"Mice lacking TRPV4 show abnormal thirst regulation in absence of renal defects in sodium water handling"

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protein aquaporin-2, a process critical to regulation of water excretion. However, whether the abundances of other proteins under parietal changes in subalial organ, mutant proctoporic area, and the organum vasculosum of the lamina terminals, where it could play a role in osmotic sensing. TRPV4 has also been evidenced in the thick ascending limb (TAL) of the nephron, where it plays a role in renal osmoregulation. Using mass spectrometry (MS)/MS coupled with stable isotope labeling (i.e., SILAC) coupled with protein mass spectrometry (LC-MSMS). A mass balance model, assuming first-order kinetics of degradation, allowed calculation of the relative translation rate for each identified protein. Initial experiments (n=3) have provided half-lives (t½) of abundance changes are unexplored. We carried out large-scale measurements of protein half-lives (t½) and relative translation rates for natively expressed proteins in cultured renal proximal tubule cells (sildA-PV, 1 U). Using metabolic labeling with stable isotopes (DILAC) using metabolic labeling with stable isotopes (DILAC) combined with protein mass spectrometry (LC-MSMS). A mass balance model, assuming first-order kinetics of degradation, allowed calculation of the relative translation rate for each identified protein. Initial experiments (n=3) have provided t½ measurements for over 1,000 proteins, a small fraction of which (<1%) was found to change significantly in response to vasopressin, including a 66% decrease in the t½ for the adenosine receptor (in the adenosine receptor family). We found that TRPV4 mRNA levels (Affymetrix arrays). Further studies are underway to define the mechanism of mRNA interactions in changes in translation rates in response to vasopressin.

Disclosure of Financial Relationships: nothing to disclose

F-PO1620

Mice Lacking TRPV4 Show Abnormal Thirst Regulation in Absence of Renal Defects Sylvie Janas, S. Sara Sseny, Olivier M. Schalman, Johannes Loffing, Philippe Gallay, Olivier Devuyst. *Nephrology, UCL; †Anatomy, University of Zurich.

TRPV4 is a polytopic channel carbon of the transient receptor potential (TRP) family. It is expressed in hypothalamic neurons of the organum vasculosum of the lamina terminals, where it is involved in sodium sensing. TRPV4 has also been evidenced in the thick ascending limb (TAL) of the nephron, where it plays a role in renal osmoregulation.

Disclosure of Financial Relationships: nothing to disclose

F-PO1621


Urea fluxes via urea transporters (UTs) help regulate urine osmolality. Hydrophytop analysis of many mammalian and bacterial UT sequences show a common motif of a highly hydrophobic region of 5 transmembrane helical passes. UT-B comprises 2 regions (hence called TM1 and TM2) linked by a hydrophilic domain. To assess the validity of the predicted topology map we placed hemagglutinin (HA) tags at various positions in the protein predicted to be intracellular or extracellular loops (Fig. 1). We expressed these tagged proteins in Xenopus oocytes and assayed for UT function by isotope uptake. We then examined the accessibility of the HA tag to its antibody in whole oocytes. Intracellular tags only become accessible when the oocyte is permeabilized by detergent. All tagged positions in Fig. 1 show urea uptake 1-2 times that of control except TM1-1, which appears to be non-functional and TM2-1, which shows ~2-fold increase. To date, antibody localization of TM1-2 and G1 tags corroborate their predicted position, while G2 tag differs in that it appears accessible with detergent permeabilization.

Disclosure of Financial Relationships: nothing to disclose

F-PO1622


Mammalian bladder epithelia (urothelia) is generally considered impermeable to urinary constituents, but high levels of urea and creatinine and presence of UTB and other transporters in bladder tissue suggest that net urea transport (Tx) may occur. We developed a in vivo oral model to study water and solute Txs across urothelia. To determine the effects of dietary proteins on ureaTx we collected 24 hour urines from four groups of rats (n=12) provided with diets differing in protein concentrations (10%, 18%, 6%, 2% protein) and bladder washout on 3.3 ml of initial urine concentration +0.2 ml. Urea concentration in initial urine averaged 680 mg/l, and urea Tx was measured by isotope dilution and concentration of urea and other solutes (as "controls") in Instilled and Retrieved (after 30 min dwell) urine. Net change (mean) Tx of solute and solutes across urothelia were calculated arc Tan. Retrieved minus Instilled, and differences within and between groups were compared. Results: After one hour bladder dwell, there was a mean 3% increase in retrieved urine volume (p<0.001) which did not differ between dietary groups. As dietary protein decreased there was a stepwise fall of Instilled UN concentration as well as net urea Tx - reflected by a decrease in change of urine urea concentration (table) and quantity (mg,not shown).

Disclosure of Financial Relationships: nothing to disclose

F-PO1623

Urea Is Important for the Urinary Concentration Capacity of the Isolated Blood Perfused Rabbit Kidney Paul S. Steele, Jerry Toelke, Yves Cuypers, Robbert Bripa. *University Hasselt, Belgium; †Anton de Kom University, Suriname.

It is generally accepted that urea plays a critical role in the urine-concentrating mechanism in mammals, but herbivores have a different handling of urea than carnivores and omnivores (Bankier in The Kidney 8th ed. Brenner p. 595). Gubler and Rabinowitz (KL 1980,17,203) have reported that urea did not enhance the concentrating ability in calf, unanesthetized, vasopressin-treated rabbits. In this study we used the isolated blood perfused rabbit kidney (Cuypers et al Phagarches Arch,2000,440,634) to test whether its urinary concentrating activity is indeed independent of urea. Left kidneys of female rabbits

Key: TH - Thursday; F - Friday; SA - Saturday; FC - Free Communication; PO - Poster; PUB - Publication Only

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