

	G (pS)	PBr	PNO3	PI	pHi sensitivity
early DCT	9.5	0.46	0.50	0.9	+
late DCT	8.8	0.41	0.48	0.62	+
CNT	10.6	0.44	0.56	0.86	+
CCD	9.8	0.60	0.54	N.D.	+
CTAL	9	0.62	0.64	N.D.	+

The table gives the unit conductance and permeabilities (relative to chloride) of the chloride channels detected in various segments of the mouse nephron. Data from refs 2,3,4 and unpublished results (CTAL)

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SA7

Acid- and volume-sensitive potassium channels in renal function

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A variety of acid- and volume-sensitive K⁺ channels have been described which are implicated in important cellular functions. In the kidney, many studies have explored the role of pH-regulated KCNJ1 (ROMK) channels in the straight and convoluted distal tubule and of volume-, pH-, and ATP-regulated K⁺ channels in the basolateral membrane of the proximal tubule. Here, we have focused on two pH- and volume-regulated K⁺ channels of the 2P domain channel family, TASK2 (KCNK5) and TASK1 (KCNK3). TASK2 is strongly expressed in proximal tubules and papillary collecting ducts. Patch-clamp experiments on proximal tubular cells indicated that the TASK2-specific K⁺ current is activated during bicarbonate transport. In *in vivo* experiments, TASK2^{-/-} mice displayed mild metabolic acidosis which was caused by an increased renal bicarbonate excretion. Activation of TASK2 by transport-induced cell swelling and basolateral export of bicarbonate appears to be an important mechanism to adapt membrane potential and osmolyte export to the needs (1,2).

Aldosterone regulates ion transport in the distal nephron which is critical for water/salt balance and the control of arterial blood pressure. TASK1 is probably the most abundant K⁺ channel in human adrenal glands. This channel has been proposed to contribute to the background conductance whose inhibition by angiotensin II stimulates aldosterone secretion. We investigated the contribution of TASK1 for this K⁺ conductance using a TASK1^{-/-} mouse as a tool. Female TASK1^{-/-} mice exhibited severe hyperaldosteronism independent of salt intake, hypokalemia, and arterial 'low renin' hypertension. The aldosterone phenotype was accompanied by a severe adrenocortical zonation defect. Aldosterone synthase

was totally absent in the zona glomerulosa but abundant in the deeper zona fasciculata. Also young male TASK1^{-/-} mice displayed a zonation defect. In contrast to females, at adulthood male TASK1^{-/-} mice had acquired normal zonation patterns highlighting the dynamics of the process of adrenocortical zonation. Interestingly, the hyperaldosteronism of female TASK1^{-/-} mice was fully remediable by glucocorticoids indicating that in those mice aldosterone secretion is under the control of ACTH. These findings are reminiscent of glucocorticoid-remediable hyperaldosteronism in humans (3).

In conclusion, proximal tubular TASK2 channels stabilize the driving force and cell volume during ongoing Na⁺ and bicarbonate transport. We propose TASK2 as a novel candidate to underlie the clinical manifestations seen in proximal renal tubular acidosis syndrome. The acid-sensitive TASK1 channel is a pivotal factor for normal adrenocortical zonation, aldosterone secretion and, thereby, regulation of renal function. The phenotype of TASK1^{-/-} mice underlines the potential of K⁺ channels to influence cell differentiation and development.

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SA8

Renal Na/Pi-cotransporter NaPi-IIa: a physiological and molecular overview

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Homeostatic balance of inorganic phosphate (Pi) has wide physiological implications. Plasma levels of Pi are kept constant by adjusting renal reabsorption/excretion to intestinal absorption. Both processes are mediated by members of the SLC34 gene family. NaPi-IIa/SLC34A1 and NaPi-IIc/SLC34A3 are responsible for renal reabsorption whereas intestinal absorption is mediated by NaPi-IIb/SLC34A2. NaPi-IIa and NaPi-IIc are expressed in the brush border membranes (BBM) of renal proximal tubules (PT) (1, 2). In the adult murine kidney, NaPi-IIa reclaims up to 80% of filtered Pi, with the 20% left attributed to NaPi-IIc (3). NaPi-IIa expression is reduced in animal models for X-linked hypophosphatemia (XLH), and *in vitro* studies indicate that similar defect could be involved in other phosphate wasting syndromes. However, such reductions on NaPi-IIa are probably secondary to defects on other factors collectively known as phosphatonins (FGF23, PHEX, FRP-4, and MEPE) (3). Recently, mutations in NaPi-IIc were linked to hereditary hypophosphatemic rickets with hypercalciuria. These findings indicate that

both cotransporters are critical for Pi homeostasis. Accordingly, the abundance of NaPi-IIa and NaPi-IIc in the proximal BBM is under strict hormonal and metabolic control.

Among the many factors that regulate renal reabsorption of Pi, parathyroid hormone (PTH), high dietary levels of Pi and FGF23 decrease the levels of NaPi-IIa and NaPi-IIc in the BBM, whereas they are upregulated in response to low dietary phosphate and 1,25 (OH)₂ vitamin D (1-3). Both cotransporters are also over-expressed in mice homozygous for a mutated form of *klotho* (4). In particular, the phosphaturic effect of PTH has been analyzed in great detail regarding NaPi-IIa downregulation (1, 5). PTH binds to its G-protein coupled receptors located on both the apical and basolateral membrane of PT and via partially characterized steps promotes the fast endocytosis and lysosomal degradation of NaPi-IIa. Binding of PTH to apical receptors activates mostly PLC/PKC-dependent signaling whereas basolateral receptors signal preferentially via cAMP. This second messenger leads to downregulation of NaPi-IIa through the classical PKA cascade, with no contribution of EPAC (6). In contrast to NaPi-IIa, endocytosis of NaPi-IIc upon PTH requires a prolonged incubation time and degradation of the internalized cotransporter is not sensitive to leupeptine (7).

At the molecular level, regulation of the apical expression of NaPi-IIa depends on the association of the cotransporter with a complex network of interacting proteins (8). A cluster of such interacting partners is represented by the NHERF family. The four members of this family are PDZ-containing proteins expressed in renal PT. They interact via their PDZ-domains with the C-terminal PDZ-binding motif of NaPi-IIa. Truncation of this motif disturbs apical expression of the cotransporter in OK cells, a proximal tubular cell culture model. Several studies in OK cells and NHERF1^{-/-} mice indicate a prominent role of this particular member of the NHERF family on the expression/regulation of NaPi-IIa. Thus, transfection of OK cells with dominant negative forms of NHERF1 hampered the apical expression of the endogenous cotransporter suggesting that NHERF1 is required for proper apical targeting/stabilization of NaPi-IIa. This hypothesis was confirmed in NHERF1^{-/-} mice that are indeed characterized by high urinary excretion of Pi as consequence of reduced expression of NaPi-IIa in the proximal BBM (9). The absence of NHERF1 also interferes with PTH signaling: activation of apical receptors with 3-34 PTH (a fragment known to signal specifically through the PKC pathway) failed to induce endocytosis of NaPi-IIa in NHERF1^{-/-} mice. Since NHERF1 can bind simultaneously to PTH receptors and to PLC, the failing of 3-34 to exert an effect of NaPi-IIa in NHERF1^{-/-} mice suggests a defective coupling of apical PTH receptors to PLC. Accordingly, the responsiveness to 3-34 PTH in NHERF1^{-/-} mice was shown to be due to lack of activation of phospholipase C (PLC) in these animals. Unlike NaPi-IIa, the pattern of expression of NHERF1 remains unaffected upon PTH treatment, suggesting that the association between both partners is negatively regulated by PTH. Indeed, PTH reduced the amount of NaPi-IIa that

coimmunoprecipitates with NHERF1 antibodies (10). In addition, PTH induced an increase in phosphorylation of NHERF1. In summary, NaPi-IIa is the major regulator of renal Pi handling. Therefore, its expression in the apical membrane is under tight control. The molecular mechanisms responsible for such control are under current investigation.

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SA9

Anion exchangers in renal health and disease

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A variety of anion exchangers perform important vectorial transport function in the polarized epithelia that constitute the renal tubule. Normal anion transport function is important for acid-base balance, urinary calcium solubility and prevention of nephrolithiasis. This talk will focus on those transporters where malfunction or mistargeting are associated with a renal phenotype.

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