Role of NH₃ and NH₄⁺ transporters in renal acid-base transport

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Weiner ID, Verlander JW. Role of NH₃ and NH₄⁺ transporters in renal acid-base transport. Am J Physiol Renal Physiol 300: F11–F23, 2011. First published November 3, 2010; doi:10.1152/ajprenal.00554.2010.—Renal ammonia excretion is the predominant component of renal net acid excretion. The majority of ammonia excretion is produced in the kidney and then undergoes regulated transport in a number of renal epithelial segments. Recent findings have substantially altered our understanding of renal ammonia transport. In particular, the classic model of passive, diffusive NH₃ movement coupled with NH₄⁺ “trapping” is being replaced by a model in which specific proteins mediate regulated transport of NH₃ and NH₄⁺ across plasma membranes. In the proximal tubule, the apical Na⁺/H⁺ exchanger, NHE-3, is a major mechanism of preferential NH₄⁺ secretion. In the thick ascending limb of Henle’s loop, the apical Na⁺-K⁺-2Cl⁻ cotransporter, NKCC2, is a major contributor to ammonia reabsorption and the basolateral Na⁺/H⁺ exchanger, NHE-4, appears to be important for basolateral NH₄⁺ exit. The collecting duct is a major site for renal ammonia secretion, involving parallel H⁺ secretion and NH₃ secretion. The Rhesus glycoproteins, Rh B Glycoprotein (Rhbg) and Rh C Glycoprotein (Rhcg), are recently recognized ammonia transporters in the distal tubule and collecting duct. Rhcg is present in both the apical and basolateral plasma membrane, is expressed in parallel with renal ammonia excretion, and mediates a critical role in renal ammonia excretion and collecting duct ammonia transport. Rhbg is expressed specifically in the basolateral plasma membrane, and its role in renal acid-base homeostasis is controversial. In the inner medullary collecting duct (IMCD), basolateral Na⁺-K⁺-ATPase enables active basolateral NH₄⁺ uptake. In addition to these proteins, several other proteins also contribute to renal NH₃/NH₄⁺ transport. The role and mechanisms of these proteins are discussed in depth in this review.

acidosis; alkalosis; ammonia

AMMONIA¹ METABOLISM AND TRANSPORT are critical components of biological processes in almost all organs. In the kidney, ammonia is a central component of the renal regulation of acid-base homeostasis. Under basal conditions, renal ammonia excretion comprises 50–70% of net acid excretion. During metabolic acidosis, increases in renal ammonia excretion comprise 80–90% of the increase in net acid excretion both in humans (89) and close to 100% of the increase in rodent models (61, 62). Decreased renal ammonia excretion independent of defects in urine acidification is present in the most common form of renal tubular acidosis in humans, type IV RTA (50).

Renal ammonia metabolism involves both intrarenal ammoniagenesis and epithelial cell-specific transport of either NH₃ or NH₄⁺. In this review, we discuss pertinent aspects of ammonia chemistry, renal ammoniagenesis, and renal epithelial segment ammonia transport, and then conclude with updated information regarding the specific proteins involved in renal epithelial cell NH₃ and NH₄⁺ transport.

Ammonia Chemistry

Ammonia exists in two molecular forms, NH₃ and NH₄⁺. The relative amounts of each are governed by the buffer reaction: NH₃ + H⁺ ↔ NH₄⁺. This reaction occurs essentially instantaneously and has a pKᵣ under biologically relevant conditions of ~9.15. Accordingly, at pH 7.4 ~98.3% of total ammonia is present as NH₄⁺ and only ~1.7% is present as NH₃. Because most biological fluids exist at a pH substantially below the pKᵣ of this buffer reaction, small changes in pH cause exponential changes in NH₃ concentration, but do not substantially change the NH₄⁺ concentration (Table 1).

NH₃, although uncharged, has an asymmetric arrangement of positively charged hydrogen nuclei surrounding a central nitrogen; this results in NH₃ being a relatively polar molecule (Fig. 1). Quantitatively, NH₃ has a molecular dipole moment, a measure of polarity, of 1.46 D. This compares with measurements of 1.85 for H₂O, 1.08 for HCl, and 1.69 for ethanol, other small, uncharged, but polar, compounds. As a consequence of this molecular polarity, NH₃ has both high water solubility and limited lipid permeability (11, 85). Indeed,
several mammalian plasma membranes have been shown to have very low NH₃ permeability, such as the stomach, colon, and thick ascending limb of the loop of Henle (TAL) (52, 95, 99). Like other small, uncharged renal solutes, such as H₂O and urea, recent evidence indicates that protein-mediated NH₃ transport contributes to the rapid rates of NH₃ transport observed in the kidney.

NH₄⁺ also has limited permeability across lipid bilayers in the absence of specific transport proteins. However, in aqueous solutions NH₄⁺ and K⁺ have nearly identical biophysical characteristics (Table 2), which enables NH₄⁺ transport at the K⁺-transport site of essentially all K⁺ transporters, including many in the kidney (106). In addition, specific Na⁺/H⁺ exchanger isoforms can function in Na⁺/NH₄⁺ exchange mode and contribute to renal epithelial ammonia transport.

Renal Ammoniagenesis

Ammonia, in contrast to most other urinary solutes, is produced in the kidney, and the sum of urinary ammonia and renal vein ammonia substantially exceeds renal arterial ammonia delivery. Thus renal ammoniagenesis is central to ammonia homeostasis. Multiple excellent reviews of ammoniagenesis have been published (24, 97), so this will not be discussed in detail here. Importantly, although almost all renal epithelial cells can produce ammonia, the proximal tubule is the primary site for physiologically relevant ammoniagenesis. Studies using microdissected renal structures have shown that the glomeruli, S₁, S₂, and S₃ portions of the proximal tubule, the descending thin limb of the loop of Henle (DTL), medullary

Table 1. Influence of pH on NH₃ and NH₄⁺ concentration

<table>
<thead>
<tr>
<th>pH</th>
<th>NH₃ Concentration, µmol/l</th>
<th>% Change</th>
<th>NH₄⁺ Concentration, µmol/l</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.00</td>
<td>0.071</td>
<td>−99.6%</td>
<td>999.9</td>
<td>1.8%</td>
</tr>
<tr>
<td>6.00</td>
<td>0.71</td>
<td>−96%</td>
<td>999.3</td>
<td>1.7%</td>
</tr>
<tr>
<td>6.50</td>
<td>2.22</td>
<td>−87%</td>
<td>997.8</td>
<td>1.6%</td>
</tr>
<tr>
<td>7.00</td>
<td>7.03</td>
<td>−60%</td>
<td>993.0</td>
<td>1.1%</td>
</tr>
<tr>
<td>7.20</td>
<td>11.1</td>
<td>−36%</td>
<td>988.9</td>
<td>0.6%</td>
</tr>
<tr>
<td>7.40</td>
<td>17.5</td>
<td>0%</td>
<td>982.5</td>
<td>0.0%</td>
</tr>
<tr>
<td>7.60</td>
<td>27.4</td>
<td>57%</td>
<td>972.6</td>
<td>−1.0%</td>
</tr>
</tbody>
</table>

Calculations were based upon solution with 1 mmol/l total ammonia and pKᵣ for NH₃ + H⁺ = NH₄⁺ buffer reaction of 9.15. The % Change columns reflect change from pH 7.40.

(mTAL) and cortical TAL, distal convoluted tubule (DCT), cortical collecting duct (CCD), outer medullary collecting duct (OMCD), and inner medullary collecting duct (IMCD) can synthesize ammonia, with glutamine being the primary metabolic substrate (25, 37). Ammoniagenesis increases in response to metabolic acidosis (Fig. 2), but predominantly in the S1 and S2 proximal tubule segments (37, 114). Metabolic acidosis may also increase ammoniagenesis in the S3 proximal tubule segment (80).

Ammonia Transport Summary

Ammonia produced in the proximal tubule is secreted preferentially into the luminal fluid, although there is some transport across the basolateral membrane. In distal proximal tubule segments, such as the S3 segment, there may also be ammonia recycling involving the DTL, which contributes to generation of an axial interstitial ammonia concentration gradient. Distal segments then secrete ammonia; the collecting duct is the site of the majority of ammonia secretion and involves parallel H⁺ and NH₃ secretion. Figure 3 summarizes ammonia transport along the various renal epithelial cell segments. Below, we will discuss the currently available information regarding the specific ammonia transport mechanisms present in the apical and basolateral plasma membranes of renal epithelial cells in each of these segments.

Proximal Tubule

**NHE-3.** The apical Na⁺/H⁺ exchanger NHE-3 is likely to be a major mechanism of apical plasma membrane NH₄⁺ secretion in the proximal tubule (Fig. 4). NHE-3 is a member of an extended family of Na⁺/H⁺ exchangers. NHE-3-mediated am-
ammoniagenesis combined with lower intracellular Na\(^+\) concentration favors preferential Na\(^+\)/NH\(_4\)\(^+\) exchange, resulting in Na\(^+\) uptake and secretion of NH\(_4\)\(^+\) secretion. Studies examining in vitro microperfused proximal tubule segments showed that combining a low luminal Na\(^+\) concentration with the Na\(^+\)/H\(^+\) exchange inhibitor amiloride decreased ammonia secretion and that ammonia secretion was not due to luminal acidification (78). Similarly, studies examining in situ microperfused proximal tubule segments showed that the addition of the Na\(^+\)/H\(^+\) exchange inhibitor EIPA to the nonselective K\(^+\) channel blocker Ba\(^{2+}\) decreased ammonia secretion by ~50% compared with rates when only Ba\(^{2+}\) was present (94). Thus multiple lines of evidence support NHE-3 mediating an important role in proximal tubule NH\(_4\)\(^+\) secretion.

Changes in NHE-3 activity may alter renal ammonia metabolism in response to metabolic acidosis. Metabolic acidosis increases both NHE-3 expression (1) and, in studies examining in vitro microperfused S2 segments, proximal tubule ammonia secretion (81). The increase in both NHE-3 expression and ammonia secretion appears to require AT\(_1\) receptor activation (81). The list of pathways and physiological conditions that alter NHE-3 activity is extensive, but with the exception of metabolic acidosis they have not been correlated with changes in proximal tubule ammonia secretion.

**Apical K\(^+\) channels.** K\(^+\) channels are a second mechanism of proximal tubule ammonia transport. Because of intracellular electronegativity, K\(^+\) channel-mediated NH\(_4\)\(^+\) transport most likely results in net ammonia reabsorption under basal conditions. Increased luminal K\(^+\) concentration increased net ammonia secretion through mechanisms not involving Na\(^+\)/H\(^+\) exchange activity (79), suggesting that luminal K\(^+\) may inhibit NH\(_4\)\(^+\) reabsorption through a common transport mechanism, most likely apical K\(^+\) channels. Furthermore, luminal Ba\(^{2+}\), a nonspecific K\(^+\) channel inhibitor, inhibited proximal tubule ammonia secretion when combined with EIPA, whereas EIPA alone did not (94). Multiple K\(^+\) channels are present in the apical membrane of the proximal tubule, including KCNA10, K\(_{\text{ATP}}\), and Kv11.1 channels. Furthermore, luminal Ba\(^{2+}\) inhibits K\(_{\text{ATP}}\) channels, whereas guanidine and metabolic acidosis sensitize these channels to Ba\(^{2+}\) inhibition (16). Consequently, the expression of K\(_{\text{ATP}}\) channels influences the role of Ba\(^{2+}\) in modulating the apical K\(^+\) conductance and the mechanism of ammonia transport.

Ammonia secretion likely involves substitution of NH\(_4\)\(^+\) for H\(^+\) at the cytosolic H\(^+\) binding site, resulting in Na\(^+\)/NH\(_4\)\(^+\) exchange activity. Studies in proximal tubule brush-border membrane vesicles show that cytosolic NH\(_4\)\(^+\) competes with cytosolic H\(^+\) for exchange with luminal Na\(^+\), enabling Na\(^+\)/NH\(_4\)\(^+\) exchange (4, 56). Although these studies showed that extracellular NH\(_4\)\(^+\) can compete with luminal Na\(^+\) for reabsorption by NHE-3, the higher intracellular NH\(_4\)\(^+\) concentration due to intracellular ammoniagenesis likely favors preferential Na\(^+\)/NH\(_4\)\(^+\) exchange, resulting in Na\(^+\) uptake and secretion of NH\(_4\)\(^+\) secretion. Studies examining in vitro microperfused proximal tubule segments showed that combining a low luminal Na\(^+\) concentration with the Na\(^+\)/H\(^+\) exchange inhibitor amiloride decreased ammonia secretion and that ammonia secretion was not due to luminal acidification (78). Similarly, studies examining in situ microperfused proximal tubule segments showed that the addition of the Na\(^+\)/H\(^+\) exchange inhibitor EIPA to the nonselective K\(^+\) channel blocker Ba\(^{2+}\) decreased ammonia secretion by ~50% compared with rates when only Ba\(^{2+}\) was present (94). Thus multiple lines of evidence support NHE-3 mediating an important role in proximal tubule NH\(_4\)\(^+\) secretion.

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TWIK-1, and KCNQ1/KCNE1; which of these mediate ammonia transport is not currently known.

Uncharacterized apical NH₃ transport. In addition to Na⁺/H⁺ exchange mediated by NHE-3 and NH₄⁺/H⁺ exchange mediated by Ba²⁺-sensitive K⁺ channels, there may also be NH₃ transport across the apical plasma membrane. Studies examining in situ microperfused PCT segments showed that ~50% of ammonia secretion persisted even after inhibition of NHE-3 and Ba²⁺-sensitive K⁺ channels (94). In other studies, luminal acidification stimulated ammonia secretion despite the presence of high concentrations of EIPA, which were sufficient to inhibit NHE-3-mediated bicarbonate reabsorption, suggesting a significant role for NHE-3-independent NH₃ secretion (93). This apparent NH₃ permeability could either reflect passive, lipid-phase NH₃ diffusion or transport by a currently unidentified apical NH₃ transport process.

Basolateral Na⁺⁺-K⁺-ATPase. Basolateral Na⁺⁺-K⁺-ATPase can enable cellular uptake of interstitial ammonia, most likely through a mechanism involving substitution of NH₄⁺ for K⁺ at the K⁺ binding site. Mathematical modeling suggests that basolateral Na⁺⁺-K⁺-ATPase-mediated NH₄⁺ uptake may mediate as much as 20–30% of net ammonia secretion, but these assumptions are highly dependent on interstitial ammonia concentration (109).

Basolateral K⁺ channels. Basolateral K⁺ channel-mediated NH₄⁺ transport is also likely, but probably has a very limited role in proximal tubule ammonia transport (109). Because of intracellular electronegativity, K⁺ channel-mediated NH₄⁺ transport is likely to facilitate cellular NH₄⁺ uptake. The specific basolateral K⁺ channels that mediate this process have not been determined.

TAL

Na⁺⁺-K⁺⁺-2Cl⁻ cotransport. The TAL is an important site for luminal bicarbonate reabsorption (Fig. 5). The Na⁺⁺-K⁺⁺-2Cl⁻ cotransporter (NKCC2) is the major mechanism for ammonia reabsorption in the TAL (36). Luminal NH₄⁺ competes with K⁺ for binding to the K⁺⁺-transport site, enabling alterations in luminal K⁺ in hypokalemia and hyperkalemia to alter net NH₄⁺ transport (32, 33). The ability of NH₄⁺ to be transported at the K⁺ binding site of NKCC2 may also contribute to TAL NaCl transport (110). Metabolic acidosis increases TAL ammonia reabsorption (35); this appears to involve increased NKCC2 protein and mRNA expression (8) and to depend on the glucocorticoid increase that occurs with chronic metabolic acidosis (9).

K⁺ channels. In the TAL, K⁺ channels can contribute to luminal NH₄⁺ uptake when apical NKCC2 cotransport is in-

Fig. 4. Ammonia transport in the proximal tubule. Ammonia is produced in the proximal tubule primarily from metabolism of glutamine and occurs primarily in the mitochondria. The enzymatic details of ammoniagenesis are not shown. Three transport mechanisms appear to mediate preferential apical ammonia secretion. These include Na⁺/NH₄⁺ exchange via NHE-3, parallel NH₃ secretion and NHE-3-mediated Na⁺/H⁺ exchange, and a Ba²⁺-sensitive NH₄⁺ conductance likely mediated by apical K⁺ channels. HCO₃⁻ is produced in equimolar amounts as NH₄⁺ in the process of ammoniagenesis and is primarily transported across the basolateral plasma membrane by NBCe1. Minor components of basolateral NH₄⁺ uptake via Na⁺⁻K⁺-ATPase and by basolateral K⁺ channels are not shown.
hibited (6). However, NKCC2 inhibitors completely inhibit TAL ammonia transport, suggesting that apical K⁺/H⁺ channels are unlikely to mediate a quantitatively important role in TAL ammonia transport (36).

K⁺/NH₄⁺ exchange activity. An electroneutral, Ba²⁺- and verapamil-inhibitable apical K⁺/NH₄⁺ (H⁺) activity has been shown to be present in the apical membrane of the TAL (7). The gene product and the protein that correlate with this transport activity have not yet been identified. However, the observation that NKCC2 inhibitors nearly completely inhibit the transcellular component of TAL ammonia transport suggests that K⁺/NH₄⁺ (H⁺) exchange activity may not have a major role in TAL ammonia reabsorption.

Apical NHE-3. Apical NHE-3 is also present in the TAL (2). However, since this transporter likely secretes NH₄⁺, and the TAL reabsorbs NH₄⁺, NHE-3 appears unlikely to mediate an important role in loop of Henle ammonia transport.

Basolateral NHE-4. Multiple lines of evidence suggest that basolateral ammonia exit in the TAL likely involves Na⁺/H⁺ exchange mediated by NHE-4. Purified mTAL basolateral membrane vesicles exhibit Na⁺/H⁺ exchange activity, which can also function in a Na⁺/NH₄⁺ exchange mode (15). Two Na⁺/H⁺ exchange isoforms are expressed in the mTAL basolateral plasma membrane, NHE-1 and NHE-4 (20). Recent studies have shown a critical role for NHE-4 in mTAL ammonia absorption, presumably by mediating basolateral Na⁺/NH₄⁺ exchange. Metabolic acidosis increased mTAL NHE-4 mRNA expression and transport activity (16), and NHE-4 gene deletion inhibited mTAL ammonia absorption, decreased generation of the medullary interstitial ammonia concentration gradient, and decreased renal ammonia excretion in response to metabolic acidosis (16). Inhibiting NHE-1 with low concentrations of peritubular EIPA did not alter ammonia reabsorption, suggesting that NHE-1 does not contribute significantly to TAL ammonia reabsorption. Higher concentrations of peritubular EIPA, sufficient to inhibit NHE-4, decreased ammonia reabsorption by ~30%, consistent with a role of basolateral NHE-4 in TAL ammonia reabsorption. NHE-4 deletion did not alter basal ammonia excretion, but did decrease basal urine pH, which may have enabled normal rates of ammonia excretion in the absence of NHE-4 (16). Thus NHE-4 appears to mediate an important role in the mTAL basolateral ammonia exit, which is necessary for normal response to metabolic acidosis.

NBCn1. A second mechanism of basolateral ammonia transport in the TAL may involve dissociation of cytosolic NH₄⁺ to NH₃ and H⁺, with basolateral NH₃ exit and buffering of the intracellular H⁺ load by bicarbonate. In this model, the method of basolateral NH₃ exit has not been experimentally defined. Basolateral bicarbonate uptake appears to buffer the associated H⁺ load, and current data suggest that the electroneutral, sodium-bicarbonate cotransporter NBCn1 is critical to this process. In animals, metabolic acidosis increased mTAL ammonia reabsorption (34) and increased mTAL NBCn1 expression and activity (60, 86). Since the electrochemical gradient for bicarbonate transport by NBCn1 favors cellular bicarbonate uptake, not exit, increased NBCn1 expression likely does not contribute to the increased bicarbonate reabsorption seen with metabolic acidosis. Instead, increased bicarbonate uptake en-
ables a “bicarbonate shuttle” mechanism which enables parallel H\(^+\) and NH\(_3\) transport (Fig. 5). Further support for this model comes from in vitro studies. In the mTAL cell line, ST-1, inhibition of NBCn1 blunted uptake of the ammonia analog \(^{14}\text{C}\)methylammonia (\(^{14}\text{C}-\text{MA}\)) (63). Finally, NBCn1 expression in Xenopus laevis oocytes increased \(^{14}\text{C}-\text{MA}\) uptake (63). Thus multiple lines of evidence suggest NBCn1 facilitates basolateral TAL ammonia transport through a “bicarbonate-shuttle” mechanism.

**TDL**

Some of the ammonia absorbed by the mTAL undergoes recycling into the DTL, resulting in countercurrent amplification of medullary interstitial ammonia concentration. Ammonia recycling predominantly involves NH\(_3\) transport, with a smaller component of NH\(_4\)\(^+\) transport (30). The molecular mechanisms of DTL NH\(_3\), and NH\(_4\)\(^+\) transport have not been determined.

**Summary of Loop of Henle Ammonia Transport**

Ammonia absorption by the TAL and ammonia secretion into the DTL produces two important elements of renal ammonia transport. The first is development of an axial ammonia concentration gradient in the medullary interstitium that parallels the hypertonicity gradient. Second, ammonia absorption by the mTAL exceeds recycling in the DTL and thereby results in net ammonia reabsorption in the loop of Henle. Thus, even though total delivered luminal ammonia at the end of the micropunctureable proximal tubule is similar to net urinary ammonia excretion, ammonia reabsorption in the loop of Henle reduces ammonia delivery to the distal tubule to only 20–40% of final urinary ammonia content (26, 40). Ammonia secretion in more distal segments is necessary for normal renal ammonia excretion.

**Distal Tubule Ammonia Transport**

Ammonia transport in the regions of the distal tubule before the collecting duct, i.e., the DCT, CNT, and initial collecting tubule (ICT), is difficult to quantify due to the difficulty in obtaining micropuncture or isolated, perfused tubule data on deep cortical nephrons or portions distal to points where nephrons merge in these segments. Net ammonia secretion occurs between the early and late portions of the distal tubule accessible to micropuncture and accounts for ~10–15% of total urinary ammonia excretion under basal conditions (92, 113). This figure likely represents an underestimate of the contribution of the CNT and ICT, because a significant portion of these segments is distal to branch points.

**Collecting Duct Ammonia Transport**

It has been recognized for years that ammonia secretion by the collecting duct accounts for the majority of urinary ammonia content. Several studies have examined the CCD, OMCD, and IMCD and have uniformly shown that collecting duct ammonia secretion involves parallel NH\(_3\) and H\(^+\) transport, with little-to-no pH-independent NH\(_4\)\(^+\) permeability (26, 57). H\(^+\) secretion likely involves both H\(^+\)-ATPase and H\(^+\)-K\(^+\)-ATPase. These proteins have been the subject of several excellent recent reviews (14, 18, 23, 39) and are not reviewed here. Intact carbonic anhydrase activity, most likely mediated by carbonic anhydrase II (CA II), is necessary for collecting duct ammonia secretion, probably by supplying cytosolic H\(^+\) for secretion (101). Although collecting duct NH\(_3\) transport was initially thought to involve diffusive NH\(_3\) movement across plasma membranes, recent studies have shown that a variety of specific proteins are essential for collecting duct ammonia secretion.

Several transporters present in the collecting duct have been examined for their potential role in collecting duct ammonia secretion, including the NKCC2, NKCC1, Na\(^+\)-K\(^+\)-ATPase, H\(^+\)-K\(^+\)-ATPase, aquaporins, and the Rh glycoproteins, Rhcg. Of these, the only transporters that clearly have important roles in collecting duct ammonia secretion are Na\(^+\)-K\(^+\)-ATPase in the IMCD, and most recently the Rh glycoproteins, Rhbg and Rhcg. Figure 6 shows our current model of collecting duct ammonia secretion.

\(\text{Na}^+-\text{K}^+-\text{ATPase}\). Na\(^+\)-K\(^+\)-ATPase is present in the basolateral plasma membrane of renal epithelial cells, and its expression is greatest in the mTAL, with lesser expression in the cortical thick ascending limb, DCT, CCD, MCD, and the proximal tubule (48). NH\(_4\)\(^+\) competes with K\(^+\) at the K\(^+\)-binding site of Na\(^+\)-K\(^+\)-ATPase, enabling Na\(^+\)-NH\(_3\) exchange (59, 103). However, the relative affinities of Na\(^+\)-K\(^+\)-ATPase for NH\(_4\)\(^+\), ~11 mM, and K\(^+\), ~1.9 mM, have important effects on Na\(^+\)-K\(^+\)-ATPase-mediated NH\(_4\)\(^+\) transport. In the cortex, interstitial ammonia concentrations are ~1 mM, suggesting NH\(_4\)\(^+\) is unlikely to be transported to a significant extent by basolateral Na\(^+\)-K\(^+\)-ATPase (59). Moreover, even in the presence of high concentrations of peritubular ammonia, basolateral Na\(^+\)-K\(^+\)-ATPase does not appear to contribute to CCD ammonia secretion (58). In contrast, interstitial ammonia concentrations in the inner medulla are high, and Na\(^+\)-K\(^+\)-ATPase-mediated basolateral NH\(_4\)\(^+\) uptake is critical for IMCD ammonia and acid secretion (59, 103). In hypokalemia, studies examining the IMCD show that decreased interstitial K\(^+\) concentration enables increased NH\(_4\)\(^+\) uptake by Na\(^+\)-K\(^+\)-ATPase and increased rates of NH\(_4\)\(^+\) secretion which do not involve changes in Na\(^+\)-K\(^+\)-ATPase expression (102). In the outer medulla, particularly in the outer stripe, interstitial ammonia concentrations are sufficiently high to postulate a role for Na\(^+\)-K\(^+\)-ATPase in ammonia secretion, but this prediction has not been experimentally tested.

**Rh glycoproteins**. In the last decade, several laboratories have contributed to the discovery of Rh glycoproteins in the kidney and the demonstration of their important contribution to renal ammonia transport. Rh glycoproteins are mammalian orthologs of Mep/AMT proteins, ammonia transporter family proteins present in yeast, plants, bacteria, and many other organisms. Three mammalian Rh glycoproteins have been identified to date, Rh A glycoprotein (RhAG/Rhag), Rh B glycoprotein (RhBG/Rhbg), and Rh C glycoprotein (RhCG/Rhcg). By convention, Rh A glycoprotein is termed RhAG in human tissues and is termed Rhag in nonhumans; a similar terminology is used for RhBG/Rhbg and RhCG/Rhcg.

**RhAG/Rhag**. Rh A glycoprotein (RhAG/Rhag) is a component of the Rh complex in erythrocytes, which consists of the nonglycosylated Rh proteins, RhD and RhCE in humans and Rh30 in nonhuman mammals, in association with RhAG/Rhag. RhAG mediates electroneutral NH\(_3\) transport (74, 88, 111, 112). However, RhAG/Rhag is an erythrocyte and erythroid-
RENAL NH₃ AND NH₄⁺ TRANSPORTERS

Fig. 6. Model of collecting duct ammonia secretion. In the interstitium, NH₄⁺ is in equilibrium with NH₃ and H⁺. NH₃ is transported across the basolateral membrane through both Rhesus glycoproteins Rhbg and Rhcg. In the IMCD, basolateral Na⁺-K⁺-ATPase is a major mechanism of basolateral NH₄⁺ uptake, followed by dissociation of NH₄⁺ to NH₃ and H⁺ (grey lines). Intracellular NH₃ is secreted across the apical membrane by apical Rhcg. H⁺ secreted by H⁺-ATPase and H⁺-K⁺-ATPase combine with luminal NH₃ to form NH₄⁺, which is “trapped” in the lumen. In addition, there may be also minor components of diffusive NH₃ movement across both the basolateral and apical plasma membranes (dotted lines). The intracellular H⁺ that is secreted by H⁺-ATPase and H⁺-K⁺-ATPase is generated by carbonic anhydrase (CA) II-accelerated CO₂ hydration that forms carbonic acid, which dissociates to H⁺ and HCO₃⁻. Basolateral Cl⁻/HCO₃⁻ exchange transports HCO₃⁻ across the basolateral membrane; HCO₃⁻ combines with H⁺ released from NH₃ to form carbonic acid, which dissociates to CO₂ and water. This CO₂ can recycle into the cell, supplying the CO₂ used for cytosolic H⁺ production. The net result is NH₃ transport from the peritubular space into the luminal fluid.

In the non-A, non-B cell, which lacks substantial basolateral Rhcg expression, Rhbg is likely the primary basolateral NH₃ transport mechanism. The B-type intercalated cell, which lacks detectable Rhbg and Rhcg expression, likely mediates transcellular ammonia secretion through mechanisms only involving lipid-phase NH₃ diffusion and thus transports ammonia at significantly slower rates.

 precursor specific protein (66), and studies in the human kidney found no evidence of renal RhAG expression (105). At present, RhAG/Rhag is thought unlikely to contribute to renal ammonia transport.

RhBG/Rhbg. RhBG/Rhbg is expressed in a wide variety of organs involved in ammonia metabolism, including kidneys, liver, skin, lung, stomach, and gastrointestinal tract (42, 44, 68, 87, 98, 107). In kidneys, the DCT, CNT, ICT, CCD, OMCD, and the IMCD express basolateral Rhbg (87, 98). In general, both intercalated and principal cells express Rhbg, and intercalated cell Rhbg expression exceeds principal cell expression. The exceptions are the CCD B-type intercalated cell, which does not express Rhbg detectable with immunohistochemistry, and the IMCD, where only intercalated cells express Rhbg (98). Rhbg’s basolateral expression appears due to basolateral stabilization through specific interactions of its cytoplasmic carboxy-terminus with ankyrin-G (69). The human kidney expresses high amounts of RhBG mRNA (68), but a recent study using a variety of antibodies did not detect RhBG protein expression (17).

RhBG/Rhbg transports both ammonia and the ammonia analog methylammonia. Most studies show that Rhbg mediates electroneutral, Na⁺- and K⁺-independent, NH₃ transport (71, 73, 119), while another identified electrogenic NH₄⁺ transport (84). The explanation for this discrepancy is not known. In all of these studies, the affinity for ammonia was ~2–4 mM. Importantly, both electroneutral NH₃ transport and electrogenic NH₄⁺ transport facilitate basolateral ammonia uptake.

Rhbg’s specific role in renal ammonia metabolism is controversial at present. Several studies suggest it can contribute to ammonia secretion in specific conditions. In the mouse, metabolic acidosis increased renal ammonia excretion and induced a progressive, time-dependent increase in Rhbg protein in the CNT, ICT, CCD, OMCD, and the IMCD in one study (12). In another study, which examined only the OMCD, metabolic acidosis increased Rhbg mRNA expression (protein expression was not examined) (22). These studies contrast with findings in the rat, where metabolic acidosi did not detectably alter Rhbg expression (90). In mice, genetic deletion of pendrin, an apical Cl⁻/HCO₃⁻ exchanger present in type B and non-A, non-B intercalated cells, decreased Rhbg expression (55). Since pendrin deletion increased urine acidification, which otherwise would increase ammonia excretion, decreased Rhbg expression may have normalized ammonia excretion rates. Finally, Rhcg deletion, either from the entire collecting duct or only from intercalated cells, increased Rhbg expression in acid-loaded mice, suggesting increased Rhbg protein expression contributed to ammonia excretion in the absence of Rhcg (61, 62). The consistent observation in the mouse that changes in Rhbg expression either parallel changes in ammonia excretion or compensate for genetic deletion of other proteins involved in renal acid-base homeostasis suggest Rhbg contributes to renal ammonia excretion.

However, studies examining genetic Rhbg deletion have reached differing conclusions as to Rhbg’s physiological role. In one study, mice with global Rhbg deletion were examined. These mice had normal basal acid-base parameters and basal ammonia excretion, normal increases in urinary ammonia excretion in response to acid loading, and normal basolateral NH₃ and NH₄⁺ permeability in microperfused CCD segments (19). These findings suggested Rhbg did not contribute to renal ammonia metabolism. Our laboratory examined mice with intercalated cell-specific Rhbg deletion (12). Basal ammonia excretion was not altered, but there was a substantial adaptive change in proximal tubule glutamine synthetase expression, which may have enabled normal rates of unstimulated ammonia excretion. With acid loading, intercalated cell-specific Rhbg deletion significantly impaired the expected increase in urinary ammonia excretion. These findings suggested Rhbg expression contributes to renal ammonia excretion and that adaptive responses to Rhbg deletion may mask Rhbg’s role under specific circumstances.
RhCG/Rhcg. There is now substantial evidence that Rh C glycoprotein (RhCG/Rhcg) is critical for renal ammonia excretion. RhCG/Rhcg is widely expressed, including in kidneys, the central nervous system, testes, lung, liver, and throughout the gastrointestinal tract (42, 44, 67, 107). In the kidney, Rhcg is expressed in the same epithelial cell distribution as Rhtub, in the DCT, CNT, ICT, CCD, OMCD, and IMCD (27, 98). With the exception of the IMCD, in which only intercalated cells express Rhcg, both intercalated cells and nonintercalated cells (i.e., DCT cells, CNT cells, and principal cells) express Rhcg, and intercalated cell expression exceeds principal cell expression (27, 98). Detectable Rhcg expression is not observed in the B-type intercalated cell (43). The presence of Rhbg and Rhcg in both intercalated cells and principal cells is consistent with functional measurements of NH3 permeability in intercalated cells and principal cells (117). Figure 7 summarizes the expression of Rhbg and Rhcg in the different intercalated cell types.

Rhcg has a complex subcellular localization. Studies in the human, rat, and mouse kidney demonstrate that Rhcg-expressing cells exhibit both apical and basolateral Rhcg immunoreactivity, with the exception of the non-A, non-B intercalated cell, which has only apical Rhcg (17, 41, 53, 90, 91). Immunogold electron microscopy in both the rat and mouse kidney demonstrated that apical Rhcg is present in both the apical plasma membrane and subapical vesicles and that basolateral Rhcg is present in the basolateral plasma membrane (53, 91). Basolateral expression can be substantial; in the rat OMCD in the inner stripe, basolateral Rhcg is ~25% of total cellular expression in intercalated cells and ~40% in principal cells (91). Although initial studies in both the rat and mouse kidney did not identify basolateral Rhcg expression, more recent studies using improved immunohistochemistry techniques and a panel of anti-Rhcg antibodies confirmed basolateral Rhcg expression and demonstrated substantial quantitative differences in the amount of basolateral Rhcg immunolabel in different mouse strains (53).

Several studies have addressed the molecular ammonia species transported by Rhcg/Rhcg. Some studies using heterologous expression in the X. laevis oocyte suggest that RhCG/Rhcg mediates electroneutral NH3 transport (71, 73, 119), while others have reported both NH3 and NH4+ transport (10). Measurement of apical plasma membrane NH3 and NH4+ permeability in the collecting duct of mice with global Rhcg deletion found decreased NH3 permeability, without a change in NH4+ permeability (13). Other studies reconstituted purified human RhCG into liposomes and demonstrated increased NH3 permeability but no change in NH4+ permeability (38, 75). Thus the majority of evidence suggests Rhcg/RhCG functions as a facilitated NH3 transporter.

Substantial evidence supports the conclusion that Rhcg mediates a critical role in renal ammonia excretion. In a variety of experimental models, Rhcg expression paralleled ammonia excretion. Metabolic acidosis significantly increased total Rhcg protein expression in both the OMCD and the IMCD, but not in the cortex (90). Rhcg mRNA expression was not altered significantly; therefore, posttranscriptional mechanisms may regulate Rhcg protein expression (90). In response to reduced renal mass, where there is increased single-nephron ammonia secretion, apical Rhcg expression increased in the CCD A-type intercalated cell and both apical and basolateral expression increased in the OMCD intercalated cell and in principal cells in the CCD and OMCD (54). Cyclosporine A nephropathy is associated with decreased Rhcg expression, which likely contributes to impaired ammonia excretion and development of metabolic acidosis in this model (65).

At least two distinct mechanisms contributed to increased apical plasma membrane Rhcg expression in response to metabolic acidosis. First, there was increased total cellular Rhcg protein expression (91). Second, there were changes in Rhcg’s subcellular location. Under basal conditions, apical Rhcg was located both in the apical plasma membrane and in subapical sites in both principal and intercalated cells. In response to chronic metabolic acidosis, particularly in the intercalated cell, apical plasma membrane expression increased and subapical expression decreased (91). The relative importance of these two mechanisms differed in principal and intercalated cells, with subcellular distribution changes being the predominant adaptive response in the OMCD intercalated cell and increased protein expression being the predominant mechanism in the OMCD principal cell (91).

Changes in basolateral Rhcg expression have also been examined in a variety of models. Chronic metabolic acidosis increased basolateral plasma membrane Rhcg expression significantly in both intercalated cells and principal cells (54, 91). In contrast to apical plasma membrane Rhcg expression, the proportion of total cellular Rhcg that was present in the basolateral plasma membrane did not change, suggesting that redistribution from cytoplasmic sites to the basolateral plasma membrane was not a major regulatory mechanism (91). Basolateral Rhcg expression has also been studied in a model of reduced renal mass (54). In this model of the early response to 5/6 renal ablation-infarction, in which there is increased single-nephron ammonia excretion, basolateral Rhcg expression in-
creased in the CCD principal cell, OMCD intercalated cell, and the OMCD principal cell (54). At present, neither genetic nor pharmacological approaches are available to assess the functional significance of these changes in basolateral Rhcg expression. However, C57BL/6 mice have substantially greater basolateral Rhcg expression than Balb/C mice (53), and this correlates with a greater ability to increase renal ammonia excretion in response to an acid load (108).

Genetic deletion studies have confirmed Rhcg’s key role in renal ammonia excretion. Global Rhcg deletion decreased basal ammonia excretion and impaired urinary ammonia excretion in response to metabolic acidosis (13). Collecting duct-specific Rhcg deletion produced similar findings, indicating that reduced ammonia excretion reflected impaired collecting duct ammonia secretion and was not an indirect effect of an extrarenal Rhcg-mediated mechanism (61). Global Rhcg deletion decreased both transepithelial ammonia permeability and apical membrane NH3 permeability in perfused collecting duct segments obtained from acid-loaded mice (13).

The observation that both intercalated and principal cells express Rhcg and that metabolic acidosis increased Rhcg in both cell types suggests that both cells contribute to transepithelial ammonia secretion. To test this prediction, recent studies examined mice with intercalated cell-specific Rhcg deletion (62). These studies showed that intercalated cell-specific Rhcg deletion did not alter the basal rate of ammonia excretion (62), suggesting principal cell Rhcg expression was sufficient for normal basal ammonia excretion. After acid loading, mice with intercalated cell-specific Rhcg deletion had an intact ability to increase urinary ammonia excretion during the first 2 days of metabolic acidosis. Because this was associated with a significantly lower urine pH, increased luminal H+ concentration, by shifting the NH3 + H+ ↔ NH4+ reaction to the right, may have decreased luminal NH3 concentration, thereby facilitating principal cell Rhcg-dependent NH3 secretion. Alternatively, the more acidic urine may result from the decreased NH3 permeability, which decreased NH3 entry and subsequent titration of secreted H+. Importantly, the finding of intact ammonia excretion in the early response to metabolic acidosis in mice with intercalated cell-specific Rhcg deletion contrasted with findings in mice with either global or collecting duct-specific Rhcg deletion, in which ammonia excretion was inhibited significantly (13, 61). Principal cell Rhcg expression appears to enable both normal basal ammonia excretion and to contribute to the increased ammonia excretion with metabolic acidosis, suggesting that the principal cell, in addition to the intercalated cell, can contribute to transepithelial ammonia secretion and thereby contribute to acid-base homeostasis.

**Tertiary structure of mammalian Rh glycoproteins.** Important studies examining the tertiary structure of Rh glycoproteins and related orthologs, Amt proteins, have led to substantial understanding of the molecular mechanism through which they transport ammonia. These studies used X-ray crystallographic approaches; initial studies examined Amt proteins, bacterial orthologs of Rh glycoproteins, and more recent studies examined first bacterial Rh glycoproteins and then human RhCG.

Bacterial Amt proteins are expressed in a homotrimeric state; each of the subunits has 11 transmembrane segments, with segments M1–M5 and M6–M10 exhibiting a quasi-twofold axis. The trimer has a net negative charge of −13.5 at the extracellular site and a net positive charge of +9 at the cytoplasmic site (51). An extracellular vestibule concentrates NH4+; in *Escherichia coli* AmtB, three highly conserved acidic residues, Phe107, Trp118, and Ser119, create a NH4+-binding site (3, 46, 118). This NH4+-binding site, and the differences in net charge at the extracellular and intracellular site, facilitate NH3 interaction with the extracellular vestibule and likely explain the membrane voltage-dependent changes in affinity for the ammonia analog methylammonia (70). A 20-Å-long, narrow, hydrophobic central channel enables NH3, but not NH4+, movement, thereby providing molecular selectivity. NH3 movement through this channel is stabilized by a unique twin in-line histidine motif. In *E. coli* AmtB, stacked phenyl rings of two highly conserved phenylalanine residues, Phe167 and Phe215, appear to regulate entry of NH3 from the extracellular vestibule into the central channel.

More recent studies examined the tertiary structure of Rh glycoproteins using similar techniques. *Nitrosomonas europaea* is an obligate chemolithoautotrophic bacterium that can oxidize ammonia as its sole energy source; it is one of only four bacteria known to express Rh glycoproteins (47), and its Rh glycoprotein, NeRh50, is known to transport ammonia (21, 104). Two reports published simultaneously detailed the crystal structure of NeRh50 (64, 72). Multiple similarities between NeRh50 and Amt proteins were identified, including the homotrimeric structure with 11 transmembrane-spanning segments of each monomer, the central pore with the twin in-line histidine motif, and a phenylalanine residue that may regulate entry into the channel. An important difference between NeRh50 and Amt proteins was the lack of critical acidic residues in the extracellular vestibule present in Amt orthologs; this absence likely accounts for the differences in ammonia affinity between Rh glycoproteins, typically 1–4 mM, and the affinity of Amt proteins, typically in the low micromolar range (64, 72).

Most recently, the structure of human RhCG was determined (38). Again, a homotrimeric structure was demonstrated. Each monomer exhibited 12 transmembrane-spanning segments; the 12th segment, termed M0, was an additional amino-terminal helix. Helices M1–M5 and M6–M10 exhibited an in-plane quasi-twofold symmetry with respect to the plasma membrane. There were multiple conserved features, including acidic residues lining the extracellular vestibules, an external aperture gated by a phenylalanine residue, a largely hydrophobic channel lumen, and the twin in-line histidine motif in the center of the channel (38). The extracellular vestibule tryptophan, which in AmtB serves to recruit NH4+, was absent in both RhCG and NeRh50, but alternative acidic residues, Glu166 in the extracellular vestibule and Asp218, Asp278, and Glu329 in the intracellular vestibule, were present (Fig. 8). RhCG exhibited another important structural feature not present in Amt proteins, a pocket extending from the cytosolic aperture to the lateral exterior surface, termed a “shunt” pathway (38). This shunt pathway has an acidic residue in its intracellular surface and may serve as an alternative pathway for NH3 entry from the hydrophobic region of the lipid bilayers.

**NKCC1.** NKCC1 is a Na+-K+-2Cl− cotransporter expressed in the basolateral region of intercalated cells in the OMCD and IMCD (31) and of IMCD cells (31, 49). However, peritubular bumetanide, an NKCC1/NKCC2 inhibitor, does not alter OMCD ammonia secretion; thus NKCC1 appears unlikely to mediate a quantitatively important role in OMCD.
ammonia secretion (101). In the IMCD, although NH₃⁺ and K⁺ compete for a common binding site on NKCC1, pharmacologically inhibiting NKCC1 did not alter peritubular NH₃⁺ uptake significantly (100). Thus NKCC1 appears unlikely to mediate a substantial role in renal ammonia secretion.

$H^+-K^+-ATPase$. $H^+-K^+-ATPase$ proteins are members of the P-type ATPase family. Various $H^+-K^+-ATPase$ subunit isoforms and activities have been reported in the apical region of collecting duct cells. The majority of data demonstrating NH₃⁺ transport by $H^+-K^+-ATPase$ suggest NH₃⁺ binds to and is transported at the K⁺ binding site. However, potassium deficiency increases expression of the colonic $H^+-K^+-ATPase$, which has been postulated to mediate increased NH₃⁺ secretion via NH₃⁺ binding and transport at the H⁺ binding site (82).

Aquaporins. Aquaporins (AQP) comprise an extended family of proteins that facilitate water transport. Because both H₂O and NH₃ have similar molecular sizes and charge distribution, several studies have examined the role of aquaporins in NH₃ transport. Importantly, some, but not all, aquaporins can transport ammonia (77). AQP1 was the first aquaporin shown to transport ammonia. Several studies have shown that expressing AQP1 in X. laevis oocytes increases NH₃ transport (77, 83). However, not all studies have confirmed NH₃ transport by AQP1 (45). AQP1 is present in the proximal tubule and in TDL; it may contribute to ammonia as well as water permeability in these segments. AQP3 is present in the basolateral membrane of collecting duct principal cells. When expressed in X. laevis oocytes, AQP3 transports NH₃ (45). Whether AQP3 contributes to renal principal cell basolateral NH₃ transport has not been determined. AQP8 is expressed in intracellular sites in the proximal tubule, CCD, and OMCD in the kidney, but not the plasma membrane (28). AQP8’s specific intracellular site in mammalian cells has not been determined, but it localizes to the inner mitochondrial membrane when expressed in yeast (96). AQP8’s role in renal ammonia metabolism is unclear. Genetic deletion alters hepatic ammonia accumulation, renal excretion of infused ammonia, and intra-renal ammonia concentrations, but does not alter serum chloride concentration, urine ammonia concentration, or urine pH either under basal conditions or in response to acid-loading (115, 116). Thus aquaporins may be able to transport NH₃, and are expressed at several renal epithelial sites in which NH₃ transport remains incompletely characterized.

Summary

Renal ammonia transport is central to acid-base homeostasis. The previous paradigm of passive, lipid-phase NH₃ diffusion and NH₃⁺ trapping is being replaced by a model in which transporter-mediated movement of NH₃ and NH₃⁺ are fundamental components of renal ammonia physiology. In the proximal tubule, preferential apical NH₃⁺ secretion involves specific transport involving NHE-3 and Ba²⁺-sensitive K⁺ channels, in the TAL ammonia reabsorption involves NH₃⁺ transport by a variety of proteins, including NKCC2 and NHE-4, in the collecting duct Rhbg and Rhcg transport NH₃, and in the IMCD basolateral Na⁺⁻K⁺⁻ATPase transports NH₃. Thus renal ammonia metabolism involves a complex interaction of multiple proteins that specifically transport the two molecularly distinct forms of ammonia, NH₃ and NH₃⁺. This complex interaction enables coordinated and highly regulated NH₃ and NH₃⁺ transport and likely contributes to the fine control of renal ammonia transport and excretion necessary for acid-base homeostasis.
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