Two Rhesus protein ammonia transporters team up to eliminate ammonium into urine

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RENAL AMMONIAGENESIS SERVES the de novo synthesis of bicarbonate consumed by metabolism and the excretion of acid. Ammonium is eventually excreted into urine after accumulation in the medullary interstitium. The process of ammonium excretion was long thought to be a mostly passive process mediated by simple diffusion of NH$_3$ and NH$_4^+$ and driven by urinary acidification and consequent trapping of ammonium in urine. The discovery of Rhesus proteins capable of transporting NH$_3$ has revolutionized our view on renal ammonium excretion. The kidney expresses two members of this family, Rh B Glycoprotein (RhBG) and Rh G Glycoprotein (RhCG), and whereas RhCG is found both in apical and basolateral membranes of cells along the collecting duct, RhBG is restricted to the basolateral membrane of the same cells. New work presented in a recent issue of the American Journal of Physiology-Renal Physiology (7) demonstrates that combined deletion of both transporters causes a more severe defect in urinary ammonium excretion than single deletion of each transporter, suggesting that both transporters cooperate in final urinary elimination of ammonium.

Renal ammoniagenesis is critical for the maintenance of acid-base homeostasis and the renal defense against acidosis. Proximal tubular glutamine metabolism can yield two molecules of NH$_3$ and HCO$_3^-$ allowing the buffering of acids generated by metabolism (5). The synthesis of ammonia involves several steps catalyzed by mitochondrial phosphate-dependent glutaminase and glutamate dehydrogenase and fuels α-ketoglutarate into gluconeogenesis where phospho-enol pyruvate carboxy kinase (PEPCK) acts as a key enzyme. At physiological pH, most of the NH$_3$ will bind a proton and thereby contribute to the elimination of acid as well as to the reabsorption of bicarbonate. A major fraction of NH$_3$ excreted into the proximal tubule lumen is reabsorbed by the Na-K-2Cl cotransporter (NKCC2) in the thick ascending limb of the loop of Henle and accumulated in the medullary interstitium, possibly by binding to sulfatides (9).

The final excretion of ammonium into urine occurs at the level of the collecting duct system. NH$_3$/NH$_4^+$ is taken up from the interstitium and secreted into urine where NH$_3$ recombines with protons secreted by H$^+$/ATPases (and H$^+$/K$^+$-ATPases), and is finally trapped as NH$_4^+$. Ammonium to be excreted from the interstitium into urine must pass two plasma membranes, the basolateral and luminal membranes of cells lining the collecting duct (11). In vitro microperfusion experiments demonstrated that passage of ammonium across the basolateral membrane cannot be mediated by Na$^+$/K$^+$-2Cl$^-$-cotransporters and Na$^+$/K$^+$-ATPases where NH$_3$ substitutes for potassium. However, mice lacking the NKCC1 cotransporter have no problem excreting ammonium into urine, suggesting that either NKCC1, the only Na$^+$/K$^+$-2Cl$^-$ cotransporter expressed in the collecting duct, is not involved in ammonium transport in vivo, or that its absence can be fully compensated (12, 13).

The discovery that members of the Rhesus family of membrane proteins, RhAG, RhBG, and RhCG, can transport NH$_3$/NH$_4^+$ suggested that these proteins may participate in renal and extrarenal ammonium transport (8). RhAG is absent from the kidney, whereas RhBG and RhCG are highly expressed in the kidney collecting duct. RhCG is found in all type A intercalated cells (Fig. 1) and segment-specific cells of the initial collecting duct system at both the luminal and basolateral membranes. RhBG is found in the same cells as RhCG but exclusively at the basolateral membrane (14). The functional relevance of these two proteins was first revealed in a series of mouse studies with complete or partial deletions of Rhbg or Rhcg KO (2). These studies showed that complete deletion of RhCG leads to a strong reduction in urinary ammonium excretion and severe metabolic acidosis. Mice with partial deletion suffer also from reduced ammonium excretion (14). Moreover, microperfusion experiments demonstrated that luminal NH$_3$ permeability was drastically reduced and that total epithelial NH$_3$ transport abolished (2, 3). Thus Rhcg is absolutely required for urinary ammonium excretion by the collecting duct and is likely the only NH$_3$ permeation pathway in the luminal membrane.

But what about the basolateral uptake pathway? In the case of RhBG, two different mouse models have been generated, a complete and a partial knockout (KO). The total Rhbg KO exhibits no impairment of urinary ammonium excretion; normal ability to regulate systemic acid-base homeostasis and to transport NH$_3$ or NH$_4^+$ across the basolateral membrane was observed (4). In contrast, a mouse model with partial Rhbg deletion had mildly reduced urinary ammonium excretion (1). A major difference between both reports was the amount of acid loaded, which was much higher in the partial Rhbg KO mouse model, suggesting that Rhbg may become limiting only under very extreme conditions.

Now, in a report published in a recent issue of the American Journal of Physiology-Renal Physiology, a mouse model lacking both Rhcg and Rhbg is presented (7). Deletion was achieved with Ksp-Cre mice driven by the cadherin promoter, which has no or only little activity in the late distal convoluted tubule and connecting tubule, leaving Rhbg and Rhcg expression intact in these early parts of the collecting duct system. Nevertheless, these mice excrete ~30–50% less ammonium than their wild-type controls. This relatively mild phenotype may be due to a massive compensatory increase in key molecules of proximal tubular ammoniagenesis such as PDG and PEPCK as well as the NHE3 Na$^+$/H$^+$ exchanger. Unfortu-
nately, the effect on thick ascending limb NKCC2 activity and expression and medullary ammonia accumulation was not tested.

Do these experiments explain basolateral ammonium transport? Conclusions are difficult. Since complete deletion of Rhcg decreased NH3 transport by the collecting duct from acidotic animals by >80%, an additional deletion of Rhbg would likely not aggravate the phenotype. Thus the relative contribution of each single transporter is difficult to infer from the combined partial deletion of Rhbg and Rhcg. It is certainly more powerful than only the partial deletion of Rhbg, but whether it has a more severe impact than the partial deletion of Rhcg is hard to tell and probably not to be expected. Direct measurement of basolateral transport activities would be a way of shedding some light on this question. In mice with complete Rhcg KO, basolateral NH3 permeability is drastically reduced in the absence of sodium, indicating that Rhcg is the main mediator of basolateral NH3 uptake, leaving little space for Rhbg (3). Direct measurements would also help to answer another question: what is the substrate of Rhbg in vivo, NH3 and/or NH4+, CO2? For a long time, this has been an open question for Rhcg, and three lines of evidence eventually demonstrated that NH3 but not NH4+ is the likely substrate: the crystal structure of the related bacterialAmt protein and human RhCG suggest a permeation pathway not permeable for charged molecules; direct reconstitution of RhCG in liposomes; and microperfusion experiments with collecting ducts from Rhcg KO mice demonstrated NH3 but not NH4+ transport (10). Based on the high similarity of Rhbg and Rhcg transport of NH3 by RhBG is likely but not proven. Moreover, a recent paper demonstrated the capability of Rhesus proteins expressed in Xenopus oocytes to also mediate CO2 permeability similar to distant relatives of Rhesus proteins in algae and that RhBG induced more CO2 than RhCG (6). Whether this is relevant in native tissues and in vivo remains to be clarified.

Thus the double KO mouse model is a very valuable tool that could provide some additional insights into the complex handling of ammonia by the kidney. However, many experiments will depend on almost extinct and cumbersome techniques such as in vitro microperfusion that can still provide many new insights. The fact that double KO mice are able to adapt might also suggest that there is cross talk between the collecting duct and the upstream segments such as proximal tubule and thick ascending limb in adapting renal ammoniagenesis and ammonium excretion. If this depends on systemic acid-base homeostasis, e.g., the degree of acidosis, or is mediated by some other signals remains to be further explored.

REFERENCES


