Title

Sites and principles of ammonia excretion in marine mussels

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P17-12
Sites and principles of ammonia excretion in marine mussels

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Question: The excretion of nitrogenous waste products in the form of ammonia (NH_3) is a fundamental process in aquatic organisms. For bivalves of the genus *Mytilus* little is known about the mechanisms and sites of excretion. We hypothesized that *Mytilus* might facilitate NH_3 excretion at physiologically alkaline environmental pH by active acid secretion to prevent back diffusion of NH_3 by NH_4^+ trapping in respiratory fluid. **Methods:** Ammonia excretion rates were determined by placing animals in individual beakers filled with seawater and measuring the increase of ammonia concentration of samples collected in regular intervals. IHC and western blotting were performed using Rh-like and V-type H^+-ATPase antibodies. **Results:** A Rh-like protein was abundantly expressed in the apical membrane of cells in the plicate organ which has been described previously as a solely respiratory organ, but represents the main site of ammonia excretion as well. In contrast, the gills and kidney were only of minor importance and the urine is not enriched in ammonia. NH_3 excretion rates were sensitive to acute exposure to elevated pH (8.5) but recovered within 24 h acclimation accompanied by increased abundance of V-type H^+-ATPase in the apical membranes of the plicate organ. However, inhibition of H^+-ATPase and Na^+-H^+-exchanger did not affect excretion suggesting that an acid-trapping mechanism is not significantly involved. In contrast, inhibition or slowing of ciliary beating by dopamine or increased seawater viscosity of gill (and plicate organ) reduced ammonia excretion rates. Therefore, the water current produced for filter feeding sufficiently ventilates the body cavity and organs and efficiently eliminates any accumulation of metabolic waste products in the boundary layer of the respiratory surfaces. **Conclusion:** In mussels, ammonia excretion is enabled by passive NH_3 diffusion from the epithelia of plicate organ and gill along an ammonia gradient between body fluids and seawater which is maintained under all environmental conditions. This principle of energy efficient ammonia excretion might be more widespread in sessile organisms which generate their own water currents for filter feeding.

P17-13
Altered activity of the epithelial sodium channel (ENaC) in the aldosterone-sensitive distal nephron (ASN) of mice with AT1a receptor deficiency

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Question: Aldosterone is thought to be the main hormone to stimulate the epithelial sodium channel (ENaC) in the aldosterone-sensitive distal nephron (ASDN) comprising the late distal convoluted tubule (DCT2), the connecting tubule (CNT) and the entire collecting duct (CD). However, we previously demonstrated that ENaC function in DCT2/CNT is largely independent of aldosterone which is in contrast to its known aldosterone sensitivity in CNT/CCD (Nesterov et al. *Am J Physiol Renal Physiol* 303: F1289–F1299, 2012). In this latter study we demonstrated that ENaC function is preserved in the DCT2/CNT but not in CNT/CCD of aldosterone-synthase deficient mice which cannot synthesize aldosterone but have high plasma levels of angiotensin II. We hypothesised that angiotensin II acting via AT1a receptors may be an important factor for maintaining ENaC activity in DCT2/CNT. **Methods:** Experiments were performed on microdissected tubules from mice with a global knockout of AT1a angiotensin II receptors (AT1a KO) or wild type (WT) mice maintained on a standard salt diet. In mice, AT1a receptors are expressed in the kidney, whereas AT1b receptors are found in the cortex of adrenal glands. Consequently, angiotensin II dependent regulation of aldosterone secretion is preserved in AT1a KO mice. To assess ENaC activity, amiloride-sensitive whole-cell currents (ΔI_{ami}) were measured using the patch-clamp technique in split-open tubular segments at a holding potential of -60 mV. **Results**: In DCT2/CNT average ΔI_{ami} was significantly lower in AT1a KO mice than in WT controls (203 ± 85 pA, n=13 versus 443 ± 98 pA, n=16, $p < 0.005$). In contrast, in CNT/CCD ΔI_{ami} was significantly higher in AT1a KO mice than in WT mice (190 ± 69 pA, n=10 versus 77 ± 37 pA, n=8, $p<0.05$). **Conclusions**: The increased ENaC activity in the CNT/CCD of AT1a KO mice can be attributed to the well-known compensatory increase in plasma aldosterone in these mice. Importantly, the reduced ENaC activity in DCT2/CNT from AT1a KO mice indicates that AT1a-mediated stimulation of ENaC activity by angiotensin II contributes to the maintenance of aldosterone-independent ENaC activity in the early part of the ASDN. The residual ENaC activity in DCT2/CNT of AT1a KO mice reveals that additional mechanisms play a role and remain to be identified.

P17-14
Function of an alternative transcript of the WNT1-inducible signaling pathway protein 1 gene in human renal cells

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The alternative transcript WISP1-OT2 (WNT1 inducible signaling pathway protein 1 overlapping transcript 2) was discovered upon treatment of renal proximal tubule epithelial cells (RPTEC) in culture with the ubiquitous mycotoxin, ochratoxin A (OTA). WISP1-OT2 is 2922 nucleotides long alternative transcript of the WISP1 gene, starting in intron 4 and ending in exon 5. It is detectable predominantly in the nuclear fraction but its function in and interaction with components of renal cells is unknown.
Therefore, we studied the interaction of WISP1-OT2 with proteins by miTRAP (microRNA and protein affinity purification) experiments. To get further insights into its function and effects on cells, WISP1-OT2 was overexpressed in human embryonic kidney 293T (HEK293T) cells. Next, we obtained the influence of WISP1-OT2 on protein-coding gene expression (BeadArray microarray technology) and on a whole transcriptome level (RNA sequencing).
Northern blot analysis confirmed the expression and length of WISP1-OT2 after OTA exposure. By miTRAP it was shown that WISP1-OT2 can bind to some proteins, mainly splicing factors and IGF2BP proteins as well as to proteins interacting with IGF2BP1 protein. Preliminary results of RNA sequencing indicate that WISP1-OT2 overexpression mostly a) downregulates transcripts encoding adhesion molecules and integral membrane components (e.g. HTR2A and CLDN4) and b) upregulates transcripts coding for immune system components (e.g. CD200 and FCAR) and ion channels (e.g. KCNK3 and TRPC3). The BeadArray microarray technology revealed that the overexpression of WISP1-OT2 affects mainly the expression of genes involved in histone demethylation. In promoter regions of these functionally enriched genes, the most abundant are response elements for SP1 and SP2 transcription factors.
These results demonstrate that the alternative transcript of the WISP1 gene seems to function as a scaffold for protein complexes. Furthermore, we speculate that it may influence transcription indirectly by pulling away splicing factors from their targets. WISP1-OT2 seems to be involved in regulation of gene expression and splicing but the exact mechanisms are not yet dissected.

P17-15
Depletion of ATP6AP2 in AS4.1 cells regulates the expression of ciliogenesis and cell-cycle genes thereby arresting cell cycle in G0/1 phase

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Question: The (pro)renin receptor (ATP6AP2) is a multifunctional protein that interacts with renin, modulates v-ATPase activity and stimulates canonical Wnt as well as non-canonical Wnt/planar cell polarity (PCP) pathways. Thus, ATP6AP2 is involved in cardiac and renal end-organ damage but also in retinal, renal, cardiac and neuronal development, neurogenesis and neuronal morphogenesis. Ubiquitous knockout of *ATP6AP2* is lethal. We observed that particularly proliferating cells do not survive in the absence of ATP6AP2. The aim of the present study was to characterize the role of ATP6AP2 in proliferation and differentiation in more detail. **Methods:** ATP6AP2 was downregulated in As4.1 cells using siRNA technique. Genes differentially regulated were determined by means of microarray methods (Affimetrix™; RT²Profiler™ Arrays). Functional consequences of downregulation were investigated with respect to proliferation (BrdU incorporation), cell cycle (propidium iodide-labelling of DNA), and expression of the primary cilium. Localization of ATP6AP2 during different stages of the cell cycle was determined by immunofluorescence. **Results:** We identified a number of novel target genes downstream of ATP6AP2 that were related to the primary cilium (*Bbs-1, -3, -7, Rabl5, Ttc26, Mks-11, -5, -2, Tctn2, Nme7)* and the cell cycle *(Pierce1, Clock, Ppif*). In association, the number of cells expressing the primary cilium was markedly increased. Proliferation was reduced and the cells were arrested in the G0/G1 phase. During mitosis, ATP6AP2 translocated from perinuclear endoplasmatic reticulum to mitotic spindle poles (pro-, meta-, anaphase) and the central spindle bundle (telophase). **Conclusions:** ATP6AP2 is necessary for cell cycle progression during mitosis and cell division by translocating to microtubular spindle apparatus. At the same time it inhibits ciliogenesis, which is a prerequisite for differentiation.

Poster Topic 18
Pathophysiology of Cancer, Diabetes, Acid-Base & Other

P18-01
The Na^+/H^+ exchanger 1 modulates pH-dependent cell-cell contacts in human melanoma

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Proteins involved in ion transport, the “transportome”, strongly contribute to various steps of the metastatic cascade in cancer. We showed previously that in human melanoma cells, the Na^+/H^+ exchanger NHE1 regulates cell migration, cell-matrix interaction and invasion by modifying the tumor microenvironment through locally extruded protons. In this study we now investigate a possible impact of NHE1 on cell-cell contacts because cell detachment from the primary tumor is an early step in the formation of metastasis.
Adhesive interaction forces between living cells were quantified using Atomic Force Microscopy (AFM). Studying human melanoma cells (MV3) with different NHE1 expression levels at different extracellular pH (pH_e) values revealed the impact of NHE1 on cell-cell adhesion. Studies on the single cell level were supported by multicellular cell aggregation assays (CAA). Molecular biology and immunofluorescence stainings were conducted to unravel a possible contribution of pH_e-dependent adhesion molecules. To study our observations in a more physiological confinement, we performed 3D emigration assays of MV3 tumor spheroids embedded in a collagen I matrix.