

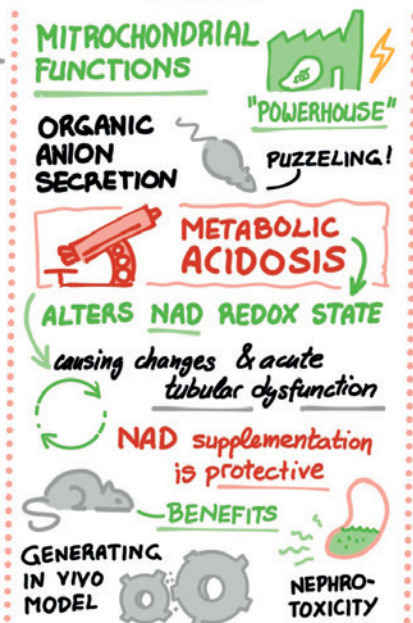
Symposium 1.3 Tubular transport

Richard Warth



TUBULAR TRANSPORT

Andrew Hall



Felix Knauf

How is OXALATE handled in the Kidney?



Potassium channels controlling tubular functions

Richard Warth, Germany

Potassium channels are involved in a broad spectrum of functions in renal tubular cells. A multitude of potassium channel genes with variable expression has been identified recently, some of which are linked to diseases in humans. For example, Bartter syndrome is a rare inherited renal tubular disorder manifesting with polyuria, hypokalemic alkalosis, hyponatremia, hypercalciuria, and hypermagnesiuria. Types 1 and 2, caused by mutations in ROMK, NKCC2, and Barttin potassium channels, are very severe and manifest antenatally. Type 3, on the other hand, is caused by CLCKB/CLCKA mutations, and has a rather mild clinical picture. Bartter 4 has the added problem of deafness, and Bartter 6 is a transient antenatal condition caused by a mutation in factor MAGED2.

The renal outer medullary potassium channel (ROMK) is the most studied member of the family and with the highest degree of expression in the kidney. ROMK is involved in potassium transport out of the cells, playing an important role in potassium secretion in the cortical collecting duct and potassium recycling in the thick ascending limb of Henle's loop. By limiting the transmembrane transport in the thick ascending limb ROMK raises the sodium load in the distal portions of the nephron

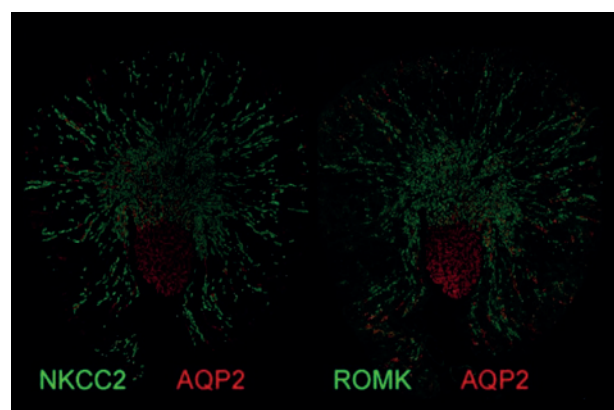


Figure 1. ROMK, aquaporin 2 (AQP2), and the Na-K-2Cl cotransporter (NKCC2) expression in renal tubular cells

where potassium is secreted in exchange for sodium. As a result, increased sodium reabsorption in the collecting system boosts potassium secretion.

KCNJ10 is a potassium channel with a much lower expression than ROMK, yet it plays a significant function in the kidney and other human organs. KCNJ10 is expressed on the basolateral membrane of the cortical thick ascending limb of Henle's loop where it inwardly rectifies potassium. Patients with epilepsy, sensorineural deafness, tubulopathy, and resulting ataxia, were found to have mutations in this potassium channel (EAST syndrome). Many other mutations have been discovered, most of which are linked to severe diseases. Still, not all patients with KCNJ10 mutation develop tubulopathy, indicating certain variability that is likely caused by the specific mutation and the context-dependence of its effects. This is supported by the fact that, in addition to the known symptoms, patients with this mutation also develop intellectual disabilities. KCNJ10 and its sub-unit, KCNJ16, are believed to mutually form channels for basolateral potassium recycling in distal tubules. A study in mice with a KCNJ16 mutation found that they had hyperkalemia as well, but strangely, they exhibited acidosis rather than alkalosis, indicating a different channel function in the distal nephron. Studies on KCNJ16 (Kir5.1) mutations revealed a common tubulopathy characterized by hypokalemia, salt wasting and acidosis, and sensorineural deafness.

Mitochondrial function and diversity along the nephron

Andrew Hall, Switzerland

In the kidney, there is a strong link between solute transport and aerobic metabolism. The transport demands are reflected in the density of mitochondria in different nephron segments. There are also certain adaptations of mitochondrial function to various transport tasks along the nephron. The in situ study with fluorescence microscopy of structure and function of mitochondria in the nephron and the effects of disease-causing insults reached interesting conclusions. Differences in the mitochondrial flavoprotein signal and the glutamate-glutamine pathway were found between segments s1 and s2 in the proximal tubule. Imaging of the collecting duct revealed heterogeneity in mitochondrial NADH signals and intercalated cells, as well as a very poor uptake of voltage-dependent dyes, implying that the mitochondria in this segment are not well energized. Therefore, other metabolic pathways are considered in intercalated cells in terms of glycolytic activity.

Mitochondria in the proximal tubules are involved in the acidosis defense system of creating ammonia and bicarbonate via an NAD⁺-dependent process. The fact that treatment of severe metabolic acidosis with bicarbonate minimizes the risk of acute kidney injury (AKI) prompted an investigation of the effects of acidosis on mitochondrial function in the proximal tubule. Considering that NAD⁺ is critical for lipid metabolism in the proximal tubule, researchers studied the effects of acidosis on lipid metabolism in an in-vivo acidosis model in mice. The acidotic animals exhibited a significant increase in tubular lipid content, and the slice model revealed large vacuoles in the proximal tubules. Electron microscopy showed that these vacuoles were multi-lamellar bodies that operate as a lipid store within the cell, particularly in the s2 segment of the proximal tubule. The acidotic mice were then treated with bicarbonate, which significantly improved their tubular function. Thus it was established that NAD⁺ is primarily used for fatty acid metabolism in the proximal tubule, but it is also required for ammoniagenesis and is dramatically increased during acidosis, implying that these processes compete for NAD⁺. Treatment of increased intracellular NAD⁺ caused a significant improvement in tubular toxicity as well as favorable effects on lipids.

Another research focused on investigating the mechanism of toxicity of the antiviral medicine Tenofovir, which causes proximal tubulopathy and may induce Fanconi syndrome and AKI. Tubular cell exposure to increasing quantities of the drug enhanced the mitochondrial footprint, with characteristically large mitochondria and damaged cristae, as well as significant transport abnormalities. The results mirrored the findings usually observed in the patients, pointing at high throughput imaging to generate more realistic in vitro models of tubular diseases.

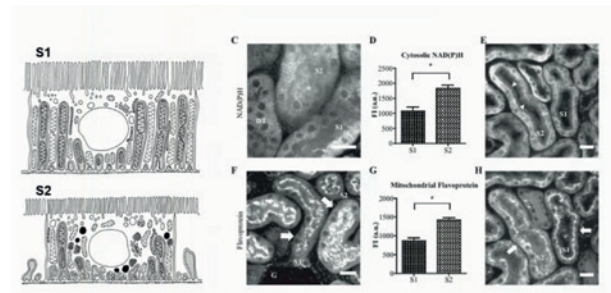


Figure 2.

Heterogeneity of mitochondrial function along the proximal tubule (from ref. 5)

Oxalate handling in health and disease-crosstalk gut and kidney

Felix Knauf, Germany

Oxalate enters the body through diet or is produced endogenously by the liver as a useless metabolic byproduct. It is predominantly excreted in the urine and, to a lesser extent, in the feces. It is also removed by microbial oxalate metabolism in the gut. The major insight into oxalate management in the proximal tubule was provided by the studies of chloride transport. The proximal tubule absorbs chloride primarily passively and paracellularly, but there is also a component of transcellular chloride absorption mediated by the SLC26A6 carrier, which is also involved in oxalate secretion, both in the kidneys and in the intestine. In the gut, oxalate predominantly binds with calcium to create a compound that is excreted in the feces. However, in physiological conditions there is never enough calcium present to combine with all the oxalate, thus some oxalate absorption occurs along with salt and water across the tight junction. In the intestine, the SLC26A6 transporter (A6) plays a key role in restricting the net absorption by back-secreting oxalate into the lumen.

Loss of kidney function leads to an increase in serum oxalate due to decreased clearance. Recent research on the oxalate handling processes in animal models of chronic kidney disease (CKD) observed an upregulation of the A6 oxalate transporter in the small intestine, but also in the colon which normally has a low A6 expression. Thus, in CKD oxalate removal through the stool increases, even eliminating the endogenously produced oxalate.

Recent research suggested oxalate was a novel risk factor for cardiovascular events. Its accumulation is associated with oxidative stress, inflammation, and elevated cardiovascular risk. Oxalate crystals can activate inflammatory cells, dendritic cells, macrophages, and monocytes by the toll-like receptor 4 inflammasome signaling that leads to cytokine release. It is believed that oxalate is pro-inflammatory, which may be the link to cardiovascular mortality. In a cohort of over one thousand hemodialyzed patients with diabetes, the risk for cardiovascular events and sudden cardiac death was significantly associated with elevated oxalate levels. Further research is expected to assess whether oxalate-lowering strategies could improve cardiovascular outcomes in dialysis patients.

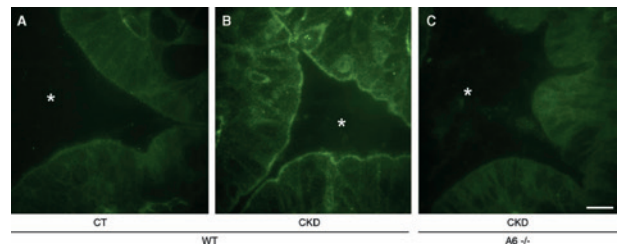


Figure 3.

The expression A6 oxalate transporter on the apical membrane of colon epithelium in mice with CKD (from ref. 11)

Further readings

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All the speakers reviewed and approved the content.