Vasopressin downregulates AQP3 function via V1aR in human colon HCT8 cells

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Introduction: Vasopressin (AVP) plays an essential role in controlling water and salt homeostasis through the activation of vasopressin receptors V1aR and V2R. Beyond kidney, the colon modulates water and salt homeostasis. An abnormal secretion of AVP can cause the syndrome of inappropriate antidiuresis which is often associated with hyponatraemia, an electrolyte disorder often observed in hospitalized and oncological patients.

Aim: In this study, we investigated the effect of vasopressin on the aquaglyceroporin AQP3, using human colon adenocarcinoma HCT8 cells as a model.

Methods: The expression of vasopressin receptors was evaluated by western blotting and immunofluorescence analysis. The glycerol uptake (measured using calcein-probe), and the cell viability (measured using crystal violet assay) were tested to analyze the effect of AVP on AQP3. In parallel, gene expression assay and western blotting analyses were performed on human colon adenocarcinoma. RNA-Seq analysis was performed as well.

Results: We found that HCT8 cells express functional V1aR and V2R. Long-term treatment with dDAVP, a vasopressin agonist, reduces the AQP3 membrane abundance, glycerol uptake, and cell viability. These effects were prevented by SR49059, a synthetic antagonist of V1aR, but not by tolvaptan, a specific V2R inhibitor. Of note, the SR49059 action was counteracted by DFP00173, a selective inhibitor of AQP3. Interestingly, compared to the normal colonic mucosa, in the colon of patients with adenocarcinoma, the expression of V1aR is significantly decreased with a partial increase in AQP3 expression. These findings were confirmed by bioinformatic analyses of RNA-Seq.

Conclusion: For the first time we demonstrated that AVP can control AQP3 expression and function through V2R and V1aR, both expressed in the colon. Specifically, the V1aR dependent pathway reduces AQP3 function, a process that is reversed in adenocarcinoma, suggesting that the AVP-dependent AQP3 pathway may represent a novel target for therapies against colon diseases associated with abnormal cell growth.

Expression and functional characterization of vasopressin receptors, V1aR and V2R, in HCT8 cells

1. a) V1aR and V2R
   b) Variations in intracellular calcium were evaluated by single-cell epifluorescence imaging. V1aR stimulation with dDAVP caused a significant increase in intracellular Ca²⁺. This calcium oscillation was prevented when cells were treated with SR49059, a selective V1aR antagonist, suggesting that HCT8 cells express a functional V1aR receptor.
   c) Data shown as mean ± S.D.

2. V2R regulates AQP3 gene expression and protein abundance

3. dDAVP treatment reduces AQP3 membrane abundance

4. V1aR pathway regulates AQP3 glycerol uptake

5. V1aR activation with dDAVP reduces cell viability

6. Vasopressin receptors are down-regulated in human colon adenocarcinoma

7. Gene expression analysis with RNA-Seq data from normal colon controls (GTEx) and primary colon cancers (TCGA)

Bioinformatic analysis of RNA-Seq showed that in human colon cancer, the gene expression of both vasopressin receptors is significantly decreased, in contrast, AQP3 gene expression is significantly increased (* vs normal colon).