Takis Anagnostopoulos Symposium: Renal and Epithelial Physiology and Pathophysiology

June 26–27, 2008 , Hôpital Necker, Paris, France

Abstracts

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Functional Interaction of Sodium-Phosphate Co-Transport (NPT2a) and Cystic Fibrosis Transmembrane Regulator (CFTR) in *Xenopus* Oocytes

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Little is known about the physiological role of CFTR in renal epithelium and bone. Of note, both CFTR and NPT2a are expressed in the renal proximal tubule and in osteoblasts. Because CFTR has been reported to regulate plethora of transporters, the aim of this work was to investigate a possible functional interaction between CFTR and NPT2a. To this end, hCFTR and/or hNPT2a were expressed in *Xenopus laevis* oocytes. In voltage clamped cells (-50mV), the current induced by 1mM inorganic phosphate (IPi) was taken as an index of NPT2a-mediated Pi transport. IPi was lower in CFTR+NPT2a-oocytes compared to NPT2a-oocytes (-28.2 ± 2.3 nA, $n = 22$ vs -43.7 ± 4.1 , $n = 21$, $p < 0.01$). A decreased $V_{max,app}$ of NPT2a (without change in the respective $K_{m,app}$ for Pi or for Na^+) and a lower membrane expression of NPT2a (assessed by Western blot of biotinylated membranes) indicated that CFTR co-expression modified NPT2a membrane expression in *X. laevis* oocytes. In CFTR+NPT2a-oocytes (but not in NPT2a-oocytes), experimental manoeuvres that increased intracellular cAMP (Forskolin 1 μ M or 8 Br cAMP, 100 μ M) induced a significant increase in IPi. Similar results were obtained in the presence of 6MB cAMP (25 μ M), but not with 8 pCPT cAMP (25 μ M). These results agree with an increase in IPi mediated by PKA stimulation, but not by EPAC stimulation. Interestingly, when $\Delta F508$ CFTR (the most frequent mutation in Cystic Fibrosis) was co-expressed with NPT2a, PKA-induced up-regulation of NPT2a was not detected, despite a reduced expression NPT2a was still observed. Thus, in *X. laevis* oocytes, coexpression of CFTR confers to NPT2a may confer to NPT2a a different regulation from that reported in renal cells. The relevance of these observations in physiopathology remains to be investigated.

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ROS-Generating NAD(P)H Oxidase 4 Interacts with the Endoplasmic Reticulum Chaperone gp96 and Controls Toll-Like Receptor-Mediated Apoptosis in Ischemic Kidney Cells

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Ischemia/reperfusion injury (IRI) induces potent inflammatory reaction and tissue damage, and cell apoptosis that may lead to severer renal damages. Toll-like receptors (TLRs) play key roles in the pathogenesis of ischemic inflammatory responses, but the mechanisms responsible for renal cell apoptosis triggered by IRI still remain poorly understood. TLR4 has been shown to interact with the non-phagocytic NAD(P)H oxidase 4 (NOX4) generating reactive oxygen species (ROS) abundantly expressed in the kidney. Using an experimental mouse model of I/R and primary cultures of renal tubule epithelial cells (RTECs), we show that IRI stimulates NOX4, but not NOX1, and activates a TLR2-, TLR4-mediated TRAF-2-apoptosis signaling-regulating kinase 1 (ASK1)-JNK apoptotic signaling pathway. Extinction of *Nox4* mRNA expression using specific siRNA fully inhibited the stimulated phosphorylated forms of ASK1 and JNK, ROS production and subsequent apoptosis in post-ischemic wild-type renal tubule cells. Silencing of the reticulum endoplasmic-resident heat shock protein gp96, which coprecipitates with NOX4 and TLR4, also prevented renal tubule cell apoptosis induced by hypoxia in wild-type RTECs. Overall, these findings have provided the first demonstration that NOX4 and gp96 play central roles in the induction of ROS-mediated cell apoptosis during IRI. The development of specific Nox inhibitors should therefore represents an attractive pharmacological approach to limit the renal tubule cell damages caused by IRI.

Self-Inhibition of the Epithelial Sodium Channel by Extracellular Sodium: Characterization of the Extracellular Sodium Sensing Site

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The Epithelial Na⁺ Channel (ENaC) is located in the apical membrane of “tight” epithelia in the distal nephron, distal colon and airways. Its activity controls the rate of transepithelial sodium transport. Among several regulatory factors, ENaC activity is modulated by the concentration of extracellular Na⁺, a phenomenon named self-inhibition. The molecular mechanism by which extracellular Na⁺ concentration is detected is not known. To investigate the properties of the extracellular Na⁺ sensing site, we studied the effects of extracellular cations on steady state amiloride-sensitive outward currents in Na⁺ loaded oocytes expressing human ENaC and compared them with self-inhibition of inward current after fast solution changes. About half of the inhibition of outward Na⁺ currents was due to self-inhibition itself and the rest might be attributed to conduction site saturation. Self-inhibition by extracellular Li⁺ was similar to that of Na⁺ except for slightly slower kinetics. Ionic selectivity of the inhibition for steady state outward current was Na⁺ ≥ Li⁺ > K⁺. We estimated an apparent inhibitory constant (K_i) of about 40 mM for extracellular Na⁺ and Li⁺, and found no evidence for a voltage dependence of the K_i. These results first support the existence of the sensing site, with features (ionic affinity and selectivity) different from the conduction properties of the channel. They also show that both self-inhibition and saturation play a significant role in the inhibition of ENaC by extracellular Na⁺ and that Na⁺ and Li⁺ interact in a similar way with the extracellular cation sensing site. Concerning the localization of this sensing site, evidences show that the extracellular loops of the channel subunits are involved in self-inhibition and plausibly contain residues responsible for the extracellular Na⁺ detection. Preliminary results highlight the central role of the α and γ subunit (and not β) in this detection and the possible localization of the sensing site in the first part (~150 amino acids) of the loops of α and γ.

Proteomics of Membrane Microdomains from Human Bronchial Cells Subjected to Proinflammatory Stimulation

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In a previous study we have observed that the cystic fibrosis transmembrane regulator (CFTR), Annexin 1 (A1) and cytosolic phospholipase A2 (cPLA2) are partially recruited in detergent insoluble microdomains (DIM) upon pro-inflammatory stimulation (PI) and that they are involved in the same macrocomplex. It is postulated that CFTR, A1 and cPLA2 participate in the regulation of inflammation by their dynamic interaction within DIM. We aimed to identify other potential partners of this complex by proteomic analysis of DIM by adapting the “in-gel tube” technique, which is compatible with membrane protein analysis. Human epithelial respiratory cells IB3/C38 (expressing either wild type or F508del CFTR) was subjected or not to PI. DIM were isolated and proteins identified by LC/MSMS. Semi-quantification of identified proteins was performed by the emPAI method.

In-gel analysis allowed the identification of more than 150 proteins in DIM. By emPAI, we established a list of proteins differentially expressed in IB3 vs C38 subjected or not to PI. These included CFTR-interacting, cytoskeletal, and TNFα-signaling proteins. As a proof of concept, some characteristic DIM-associated proteins, such as caveolin-1 and flotillin-1 were detected in all samples subjected to in-gel analysis. A number of membrane proteins were also identified. In-gel tube reveals as a useful method for membrane microdomain proteomic analysis. PI induces differential expression of several proteins in DIM from normal versus mutated CFTR-expressing cells. These results will provide valuable information about potential components of a DIM-associated CFTR-containing dynamic macrocomplex, as well as new hypotheses on the participation of CFTR in the regulation of the inflammatory response.

(This work has been supported by Vaincre la Mucoviscidose and by the European Commission).

The Basolateral Na/H Exchanger NHE4 Is an Ammonia Transporter which Is Critical for Renal Adaptation to Metabolic Acidosis

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Ammonia absorption by the medullary thick ascending limb of Henle's loop is thought to be a critical step in renal ammonia

handling and ammonia excretion in final urine. We made the assumption that the basolateral Na^+/H^+ exchange NHE4 may be involved in ammonia efflux out of the MTALH cell. Using *in vitro* microperfusion, we demonstrated that NHE4 is functional in intact MTALH cells. Both pharmacological inhibition and genetic disruption of NHE4 were responsible for a 40 % decrease in ammonia absorption by the *in vitro* microperfused MTALH. Metabolic acidosis increased NHE4 activity by 50 % and ammonia reabsorption related to NHE4 in MTALH cells. As compared with wild-type littermates, young (2-month old) NHE4^{-/-} mice displayed a normal plasma acid-base status and similar urinary net acid excretion on baseline. However, when challenged with a 7-day NH_4Cl -load, they were unable to increase ammonia and net acid excretion as much as their wild type counterparts. Adult (5-month old) NHE4^{-/-} mice displayed hyperchloremic metabolic acidosis (plasmatic HCO_3^- is equal to 25.7 ± 1.0 mM for NHE4^{+/+} mice versus 22.7 ± 0.7 mM for NHE4^{-/-} mice, $p < 0.01$) together with inappropriate urinary net acid excretion (Unet acid/Ucreatinin (mEq/mmol) is equal to -11.5 ± 5.9 for NHE4^{+/+} mice versus -1.2 ± 2.8 for NHE4^{-/-} mice, $p < 0.01$).

We conclude that NHE4 is responsible for a significant part of ammonia absorption by the MTALH. Moreover, the lack of NHE4 induces an inability to excrete an acid load and, eventually, a systemic hyperchloremic metabolic acidosis.

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Modulation of Cx43 Expression during Progression of Hypertension-Induced Chronic Renal Failure

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Chronic renal failure (CRF), one of the main causes of disability in western countries, is promoted by a variety of factors that are linked by their ability to promote chronic inflammation and fibrosis. Inflammation and excessive scarring have been recently associated with disruptions of gap junction-mediated intercellular communication. In particular, alterations of the expression of the major gap junction protein connexin 43 (Cx43) in the vascular level have been associated to the development of inflammation in chronic vascular pathologies such as atherosclerosis.

The objective of this study was to investigate whether Cx43 alterations were involved in the early phenotypic changes of the renal vasculature leading to the development of chronic renal disease. For this purpose we used the RenTg mice expressing high steady levels of renin. Consequently, these mice display high blood pressure, enhanced proteinuria and kidney damage. Kidneys from RenTg mice and their counterparts were collected at 12, 21, 40 and 53 weeks (w) after sacrifice of the animals in order study modulation of Cx43 expression during progression of hypertension-induced CRF. Analysis of cross sections of the kidneys revealed a progressive increase in Cx43 immunostaining on the 12w RenTg mice, in the peritubular as well as in the glomerular capillaries. The Cx43 expression pattern was paralleled closely by that of the endothelial

vascular cell adhesion molecule-1 (VCAM-1). CD68 immunostaining, showed enhanced macrophage infiltration in RenTg mice from 21w, whereas CD3 staining was detected after 40w. Thus, alterations of Cx43 expression precede the inflammatory process during the early stages of the CRF. These findings underline the importance of cell-to-cell communication in renal failure, and may open towards therapeutic strategies targeting gap junctional communication to evaluate the evolution of this common disease.

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TLR4-Mediated Cell Activation Facilitates the Translocation of Uropathogenic *E. Coli* across Renal Medullary Collecting Duct Cells

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Urinary tract infection (UTI) and acute pyelonephritis (APN), which constitute a major cause of morbidity and mortality in humans, are mainly due to uropathogenic *Escherichia coli* (UPEC). We have shown that renal medullary collecting duct (MCD) cells are a preferential intrarenal site for UPEC adhesion and initiation of inflammatory response. However, the ability of UPECs to translocate across impermeant MCD to invade the renal interstitium had not been unambiguously demonstrated. Here we show that UPEC strains adhered and translocated across confluent layers of inner MCD cells, mpkIMCD. UPECs expressing cytolytic and vacuolating cytotoxins disrupted cell layers whereas non cytolytic UPECs passed through intact cell layers. Transcellular translocation of UPECs was dramatically reduced after extinction of the Toll-like receptor 4 (TLR4) and of the lipid raft marker caveolin-1. Disruption of the lipid raft integrity significantly reduced the concomitant stimulation of proinflammatory mediators caused by UPECs, and their transcellular translocation across mpkIMCD cell layers. Bacterial translocation was also significantly reduced in confluent cultures of MCDs dissected from kidneys of TLR4-deficient C3H/HeJ mice compared to those from wild-type C3H/HeOuJ mice. Also UPECs were detected in the renal interstitium of C3H/HeOuJ mice inoculated with UPECs. Benzyl alcohol, an anesthetic which enhances membrane fluidity, favored the recruitment of caveolin-1 in lipid rafts and increased the translocation of UPECs across cultured C3H/HeJ MCDs. These findings have provided the first demonstration that the translocation of UPECs across impermeant medullary collecting duct cell layers may occur through lipid rafts *via* a TLR4-facilitated process.

Prorenin Receptor [(P)RR] Is Highly Expressed in Developing Kidney and the Soluble Form has a Proliferative Effect

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The (pro)renin receptor exists as a 39 kD-full length transmembrane protein responsible for renin and prorenin binding and signaling and as a 28 kDa-soluble form generated by intracellular cleavage.

Aims of the Study: To determine the expression of (P)RR in kidneys of *E15* and new born mice by *in situ* hybridization and to study the proliferative effect of soluble (P)RR.

Results: In *E15* (P)RR is highly expressed in vessels and ureteric buds whereas renin expression is restricted to vessels. (P)RR expression showed a gradual decrease of intensity from the nephrogenic cortex to the papilla. In non-condensing and condensing mesenchyme (P)RR expression was low but increased dramatically after mesenchymal-to-epithelial conversion i.e. in vesicles and S shape bodies. In new born mouse (P)RR expression is high in distal, proximal and collecting tubules and low in glomeruli and arteries. In new born mice, a strong expression of (P)RR in highly dividing cells of intestinal crypt vili and bone marrow lead us to study the possibility for soluble (P)RR to act as a proliferative factor. We tested the effect of conditioned medium of HEK cells control (CM) or of HEK cells over-secreting soluble (P)RR [CMs-(P)RR] on mesangial cells proliferation using Cell titerGlo Luminescent Proliferation kit. The results showed that proliferation of cells incubated 48 h with control CM was similar to culture medium alone ($5,700 \pm 1,185$ and $5,480 \pm 1,034$ RFU) whereas incubation with CMs-(P)RR provoked a significant increased proliferation ($10,300 \pm 1,643$ RFU) comparable to 1% fetal calf serum ($10,084 \pm 1,638$ RFU) ($P < 0.05$ vs control).

Conclusion: Our results showed an unexpected pattern of expression of the (pro)renin receptor in mouse embryo and suggest that it may have other functions during development, such as a growth factor function.

Functional Consequences of the Sodium/Hydrogen Exchanger Regulatory Factor 1 (NHERF1) E68A Mutation on Renal Phosphate Transport

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Introduction: Kidney plays a central role in phosphate homeostasis. Phosphate is freely filtered at the glomerulus then

reabsorbed in the proximal tubule by apical sodium-phosphate transporters NPT2a. NPT2a expression is controlled by the parathormone (PTH). NHERF1 is a regulatory factor that binds to NPT2a and PTH receptor. NHERF1-NPT2a interaction results in an increase in NPT2a apical expression. Binding of NHERF1 to PTH receptor decreases cAMP production in response to PTH. Targeted inactivation of NHERF1 gene in mice results in a phenotype similar to NPT2a^{-/-} mice associating: renal phosphate leak, nephrolithiasis and bone demineralization. In a patient exhibiting hypophosphatemia and nephrolithiasis, we identified a mutation in NHERF1 gene.

The aim of the present study is to characterize the consequences of the identified NHERF1 E68A mutation on phosphate transport in human cultured cells.

Results: NHERF1 wild type or E68A mutant proteins were expressed with NPT2a in human epithelial HELA cells. Phosphate uptake was significantly lower in cells expressing E68A than in those expressing the wild type protein. Co-immunoprecipitation experiments showed a marked decreased in NPT2a-NHERF1 mutant interaction in HELA cells. We are studying the effects of the E68A mutant on NPT2a membranous expression and on PTH receptor signalling.

Conclusion: Our results support the role of the E68A mutant in the patient phenotype.

A Soluble Form of the (Pro)renin Receptor Is Generated by an Intracellular Processing by Furin

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The (pro)renin receptor is a single transmembrane domain receptor which specifically binds renin and prorenin. Comparison of (P)RR sequence between (P)RR orthologs in different species indicates that the C-terminus domain corresponding to the transmembrane and the cytoplasmic domains is highly conserved in invertebrates and in vertebrates whereas the N-terminus coding for the ectodomain is conserved only in vertebrates. In support of the hypothesis that (P)RR gene results from the fusion of one ancestral gene coding for the C-terminus and a recent gene coding for the N-terminus, we found by western blot that (P)RR exists under 2 main forms, a 36 kDa full-length found in cell lysate and in conditioned medium and a 28kDa-soluble form [s(P)RR] in the conditioned medium, indicating that s(P)RR is cleaved intracellularly and is constitutively secreted by human glomerular epithelial cells. To study the possibility of a cleavage of (P)RR at a furin-like cleavage site in the ectodomain, we performed site-directed mutagenesis in the sequence R⁽²⁷⁵⁾KTR⁽²⁷⁸⁾ and we analyzed the molecular forms of (P)RR in human colon carcinoma LoVo cells devoided of active furin. The results showed that LoVo cells did not generate s(P)RR and that mutation in the furin cleavage site completely prevented the generation of s(P)RR, thereby confirming the importance of the furin site for the processing of (P)

RR. Preincubation of renin with s(P)RR inhibited renin-induced ERK1/2 activation, suggesting that s(P)RR may act as a trap for renin. These data would be important if we can show the existence of circulating s(P)RR in plasma and/or in urine.

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Luminal Albumin Inhibits Na Transport via NF- κ B Activation and Down-Regulation of SGK1 in Collecting Duct Cells

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Albuminuria is generally associated with Na retention by the collecting duct (CD) and also accelerates the progression of chronic renal failure. The aims of our study were to assess whether CD principal cells internalize luminal albumin and whether luminal albumin induces pro-inflammatory signaling and/or alters transepithelial sodium transport. For this purpose we studied mCCD_{cl1} and mpkCCD_{cl4} cells, two differentiated mouse collecting duct cell lines. Results showed that mCCD_{cl1} cells quickly internalized FITC-conjugated albumin in intracellular vesicles. In addition, albumin induced a dose-dependent inhibition of the amiloride-sensitive transepithelial current with a threshold at 1 mg/ml and a maximal effect at 10 mg/ml both in the absence and presence of aldosterone. This effect of albumin on transepithelial Na transport was associated with decreased SGK1 mRNA and protein expression levels both in the absence and presence of aldosterone. Since we have previously shown that NF- κ B activation leads to inhibition of Na transport via decreased SGK1 transcription, we assessed the effect of luminal albumin on NF- κ B activity by measuring luciferase reporter gene expression and accumulation of mRNA from marker genes, i.e. I κ B α and TNF α . Results showed that luminal albumin indeed activated NF- κ B with threshold and maximal effects observed with 1 and 10 mg/ml albumin, respectively. The effect of luminal albumin on NF- κ B activation was partially prevented by expression of either dynamin-1 or -2 dominant negative K44A mutants. In contrast, inhibition of lysosomal protein degradation by either chloroquin or leupeptin did not prevent the luminal albumin-induced down-regulation of SGK1. In conclusion, our results show that cultured CD principal cells internalize luminal albumin via clathrin-dependent endocytosis which leads to NF- κ B activation, SGK1 down-regulation and inhibition of transepithelial Na transport.

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Specific Effects of Tubular Calcium-Sensing Receptor (CaSR) Activation on Renal Handling of Calcium, Phosphate, Magnesium, Sodium, Potassium and Water

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Background: The calcium-sensing receptor (CaSR), a G protein-coupled receptor, has been located on parathyroid, C thyroid, kidney, gastro-intestinal, bone and cerebral cells. CaSR is best known for its role in regulating the secretion and synthesis of parathyroid hormone (PTH) and calcitonin in response to the change in extracellular calcium. In kidney, CaSR is mainly located in thick ascending limb of Henle loop where it may inhibit calcium reabsorption. Direct effects on renal handling of phosphate, magnesium, sodium, potassium and water are suspected but have not been defined.

Purpose of Study: To define the specific role of renal tubular CaSR on renal handling of calcium, phosphate, magnesium, sodium, potassium and water, using a specific allosteric agonist of CaSR, namely cinacalcet.

Materials and Methods: Normal and thyro-parathyroidectomized (TPTX) PTH-supplemented (using subcutaneous osmotic minipumps) Sprague-Dawley male rats were orally treated with a single dose of cinacalcet (10 mg/kg) or vehicle. All rats were studied in metabolic cages. Evolution of serum calcium, and urinary calcium, phosphate, magnesium, sodium, potassium and water was recorded.

Preliminary Results: In normal rats, a single dose of cinacalcet decreased serum calcium, and significantly and transiently increased urinary calcium but we had no effect on urinary phosphate, magnesium, sodium, potassium and water. In TPTX PTH-supplemented rats, cinacalcet also decreased serum calcium. However no significant effect on urinary calcium, phosphate, magnesium, sodium, potassium and water was found.

Conclusion: This suggests that a single oral dose of cinacalcet (10 mg/kg) decreases renal tubular reabsorption of calcium by a specific PTH-independent effect, but has no effect on renal handling of phosphate, magnesium, sodium, potassium and water.

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Modulation of WNKs Expression by Sodium, Potassium and Aldosterone

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Introduction: WNK1 and WNK4, two serine-threonine kinases, are responsible for Familial Hyperkalemic Hypertension (FHHt), a rare form of human hypertension caused by an altera-

tion in renal ion handling. *WNK1* gives rise to an ubiquitous long isoform (*L-WNK1*), expressed at a low level in the entire kidney, and to a shorter kidney-specific isoform (*KS-WNK1*) lacking kinase activity, which is highly expressed in the distal tubule and acts as a dominant-negative form of *L-WNK1*. *WNK4* is expressed in the entire Aldosterone Sensitive Distal Nephron (ASDN). *In vitro* and *in vivo* studies have shown that *WNK4*, *L-WNK1* and *KS-WNK1* play a key role and interact to regulate Na⁺ and K⁺ transport in the ASDN.

Methods and Results: We have studied *L-WNK1*, *KS-WNK1* and *WNK4* expression by real-time PCR in mice fed a low-or high-sodium diet, a high-potassium diet or submitted to a chronic aldosterone infusion during 10 days. While unaffected by a low- or high-Na⁺ diet, *KS-WNK1* but not *L-WNK1* expression was increased 2-fold by a high-K⁺ diet or a chronic infusion of aldosterone. *WNK4* expression was stimulated by both a high Na⁺ and K⁺ intake, but not by aldosterone infusion. We also studied the expression of *L-WNK1* and *WNK4* in the colon, where *WNK4* has been shown to inhibit ENaC activity. A high-Na⁺ diet stimulated *L-WNK1* (1.5-fold) but not *WNK4* expression. *L-WNK1* expression was also stimulated by aldosterone infusion in the heart and the cerebellum, two tissues where it is highly expressed.

Conclusions: These results show that aldosterone differentially regulates the two *WNK1* isoforms. In the kidney, it stimulates *KS-WNK1* expression but another unknown regulator, possibly Ang II, counteracts this stimulation under salt restriction. Potassium itself, independently of aldosterone, is responsible for *WNK4* stimulation.

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Stable Knockdown of *NPHP1* and *NPHP4* Leads to Polarity Defects in Madin-Darby Canine Kidney (MDCK) Cell Lines

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Nephronophthisis is an autosomal recessive disorder causing pediatric end-stage renal disease characterized by tubular atrophy, thickened basement membranes, and the development of medullary cysts, which arise via unknown mechanisms. The disease is caused by mutations in *NPHP1-9* genes encoding nephrocystins which have been localized at cell-cell junctions, primary cilia, and centrosomes. In this study, we examine the function of nephrocystin-1 and nephrocystin-4 and uncover their role in epithelial polarity. Nephrocystin expression level was increased several-fold in MDCK cells as they become progressively more polarized. shRNA-mediated knockdown of either *NPHP1* or *NPHP4* resulted in delayed tight junction formation and abnormal cilia formation, further indicating a role for these genes in the formation of fully-polarized epithelium. Furthermore, growth of nephrocystin-depleted cells in a three-dimensional culture model led to the formation of abnormal, multi-lumen cysts. These phenotypes were

intriguingly similar to those reported for shRNA-mediated knock-down of the tight junctional proteins PALS1 and PATJ, and indeed we demonstrate here a physical interaction between these nephrocystins and PALS1/PATJ via coimmunoprecipitation. Taken together, these results demonstrate that the nephrocystins play an essential role in the maintenance of apico-basolateral polarity and suggest a plausible mechanism by which the *in vivo* histopathologic features of nephronophthisis might develop.

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Mice Overexpressing the Mineralocorticoid Receptor (MR) in Keratinocytes Exhibit Altered Gene Expression and Skin Properties

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The MR regulates renal sodium reabsorption; it is also expressed in the human and mouse skin, with unknown pathophysiological relevance. Epidermal and hair follicle keratinocytes are highly susceptible to regulation by some nuclear receptors including retinoids and glucocorticoids. We have shown that epidermal MR over-expression (conditional tet OFF system) leads to impaired epidermal maturation, skin hypoplasia and perinatal death (Sainte Marie *et al*, Am. J. Pathol 2007). Using this mouse model, we now ask whether skin MR could affect classical MR/aldosterone regulated genes, despite lack of evidence for patent ion/fluid transepidermal movements. Real-time PCR analyses of skin explants from neonates (double-transgenic, DT, with MR over-expression compared to their control littermates) revealed an increased expression of epithelial sodium channel ENaC, of *sgk1* and of the Channel Activating Proteases Cap1 and Cap3. In addition, primary cultures of skin patches from DT exhibited impaired keratinocyte growth. Efforts are made to understand the links between epidermal proliferation/differentiation and MR-dependent signaling. This model will allow evaluating whether MR agonism (by aldosterone or by glucocorticoids) and antagonism can be used to modify epidermal homeostasis and growth. Comparisons will be made between MR over-expression (in face of normal aldosterone/glucocorticoid plasma levels) and exposure of normal skin to exogenous ligands in mouse skin. It will also allow progress in elucidating novel roles for MR-dependent cascades in these non classical target cells.

Gamma-Hydroxybutyrate Is Metabolized by Rat Renal Proximal Tubules: A Carbon 13 NMR Study

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Gamma-hydroxybutyrate (GHB) is both a physiological brain metabolite and a drug of abuse (date rape drug). We have conducted a study to evaluate the capacity of the kidneys to eliminate such a compound that, when ingested in gram amounts, remains at high concentrations in the blood for hours and exerts toxic effects. For this, renal proximal tubules isolated from fed and 48h-fasted Wistar rats were incubated at 37°C with 1 and 5 mM of unlabelled GHB or with [U-¹³C]GHB in Krebs-Henseleit medium with an atmosphere of 5%CO₂/95%O₂. Precision-cut liver slices prepared from fed and 48h-fasted rats were also incubated under the same experimental conditions. At the end of incubation, substrate removal and product formation were measured by ¹³C NMR spectroscopy using a high field NMR spectrometer (500 MHz). At both 1 and 5 mM concentration, unlabelled and labelled GHB was shown to be metabolized not only by liver cells, which are considered to be the main cells responsible for GHB disposal *in vivo*, but also at significant rates by proximal tubular cells. The nutritional state did not influence GHB utilization either in liver or in renal cells. The NMR spectra obtained did not reveal any labelled product of GHB metabolism in renal proximal tubules whereas some glucose became labelled after incubation of liver slices; although GHB was not a good gluconeogenic precursor, this reveals that GHB carbons were incorporated into the oxaloacetate pool. The latter data indicate that, in both liver and renal proximal cells, most of the GHB metabolized was converted into CO₂, presumably by the beta-oxidation pathway.

This study establishes that, beside the liver, the kidneys may significantly contribute to the removal and metabolism of GHB *in vivo*.

Tissue Kallikrein Deficient Mice Have a Renal Defect in Base Excretion

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Tissue kallikrein (TK) is a serine protease that generates kinins locally in many organs. In the kidney, TK is synthesized in large amounts by connecting tubule cells and then, is mainly secreted into the urinary fluid and to a lesser extent to the peritubular

interstitium. A highly significant relationship between bicarbonate excretion and kallikrein activity in the urine has been reported and suggests that renal kallikrein is involved in the regulation of acid-balance balance (i.e., renal bicarbonate handling). To test that hypothesis, we used a mouse model with TK gene disruption (TK^{-/-}). We looked at their acid base balance under basal condition and acid or alkali challenges. TK^{-/-} mice did not exhibit overt alteration in acid base balance. At basal state, there was no difference in urine pH, ammonium and citrate excretion between genotypes. However, phosphate excretion, titratable acid and net acid excretion were significantly increased in TK null mice relative to wild type mice. TK null mice were not able to adapt to an alkali load; after 6 days, they kept high blood pH and bicarbonate concentration while wild type mice restored normal blood pH and bicarbonate concentration. In contrast, TK null mice develop less severe acidosis than wild type mice following 2 days acid load. H⁺/HCO₃⁻ transporter expression was examined by semiquantitative immunoblotting in TK^{-/-} and wild type mice. Under basal conditions, TK^{-/-} mice had lower proximal tubule NHE3 and NBC1 protein expression suggesting that these mice minimized changes in systemic pH by reducing proximal bicarbonate reabsorption. H⁺/HCO₃⁻ transporter expression within the cortical part of the distal nephron of TK^{-/-} mice (i.e., NHE2, NBC3, Pendrin, vacuolar H⁺-ATPase, and α1HKATPase) was unchanged. Our data indicates that TK is a factor modulating HCO₃⁻/H⁺ transport in the kidney. The molecular target of this effect remains to be identified.

Cadmium Inhibits Lactate Gluconeogenesis in Human Renal Proximal Tubules

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Cadmium is a nephrotoxic heavy metal that disturbs the function of the renal proximal tubule, but it remains unknown if it interferes with the metabolism of the latter nephron segment. Given the importance of lactate as a physiological substrate of the human kidney *in vivo*, we have conducted an *in vitro* study to characterize the effect of cadmium on lactate metabolism.

For this, isolated human proximal tubules were incubated with unlabeled or variously ¹³C-labelled lactates or lactate plus ¹³C-bicarbonate. After incubation, substrate removal and product formation were measured by enzymatic and ¹³C MRS methods.

CdCl₂ inhibited gluconeogenesis from 1 mM lactate in a dose-dependent manner with a IC₅₀ of 0.1 mM. The labelling patterns of the glucoses synthesized from [1-¹³C]-, [2-¹³C]- and [3-¹³C]-lactate as well as from lactate plus ¹³C-bicarbonate indicated that lactate gluconeogenesis involved not only the passage of carbons through pyruvate carboxylase and the reversible equilibration of oxaloacetate with fumarate but also pyruvate dehydrogenase and the entire tricarboxylic acid cycle. Combination of enzymatic and MRS

methods with a mathematical model of lactate metabolism, which provides a panoramic view of lactate metabolism, reveals that 0.1 mM CdCl₂ inhibited lactate removal and fluxes through pyruvate carboxylase, phosphoenolpyruvate carboxykinase and the subsequent steps of lactate gluconeogenesis. The cellular level of acetyl-CoA level was also diminished by 0.1 mM CdCl₂. By contrast, 0.1 mM CdCl₂ did not alter fluxes through pyruvate dehydrogenase and through the entire tricarboxylic acid cycle although it significantly decreased the cellular coenzyme A and ATP levels.

It is concluded that cadmium strongly inhibits human renal lactate gluconeogenesis, an effect consistent with the fall of the cellular levels of not only acetyl-coenzyme A, a cofactor of pyruvate carboxylase, but also of ATP whose mitochondrial synthesis appears to be inhibited.

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Towards Identifying Possible Signaling Pathways Regulating Potassium Handling in the Cortical Collecting Duct

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Background: The cortical collecting duct (CCD) plays a key role in the regulated K⁺ secretion, which is mainly mediated through ROMK (Renal Outer Medullary K⁺ channel) on the apical membrane. Several factors (Na⁺, pH, mineralcorticoids) are known to modulate K⁺ transport in the CCD, however, little is known about the sensor and signal transduction mechanisms that are able to detect excess or low level of K⁺ reserves and act on effectors in the CCD.

Aim: This project focuses on identifying possible kaliuretic and/or antikaliuretic factors, and their signaling pathway(s), regulating potassium handling in the CCD.

Material and Methods: We take advantage of a mouse CCD cell line (mCCD_{cl1}), which has the ability to secrete potassium. Serum, a source of possible secreted kaliuretic and/or antikaliuretic factors, was used for stimulating the cells. The cells were pre-treated with 20% fetal calf serum (FCS) and thereafter exposed to 20% FCS, 20% rat serum or 2% FCS. Functional (transepithelial electrophysiological measurements), transcriptional (qRT-PCR) and transcriptome-wide expression (mouse 430 2.0 Affymetrix array) studies have been done on the cells treated under these conditions.

Results and Perspectives: For mCCD cells exposed to 20% FCS or rat serum we observed a 5-fold decrease in the K⁺ transport, measured as the barium-sensitive conductance, in the rat serum treated cells compared to the FCS treated cells. This modulation also involved a 2- to 3-fold transcriptional down-regulation of ROMK, and of some other transporter subunits, by rat serum under the same treatment conditions. Based on these data we thought that the difference in potassium channel function and transcriptional regulation between the rat serum and the control serum (FCS) treated mCCD cells could involve signal transduction pathway(s) important for potassium channel regulation. Indeed,

the transcriptome-wide data confirmed our previous observations and enabled us to identify novel genes, which are potentially interesting candidates involved in potassium handling. Further data analysis and functional studies will be needed to characterize the possible role and involvement of these candidate genes in a potassium signaling pathway in the CCD.

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Expression, Localization, and Regulation by Testosterone of Cysteine Sulfinate Decarboxylase Gene in Male and Female Mouse Kidney

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Taurine is synthesized by cysteine sulfinate decarboxylase (CSD). A sexual dimorphism in the expression of CSD gene (*Csad*) occurs in the rat liver. Estradiol down-regulates its expression. Given that the expression of *Csad* in the mouse kidney is poorly documented, we tested whether sex-hormones control the expression of *Csad*. In addition, we verified whether the renal distribution of CSD was sex- and species-specific. Adult male and female OF1-Swiss and C57BL/6J mice were used. RNAs and proteins were isolated from kidneys and dissected renal zones for RT-PCR and Western blot analyses, respectively. Mice were subjected to orchidectomy or ovariectomy. Female and orchidectomized mice were treated with testosterone or dihydrotestosterone. In both strains, the expression of *Csad* was two-fold higher in male than in female kidneys. Orchidectomy but not ovariectomy diminished the expression of *Csad* by ≈2-fold. At the mRNA level, the effect of orchidectomy was reversed by testosterone but not DHT. Testosterone enhanced the level of CSD mRNA in female kidneys. CSD was unevenly distributed with 90% in the cortex and the outer stripe of the outer medulla. Male superficial cortex exhibited a higher level of CSD than that of the female (20% vs. 6%, respectively). The results support the view of a sexual dimorphism in the expression of *Csad* in kidneys of these two mouse strains. The expression of *Csad* is constitutive and inducible. The up-regulation of *Csad* by testosterone is organ- and species-specific. In contrast to rats, the proximal convoluted tubules of mice might highly express *Csad*.

Cyclosporine Causes Epithelial to Mesenchymal Transition *in vivo*

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Background: Cyclosporine A (CsA) is a major immunosuppressive drug used in transplantation: it improves patient and graft survival. Meanwhile, chronic administration of CsA is nephrotoxic, eventually leading to renal fibrosis. It is well known that fibrogenesis is largely mediated by interstitial fibroblast activation. Evidence recently emerged that these myo-fibroblasts may derive from tubular epithelial cells, by a process called epithelial-to-mesenchymal transition (EMT). In human beings, some epithelial phenotypic changes were reported to occur in various nephropathies, which are highly suggestive of the early stages of EMT. The aim of our study was to determine whether CsA could induce EMT of tubular cells *in vivo*.

Methods: CsA (from 15 to 30 mg/kg) was administered subcutaneously to adult Sprague Dawley rats, and to CD1 mice, subcutaneously, and daily, for 2 to 4 weeks. Hemodynamic and urinary biochemical parameters were evaluated during the treatment. Gene expression was analyzed by quantitative RT-PCR at day 7. Renal histopathologic alterations, including vascular tubular damages, interstitial infiltrates and fibrosis, were characterized by Trichrome Masson's staining. EMT was examined by immunostaining using specific antibodies against vimentin and β -catenin.

Results: When compared to animals treated with a vehicle, animals treated with CsA developed a dose and time-dependent increase in serum creatinine, with tubular damage (cytoplasmic vacuoles). By RT-PCR, in renal cortex, some epithelial genes were down-regulated and mesenchymal genes up-regulated. By immunohistochemistry, a major increase of vimentin staining was found in tubular cells. This increase correlated with the increased levels of serum creatinine. In vimentin positive tubular cells, β -catenin expression was confined to the baso-lateral membranes, a phenotype that was never observed in vehicle-treated rodents.

Conclusion: CsA causes the apparition of mesenchymal markers in the tubular epithelium *in vivo*, suggesting that EMT could be instrumental in CsA-induced renal fibrosis.

Proliferation and Regeneration of Intrarenal Urothelium (RUTEC) after Unilateral Ureteral Obstruction

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Introduction: During Unilateral Ureteral Obstruction (UUO) we observe a dilation of intrarenal cavity and a destruction of the renal parenchyma. Curiously, very few studies are dedicated to

intrarenal urothelium injuries while it constitutes the first structure subjected to hyperpressure. Within the kidney, contrary to the bladder, the urothelium is present in a single layer both facing the papilla and the fornix and express different uroplakines who contribute to the impermeability of this structure. We wonder that transient UUO could lead to a structural change of intrarenal urothelium.

Materials and Methods: In C57/B6 mice, unilateral UUO was performed using two different models: a nylon suture to induce a severe obstruction (model 1), a surgical clip (model 2). Mice received BrdU in drinking water throughout all experiments. The cellular proliferation was estimated by measure of BrdU incorporation and anti KI67 marking. Immunohistochemistry (IHC) and immunofluorescence (IF) were used for proliferation and differentiation analyses.

Results: In model 1, desquamation of RUTEC was detected on D1 with a marginal proliferation. A denudation of the basement membranes was present by D3 after UUO. In model 2, proliferation of RUTEC appears as soon as D1 with a peak at D2 (Proliferation index 33 %) associated with a decrease of the expression of uroplakines from D2. The monolayer urothelium moved into a multilayered epithelium and returns to the normal state (D12) after the removal of the clip in contrast to persistent obstruction. Oral administration of EGFR inhibitor does not inhibit RUTEC proliferation. RUTEC proliferation was associated with a decrease of FGFR2 expression by IHC and Western Blot indicating a potential role of FGF/FGFR2 pathway. Among the ligands of FGFR2, FGF7 is a good candidate in this process of proliferation as suggested by preliminary data in RT PCR.

Conclusion: Our results suggest that severe UUO (model 1) induces a destruction of the intrarenal urothelium. In contrast, less severe UUO (model 2) induces an intense proliferation of the RUTEC. This proliferation is EGFR independent and may involve the FGFR2 pathway.

The LPA1 Receptor in Renal Interstitial Fibrosis: A Follow-Up Study

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Lysophosphatidic acid (LPA) is a bioactive phospholipid known to be involved in a number of biological processes via the activation of G-protein coupled receptors (LPA1-4). The aim of the present study was to analyze the possible involvement of LPA and its receptors in the development of renal tubulo-interstitial fibrosis (TIF) induced by unilateral ureteral obstruction (UUO) in mice.

UUO induced a 5-fold increase in LPA1 receptor- and no change in LPA2 receptor- mRNA levels. LPA3 and LPA4 recep-

tors were not detected. Furthermore, LPA1 null mice and wild type mice treated with LPA1 receptor antagonist (Ki16425) display significantly lower UO₂-induced TIF than non-treated wild type mice. This protection might involve inhibition of connective tissue growth factor (CTGF), since LPA treatment of proximal tubular cells (MCT) rapidly and significantly increased CTGF expression. Currently we explore the effects of delayed pharmacological blockade of the LPA1 receptor and co-treatment with an angiotensin converting enzyme inhibitor in the UO₂ model.

In conclusion these data should make it possible to determine if LPA1 receptor blockade might become an alternative therapeutic strategy to slow down the progression of renal tubulointerstitial fibrosis.

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Functional Analysis of CLC-5 Mutations Related to Dent's Disease

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CLC-5, a Cl⁻/H⁺ antiporter member of the CLC family, is expressed on early endosomes in proximal tubule cells, where it is thought to play a critical role in endosomal function. Recent experiments suggest that CLC-5 is also expressed at the cell surface of these cells. Several key studies on CLC-5 knockout mice have shown that mutations of *CLCN5*, the gene encoding CLC-5, are associated with Dent's disease, an X-linked renal disease associated with low molecular weight proteinuria, hypercalciuria and nephrolithiasis. The impact of Dent's disease-causing mutations on CLC-5 has not yet been fully investigated in terms of electrical activity, and trafficking. Thus, to further understand the CLC-5 dysfunction in Dent's disease, we performed functional studies of CLC-5 wild-type and mutants in *Xenopus laevis* oocytes.

CLC-5 currents and surface expression were analysed using two-electrode voltage-clamp recording and chemiluminescence respectively. One mutation, K115R resulted in a reduction to about 30% of wild-type CLC-5 currents. These mutants trafficked normally to the plasma membrane, like the CLC-5 wild-type. No significant currents were recorded with the mutants G179D and L469P. The loss of currents for the mutant L469P correlated well with a loss of surface expression. We found no significant difference between surface expression of the mutants G179D and wild-type CLC-5.

Our results suggest that several mechanisms are able to impair CLC-5 function. More functional studies are necessary to better understand the defects caused by these mutations.

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9-Phenanthrol Selectively Inhibits the TRPM4 Cationic Channel

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Calcium-activated non-selective cation (NSC_{Ca}) channels have been described in a variety of tissues, including most parts of the renal tubule. However, their physiological functions are poorly established because of the absence of specific pharmacology. The Transient Receptor Potential Melastatin 4 (TRPM4) protein corresponds to NSC_{Ca} channel widely distributed with a high expression in the kidney. It is expected to support the NSC_{Ca} current recorded in the kidney and in other tissues such as heart and immune cells.

In the aim to identify new pharmacological tools specific for TRPM4, we investigated the effect of hydroxytricyclic compounds that modulate ABC transporters, as these proteins share similarities with TRPM4. Patch-clamp experiments were done on TRPM4-transfected HEK-293 cells.

We tested MPB-104, a benzo[c]quinolizinium known to activate the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). TRPM4 current was inhibited by MPB-104 with an IC₅₀ estimated at 2.10⁻⁵ M. Thus, we investigated the effect of 9-phenanthrol that lacks the chemical groups necessary for CFTR activation. While inactive on CFTR current, 9-phenanthrol inhibited TRPM4 current (IC₅₀ = 2.10⁻⁵ M). Moreover, 9-phenanthrol (even at 10⁻⁴ M) had no effect on TRPM5, the most closely related channel in the TRP protein family.

In conclusion, 9-phenanthrol is a new TRPM4 inhibitor that may allow elucidation of the physiological function of TRPM4. It is an appropriate molecule to differentiate TRPM4 over TRPM5 currents on native cells and thus determine the identity of NSC_{Ca} in a variety of tissues including kidney.

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Atp6v1b1 Deficient Mice Develop a Bartter-Like Syndrome

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Human patients suffering from type 1 distal renal acidosis often exhibit NaCl, K and water loss. These features are generally believed to be the consequence of nephrocalcinosis. The

B1-subunit deficient mice (*Atp6v1b1*^{-/-}), which display type 1 distal renal acidosis don't develop nephrocalcinosis. The purpose of this study was to examine whether these mice have alterations in Na and water balance. Sodium-replete deficient mice exhibited a mild polyuria, hypokalemia, and hypercalciuria. When submitted to a sodium-depleted diet, knockout mice exhibited major polyuria, along with renal loss of NaCl leading to a marked secondary hyperaldosteronism. Abundance of Na and Cl transporters along the nephron was examined by semiquantitative immunoblotting in *Atp6v1b1*^{-/-} and control mice. NKCC2 and AQP2 protein abundances were dramatically decreased. In contrast, the abundance of the proximal NHE3 was increased, as well as the three subunits of the epithelial sodium channel ENaC in the medullary collecting duct. Abundance of NCC was unchanged. In conclusion, *Atp6v1b1*^{-/-} mice have a phenotype resembling to Bartter syndrome. Hypercalciuria, NaCl and water losses are likely consecutive to the down-regulation of NKCC2 and AQP2. Increased ENaC abundance might drive higher K secretion through ROMK and thus might account for renal hypokalemia in *Atp6v1b1*^{-/-} mice.

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Plasmin Activates the Epithelial Sodium Channel (ENaC)

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The epithelial sodium channel (ENaC) can be activated by serine proteases, but (patho-)physiologically relevant proteases involved in ENaC activation remain to be identified. Nephrotic syndrome is a kidney disease characterized by proteinuria and increased renal Na⁺ reabsorption. In nephrotic syndrome, plasminogen, the precursor of plasmin, appears in the urine where it is likely to be converted to plasmin by urokinase-type plasminogen activator (uPA). We tested the effect of uPA and plasminogen on ENaC heterologously expressed in *Xenopus laevis* oocytes and on endogenously expressed ENaC in M-1 mouse collecting duct cells. To prevent ENaC activation by endogenous proteases, the M-1 cells were pretreated with furin inhibitor. In oocytes and in M-1 cells the combined application of plasminogen and uPA significantly increased the amiloride-sensitive ENaC whole-cell currents and short-circuit currents, respectively. In contrast, individual application of either uPA or plasminogen alone had little or no effect. Biotinylation experiments with oocytes demonstrated that the combined application of uPA and plasminogen resulted in the appearance of a γ -ENaC cleavage product at the cell surface. Furthermore, urine samples from PAN nephrotic rats significantly increased the amiloride-sensitive ENaC whole-cell currents in oocytes. In contrast, the corresponding heat inactivated nephrotic urine samples or non-nephrotic urine showed no significant stimulatory effect. Our data suggest that plasmin generated in nephrotic urine can proteolytically activate ENaC in the luminal

membrane of the distal nephron which is likely to contribute to renal Na⁺ retention in nephrotic syndrome.

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Effect of Estrogens and Estrogens Derivatives on Cystic Fibrosis Transmembrane Regulator Expression in MDCK Cells

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Cystic Fibrosis Transmembrane Regulator (CFTR) is a c-AMP dependent chloride channel that drives epithelial chloride secretion. Mutations in CFTR are responsible for Cystic Fibrosis. CFTR is also involved in renal cysts growth and development during autosomal dominant renal polycystic disease. Despite CFTR wide role in physiopathology, little is known about hormonal regulation of CFTR in epithelial cells. Our aim was to study the effect of estrogens on CFTR in MDCK cells.

MDCK1 cells were treated with 17 β - or 17 α -Estradiol, diethylstilbestrol (a synthetic estrogen now used in prostate cancer therapy), Resveratrol (a natural stilbene from grapes, reported to have pleiotropic beneficial effects), or genistein. Quantitative PCRs were used to detect CFTR mRNAs. Western blots were performed on total or on membrane proteins using the CFTR 24-1 antibody. CFTR localization was analysed by cyto-immunochemistry and confocal microscopy using the CFTR 24-1 Ab and Alexa fluor 594 as the secondary Ab. CFTR function was assessed studying cyst growth in 3D cell cultures.

Western blot analyses showed a faint CFTR expression in untreated MDCK cells. PCR confirmed the presence of CFTR mRNA. All tested compounds (except 17 α -Estradiol) increased CFTR mRNA and protein expression. Maximal efficiency was observed using 17 β -Estradiol and Resveratrol. Immunofluorescence confirmed an increased CFTR labelling on apical membranes of 17 β -Estradiol or Resveratrol treated cells. Cyst growth was enhanced in the presence of these compounds.

These data suggest that estrogens and estrogens derivatives modulate CFTR expression in MDCK cells. The mechanism involved in this effect has to be elucidated. Also, the role of estrogens (or estrogen deprivation) during polycystic kidney disease (or during cystic fibrosis) has to be further investigated.

Re-Examination of the Immunolocalization of the Cystic Fibrosis Transmembrane Regulator in Mouse Kidney: Evidence for Its Basolateral Location in Renal Epithelial Cells

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It is known that the immunolocalization of the cystic fibrosis transmembrane regulator (CFTR) may differ among studies because of differences in the sample preparation, the use of different specific CFTR antibodies and of different staining procedures. In our study, the distribution of the CFTR was re-examined by immunohistochemistry and immunofluorescence in paraffin embedded sections of mouse kidney after fixation in Bouin's fluid and using the monoclonal anti-human CFTR C-terminus antibody (clone 24-1) from R&D Systems. As already described in the literature, most of the CFTR labelling was associated to the straight segment of the proximal tubule which presented a strong labelling of the brush border but also a strong labelling associated to large vesicles scattered in the cytoplasm of the epithelial cells. The proximal convoluted tubule also displayed a strong apical CFTR labelling of the brush border and a vesicle associated CFTR labelling but in small vesicles and much less abundant than in the S3 segments. The vesicle associated CFTR labelling was observed in all the following segments of the renal tubule but in scarce and small vesicles and no more apical CFTR labelling was noted. A basolateral CFTR labelling was clearly observed in the connecting segment and the initial collecting duct, associated to principal cells and intercalated cells. In the cortical and especially the medullar collecting duct, the basolateral CFTR labelling was restricted to the type A intercalated cells. Our study confirmed previous immunohistochemical observations on the CFTR location in mouse kidney, nevertheless with some differences, but it also pointed out an original basolateral location of the CFTR in specific cell of the renal tubule. Results are discussed according to already known or suspected locations of the CFTR in mammal kidney.

A Major Role of Lutheran/Basal Cell Adhesion Molecule (Lu/BCAM) in the Control of Glomerular Permeability during Hypertension

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Background: The Lutheran (Lu) blood group/ basal cell adhesion molecule (BCAM) antigen is highly expressed in

endothelial cells and has been recognized as the receptor for the α_5 chain of laminins 10/11. Lu^{-/-} animals display mild abnormalities of the Glomerular Basement Membrane. We investigate whether Lu/BCAM could limit endothelial permeability.

Methods and Results: Hypertension and glomerulosclerosis were induced by chronic infusion of Angiotensin II for 28 days in Lu^{-/-} KO mice (n=13) and their wild-type male littermates (n=10). Both group developed similar degree of systolic hypertension throughout the course of the experiment (mean SBP: 172±9 vs. 186±6 mmHg on day 28 in KO and WT, respectively). Upon AngII infusion, Lu^{-/-} mice, that displayed baseline microalbuminuria within normal range, exhibited a larger increase in albuminuria (541±177 g/mol creatinine on day 14 and 1722±601 g/mol on day 21) than WT mice (163±43 g/mol creatinine on day 14 and 461±179 g/mol on day 21, p<0.01). The albumin urinary excretion rate was exceptionally high in Lu^{-/-} animals. A massive precipitation of proteins was even observed in the glomerular chamber in hypertensive KO mice, suggesting that increased albuminuria was due to a massive glomerular defect rather than to defective tubular reabsorption of albumin.

Conclusion: Lu/BCAM that is highly expressed in the renal microvascular endothelium didn't affect baseline blood pressure and AngII-induced hypertension but unraveled a critical role in limiting glomerular permselectivity under hypertensive condition.

A Novel Hypertensive Transgenic Mouse to Study Progression and Regression of Chronic Renal Failure

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The objective of the present study was to investigate gene regulation during progression and reversal of hypertension-associated renal failure. We used a new model of transgenic mice, the RenTg strain, expressing a renin transgene at a constant high level.

RenTg started displaying high blood pressure and abnormal albuminuria after 3 months. At 12 months, kidneys of RenTg showed severe histological alterations of all renal compartments. These changes were associated to decreased expression of nephrin, podocin, E-cadherin and megalin, while profibrotic genes of the TGF β superfamily (particularly TGF β and GDF7) were activated.

Treatment of RenTg for 6 weeks with a low dose of an ATI antagonist (irbesartan) did not reduce systolic pressure. However, albuminuria markedly dropped to normal levels. The normalization of albuminuria was accompanied by reversal of vascular lesions and reappearance of podocin, nephrin, E-cadherin and megalin. In addition, expression of TGF β and GDF7 was normalized.

Using this novel transgenic strain of mice, we showed that long lasting proteinuria and alterations of the expression of key proteins of the integrity and function of podocytes and tubules are reversible. RenTg is a useful tool adapted to provide clues for a better understanding of mechanisms of progression and reversal of renal disease.

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Sodium Retention in Acromegaly Results from Growth Hormone-Induced Epithelial Sodium Channel Activation in the Late Distal Nephron

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Acromegalic patients present with volume expansion and arterial hypertension but the nephronic sites and molecular mechanisms of direct antinatriuretic action of growth hormone (GH) remain unclear. Here, we show that acromegalic GC rats, which are chronically exposed to high levels of GH, exhibited a decrease of furosemide-induced natriuresis and an increase of amiloride-stimulated natriuresis compared to controls. Enhanced Na⁺,K⁺-ATPase activity and altered proteolytic maturation of epithelial sodium channel (ENaC) α and γ subunits in the cortical collecting ducts (CCD) of GC rats provided additional evidence for an increased sodium reabsorption in the late distal nephron under chronic GH excess. *In vitro* experiments on KC3AC1 cells, a murine CCD cell model revealed the expression of functional GH receptors (GHR) and IGF-1 receptors coupled to activation of JAK2/STAT5, ERK and AKT signaling pathways. That GH directly controls sodium reabsorption in CCD cells is supported by i) stimulation of transepithelial sodium transport inhibited by GHR antagonist pegvisomant ii) induction of α ENaC mRNA expression iii) identification of STAT5 binding to a response element located in the α ENaC promoter, indicative of the transcriptional regulation of α ENaC by GH. Our findings provide first evidence that GH, in concert with IGF-1, stimulates ENaC-mediated sodium transport in the late distal nephron, accounting for the pathogenesis of sodium retention in acromegaly.

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Mitochondrial Glutathion and Complex I Activity in Cells with a Non Functional CFTR

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Cystic fibrosis (CF) is an autosomal recessive disorder associated with mutations in the gene encoding for the cystic fibrosis transmembrane regulator protein (CFTR). CFTR transports chloride and other organic anions including glutathione (GSH) across apical cell membranes. Previous studies show a deficit in mitochondrial GSH (mGSH) in cells with a non functional CFTR. Furthermore, in several cellular models a deficit in mGSH has been known to lead to complex I (CI) inhibition. In the present study, we aim to investigate the relationships between a non functional CFTR, mGSH and CI activity. Mitochondria were obtained from human cells derived from two bronchial epithelial cell lines from patients with CF vs. corrected cells: IB3 vs the corrected C38 cells and also CFBE Δ F vs CFBE WT cells. mGSH modulation was studied using a glutathione mono ethyl ester, compound increasing preferentially mGSH. HPLC coupled with electrochemical detection was used for mGSH measurements and CI activity was measured by spectrophotometry. Blue native PAGE was performed to study CI expression. The results show a significant and specific decrease in CI activity in CFBE Δ F cells compared to WT cells, interestingly when WT cells were treated with a CFTR inhibitor (Inh 172) CI activity dropped significantly, mimicking the activity in Δ F cells. Blue native PAGE showed that this inhibition was not due to differential expression. mGSH assays showed a significant decrease in IB3 cells compared to C38 cells. Treatment with glutathione ester was able to restore IB3 mGSH to the same level as C38 cells and this allowed partial restoration of CI activity. When isolated mitochondria were incubated with DTT similar recovery of activity was observed suggesting that oxidation and possibly glutathionylation of certain CI proteins could be the mechanism involved in complex I inhibition in cells with a non functional CFTR.

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Targeting the Kinin B1-Receptor Attenuates Renal Interstitial Fibrosis *in vivo*

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The bradykinin B1-receptor (B1R) receptor is weakly expressed under physiological conditions but induced by inflammatory stimuli. Here we report a role for the B1R in renal fibrosis using two different animal models.

Using a model of accelerated tubulointerstitial fibrosis induced by unilateral ureteral obstruction (UUO) we showed that genetic and pharmacological blockade of the B1R, either before or after UUO, reduced the induction of several markers of renal fibrosis: macrophage infiltration, myofibroblast accumulation and collagen deposition.

Since the majority of chronic kidney diseases are initiated by glomerular injury we also used the model of nephrotoxic serum (NTS) induced-glomerulonephritis and investigated whether blocking the B1R was effective in slowing-down the fibrotic process in this model. B1R antagonist treatment, starting 2 weeks after NTS administration, improved significantly renal function and histology.

Mechanistic studies showed that B1R blockade is reducing macrophage infiltration most likely by acting on chemokine expression.

In conclusion we demonstrate that delayed treatment with a B1R antagonist is leading to a significant decrease in the progression of renal fibrosis in two different animal models. Our findings show that blocking the B1R is a promising antifibrotic therapy and that this is feasible as orally active and stable antagonists are available.

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Heterotetrameric Kir4.1/Kir5.1 Channel Forms the Major Basolateral K⁺ Channel in Mouse CCD Principal Cells

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The cortical collecting duct (CCD) plays a key role in K⁺ secretion and Na⁺ reabsorption. In CCD principal cells, a basolateral K⁺ conductance (i) recycles the K⁺ carried into the cell by the Na⁺/K⁺-ATPase, (ii) contributes to setting the resting basolateral membrane potential difference, and (iii) may determine the direction of the net basolateral K⁺ flux, depending on physiological status. Little is known about the molecular nature of native basolateral K⁺ channels of CCD principal cells.

A K⁺ channel with an intermediate conductance of 40 pS predominated in cell-attached patches of basolateral membrane of mouse CCD principal cells. In inside-out membrane patches exposed to an Mg²⁺-free medium, its current-voltage relationship was linear with a conductance of 38 pS, and internal Mg²⁺ reduced its outward conductance to 13 pS. Spermine (6x10⁻⁷ M) reduced its activity by 50 % at V_c = 60 mV. A sigmoid relationship between intracellular pH (pH_i) and channel activity (NP_o) was observed with a pK of 7.24 and a Hill coefficient of 1.7. These properties are consistent with either Kir4.1/Kir5.1 or Kir4.2/Kir5.1 channels. By real-time PCR, Kir4.1 and Kir5.1, but not Kir4.2, mRNAs were detected. Kir4.1 and Kir5.1 proteins cellularly co-localized with AQP2, while AQP2-negative cells (*i.e.* intercalated cells) were unstained. Dietary K⁺ had no influence on the properties of

the intermediate conductance channel, but a Na⁺-depleted diet increased its open probability by ~25 %.

K⁺ conductance in the basolateral membrane of mouse CCD principal cells is therefore mainly constituted by heteromeric Kir4.1/Kir5.1 inwardly rectifying channel. These results, together with previous data showing the presence of a similar channel in basolateral membrane of thick ascending limb and distal convoluted tubule cells, indicate that this channel may be a major component of the basolateral K⁺ conductance along the mouse distal nephron.

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Changes in Cardiac Transcriptome Induced by Altered Corticosteroid Receptor Signaling in the Heart

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Aldosterone plays a major role in regulation of extracellular volume and blood pressure, through its renal action via the mineralocorticoid receptor (MR); in addition the extra-renal roles of MR/aldosterone in non epithelial cells (neurones, cardiomyocytes, vessels, adipocytes) are now intensively investigated. Animal models (aldo/DOCA-salt) and clinical trials (RALES, EPHESUS) evidenced a major pathophysiological contribution of aldosterone/MR in the cardiovascular system. To evaluate MR cardiac signaling, we analyzed the transcriptomic profile of 2 transgenic mouse models with conditional inducible overexpression of the MR and of the glucocorticoid receptor (GR) in cardiomyocytes (MHC-MR and MHC-GR). Using MWG and dedicated cardiochips, we have identified 438 cardiac genes (out of 9406) that are differentially expressed between control and MHC-MR mice, and 351 genes (out of 3927) differentially expressed between control and MHC-GR mice. After real-time PCR validation of some modulated genes (ANF, periostin, troponinT3), and establishment of the time-course of changes in expression, we now focus on gene alterations that are sensitive only to MR (not to GR) overexpression to identify specific MR signaling cascades in the heart in our transgenic models and that can be validated in other models (as aldo/DOCA-salt). Importantly, some of the MR-specific genes encode for secreted proteins that may represent novel markers of MR activation in circulating blood, including in human pathology (patent deposited).

A Mitotic Transcriptional Switch in Polycystic Kidney Disease

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HNF1 β is a transcription factor expressed in tubular epithelia. Its renal specific inactivation leads to a severe polycystic kidney disease in mice due to the defective expression of Pkhd1, Pkd2 and Tamm-Horsfall, genes whose mutations are responsible for renal cystic diseases.

To investigate the role of HNF1 β in maintenance of tubular shape, we made use of an inducible gene inactivation system. We found that the lack of HNF1 β results in cyst formation only if carried out in the first days after birth (P0-P2) and not during a refractory period that is set two weeks later. The sensitive period is characterized by an intense, cell-synchronized proliferative pattern that lengths tubules during morphogenesis. On the contrary, cell proliferation is rare and non-synchronized in the refractory period. An intense and synchronized cell proliferation can be induced by pathological conditions such as an ischemic injury. Interestingly, when normally shaped HNF1 β -deficient tubules (deleted during the refractory period) are forced, in this way, to re-enter the cell cycle they give rise to cystic dilations. By measuring the mitotic angles according to the tubular axes, we demonstrated that mitosis are distorted (average = 38°) in mutant post-ischemic kidneys as compared to wild type ones (average = 15°). Moreover, the expression of PKD2 is decreased in kidneys of mice deleted at P0-P2 or adult mice submitted to ischemia but not in kidneys deleted at P15. Interestingly, we showed that PKD2 expression is decreased specifically in cells that have proliferated, suggesting a role of HNF1 β in the re-expression of this gene after the cell cycle.

Our results reveal that the lack of HNF1 β per se, is not sufficient to elicit tubular dilation and cyst formation. In this context, in a cystic gene deficient epithelium, synchronized and locally intense proliferation might be the crucial event that leads to cystogenesis by unmasking transcriptional silencing of target genes, leading to planar cell polarization defects.

Inhibition of Insulin-Stimulated Hydrogen Peroxide Cell Production Prevents Stimulation of Sodium Transport in A6 Cell Monolayers

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Insulin-stimulated sodium transport across A6 cell monolayers involves the activation of PI 3-kinase. We have previously demonstrated that exogenous addition of H₂O₂ also provokes an increase in PI 3-kinase activity leading to subsequent rise in sodium transport. As a burst of H₂O₂ production following insulin stimulation has been detected in various cell types, it raises the question whether such a mechanism is responsible for the activation of PI 3-kinase and the subsequent stimulation of sodium transport. In this present study, we therefore incubated A6 cell monolayers with the oxidation-sensitive fluorescent probe 5,6-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H₂DCF-DA) under various experimental conditions. Insulin induced an immediate and sustained increase in fluorescence, indicative of H₂O₂ production that begins within two minutes after insulin stimulation. We also tested the effect of preincubation of the A6 cell monolayers with different chelators of either superoxide (O₂^{•-}) (Nitro Blue Tetrazolium, 100 μ M) or H₂O₂ (Ebselen, 50 μ M), or blockers of NADPH oxidase (Nox) enzymes (diphenyleneiodonium, 5 μ M; phenylarsine oxide, 1 μ M and Plumbagin, 30 μ M). They all prevented both the insulin-induced stimulation of H₂O₂ production and the insulin-induced stimulation of sodium transport. Finally, pretreatment of the A6 cell monolayers with diphenyleneiodonium prevented p85 docking at the plasma membrane and inhibited insulin-stimulated PI 3-kinase activity. In conclusion, insulin induces an acute burst of H₂O₂ production in A6 epithelial cells which appears to be responsible for activation of PI 3-kinase, PIP₃ production and subsequent increase in sodium transport.

MEPE: A Novel Regulator of Phosphate Balance?

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Matrix extracellular phosphoglycoprotein (MEPE) is one of a group of putative phosphaturic compounds (phosphatonins) isolated originally from tumours in patients with oncogenic osteomalacia, and subsequently identified in normal plasma. Here we

provide *in vivo* evidence for an inhibitory action of MEPE on two key epithelia involved in phosphate homeostasis. Anaesthetised rats, prepared surgically for clearance measurements, were infused intravenously with one of 3 doses of MEPE, or with vehicle alone, for 2 hours. MEPE caused a dose-dependent inhibition of fractional phosphate reabsorption: fractional phosphate excretion increased from $12 \pm 3\%$ in vehicle-infused rats to a maximum of $27 \pm 3\%$ during MEPE infusion ($P < 0.01$). Subsequent renal micropuncture studies localised its effect to the proximal tubule. To assess the effect on the proximal tubular phosphate transporter NaPi-IIa, kidneys were fixed *in vivo* and a standard avidin-biotin protocol using nickel-DAB or Texas Red was used for immunohistochemical staining of NaPi-IIa. MEPE caused a reduction in NaPi-IIa staining throughout the renal cortex, suggesting its internalisation (by endocytic retrieval) from the apical membrane.

In separate experiments in MEPE- and vehicle-infused anaesthetised rats, segments of duodenum and jejunum were perfused with buffer solution containing ^{32}P -labelled phosphate, and blood samples taken at intervals up to 30 min, to assess ^{32}P absorption. MEPE had no effect in the duodenum, but caused a dose-dependent inhibition of phosphate absorption in the jejunum (maximum inhibition at 30 min = 27%; $P < 0.05$), where phosphate uptake is sensitive to vitamin D.

These findings suggest that MEPE may be a novel and physiologically important regulator of phosphate transport in both the kidney and intestine.

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Invariant Natural Killer T Cells Confer Resistance to Experimental Anti-Glomerular Basement Glomerulonephritis

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Invariant natural killer T (iNKT) cells are a subset of T lymphocytes that, upon TCR stimulation, can rapidly secrete a wide variety of cytokines. These cytokines then exert a regulatory or effector function depending on the immunological context. In this study, we showed that iNKT cells play an important role in resistance to anti-glomerular basement membrane-induced glomerulonephritis (anti-GBM GN). The administration of a sheep anti-rat glomerular basement membrane serum to C57BL/6J mice resulted in the influx of large numbers of iNKT lymphocytes into the renal tissue. To determine the role of these cells, we examined

C57BL/6J $\text{J}\alpha 18^{-/-}$ mice, which lack iNKT cells. We found that these mice had higher levels of blood urea nitrogen and proteinuria than wild-type mice. In addition, the $\text{J}\alpha 18^{-/-}$ mice showed more intense tissue wounding and greater glomerular and tubular injury than wild-type mice. Quantitative PCR analyses of renal tissue revealed elevated expression of transforming growth factor (TGF)- β and TGF- β -induced gene product in the kidney of wild-type but not in $\text{J}\alpha 18^{-/-}$ animals, suggesting that TGF- β plays an important role in protection from anti-GBM GN. Indeed, administration of a TGF- β -neutralizing antibody reduced resistance to anti-GBM GN. Together, these results show that iNKT cells play a regulatory role in experimental anti-GBM GN and suggest that TGF- β plays a key role in acute glomerulonephritis.

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Protease-Activated Receptors Type 2 (PAR2) Stimulate Trans-Cellular and Para-Cellular Sodium Reabsorption in the Rat Cortical Thick Ascending Limb of Henle's Loop (cTAL)

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Expression of PAR2 in the kidney has been established at the mRNA level but the presence and role of the protein in native renal epithelial cells is still a matter of discussion. In the present study, we searched for a role of PAR2 on ion transport in freshly microdissected rat cTAL.

PAR2 was challenged with either trypsin, or the specific agonist peptide SLIGRL-NH₂ (AP).

Basolateral trypsin (0.05-5U/ml) dose-dependently induced recruitment of intracellular calcium. This response was mimicked by AP (40 μM), but not by the inactive reverse peptide LRGILS-NH₂, and it was abolished by soybean trypsin inhibitor. AP also increased 1.9-fold the phosphorylation of ERK_{1,2}. Incubation with trypsin (3U/ml) or AP 1) increased the lumen positive transepithelial voltage (PD) by approximately 1.5 mV, 2) stimulated Na,K-ATPase maximal activity by >80% (an effect not altered when apical sodium entry was blocked with furosemide), 3) increased 2 fold Na,K-ATPase affinity for sodium, and 4) enhanced paracellular conductance to sodium. Inhibition of phospholipase C (PLC) with Et-18-OCH₃ prevented both the stimulation of Na,K-ATPase and paracellular conductance. However, inhibition of classical protein kinases C (PKC) with Gö6976, and ERK_{1,2} kinase (MEK) with U0126 abolished the effect of AP on paracellular conductance and Vmax of Na,K-ATPase, but reversed neither the increase of Na,K-ATPase affinity for Na, nor the increase of PD. PD increase persisted in the presence of intracellular BAPTA. Total inhibition was achieved with staurosporin.

In conclusion, PAR2 activation increases both transcellular and paracellular sodium reabsorption in rat cTAL. PAR2 effect on transcellular sodium reabsorption results from a primary effect on Na,K-ATPase involving a PLC/Ca/cPKC dependent increase of

the number of active units at the plasma membrane; and a PLC-dependent but Ca-independent increase of Na,K-ATPase affinity for Na through a yet unknown kinase.

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Novel Amiloride-Resistant Thiazide-Sensitive Electroneutral NaCl Transport Pathway in Mouse CCD

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Mouse model of Gitelman syndrome (NCC^{-/-} mice), are not hypokalemic in spite that the NaCl loss in the DCT should be compensated by increased Na⁺ absorption, which promotes K⁺ secretion, in the CNT and CCD. To understand how these mice limit renal K⁺ loss, Na⁺, Cl⁻, and K⁺ transports (J_{Na}, J_K, J_{Cl}) were examined in freshly isolated CCDs from different mouse strains, including NCC^{-/-} mice.

As expected, Na⁺-deplete NCC^{+/-} mice CCDs, showed electrogenic Na⁺ absorption and K⁺ secretion. Amiloride 10⁻⁵ M abolished V_{Te} and J_K, but decreased J_{Na} only by 65 %, and amiloride-resistant NaCl absorption was abolished by adding 10⁻⁴M luminal hydrochlorothiazide (HCTZ). In NCC^{-/-} mice, only the amiloride-resistant, HCTZ-sensitive component of NaCl absorption is detectable, while V_{Te} and J_K were not distinguishable from zero. This electroneutral NaCl transport was also detected in CD-specific ENaC KO mice CCDs confirming that this transport is ENaC-independent.

The electroneutral NaCl reabsorption is bicarbonate dependent and interestingly J_{Cl} but not J_{Na} was found to be inhibited by acetazolamide, a carbonic anhydrase blocker, indicating that Na⁺ and Cl⁻ are transported by two distinct molecules in the intercalated cells. In CCD intercalated cell of Na depleted mice, luminal removal of Na⁺ elicited a significant decrease in pHi that was reversed upon re-addition of Na⁺. These effects were largely blunted when either Cl⁻ or HCO₃⁻ were omitted in the bath or when HCTZ 10⁻⁴M was present in the lumen demonstrating the presence of the Na-driven Cl/HCO₃ exchanger activity (NDCBE). RT-PCR on isolated mice CCDs confirmed the expression of the NDCBE SLC4A8 transcripts.

In conclusion, NCC^{-/-} mice avoid K⁺ loss by promoting an ENaC independent electroneutral NaCl reabsorption in CCD intercalated cells. This transport pathway requires the coupling of two transporters; one could be NDCBE to transport Na⁺ while pendrin is expected to transport Cl⁻.

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The Farnesyltransferase Inhibitor R115777 Attenuates Atherosclerosis and Vascular Calcification in Apolipoprotein E Deficient (ApoE^{-/-}) Mice with Chronic Renal Failure (CRF)

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Both atherosclerosis and vascular calcification are accelerated in chronic kidney disease patients, in association with risk factors such as dyslipidemia and inflammation. The mevalonate pathway plays a role in these processes via several key proteins like Ras protein which controls cell proliferation and apoptosis. We tested inhibitor of farnesyl transferase - R115777, to interfere with the progression of atherosclerosis and vascular calcification in CRF Apo E^{-/-} mice. Female non-CRF or CRF mice were assigned to four groups, receiving either R115777 or vehicle for 8 weeks. Serum urea, total Ca, phosphorus (P), Ca x P product, and cholesterol were increased in CRF as compared to non-CRF mice. Changes in serum polypeptide patterns in response to R115777 were screened using proteomic analysis. Atheromatous lesion area on longitudinal aorta was markedly smaller in R115777-treated CRF mice compared with vehicle-treated mice. Both plaque and non-plaque calcification at aortic root were lower in R115777 than in vehicle treated CRF mice. Among mechanisms involved, we found a decrease of apoptotic macrophages in the lesions also both nitrotyrosine staining as a measure of oxidative damage and collagen content as an expression of vascular fibrosis were decreased in response to treatment. The R115777 decreased serum Urea and total Ca levels in CRF mice. Proteomic analysis of serum showed changes in polypeptide patterns of fetuin, apolipoprotein IV and alpha-HS globin in response to CRF and R115777. The farnesyl transferase inhibitor R115777 delayed the progression of both atheromatous lesions and calcification of the aorta in apoE^{-/-} mice with CRF. This beneficial effect was probably mediated by blockade of mevalonate pathway activity and concomitant changes in macrophage apoptosis and oxidative stress.

Impact of *VKORC1* Haplotypes on Long-Term Graft Function in Kidney Transplantation

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Background: Chronic allograft injury is the major cause of renal allograft loss. Vitamin K epoxide reductase complex subunit 1 (*VKORC1*) haplotype combinations were found to be associated with the risk of developing vascular diseases. We aimed to study the effect of *VKORC1* haplotypes on long-term graft function in a cohort of kidney transplant recipients.

Method: A total of 288 renal allograft recipients participated in the study. Long-term renal graft function was measured by the estimation of the glomerular filtration rate (eGFR). *VKORC1* C+1173T single nucleotide polymorphism (rs9934438) was used as a tagging SNP for *VKORC1**2 haplotype.

Results: Patients homozygous for *VKORC1**2 haplotype showed less deterioration of renal graft function compared to the other patients (HR: 0.34, 95% CI: 0.26 - 0.87, $P=0.02$). Among the patients who experienced a renal graft biopsy for renal dysfunction ($n=113$), those who had *VKORC1**2/*2 genotype presented a lower degree of moderate to severe arterial changes (26% vs. 48% respectively, $P=0.1$).

Conclusion: Our results suggest that *VKORC1* haplotypes may play a role in the long-term renal allograft function. These findings need to be replicated in prospective clinical studies.

Endothelin Promotes Vasculopathy in Sick Cell Disease through Leukocyte Adhesion to the Endothelium

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Interactions between leukocytes and the endothelium can increase sickle cell disease (SCD)-induced organ damages. Hyperleukocytosis and high neutrophil counts are independent predictors of adverse outcome in SCD. Human and mouse leukocytes express ETRs on their surfaces, but the role of ETRs has not been completely defined. We previously observed a decrease of systemic hyperleukocytosis, and neutrophil activation in the

kidneys of sickle transgenic SAD mice during experimental vaso-occlusive crisis (VOC) with administration of bosentan, a dual endothelin receptors antagonist. We thus hypothesized that the endothelin receptors could directly stimulate leukocyte adhesion to endothelium in sickle mice. We isolated mononuclear cells from bone marrow or blood and measured their adhesion to isolated-perfused carotids from wild-type and SAD mice. In vitro, we quantified the number of adhesive mononuclear cells to VCAM-1 and E-selectin in static condition. Leukocytes adhesion to SCD endothelium of SAD mice was increased by 66 % ($p<0.05$). Bosentan, in vivo or in vitro, diminished significantly leukocytes adhesion to the endothelium in SAD mice. ETR blockade reversed the increased adhesion of SAD mononuclear cells to VCAM1. In vitro, leukocytes adhesion from SAD mice to VCAM-1 is twice as much as adhesion of normal mononuclear cells.

In conclusion, transgenic sickle cell SAD mice display abnormal endothelium-leukocyte interactions. A treatment with a dual endothelin receptor antagonist could prevent leukocyte adhesion, suggesting a direct proinflammatory mechanism of endothelin involving both, actions on the endothelium and on leukocytes. In particular, a tonic stimulation of V-CAM1/VLA4-mediated adhesion and E-selectin dependent adhesion was observed in SAD animals only.

These findings may explain in part the potent beneficial role of endothelin antagonism in the sickle cell disease.

Mechanism of Endogenous Renal Repair Following Release of Unilateral Ureteral Obstruction: Role of Thrombospondin-1 and TGF- β 1

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Chronic kidney diseases are characterized by the development of progressive glomerulosclerosis and interstitial fibrosis. Although numerous studies have studied the events that lead to the development of renal fibrosis, less is understood about the process of endogenous renal repair. Our goal is to study the mechanisms of renal repair following ureteral obstruction relieve (R-UUO). Here, we focused on thrombospondin-1 (TSP-1), the endogenous activator of TGF- β 1, a major profibrotic cytokine implied in the development of renal fibrosis in this model.

Male Sprague Dawley rats (250 g) underwent 10 days UUO. UUO was relieved and rats were allowed to recover for 30 days. UUO resulted in cortical tubular cell atrophy and tubular dilatation, associated with marked myofibroblast proliferation, macrophage cellular infiltration and cellular apoptosis leading to interstitial fibrosis. Peritubular capillary (PTC) network decreased with a reduced endothelial cell density after UUO. TSP-1 protein expression was expressed predominantly in the interstitium around the dilated atrophic tubes. We assessed the renal repair 30 days after R-UUO and demonstrated regeneration of the PTC, re-epithelialization and reappearance of the proximal tubular brush

border by both Masson trichrome and PAS staining. Increased cell viability was confirmed by MTT assay after R-UUO by comparison to UUO rats. This renal repair coincided with a reduced number of macrophages and myofibroblasts, and decreased cellular apoptosis compared to UUO rats. No more expression of TSP-1 was observed in R-UUO rats.

Our study shows the renal potential of regeneration after established interstitial fibrosis induced by UUO. The link between TSP1 expression and renal fibrosis suggests a potential beneficial effect of pharmacological TSP-1 inhibition against the progression of chronic kidney diseases. Inhibition of TSP-1 dependent activation of TGF- β in our model is currently investigated.

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Phosphorylation and Ankyrin-G Binding of the C-Terminal Domain Regulate Targeting and Function of the Ammonium Transporter RhBG

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RhBG, a human member of the Amt/Mep/Rh/superfamily of ammonium transporters, has been shown to facilitate NH₃ transport and to be anchored to the basolateral plasma membrane of kidney epithelial cells, via ankyrin-G. We showed here that triple alanine substitution of the 419-FLD-421 sequence, which links the cytoplasmic C-terminal domain of RhBG to ankyrin-G, not only disrupted the interaction of RhBG with the spectrin-based skeleton but also delayed its cell surface expression and abolished its NH₃ transport function in epithelial cell lines. Similarly, we demonstrated that both anchoring to the membrane skeleton and ammonium transport activity are regulated by the phosphorylation status of the C-terminal tail of RhBG. Tyrosine 429, which belongs to the previously reported YED basolateral targeting signal of RhBG, was demonstrated to be phosphorylated *in vitro* using purified Src and Syk kinases and *ex vivo* by analyzing the effect of pervanadate treatment on wild-type RhBG or Y429A mutants. Then, we showed that Y429D and Y429E mutations, mimicking constitutive phosphorylation, abolished NH₃ transport and enhanced Triton X-100 solubilization of RhBG from the cell membrane. In contrast, the non-phosphorylated/non-phosphorylatable Y429A and Y429F mutants behaved the same as wild-type RhBG. Conversely, Y/A or Y/F but not Y/E or Y/D mutations of residue 429 abolished the exclusive basolateral localization of RhBG in polarized epithelial cells. All these results led to a model in which targeting and ammonium transport function of RhBG are regulated by both phosphorylation and membrane skeleton binding of the C-terminal cytoplasmic domain.

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Cyclosporine A Impairs Cellular Activation and Favors Renal Bacterial Colonization through Inhibition of TLR4-Mediated Signaling in Renal Collecting Duct Cells

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Urinary tract infections (UTIs) and pyelonephritis (PN), mainly due to uropathogenic *Escherichia coli* (UPEC), is the most common bacterial infection observed in renal transplanted patients that may compromise renal graft function. Immunosuppressive therapy post-transplant tends to increase the risk of bacterial infection, but the mechanism by which they favor UTI remains elusive. Renal collecting duct (CD) cells are a preferential site of UPECs adhesion and initiation of Toll-like receptor 4 (TLR4)-mediated inflammatory response for host defense. Here we analyzed the effects of immunosuppressive drug cyclosporine A (CsA) on CD cell activation and its impact on renal bacterial invasion. CsA impaired lipopolysaccharide (LPS)- and UPECs-induced cell activation by inhibiting TLR4 mRNA expression, TLR4-mediated activation of the transcription factor NF- κ B and mitogen-activated protein (MAP) kinases ERK1/2 and JNK, and subsequent production of proinflammatory cytokines, in cultured collecting duct mpkCCD_{cl4} cells. Using an experimental murine model of ascending pyelonephritis, we also show that chronic administration of CsA significantly inhibited the stimulated expression of proinflammatory mediators and greatly enhanced renal bacterial colonization in TLR4-expressing C3H/HeN mice, but not in TLR4-defective C3H/HeJ mice, 24 h after transurethral inoculation of UPECs. Therefore the direct inhibition caused by CsA of TLR4-mediated signaling in collecting duct target cells favors the colonization of kidneys by uropathogenic bacteria. These findings may explain in part the high frequency of UTIs and APNs observed in transplanted patients.

Antioxidant Imbalance in the Lung from Cystic Fibrosis Mice: Abnormal Peroxiredoxin 6 Expression Levels and Lipid Peroxidation

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Cystic fibrosis is a fatal genetic disorder involving dysfunctions of the CFTR protein, an ABC protein located on the apical cell membrane of secretory epithelia. The aim of the current study was to examine the cellular balance between antioxidant defenses and oxidative damages in the lung from just weaned CFTR-knockout mice (CF). GSH-peroxidase activity and lipid oxidation index were found significantly increased in lung extracts from CF mice as compared to wild-type (WT). In addition, proteomic analysis revealed that a major cellular pulmonary GSH-peroxidase, Peroxiredoxin 6 (PRDX6) that is involved in the repair of damaged cell membranes by reducing phospholipid hydroperoxides is differentially expressed in CF. Immunoblot and immunohistochemistry analyses revealed that PRDX6 expression is increased in CF as compared to WT lungs. Consistent with this result, a specific PRDX6 assay, using phosphatidylcholine hydroperoxide (PC-OOH) as substrate, showed that PRDX6 activity is similarly increased in CF lung. The increased lipid oxidation index and PRDX6 antioxidant activity suggest the presence of a constitutive redox imbalance in CF pulmonary cells and raise the possibility that the intracellular antioxidant defense mechanism could be overwhelmed when exposed to a pro-oxidative environment. To assess this hypothesis we evaluated the level of PC-OOH together with PRDX6 expression in the lung from mice exposed to an oxidative challenge induced by paraquat (PQ). After PQ treatment, CF lungs exhibited a dramatic PC-OOH increase and a marked decrease in PRDX6 protein expression. This impaired PL-OOH detoxification mechanism may enhance oxidative stress-related signalling and damage contributing to an exaggerated inflammatory response in CF lung.

The Na⁺/H⁺ Exchanger NHE4 Is Required for Adequate Intestinal Ca Absorption and Maintenance of Bone Mineral Density

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NHE4 is expressed in many tissues, including stomach, kidney, small intestine, and colon. Recently we have shown that NHE4

participates in NH₄ absorption in the medullary thick ascending limb cells and is required for the renal adaptation to metabolic acidosis. NHE4 is widely expressed in the gastric parietal cells, where it is supposed to play a role in ion gastric secretion and also along the small intestine where its function is unknown. We made the assumption that, like in the kidney and stomach, NHE4 could participate in ion transport across the intestinal epithelium. To address this issue, *in vivo* metabolic studies were performed in adult NHE4^{+/+} and ^{-/-} littermate mice on a normal diet (0.72% P, 1.1% Ca), followed by a 1-week period on a low PO₄ diet (0.11% P). Bone densitometry was performed in adult NHE4^{+/+} and ^{-/-} littermate mice at 4 and 7 months. Under baseline conditions, NHE4^{-/-} mice, as compared to NHE4^{+/+} mice, had similar urinary excretions of water, creatinine, K, Cl and Mg, whereas they exhibited a lower urinary Ca excretion (0.68±0.05 vs. 0.99±0.08 mmol/mmol creat, *p*=0.01) and a higher urinary PO₄ excretion (41.8±1.6 vs. 24.1±2.4 mmol/mmol creat, *p*<0.001). Accordingly, stool Ca excretion was higher and stool PO₄ excretion was lower in NHE4^{-/-} than in NHE4^{+/+} mice. The difference in urinary Ca excretion remained on low PO₄ diet (2.1±0.3 vs. 3.7±0.4 mmol/mmol creat, *p*<0.01) suggesting that this phenomena was not secondary to a hyperabsorption of PO₄. The difference in PO₄ excretion almost completely disappeared on low PO₄ diet. NHE4^{-/-} mice have higher plasma levels of 1,25 dihydroxy-vitamin D, without difference in plasma levels of 25hydroxy-vitamin D, PTH, Ca or PO₄. Total body and vertebral BMD were found to be lower in NHE4^{-/-} mice than in NHE4^{+/+} mice. Altogether, the results support the view that NHE4 is required for adequate intestinal Ca⁺⁺ absorption and is a new genetic determinant of bone mineral density.

Lipocalin 2 in Chronic Kidney Disease Progression

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Chronic kidney diseases (CKD) are characterized by a progressive decline of renal function, occurring once a critical number of nephrons has been lost. We previously showed that activation of epidermal growth factor receptor (EGFR) plays a key role in these events. Here, we combined an experimental model of nephron reduction (3/4 nephrectomy, Nx), mice from different genetic backgrounds and microarrays to identify the critical targets of EGF pathway during CKD progression. The transcriptomes of remnant kidneys of B6D2/F1 mice, which undergo compensatory growth alone, and of FVB/N mice, which upon EGF-R activation additionally develop severe renal lesions 2 months after Nx, were compared. The gene maximally induced in damaged FVB/N kidneys is *lcn2*, encoding lipocalin 2 (Lcn2), a secreted binding protein. Quantitative real-time RT-PCR and western blot confirmed the increase of Lcn2 and *in-situ* hybridization localized mRNA to proximal tubules. Moreover, we demonstrated that addition

of EGF to renal IMCD cells stimulates the transcription of *lcn2* gene.

We next investigated the mechanisms by which *Lcn2* may favor lesion progression. It has been shown that *Lcn2* modulates iron transport and cell proliferation, two key events in renal deterioration process. We showed that, in FVB/N Nx mice, *Lcn2* overexpression is associated to iron deposition. However, iron chelation by desferoxamine resulted in more severe renal lesions and higher *Lcn2* expression, demonstrating that iron overload did not account for renal deterioration in our model. Immunohistochemical analysis revealed that *Lcn2* overexpression is also associated to increased cell proliferation in tubules of FVB/N Nx mice. More importantly, *Lcn2* silencing by shRNAs inhibits EGF-induced cell proliferation in IMCD cells. The ongoing experiments on *lcn2* FVB/N knock-out mice should define the role of this pathway *in vivo*.

In conclusion, we have identified several novel mediators of CKD progression. Among these, *Lcn2* might play a key role by mediating the mitogenic effect of EGF-R pathway in renal tubular cells.

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Insights into the Molecular Mechanisms Underpinning Bartter's Syndrome Type 1

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Mutations in the apically located Na-K-2Cl cotransporter, NKCC2, lead to Bartter's Syndrome Type 1 (BS type 1), and inherited kidney disorder associated with salt wasting, hypokalemia, and metabolic alkalosis. Yet little is known about the mechanisms regulating mutated NKCC2 proteins. The aim of the present work was to examine the effect of Y998X mutation on NKCC2 expression in cultured renal cells. Wild Type (WT) NKCC2 was detected as two bands representing the core-glycosylated and the complex-glycosylated forms of the protein, whereas only the core-glycosylated form of Y998X protein was detectable. Immunofluorescence analysis showed that WT NKCC2 localized predominantly to plasma membrane. In contrast, Y998X proteins were observed only intracellularly in a perinuclear and reticular pattern typical of the endoplasmic reticulum (ER), indicating that Y998X is most likely to be retained within the ER and thus regulated by the ER-associated protein degradation (ERAD). To identify the protein partners involved in ERAD of NKCC2, we screened a kidney cDNA library through yeast two-hybrid and identified OS-9, an ER-resident lectin, as a specific binding partner of NKCC2 C-terminus. In cells over-expressing OS-9, total cellular WT NKCC2 and Y998X protein levels were markedly decreased, an effect blocked by the proteasome inhibitor MG132. Pulse-chase experiments demonstrated that the marked reduction in the cotransporter protein levels was due to increased protein degradation. Furthermore, mutations of NKCC2 N-glycosylation sites abolished OS-9 effect indicating that OS-9-induced protein degradation is N-glycan dependant. In summary: 1) Y998X muta-

tion results in ER retention of the protein and its degradation by the ERAD system. 2) ERAD of NKCC2 specifically involves OS-9. These data provide therefore a mechanism for the cotransporter dysfunction and the first insight into the molecular basis of BS type 1. The identification and selective modulation of ERAD components specific for NKCC2 might provide novel therapeutic strategies for the treatment of BS type 1.

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A Structure/Function Study of the Human RhCG Ammonium Channel

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Rh glycoproteins, the mammalian members of the Amt/Mep/Rh super-family, facilitate movement of ammonium across plasma membranes. Homology models based on the experimental structures of *Escherichia coli* AmtB and *Nitrosomonas europae* Rh50 confirms a channel structure for human RhAG, RhBG, RhCG glycoproteins. While RhAG expression is erythroid-restricted, epithelial expression of RhBG and RhCG in the kidney is restricted to cells involved in ammonium secretion (connecting tubules and collecting ducts).

Using site-directed mutagenesis and stopped-flow spectrometry applied to RhCG-expressing HEK293E cells, the present study highlighted critical residues involved in Rh channel activity and specific mechanistics of NH₃ transport as compared to AmtB. First, the lack of the aromatic feature in the external vestibule suggested that Rh function is independent of the presence of NH₄⁺-binding sites. Secondly, whereas only one of the two phenylalanines of the "gate" is described as absolutely required for the AmtB channel activity, both were necessary for the Rh transport activity. Thirdly, while the removal of both phenylalanines was reported to induce a loss of the AmtB function, a similar alteration resulted in a recovery of the Rh activity.

Finally, osmotic water permeability, measured by a light scattering method on wild type and Phe-mutated RhCG expressing cells, did not reveal any role of the "phenylalanine-gate" in substrate selectivity.

Functional differences with AmtB could account for respective biological relevance of ammonium required as nutriment for bacteria and fundamental for acid-base homeostasis in human tissues.

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