L- lactate in rat astrocytes originates from glycogen stores

Katja Fink[1], Jelena Velebit[1, 2], Nina Vardjan[1, 2], Robert Zorec[1, 2], Marko Kreft[1, 2, 3]

[1]Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Zaloška 4, Ljubljana, Slovenia, [2] Celica Biomedical, Tehnološki park 24, Ljubljana, Slovenia,
[3] Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, Ljubljana, Slovenia

ABSTRACT

Astrocytes play a key role in providing homeostasis in the central nervous system. D-glucose uptake in astrocytes is an essential source for the noradrenaline induced increase in intracellular L-lactate. We found that intracellular L-lactate arises exclusively from the glycogen – a temporary energy store in the brain. The glycolytic pathway intermediates also support oxidative phosphorylation in mitochondria. At rest, a large proportion of D-glucose is metabolized in the Krebs cycle, since the resting L-lactate concentration is low. This is increased when the Krebs cycle is blocked or cells are stimulated with noradrenaline.

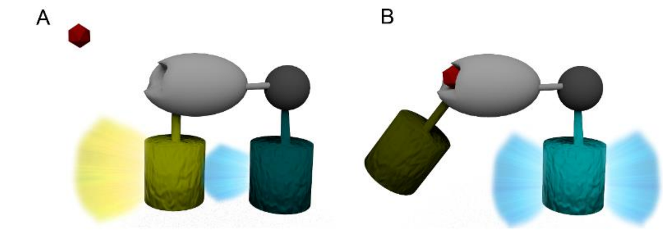
INTRODUCTION

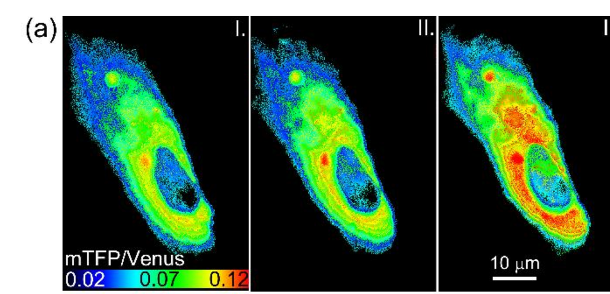
Astrocytes are numerous neuroglial cells of the central nervous system with ideal anatomical position between neurons and vasculature, which enables them to provide glucose for neurons. During cognitive efforts approximately 20% of additional energy is required. This mediates chemical messengers, such as noradrenaline (NA). NA targets astroglial aerobic glycolysis, the hallmark of which is the end-product L-lactate. Astrocytes exhibit a prominent glycogen shunt, in which a portion of D-glucose molecules entering the cytoplasm is transiently incorporated into glycogen, a buffer and source of D-glucose during increased energy demand.

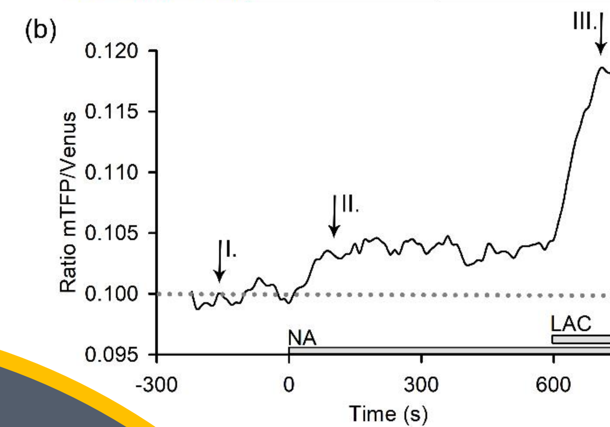
We studied single astrocytes by measuring cytosolic L-lactate ([lac]i) with the FRET nanosensor Laconic. We examined whether NA-induced increase in [lac]i is influenced by: i) 2-deoxy-D-glucose (2-DG, 3 mM), a molecule that enters the cytosol and inhibits the glycolytic pathway; ii) 1,4-dideoxy-1,4-imino-d-arabinitol (DAB, 300 µM), a potent inhibitor of glycogen phosphorylase and glycogen degradation; and iii) 3-nitropropionic acid (3-NPA, 1 mM), an inhibitor of the Krebs cycle.

FRET METHOD

FRET = Förster resonance energy transfer.
Nanosensor Laconic shows changes in lactate concetration through change mTFP/Venus ratio
(a) Yellow protein emition – no L-lactate binded
(b) Cyan protein emition – L-lactate binded

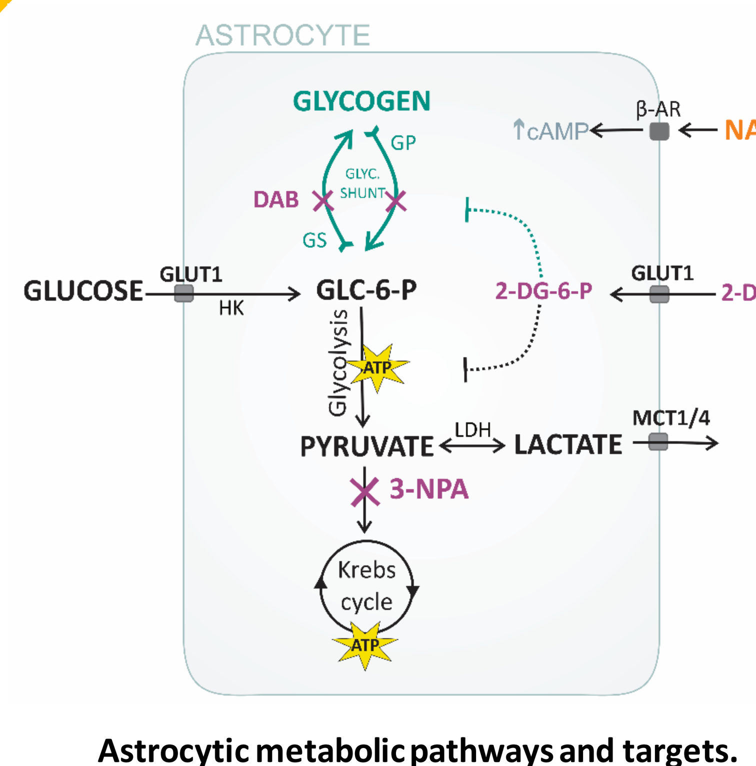






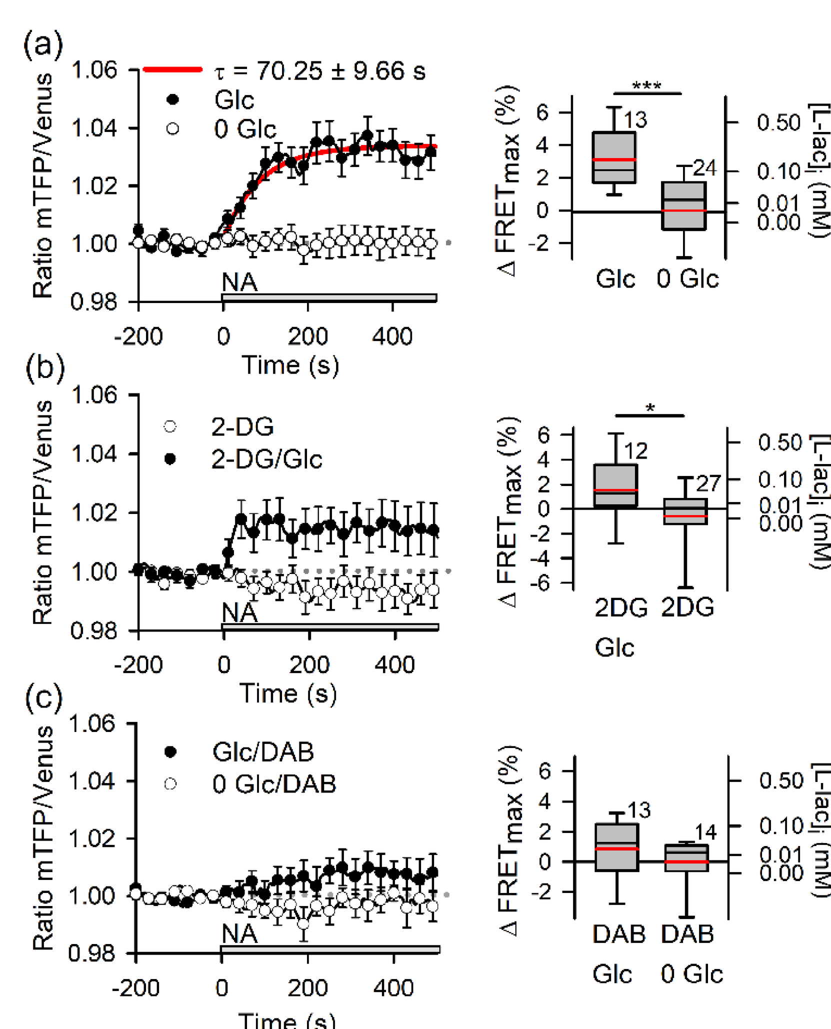
Stimulation of Laconic-transfected astrocytes with noradrenaline and extracellular L-lactate.
(a) Pseudocolored micrographs display time-dependent changes in the mTFP/Venus ratio in a single astrocyte, reporting [lac]i. Laconic expression is predominantly present in the cytosol. The representative images display the astrocyte at the beginning of the experiment (I.) and after the application of 200 µM noradrenaline (NA) (II.) and 10 mM L-lactate (III.).

(b) The mTFP/Venus ratio acquired from a time series of micrographs of the cytoplasmic region. Note the rise in the signal after the application of NA and L-lactate (LAC; horizontal bar). The arrows denote the frames shown in (a).

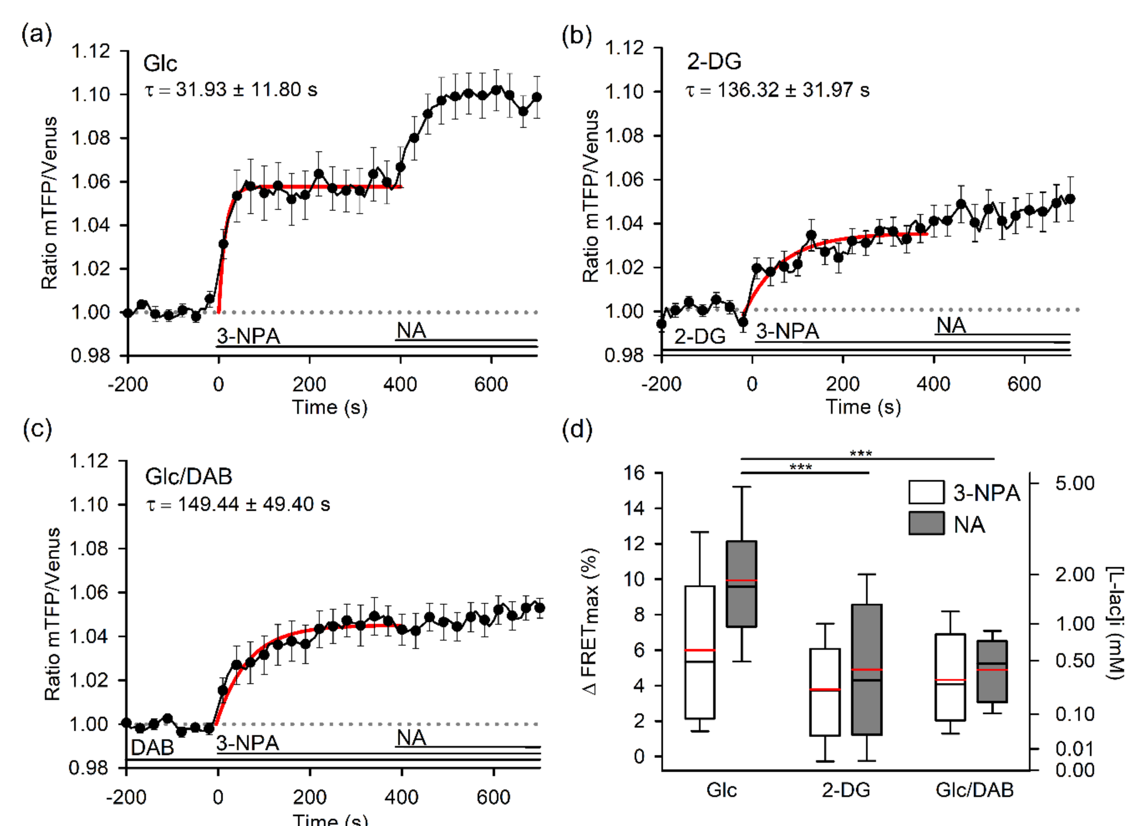


Astrocytic metabolic pathways and targets.

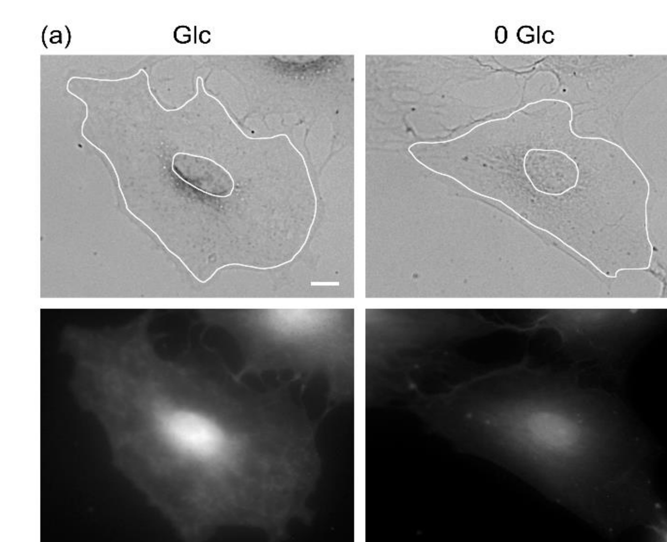
RESULTS

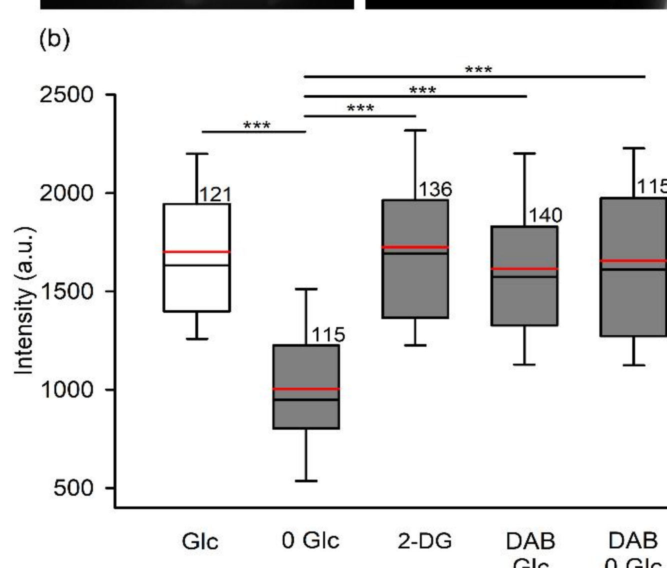


Noradrenaline-evoked L-lactate elevations in astrocytes.
The left panels represent the time courses of normalized mTFP/Venus ratios after adrenergic stimulation of astrocytes with 200 µM noradrenaline (NA), with their corresponding mean amplitudes on the right panels.
(a) Astrocytes preincubated 3 mM D-glucose respond with a rise in the mTFP/Venus ratio. Astrocytes starved in 0 mM D-glucose, do not respond to adrenergic stimulation. The exponential curve (red line) shows the time constant for positive control experiments.
(b) The combination of 1.5 mM D-glucose and 1.5 mM 2-DG increases the NA-evoked mTFP/Venus ratio. Preincubation in 3 mM 2-DG results in no NA-evoked L-lactate concentration increases.
(c) Preincubation in 300 µM DAB results in reduced L-lactate production both in the presence or absence of D-glucose.



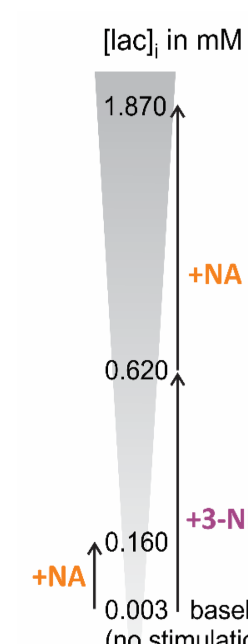
Acute 3-nitropropionic acid increases intracellular L-lactate concentrations.
(a–c) Acute 3-nitropropionic acid (3-NPA) application increases $[lac]_i$ in all tested extracellular solutions: (a) 3 mM D-glucose (Glc), (b) 3 mM 2-deoxy-D-glucose (2-DG), and (c) 3 mM Glc and DAB. Noradrenaline (NA) was applied at 400 s in all experiments. The red curves represent exponential curves that were fit to the data.
a) The amplitude of normalized mTFP/Venus ratios of astrocytes in 3 mM D-glucose increases. Subsequent stimulation with NA evokes an additional increase.
(b) The amplitude in 2-DG-treated astrocytes increases. Subsequent stimulation with NA does not evoke any additional increase.
(c) The amplitude in DAB-treated astrocytes increases. Subsequent stimulation with NA does not evoke any additional increase.
(d) The mean amplitudes of normalized mTFP/Venus ratios in all tested solutions after 3-NPA and NA stimulation. In the presence of DAB or 2-DG, there is no response to NA. The right scale in (d) displays the calculated $[lac]_i$ based on the calibration curve.





Glycogen content in single astrocytes determined by PAS staining
a) Transmitted light (top row) and fluorescent micrographs (bottom row) show astrocytes with intracellular glycogen (PAS staining). Cell incubated in 3 mM D-glucose (left) display more glycogen (brighter fluorescence) compared to the cell incubated in D-glucose free solution (right). The intensity of PAS staining is related to the glycogen content in the cell. White line is encompassing region-of-interest excluding nucleus for analysis. Scale bar: 10 mm.
b) Mean fluorescence intensity of PAS staining per cell area. White bar represents astrocytes in 3 mM D-glucose (Glc) and is used as contorol. In D-glucose-free solution (0 Glc) intensity mean is statistically different from all other series. Astrocytes have similar level of glycogen in 2-deoxy D-glucose solution (2-DG) incubated cells as in control solution. DAB treatment resulted in similar glycogen content in cells incubated in D-glucose-rich (DAB/Glc) and D-glucose-free (DAB/0 Glc) solution.

CONCLUSIONS



Summary of effects of noradrenaline (NA) and 3-NPA on intracellular L-lactate concentration $[lac]_i$ in astrocytes

We conclude that L-lactate production via aerobic glycolysis is an essential energy pathway in NA-stimulated astrocytes, as glycogen degradation and L-lactate production are targets of noradrenergic stimulation. We have demonstrated that all additional L-lactate derives from D-glucose originating from the glycogen shunt after NA stimulation. Nevertheless, we found that oxidative metabolism is important at rest, as Krebs cycle blockage resulted in increased L-lactate production.

LITERATURE

Pellerin, L., & Magistretti, P. J. (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci U S A, 91*(22), 10625-10629.
Prebil, M., Chowdhury, H. H., Zorec, R., & Kreft, M. (2011a). Changes in cytosolic glucose level in ATP stimulated live astrocyte cells. *Biochem Biophys Res Commun.* doi:S0006-291X(11)00054-4 [pii] 10.1016/j.bbrc.2011.01.035
San Martín, A., Ceballo, S., Ruminot, I., Lerchundi, R., Frommer, W. B., & Barros, L. F. (2013). A genetically encoded FRET lactate sensor and its use to detect the Warburg effect in single cancer cells. *PLoS One, 8(2)*, e57712. doi:10.1371/journal.pone.0057712
Vardjan, N., Kreft, M., & Zorec, R. (2014). Dynamics of beta-adrenergic/cAMP signaling and morphological changes in cultured astrocytes. *Glia, 62(4)*, 566-579. doi:10.1002/glia.22626

ACKNOWLEDGEMENT

We thank Dr. L. F. Barros for providing the Laconic nanosensor (Addgene, 44238). This work was supported by the grants from the Slovenian Research Agency (P3-310, J3-4146, J3-6790, I0-0034) and COST action ERNEST (CA18133).