C. Perfusion was applied for 90 minutes. Group 1: normal saline+ HIPEC+ ketamine Group 2: Cisplatin 7mg/kg + HIPEC+ ketamine Group 3: Cisplatin 7mg/kg+HIPEC+%3 sevoflurane (2 hours) Group 4: Cisplatin 7mg/kg+HIPEC+%6 desflurane (2 hours) 24 hours after anaesthesia procedure, all rabbits were euthanasia under anesthesia and heparinized total blood samples were used to prepare erythrocyte packs. These erythrocyte suspensions were used for the measurement of deformability.

Results and Discussion: HIPEC application is determined as it increases resistance relatively more than control group (p< 0.0001). Erythrocyte deformability index is determined as increased significantly in all of the groups more than control group (p< 0.0001, all groups). Sevoflurane and desflurane use in rabbits which were predisposed to HIPEC is determined as it does not change erythrocyte deformability (p=0.846, p=0.734).

Conclusion: HIPEC application is determined as it decreases erythrocyte deformability in our study. Also we observed that inhalation agents sevoflurane and desflurane has no effect on deformability during HIPEC.

D11-11

The effects of HES 130 / 0,4 application on erythrocyte deformability in ureteral obstructed rats

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Aim: The administration of plasma expanders is crucial for managing critically ill patients across a range of clinical conditions, including major surgery, hemorrhagic shock, and trauma. However, the effects of hydroxyethyl starch (HES) on erythrocyte aggregation remain controversial. For this reason, we aimed to investigate the effects of HES 130/0.4, performed renal insufficiency by experimentally unilateral ureteral obstruction, on erythrocyte deformability.

Materials and Methods: 24 rats were divided into 4 group Control, HES 130/0.4, Ureter Obstruction (UO) and UO-HES130/0.4. Urethral obstruction groups were treated with ketamine anesthesia with a low abdominal incision and reached to the distal of right ureter and sutured with 2.0 mersilen then waited for 3 weeks for late term renal insufficiency. 20 ml/kg of HES 130/0.4 (Voluven) were infused intravenously to the HES 130/0.4 and UO-HES130/0.4 group. After 24-hour, rats were sacrificed. Deformability measurements were performed using 5% haematocrit in a phosphate-buffered saline (PBS) buffer.

Results and Discussion: Relative resistance was increased in all groups compared to the control group (p < 0.0001). In all groups, erythrocyte deformability index was found to be significantly higher than the control group (Group C-Group HES, p=0.023, Group C- Group UO, p<0.0001, Group C- Group UO-HES, p<0.0001). UO-HES group had significantly increased erythrocyte deformability index compared with the HES group (p=0.031, p=0.021, respectively). It was determined that HES 130/0.4 application did not change erythrocyte deformability in ureteral obstructed rats (p=0.785).

Conclusion: The use of HES 130/0.4 has no negative effects on erythrocyte deformability in ureteral obstructed rats. We think that indications should be used correctly when using HES 130/0.4.

D11-12

IkB Kinase 2 impairs Platelet Activation

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QUESTION: Megakaryocytes can sense inflammatory signals, but little is known how this might change platelet function. Most inflammatory signaling pathways converge at the kinase IKK2 (IkB kinase 2) activating the transcription factor NF-κB. The aim of this study was to determine the effect of persistent inflammation on platelet function, by altering NF-κB activity in megakaryocytes with a constitutively active IKK2.

METHODS: Mice with a megakaryocyte-specific constitutively active IKK2 were compared to littermate controls. Platelet count and lifespan was determined. Platelet function was tested *in vivo* by tail bleeding and induction of thrombi via mechanical injury and via ferric chloride, together with intravital imaging. *In vitro* assays included agonist-induced degranulation and aggregation.

RESULTS: Platelet count and lifespan is unaltered, however bleeding time increased and *in vivo* thrombus formation is impaired in mice with constitutively active platelet IKK2. Consistently, *in vitro*

platelet aggregation, degranulation and GPIIb/IIIa activation were decreased in platelets with constitutively active IKK2 upon stimulation with ADP and PAR4 receptor agonist peptide.

CONCLUSIONS: Our data indicates that active IKK2 or NF- κ B interferes with platelet activation, either directly through kinase activity in platelets or via constitutively active NF- κ B signaling in megakaryocytes.

D15: Other

D15-1

The physiological reaction by interaction of human body anatomical axes results in tissue function normalization - a feature of human body axis sensation

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Question: Does human body anatomical axis (HBAA) impart the polarity of individual cell as embryonic axes do (Proc Physiol Soc, 2016)?

Methods: Ou MC decrescendo phenomenon treatment (OuDPt) was performed by patients themselves for neoplastic diseases treatment, the contralateral hand is placed over the lesion along HBAA of left-right, dorsoventral or vertical axis (Proc Physiol Soc, 2015). Three patients separately with endometrial cancer, ovarian cancer with carcinomatosis and suspected pancreatic cancer had been improved by OuDPt via 2 HBAA (2 dimensions) but progressed clinically later (Cancer Res, 2016). Thus, all these 3 patients performed OuDPt with all 3 HBAA which formed a 3 dimensional (3D) polarity system.

Results: The endometrial cancer regressed from vagina to uterine cervix as stage IIIB to II by treatment for about 3 months. The top of the ovarian tumor shrank from the third to the level beneath the fourth lumbar spine, with trapped intestinal loop detaching from the tumor after treatment for about 3 months. The size of the pancreatic isodense tumor decreased from 1.6 × 1.7 cm to 1.02 × 0.96 cm with measurement at its greatest dimension by treatment about 4 months, and no tumor was visible by sonography about 1 year later.

Conclusions: Three dimensional tissue organization has shown to be a potential non-canonical tumor suppressor, which is hypothesized as via normalizing polarity of mutant cells and, ultimately, suppressing tumor development and progression (J Cell Sci, 2008). Our study shows that 3D human body polarity system formed by HBAA interactions suppresses the development of the neoplasm more efficiently than OuDPt with 2D body polarity system (TJOG, 2017), which may indicate HBAA imparts cell polarity.

D15-2

Hypoglycemic and hypolipidemic effects of apple cider vinegar in Tunisian type 2 diabetic patients.

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Type 2 diabetes is one of the most prevalent endocrine disorders worldwide. Traditionally, herbal plants and their derivatives are used to lessen complications of type 2 diabetes. In the current study the hypoglycemic and hypolipidemic properties of apple cider vinegar have been reported for vinegar in type 2 diabetic patients were investigated. In this trial study, sixty patients from both sex (30 females and 30 males) with type 2 diabetes were divided into two groups. The first group took 15 ml of apple cider vinegar with their middle meal for one month. The second group received water as placebo. At the beginning and end of the study, blood samples were collected and biochemical factors including fasting blood sugar (FBS), triglycerides (TG), total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), very low density lipoprotein (VLDL) and atherogenic index were evaluated. Findings showed that FBS, TG, TC, LDL-C, VLDL and atherogenic index decreased where HDL-C concentrations increased significantly in the first group. There was no significant difference in the studied parameters in placebo group. Based on the results of this study, it can be concluded that apple cider vinegar is a hypoglycemic and hypolipidemic agent that can be applied for treatment of type 2 diabetes.

D15-3

The BMI1 inhibitor PTC-209 is a potential compound to halt cellular growth in biliary tract cancer cells

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Questions

Biliary tract cancer (BTC) is a deadly disease with limited therapeutic options. BMI1 is a core component of the polycomb repressive complex 1 (PRC1), a major epigenetic regulator. Current literature indicates that aberrant PRC1 activity contributes to BTC. Recently, a specific BMI1 inhibitor called PTC-209 was developed. The aim of this study was to investigate the effect of PTC-209 using a comprehensive BTC cell model.

Methods

Expression of BMI1 in BTC cells was measured by quantitative real-time PCR and western blot. Cytotoxic effects of PTC-209 were evaluated by Resazurin assay, confluence measurement, Caspase-Glo® 3/7 Assay, immunostaining and cell cycle analysis. Effects of PTC-209 on cancer stem cell characteristics were investigated by sphere formation and ALDEFLUOR™ Kit. RT2 Profiler PCR Array was used for comprehensive gene expression analysis. Synergistic effects of PTC-209 treatment with standard chemotherapeutic cisplatin were evaluated according to combosyn.com.

Results

BMI1 was expressed in BTC cells. Treatment with PTC-209 resulted in diminished BMI1 protein levels and cell cycle arrest at G1/S. Gene expression analysis revealed diminished expression of cell-cycle promoting genes as well as of DNA synthesis initiation factors following PTC-209 treatment, accompanied by up-regulation of cell cycle inhibitors CDKN1A and CDKN2B. In addition, PTC-209 caused reduction of cancer stem cell characteristics: tumor spheres as well as percentage of cells positive for stem cell enzyme aldehyde-dehydrogenase were reduced. Lastly, specific combinations of PTC-209 with cisplatin showed a synergistic cytotoxic effect.

Conclusion

PTC-209 is a promising drug for future *in vitro* and *in vivo* studies in BTC.

D15-4

The effect of Napabucasin on cancer stem cells in biliary tract cancer

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Questions:

Napabucasin (BBI608) is a newly developed anti-cancer stem cell drug and currently used in several clinical trials. Involvement of cancer stem cells (CSCs) has been demonstrated for biliary tract cancer (BTC) - a deadly disease with poor chance of survival and limited therapeutic options. Targeting CSCs in BTC might be a beneficial and very promising therapeutic route. Up to now, scientific data regarding Napabucasin and cancer are very sparse - for BTC, no data are available. Therefore, the aim of the present study is to investigate the cytotoxicity of Napabucasin in an *in vitro* BTC cell model as well as the effect on expression of stem cell genes, pathways and functional CSC characteristics.

Methods and Results:

Cytotoxic effect of Napabucasin on BTC cell lines was evaluated by Resazurin assay. Time course experiments for 72h to investigate the proliferation and cytotoxic rate have been performed. Napabucasin showed a cell line and concentration-dependent cytotoxic effect on BTC cell lines. Time course experiments indicate cell death rather than proliferation stop as mode of cytotoxicity.

Outlook:

Further experiments in the present study will clarify the potential of Napabucasin as a possible therapeutic drug in BTC. Specifically, changes in CSC specific gene expression will be analyzed using RT² Profiler PCR Array and western blot following Napabucasin treatment. Moreover, the effect of Napabucasin on functional CSC characteristics such as migration, invasion, colony formation and aldehyde-dehydrogenase positivity will be investigated. Lastly, the potential of Napabucasin as an adjuvant substance with conventionally applied drugs cisplatin and gemcitabine will be determined.

D15-5

The histone-modification complex G9a and its role in biliary tract cancer/cholangiocarcinoma

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Questions:

Biliary tract cancer (BTC) is a deadly malignancy with limited therapeutic options, making the identification of new therapeutic targets a primary scientific goal. The histone-methyltransferase G9 specifically dimethylates lysine 9 at histone 3 (H3K9me2) and is overexpressed in cancer. However, the role of G9a in BTC is not described yet. Therefore, the aim of this study is to test G9a expression in BTC specimens and cell lines and to investigate the effect of G9a inhibition in BTC cells.

Methods:

G9a expression in FFPE BTC specimens (n=78) was determined via immunohistochemistry (IHC) and correlated with clinicopathological data. In BTC cell lines (n=9), G9a, as well as H3K9me2 expression were examined on mRNA and/or protein level. Cytotoxicity of G9a inhibition was evaluated via the resazurin assay using established small-molecular G9a inhibitors BIX01294, BRD4770 and UNC0642.

Results:

G9a was detectable in approximately 50% of BTC specimens. G9a expression significantly positively correlated with higher tumor grading. Additionally, G9a expression was diametrically associated with Vimentin (positive) and E-Cadherin (negative) protein expression. In BTC cells, G9a and H3K9me2 are expressed in a cell line-dependent manner. Statistical analysis revealed significant correlation between G9a mRNA, G9a protein and H3K9me2 expression levels. G9a inhibition resulted in drastic reduction of viable cells.

Conclusion:

The expression of G9a in BTC specimens and BTC cells as well as the cytotoxic effect of G9a inhibition provides first evidence that G9a may be an important regulator for BTC development and progression, making it an attractive, potential therapeutic target for epigenetic-related treatment of BTC in future.

D15-7

Development and validation of body composition prediction equations for the prediction of total body water and fat-free mass in North African Arabic children

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Background/Objectives: Accuracy of the impedance analysis depends on population-specific prediction equations. The aim of this study was to propose new prediction equations to estimate body composition using impedance analysis for healthy Tunisian children and validate them using the deuterium oxide dilution (D2O) technique.

Subjects/Methods: The body composition was estimated by the Tanita impedance analyzer. The validity of this system was investigated in 134 school children aged between 8 and 11 years. Total body water (TBW) and Fat Free Mass (FFM) were determined using the D2O technique. Participants were divided equally into development (n=67) and validation groups (n=67) to develop prediction equation using linear regression models.

Results: The comparison between body composition obtained by Tanita system and by D2O technique illustrated a significant difference (p<0.01). Compared to D2O technique, the impedance analysis underestimates fat mass and overestimates FFM and TBW. The prediction equations for TBW and FFM were developed with sex, age, weight and resistance index as possible predictor variables. The selected equations presented the highest adjusted coefficient of determination (R²), the lowest standard error of the estimate (SEE) value and the lowest P values. The pure error was 1.263 for the TBW equation and 1.646 for the FFM. The Bland Altman plot illustrated the good level of concordance between the TBW and FFM predicted by the new equations and measured by isotope dilution.

Conclusions: Our study provides valid prediction equations for estimation of TBW, FFM from impedance analysis measures for Tunisian children. These equations are applicable to children aged between 8 and 11 years.

D15-8

The effect of kisspeptin fragments in late pregnant uterine function *in vitro*

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Questions: Oxytocin (OT) has an important role in the regulation of smooth muscle contractility. Several adipokines are known which can activate the OT neurons in the central nervous system such as kisspeptin (KISS1). Our aims were to clarify the myometrial effects of KISS1 58-65 and KISS1 94-121 and to determine their receptors in the pregnant rat uteri throughout gestation.

Methods: Contractions of uterine rings from non-pregnant and 22-day-pregnant rats were measured in an organ bath. The contractions were stimulated with 25 mM KCl and cumulative-dose response curves were elicited in the presence of the kisspeptin fragments (10⁻¹² – 10⁻⁷ M), and the kisspeptin antagonist Kisspeptin-234 trifluoroacetate (10⁻⁹ M) and after removing the endometrium. The KISS1 receptor expression was determined by RT-PCR and Western blot analysis.

Results: The KISS1 receptors were expressed both in the non-pregnant and pregnant uteri. The highest expression (P=0,017) was found on the 5th day of pregnancy, which refers to the effect of kisspeptin in implantation. The expression of the receptors was higher in the endometrium than in the myometrium. Both kisspeptin fragments caused myometrial relaxation (50 %), however after removing the endometrium the relaxing effect of kisspeptin was ceased, as in the presence of kisspeptin antagonist.

Conclusions: The expression of kisspeptin was decreased throughout gestation. The effect of KISS1 58-65 fragment is endometrium-dependant while the effect of KISS1 94-121 fragment in the endometrium might be independent from the receptor. We hypothesize that the adipocyte-produced peptides in the uterus have a crucial role in the process of early or prolonged delivery.

Key words: gestation, kisspeptin, uterus

D15-9

Interaction of alpha-tocopherol and cyclooxygenase-inhibitors on smooth muscles of rats: the significance of cyclooxygenase-activity in uterus and trachea

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Key words: COX-inhibitor, antioxidant, smooth muscle

Questions: The use of antioxidant supplements is popular. However, the effects of these materials on various medications have only been scarcely investigated. The cyclooxygenase enzymes (COX) liberate reactive oxygen species. Our aim was to investigate how the antioxidant tocopherol influences the effects of COX inhibitors (COXi) in the smooth muscles in rats *in vitro*.

Methods: Contractility of smooth muscle tissues from 22-day-pregnant and non-pregnant Sprague-Dawley rats were measured in isolated organ bath *in vitro*. α -Tocopherol-succinate (10⁻⁷ M) was applied as antioxidant, while non-selective diclofenac (10⁻⁹ – 10⁻⁵ M) and COX-2 selective rofecoxib (10⁻¹⁰ – 10⁻⁵ M) were used in cumulative doses as COXi. The COX activities of the samples were measured by enzyme-immunoassay kit.

Results: In the presence of tocopherol, the uterus relaxant effects of the diclofenac and rofecoxib are increased on the pregnant uteri along with COX-activity. The increase in COX-2 activity was higher than that of COX-1. The diclofenac action was reduced after rofecoxib pre-treatment. Tocopherol influenced neither COX-activity nor the effect of COXi in the non-pregnant uteri. Tocopherol slightly

intensified the tracheal tone-reducing effects of rofecoxib and diclofenac without alteration of COX-activity. The lowest COX-activity was found in non-pregnant uteri while the highest one was in the trachea.

Conclusions: Tocopherol modifies the smooth muscle effect of COXi. Its effect is the most intensive in smooth muscle with medium COX activity. Tocopherol itself can induce the COX activity (especially COX-2) in pregnant uteri. The relaxing effect mainly belongs to COX-2 inhibition.

D15-10

The effects of the amoxicillin, fosfomycin and doxycycline on the aquaporin 5 expression in rat uterus before delivery.

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Questions: The aquaporin (AQP) water channels are expressed in the female reproductive tissues and they play important role during pregnancy. Earlier we proved that AQP 1, 2, 3, 5, 8 and 9 are detectable in the late-pregnant rat uterus and the AQP5 expression showed a dramatic down-regulation on the last day of pregnancy that is regulated by oxytocin and may play a role in delivery. Since antibiotics are among the drugs used to stop preterm labor, therefore our aim was to study the changes the AQP5 expression and uterine contractility after antibiotic treatment.

Methods: 3 groups of pregnant rats were used for the study. Amoxicillin (Group 1, 40 mg/kg) or doxycycline (Group 2, 30 mg/kg) was given orally for 1 week from day 16 of gestation. Fosfomycin (Group 3, 40 mg/kg) was given orally on day 21 of gestation. On pregnancy day 22 uterine samples were collected. We used reverse-transcriptase PCR and Western blot techniques for the detection of the changes in AQP5 expression. Uterine contractility was investigated in an isolated organ bath system.

Results: Fosfomycin and amoxicillin pretreatment caused a significant increase of AQP5 mRNA and protein levels on the last day of pregnancy. Doxycycline pretreatment caused a significant decrease of AQP5 mRNA and protein levels. The fosfomycin and amoxicillin pretreatment significantly reduced the uterine contractions both to potassium chloride and oxytocin treatment.

Conclusion: We suppose that the AQP5 expression is inversely related to the uterine contractility because the increased AQP5 expression is accompanied with decreased of the contractions. Fosfomycin and amoxicillin treatment during pregnancy may be favorable in the therapy of preterm labor.

D15-11

Uterine expressions and pharmacological influences of RhoA and Rho-kinases during pregnancy in rats

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Question: Activations of RhoA and Rho- associated kinases (ROCK I,II) play a pivotal role in the regulation of smooth muscle contraction via phosphorylation of myosin-light chain and myosin phosphatase. There are only few data on the RhoA and ROCKs expression levels in rat uteri. Our aim was to investigate the mRNA and protein level of RhoA and ROCKs during pregnancy, parturition and post-partum period and to evaluate the effects of ROCK (Y-27632, fasudil and RKI 1441) and RhoA inhibitors (simvastatin) on uterine contractility.

Methods: The mRNA and protein expressions of RhoA and ROCKs were measured in non-pregnant, on days 5, 15, 18, 20, 22 of pregnancy, during parturition and on days 1, 3, 5, 7 after delivery by Real-time PCR and Western blot analysis. Furthermore, the effects of RhoA and ROCK inhibitors were investigated on oxytocin induced uterine contractions.

Results: The mRNA and protein levels of RhoA did not change significantly from pregnancy day 15, while a sharp increase was detected during parturition. The ROCKs were down-regulated in the early stage of pregnancy, while it sharply increased in delivery. Simvastatin relaxed the myometrial contractions, although its inhibitory effects were not followed by the alteration of RhoA. The strongest inhibitory effect of fasudil was found on non-pregnant uteri. The maximum relaxing effects of Y-27632 and RKI 1441 were altered in a proportional way with the target protein expressions.

Discussion: The lower expression of ROCKs during pregnancy may contribute to the maintenance of relative quiescence of pregnant myometrium and the sudden increase of RhoA and ROCKs during labour suggests that they may contribute to the enhanced contractility and the initiation of delivery.

Key words: RhoA/ Rho-kinases, uterus, pregnancy, rat

D15-12

The Relation between Heavy Metals and Lipid Peroxidation Marker in Laryngeal Cancer

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Background: Heavy metals are present in the environment in greater or lesser amounts it is the concentration and their dosage which cause mutagenic and carcinogenic effect. Numerous investigations have revealed an association between heavy metal exposure and the incidence and mortality of cancer. Larynx cancer is one of the most frequently seen cancer type between head and neck cancers. In present study we investigated the relation between heavy metals such as zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd) levels and malondialdehyde (MDA) concentration as lipid peroxidation marker in laryngeal cancer.

Method: In present study, considered element measurements and malondialdehyde concentration determinations were realized in blood samples of larynx cancer patients (n: 20) and healthy controls (n: 15). Element concentrations were determined by atomic absorption spectrophotometer. MDA measurements were done according to Stokes Dormandy method in erythrocytes and serum

Findings: Serum Cu levels found to be higher in cancer group ($p < 0.001$) than controls, but Zn levels were determined at lower level ($p < 0.01$). Lead and cadmium levels in cancer group were statistically higher compare to control values in blood samples (respectively, $p < 0.01$; $p < 0.001$). On the other hand MDA concentrations were found higher both in plasma and blood samples of cancer group than control group (respectively, $p < 0.001$; $p < 0.001$).

Results: Our results show that higher MDA levels may lead to development of malign tumors. Possible reason of such a situation could explained be with the inhibitory effect of heavy metals on antioxidant defense of body.

D15-13

Can projects-based learning in medical biophysics create precondition for better understanding clinical teaching subjects?

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Questions

A specific project-based learning model for Medical Biophysics is applied at the Comenius University Faculty of Medicine in Bratislava in order to acquaint first-year students with the physical principles of the modern diagnostic and therapeutic methods.

Methods

Students identify the topics of the projects at the beginning of semester and work on them with timetable agreed by the teacher. Final stage involves the presentation of the results in students auditorium followed by discussion and finally submission.

The content analysis of semester projects was undertaken with the aims: to compare obtained results with analysis done in previous academic years; to summarize the wide spectrum of physical and biophysical applications in medical diagnostic and therapeutic methods, safety problems related to physical principles of medical equipment; to confirm usefulness of project-based learning.

Results

We analyzed 234 semestral projects submitted by first-year medical students in the interval 2013/2014–2016/2017 according to their content. 18,8% of them dealt with the physical topics, 50% belonged to diagnostic applications and 32% described applications of medical biophysics in therapy. We have created a database of semestral projects containing a wide spectrum of partial physical topics connected with medical applications reflecting actual needs of medical practice.

Conclusions

Based on obtained results we have confirmed the relevance of project-based learning in medical biophysics. It represents an effective teaching tool that is positively evaluated by students. We also believe that project-based learning represents important motivating factor.

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Key words: medical biophysics, project-based learning, semester projects

D15-14

Prevalence of underweight, thinness, overweight and obesity according to WHO standards, in a group of 100 female Tunisian students

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Introduction and purpose of the study: Today, around the world, obesity affects nearly 650 million adults, or 13% of the world's adult population. This problem also seems to affect the students' milieu. The objective of our study is to assess the prevalence of leanness, overweight and obesity in a group of students aged 19-25 years in Tunis (Tunisia) in 2016 using WHO references.

Subjects and methods: A cross-sectional descriptive study of 100 students, volunteers and motivated to be part of the study. They all underwent clinical examination, anthropometric measurements, impedance measurements and food surveys.

Results: According to WHO references, 10.5% of female students are meager, 15.9% are overweight and 9% are obese. These results are confirmed by the impedancemetry which shows that 37% of the population studied has a high percentage of fat mass. The analysis of the food survey shows that the contributions of 56.5% of the students are unbalanced both quantitatively and qualitatively. Meals were also de-structured with absence of breakfast 2 to 4 times per week in 64.7% of the students. A predominance of meals taken at fast food was also noted in particular, the "melawi", a preparation made from dough made with oil and stuffed with cheese, mayonnaise and tuna.

CONCLUSION: These results highlight the existence of high prevalences of overweight and obesity in a sample of the Tunisian female student population. These results are confirmed by the impedance measurement and analysis of the food survey. More efforts are strongly recommended to target this population in terms of nutritional education.

Keywords: Obesity, students, eating behavior, imbalance.

D15-15

Treatment with estrogen receptor agonist ER β , but not ER α , improves torsion-induced oxidative testis injury in rats

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Torsion of spermatic cord impairs testicular function. In order to examine the effect of estrogen receptor (ER) agonists and 17 β -estradiol (E₂) on torsion-induced injury, male Sprague-Dawley rats (6-8 week-old; n=40) underwent testicular torsion for 2 h or had scrotal incision (sham) under anesthesia. Before and after torsion, testicular blood flow was monitored by a Doppler flowmeter. Following detorsion, vehicle (oil) or E₂, ER β (DPN) or ER α (PPT) agonist was given subcutaneously (each 1mg/kg/day) for 3 days. At 72nd h of torsion, rats were decapitated and serum testosterone levels were measured. Myeloperoxidase activity (MPO), malondialdehyde (MDA), superoxide dismutase (SOD) levels, and reactive oxygen species (ROS) in testes were measured. Scoring of seminiferous tubules was made microscopically. Data were analyzed using ANOVA and Student's t-test. Blunted testicular blood flow upon 2-h torsion was recovered following reperfusion, while recovery was significantly greater in E₂ or ER agonist-treated groups. MPO and ROS levels were increased in vehicle-treated torsion group (p<0.05-0.001), but elevations in SOD and MDA were not different than sham group. Elevated ROS levels were depressed by DPN or E₂, while PPT further elevated ROS levels (p<0.05). Number of seminiferous tubules was significantly lower in vehicle- or PPT-treated rats (p<0.001), but the tubule count was higher in testes of DPN- or E₂-treated rats. Although reduction in serum testosterone level of sham group was not significant, PPT and E₂ further depressed testosterone level (p<0.01). The results demonstrate that E₂ or ER β , but not ER α , reduced testicular injury by inhibiting generation of ischemia-reperfusion-induced release of ROS.

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