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The rhesus protein RhCG: a new perspective in ammonium transport and distal urinary acidification

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Urinary acidification is a complex process requiring the coordinated action of enzymes and transport proteins and resulting in the removal of acid and the regeneration of bicarbonate. Proton secretion is mediated by luminal H^+-ATPases and requires the parallel movement of NH_3, and its protonation to NH_4^+, to provide sufficient buffering. It has been long assumed that ammonia secretion is a passive process occurring by means of simple diffusion driven by the urinary trapping of ammonium. However, new data indicate that mammalian cells possess specific membrane proteins from the family of rhesus proteins involved in ammonia/μm permeability. Rhesus proteins were first identified in yeast and later also in plants, algae, and mammals. In rodents, RhBG and RhCG are expressed in the collecting duct, whereas in humans only RhCG was detected. Their expression increases with maturation of the kidney and accelerates after birth in parallel with other acid–base transport proteins. Deletion of RhBG in mice had no effect on renal ammonium excretion, whereas RhCG deficiency reduces renal ammonium secretion strongly, causes metabolic acidosis in acid-challenged mice, and impairs restoration of normal acid–base status. Microperfusion experiments or functional reconstitution in liposomes demonstrates that ammonia is the most likely substrate of RhCG. Similarly, crystal structures of human RhCG and the homologous bacterial AmtB protein suggest that these proteins may form gas channels.

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The kidneys excrete ~70 mmol of acids per day from the body. Only a minute fraction is excreted as free protons, but most acids are in the form of ammonium (about 2/3) and titratable acids (about 1/3), such as phosphate. The importance of renal acid elimination is underlined by a variety of syndromes of acquired or inherited forms of renal tubular acidosis.[1,2] Chronic metabolic acidosis represents a major morbidity and mortality risk factor and may even accelerate deterioration of renal function in patients with early stages of renal disease.[3,4]

MECHANISMS OF DISTAL ACID EXCRETION

Type A intercalated cells (A-ICs) in the collecting duct system (late distal convoluted tubule to the initial third of the inner medullary collecting duct) mediate the removal of acids (protons and ammonium), as well as the *de novo* generation of bicarbonate.[2] Cytosolic carbonic anhydrase II hydrates CO_2 to form H^+ and HCO_3^-, which in turn is released into the interstitium involving the basolateral and A-IC-specific chloride/bicarbonate exchanger AE1.[5,6] H^+-ATPases localized at the luminal pole of A-IC excrete protons,[7] thereby acidifying urine. However, H^+-ATPases can establish a maximal pH gradient of about 2–2.5 units pH between the intracellular compartment (~pH 7.2) and urine, thus limiting removal of hydrogen ions. The daily amount of acids removed is about 1 mEq per kg body weight (about 70 mEq in a healthy adult person). The excretion of this amount of acid in an unbuffered solution would thus require several hundred liters of urine (1 l of unbuffered urine, pH 4.5, containing maximally 30 μM protons). Titratable acids (mainly phosphate, to a lesser extent citrate, and creatinine) can help buffer protons (about 1/3 of the daily acid load). A major fraction of protons, however, is buffered by ammonia after parallel secretion into urine (approximately 2/3 of the daily acid load). Ammonia secretion occurs along the entire length of the collecting duct system, but increases substantially in the later parts.[8,9]

In 1945, Robert Pitts[10,11] had described in two seminal papers the role of ammonium in renal acid secretion and postulated that ammonium secretion is a passive process driven by the ammonia concentration gradient, the diffusion of ammonia across the luminal membrane, and the

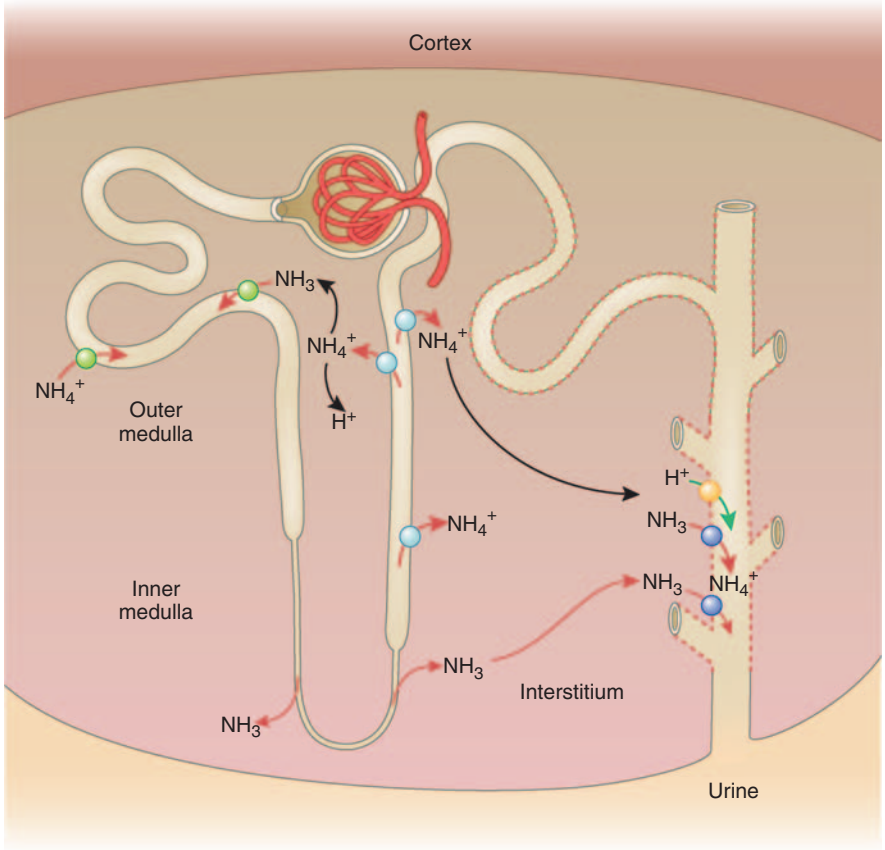

Figure 1 | Urinary ammonium generation and transport along the nephron. Ammonium is generated in the cells of the proximal tubule from metabolism of glutamine and secreted into primary urine. At the level of the thin descending and ascending limb of the loop of Henle, low amounts of NH_3 are absorbed into the interstitium, generating a cortico-papillary gradient (shaded in red). Massive absorption of NH_4^+ occurs in the thick ascending limb of Henle via the furosemide-sensitive $Na^+/K^+/2Cl^-$ cotransporter NKCC2 accumulating high interstitial concentrations of NH_4^+. Finally, NH_3 is secreted into urine along the collecting duct, protonated, trapped as NH_4^+, and excreted with urine. Colored circles indicate carrier-mediated transport of NH_4^+ or NH_3, red and green dotted lines indicate expression of RhCG in intercalated (red) or principal cells (green) (for details see text).

subsequent trapping of ammonium in urine after protonation. This hypothesis remained textbook knowledge until recently. Further work demonstrated that ammoniagenesis occurs from metabolism of glutamine in the proximal tubule regenerating the bicarbonate lost while buffering protons stemming from metabolism.[12–14] Ammonium is then secreted into urine at the level of the proximal tubule, is mostly actively reabsorbed in the thick ascending limb, and finally accumulates in the interstitium with a high cortico-medullary gradient (Figure 1).[8] This interstitial high concentration, together with a pH gradient (from inside the cells of the collecting duct into urine), provides the driving force for ammonia transport by intercalated cells. Ammonia secretion in the collecting duct is mediated by intercalated cells, but principal cells may also contribute although their permeability is lower.[15]

NH_3/NH_4^+ uptake from interstitium by intercalated cells may be mediated by several pathways, including the $Na^+/K^+/2Cl$-cotransporter NKCC1, the Na^+/K^+-ATPase, and might involve also the RhCG protein (for a review, see Weiner and Hamm[9]). Preliminary data from our group show reduced basolateral NH_3 permeability in RhCG KO mice. The final step of ammonia secretion into urine had been studied in great detail by Knepper and colleagues[8] using microperfusion experiments and demonstrating that it involved high apical ammonia permeability. Whether this is an active process or involves transport proteins has remained elusive.

RHESUS PROTEINS: NOVEL AMMONIUM TRANSPORT PROTEINS?

The discovery of Marini *et al.*[16] that the mammalian homologs (RhAG and RhGK/RhCG) of the yeast methyl-ammonia permeases ammonium transporters could also mediate transport of ammonia/um opened the possibility that these molecules might participate in renal ammonium elimination. Similar molecules were also found in plants, algae, and fish. Expression of rhesus proteins RhAG, RhBG, and RhCG in various heterologous cell models induced ammonia/ammonium transport. However, the mode of transport and exact substrate (NH_3 or NH_4^+), as well as the coupling to other ions (counter- or cotransport of protons) and stoichiometry, have remained controversial