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Renal Ammonia Metabolism and Transport

I. David Weiner[*,1,2] and **Jill W. Verlander**[2]

[1]Nephrology and Hypertension Section, NF/SGVHS, Gainesville, Florida

[2]Division of Nephrology, Hypertension and Transplantation, University of Florida College of Medicine, Gainesville, Florida

Abstract

Renal ammonia metabolism and transport mediates a central role in acid-base homeostasis. In contrast to most renal solutes, the majority of renal ammonia excretion derives from intrarenal production, not from glomerular filtration. Renal ammoniagenesis predominantly results from glutamine metabolism, which produces 2 NH_4^+ and 2 HCO_3^- for each glutamine metabolized. The proximal tubule is the primary site for ammoniagenesis, but there is evidence for ammoniagenesis by most renal epithelial cells. Ammonia produced in the kidney is either excreted into the urine or returned to the systemic circulation through the renal veins. Ammonia excreted in the urine promotes acid excretion; ammonia returned to the systemic circulation is metabolized in the liver in a HCO_3^--consuming process, resulting in no net benefit to acid-base homeostasis. Highly regulated ammonia transport by renal epithelial cells determines the proportion of ammonia excreted in the urine versus returned to the systemic circulation. The traditional paradigm of ammonia transport involving passive NH_3 diffusion, protonation in the lumen and NH_4^+ trapping due to an inability to cross plasma membranes is being replaced by the recognition of limited plasma membrane NH_3 permeability in combination with the presence of specific NH_3-transporting and NH_4^+-transporting proteins in specific renal epithelial cells. Ammonia production and transport are regulated by a variety of factors, including extracellular pH and K^+, and by several hormones, such as mineralocorticoids, glucocorticoids and angiotensin II. This coordinated process of regulated ammonia production and transport is critical for the effective maintenance of acid-base homeostasis.

Introduction

Acid-base homeostasis is critical for normal health, growth, and development. Acid-base disorders lead to a wide variety of clinical problems, including growth retardation in neonates and children, nausea and vomiting, electrolyte disturbances, increased susceptibility to cardiac arrhythmias, decreased catecholamine sensitivity, particularly in the cardiovascular system, osteoporosis and osteomalacia, recurrent nephrolithiasis, skeletal muscle atrophy, paresthesias, and coma.

[*]Correspondence to weineid@ufl.edu.

The kidneys have two major functions in acid-base homeostasis, reabsorption of filtered bicarbonate and generation of new bicarbonate. First, daily filtered bicarbonate averages approximately 4200 mmol/d for the adult with normal renal function, and is reabsorbed almost completely by renal epithelial cells. Second, both intracellular and extracellular buffers are used to buffer endogenous and exogenous acid loads and must be replenished to maintain acid-base homoeostasis. The kidneys produce “new bicarbonate” to do so, and the primary mechanism of new bicarbonate generation involves renal ammonia metabolism. This manuscript’s purpose is to review the mechanisms and the regulation of renal ammonia metabolism and transport. Ammonia exists in two different molecular forms, NH_3 and NH_4^+. In this manuscript, we use the terms “ammonia” and “total ammonia” to refer to the combination of NH_3 and NH_4^+. When referring specifically to NH_3, we state “NH_3.” When discussing NH_4^+ we state “NH_4^+” specifically.

Role of Ammonia Metabolism in Acid-Base Homeostasis

The major acid-base disturbance that mammals face on a daily basis reflects the acid load resulting from metabolism of proteins. For the average human, this acid load approximates 0.8 mEq/kg/d, or approximately 56 mEq for a 70 kg individual. The correlation between endogenous acid production and body mass presumably occurs because protein metabolism often parallels body mass, and endogenous acid production results from endogenous protein metabolism. This acid load is buffered by intracellular and extracellular buffers, primarily bicarbonate. Long-term maintenance of acid-base homeostasis requires production of new alkaline buffers from the process of net acid excretion to replace those “consumed” in buffering this daily acid load. In humans and in rodents, urinary ammonia excretion comprises approximately 50% to 70% of basal net acid excretion (15, 36, 92, 132, 177).

Understanding the role of renal ammonia metabolism in acid-base homeostasis involves consideration of the effects of renal and extrarenal ammonia metabolism. Renal ammoniagenesis, utilizing glutamine as the primary metabolic substrate, generates equivalent amounts of NH_4^+ and HCO_3^-. The HCO_3^- produced is preferentially transported across the basolateral plasma membrane, absorbed into peritubular capillaries and returned to the systemic circulation. Renal NH_4^+ excretion thereby quantitatively indicates renal HCO_3^- production. Although a substantial component of renal NH_4^+ that is produced is returned to the systemic circulation via the renal vein, this $NH_4^+$is metabolized in the liver, predominantly via the urea cycle, in a process that utilizes HCO_3^-. Accordingly, the component of NH_4^+ produced by the kidney that is then metabolized by the liver does not contribute to “new” HCO_3^- production.

Regulation of renal ammoniagenesis, transport, and excretion are integral to acid-base homeostasis. In response to metabolic acidosis (Fig. 1), increases in urinary ammonia excretion are the primary component of the increase in net acid excretion (36, 92, 177); indeed in studies in the mouse using HCl addition to food to induce metabolic acidosis, we observed no change in titratable acid excretion; increased ammonia excretion was almost entirely responsible for the increase in net acid excretion (15, 92). In hypokalemia, urinary ammonia excretion increases despite concurrent metabolic alkalosis (60), and may be a major factor in the development of metabolic alkalosis. Thus, in many of the most common

clinical conditions changes in urinary ammonia excretion are the predominant mechanism through which the kidneys regulate net acid excretion.

Overview of Ammonia Metabolism

Ammonia metabolism involves integrated function of multiple portions of the kidney. Only a minimal amount of urinary ammonia derives from glomerular filtration, making ammonia unique among the major compounds present in the urine. Quantitatively, assuming a plasma ammonia concentration of 20 μmol/L and a GFR of 120 mL/min/1.73 m^2, the filtered load of ammonia in a normal individual is approximately 3 to 4 mmol/d, 60 to 80% of which is reabsorbed prior to the collecting duct, leading to filtered ammonia contributing only approximately 1 mmol/d of total renal ammonia excretion. In most individuals, the normal urinary ammonia excretion is 30 to 40 mmol/d, indicating that filtered ammonia is only a minor component of total excreted ammonia. In response to metabolic acidosis, where urinary ammonia can increase to more than 200 mmol/d, plasma ammonia levels, and consequently the filtered load of ammonia, do not change and may even decrease slightly (126). Renal ammonia excretion almost entirely reflects intrarenal ammoniagenesis and selective renal epithelial cell ammonia transport into either the urine or into the circulation.

Ammonia's selective transport into either the tubule fluid, where it can be excreted, or into the peritubular fluid, when it can be returned to the systemic circulation, determines its effect on acid-base homeostasis. Ammonia excreted into the urine results in equimolar new bicarbonate formation. Ammonia returned to the systemic circulation is metabolized by the liver; this process utilizes equimolar amounts of bicarbonate, thereby consuming the bicarbonate produced during renal ammoniagenesis. This process of selective, directional transport is a critical component of the renal regulation of acid-base homeostasis. Under basal conditions, approximately 50% of ammonia generated by the kidney is excreted in the urine and approximately 50% returns to the renal vein (126). With metabolic acidosis, total renal ammoniagenesis increases, urinary ammonia excretion increases, but ammonia return to the circulation does not change proportionately, and may not even change at all (126). Selective transport of ammonia into either the urine or the circulation involves integrated transport in the proximal tubule, thick ascending limb of the loop of Henle, and the collecting duct (Fig. 2).

Ammonia Chemistry

Ammonia exists in two molecular forms, NH_3 and NH_4^+. The relative amounts of each are governed by the buffer reaction: $NH_3 + H^+ \leftrightarrow NH_4^+$. This reaction occurs essentially instantaneously and has a pKa' under biologically relevant conditions of approximately 9.15. Accordingly, the majority of ammonia is present as NH_4^+; at pH 7.4 only approximately 1.7 % of total ammonia is present as NH_3. The pH of most biological fluids is substantially below the pKa' of this buffer reaction; consequently changes in pH cause exponential changes in NH_3 concentration, but almost no change in NH_4^+ concentration (Fig. 3). Because there are two molecular forms of ammonia, one needs to consider the biophysical characteristics of each to understand the differences in transport of each.

NH_3 is a small, uncharged molecule. These characteristics led initially to the belief that NH_3 is highly lipid permeable and essentially in diffusion equilibrium across lipid membranes. However, a substantial body of evidence indicates that this is not the case. In particular, NH_3, although uncharged, has an asymmetric arrangement of positively charged hydrogen nuclei surrounding a central nitrogen, which makes NH_3 a relatively polar molecule (Fig. 4). Quantitatively, NH_3 has a molecular dipole moment, a measure of the degree of separation of positive and negative charge, of 1.47 D (73). For comparison, the molecular dipole moments of HCl and H_2O are 1.08 and 1.85, respectively. Functionally, this charge polarity results in NH_3 actually having only a limited lipid permeability (14,124), which leads to limited, sometimes only minimal, plasma membrane NH_3 permeability (18,152,168,193). Importantly, limited plasma membrane NH_3 permeability causes the development of transepithelial NH_3 gradients, which have been demonstrated to be present in the kidney (50).

NH_4^+ also has limited permeability across lipid bilayers in the absence of specific transport proteins. However, the model of ammonia transport with "NH_4^+ trapping" due to lack of NH_4^+ transport across plasma membranes is now known to not be fully correct. In the absence of specific transporters, NH_4^+ has a very low permeability across plasma membranes. Thus, in some tissues, such as the apical membrane of collecting duct segments, there is essentially no detectable NH_4^+ permeability. However, in many tissues, specific proteins are able to transport NH_4^+ across plasma membranes, and this process is critical to renal ammonia excretion. The ability of these proteins to transport NH_4^+ is due, in large part, to the specific biophysical characteristics of hydrated NH_4^+ ions. When examined in aqueous solutions, NH_4^+ and K^+ have nearly identical biophysical characteristics (177, 180). This effective molecular mimicry enables NH_4^+ to be transported at the K^+-transport site of essentially every known K^+-transporting protein (177). As will be discussed below, NH_4^+ transport by specific membrane proteins contributes importantly to renal ammonia excretion.

Ammonia Production

Ammonia is produced by almost all renal epithelial cells, but the proximal tubule is the primary site for physiologically relevant ammoniagenesis. Studies examining dissected renal segments, including the glomeruli, S1, S2, and S3, descending thin limb of the loop of Henle, medullary and cortical thick ascending limb of the loop of Henle, distal convoluted tubule (DCT), cortical collecting duct (CCD), outer medullary collecting duct (OMCD), and inner medullary collecting duct (IMCD), show that all have the capability to synthesize ammonia using glutamine as their primary metabolic substrate (49). Quantitatively, metabolism of each glutamine molecule leads to generation of 2 NH_4^+ and 2 HCO_3^- ions when glutamine is metabolized through phosphate-dependent glutaminase (PDG), glutamate dehydrogenase (GDH), and phosphoenolpyruvate carboxykinase (PEPCK) during metabolic acidosis and hypokalemia. Although essentially all renal epithelial cells have the capability of producing ammonia, most studies have shown that metabolic acidosis only increases the rates of ammoniagenesis in the S1 and S2 proximal tubule segments (49, 190), and under certain *in vitro* conditions also in S3 segments (116). Taking into consideration the production rates per unit length and the relative lengths of the different nephron segments,

the proximal tubule accounts for 60% to 70% of total renal ammonia production under basal conditions and at least 70 to 80% under conditions of metabolic acidosis (49) (Fig. 5).

Multiple pathways for ammoniagenesis are present in the proximal tubule (Fig. 6), but the predominant pathway involves the enzyme, PDG. PDG is present inside mitochondria and metabolizes glutamine to glutamate, producing NH_4^+. Glutamate then undergoes further metabolism through multiple pathways. The major pathway involves GDH, resulting in production of α-ketoglutarate (α-KG) and NH_4^+. GDH-mediated metabolism of glutamate to form α-KG is the quantitatively predominant mechanism, and is regulated in parallel with changes in total renal ammoniagenesis. Although transamination of glutamate via glutamic-oxaloacetic transaminase (GOT) does not release NH_4^+, the aspartate produced can be metabolized through the purine nucleotide cycle (PNC), forming fumarate and releasing NH_4^+. Quantitatively, the PNC pathway mediates only a minor role in overall ammoniagenesis (159). Glutamate can also be metabolized through glutamate decarboxylase to form γ-aminobutyric acid (GABA), and this may account for as much as 25% of glutamate oxidation in the renal cortex (89), but this pathway does not alter net ammoniagenesis since GABA metabolism to succinate semialdehyde via the enzyme γ-aminobutyrate transaminase results in regeneration of glutamate.

α-KG produced from GDH is then sequentially metabolized resulting in HCO_3^- production. α-KG is metabolized through the enzymes α-KG-dehydrogenase and succinate dehydrogenase to form oxaloacetic acid (OAA). OAA then serves as a substrate for PEPCK to form PEP, which can then be utilized for gluconeogenesis (157). Thus, ammoniagenesis results in renal gluconeogenesis and renal gluconeogenesis is increased by many conditions which increase ammoniagenesis, including metabolic acidosis, respiratory acidosis, and hypokalemia (4).

Glutamate can also be converted back to glutamine via the enzyme glutamine synthetase. Because this reaction utilizes NH_4^+ as a cosubstrate, it results in decreased net NH_4^+ formation. Glutamine synthetase is expressed primarily in the proximal tubule of the rat and mouse (23, 130). Metabolic acidosis in the rat decreases glutamine synthetase activity (95), but not protein expression (161), and in the mouse decreases both activity and expression (15, 29, 92, 93).

Glutamine can also be metabolized by γ-glutamyl transpeptidase (γ-GT), also known as phosphate-independent glutaminase, although this is unlikely to be a major mechanism of renal ammoniagenesis. The glutamate resulting from γ-GT-dependent glutamine metabolism is then transported into the cell, where it can undergo further metabolism through GDH, resulting in further NH^{4+} production. Another pathway involves sequential metabolism through glutamine ketoacid aminotransferase and ω-amidase, forming α-KG and releasing NH_4^+. Quantitative assessments suggest this pathway does not contribute substantially to net ammoniagenesis either under basal conditions or in response to either acute or chronic metabolic acidosis (159).

Glutamine Transport in Ammoniagenesis

Glutamine is the primary metabolic source for renal ammoniagenesis (161,166). Under conditions of normal acid-base balance, renal glutamine uptake is low, less than 3% of delivered glutamine. During acute metabolic acidosis there is a rapid, approximately 2×-fold increase in plasma glutamine levels, primarily from increased skeletal muscle glutamine release (161). There is a parallel increase in renal glutamine uptake, to approximately 20% of delivered glutamine (68, 161). With chronic metabolic acidosis, renal glutamine uptake increases substantially, to as much as 50% of delivered glutamine, even though plasma glutamine levels often decrease (68). Increased renal glutamine utilization is balanced by increased skeletal muscle and liver glutamine release (157, 161). As will be discussed below, renal glutamine uptake involves both apical and basolateral transport processes.

Plasma glutamine is effectively 100% filtered by the glomerulus and the filtered glutamine is almost completely reabsorbed by the proximal convoluted tubules (145). The mechanism(s) of proximal tubule glutamine transport remain incompletely characterized, and there is a degree of contradictory evidence about the specific proteins involved in this process. Apical glutamine transport, when assessed in brush border membrane vesicles, is Na^+ dependent and has inhibitor characteristics similar to those identified for SNAT3/SN1 (SLC38A3) (76) and SNAT3 has been identified by immunoblot analysis in brush border membrane vesicles (76). However, immunohistochemistry has only identified basolateral SNAT3 immunoreactivity in the proximal tubule (25). Thus, there is conflicting evidence regarding SNAT3's role in apical glutamine transport. Other glutamine transporters expressed in the apical membrane in the proximal tubule include the Na^+-dependent neutral amino acid transporters B^0AT1 (SLC6A19) and B^0AT3 (SLC6A18). Under basal conditions, relatively little of the glutamine reabsorbed in the proximal tubule is metabolized, and instead is transported across the basolateral membrane into the peritubular space, resulting in net glutamine reabsorption. The mechanism of basolateral glutamine exit has not been well characterized, but may involve the y^+L amino acid-transport system.

Whether apical uptake of glutamine is enhanced during acidosis is not clear. Some studies have found that chronic metabolic acidosis increases both apical glutamine transport and SNAT3 protein expression in brush border membrane vesicles (76). Other studies have found no change in apical Na^+-dependent glutamine transport (186). Chronic metabolic acidosis does not alter expression of the other apical glutamine transporters, B^0AT1 (SLC6A19) and B^0AT3 (SLC6A18) (107). However, because filtered glutamine is essentially completely reabsorbed in the proximal tubule under basal conditions, increased rates of apical glutamine transport with metabolic acidosis do not increase the net amount of uptake.

Basolateral glutamine uptake is also present. Mechanistically, basolateral glutamine uptake appears to be mediated by SNAT3 expression, at least in the proximal tubule S3 segment under basal conditions and throughout the entire proximal tubule in response to metabolic acidosis (76, 153). Basolateral glutamine uptake by proximal tubule cells is regulated by a variety of conditions. Both metabolic and respiratory acidosis increases basolateral glutamine uptake (76, 186, 187). Immunohistochemistry shows that multiple conditions that

increase ammoniagenesis, that is, chronic metabolic acidosis, hypokalemia and high protein intake, also increase basolateral SNAT3 immunolabel in the S3 proximal tubule, and also induce detectable expression in the basolateral plasma membrane in the proximal tubule S1 and S2 segments (25, 107). Increased basolateral SNAT3 expression presumably mediates increased glutamine uptake from the peritubular fluid, enabling increased ammoniagenesis and explaining the observation that renal glutamine extraction can exceed glutamine filtration.

Multiple other transporters may also contribute to glutamine transport, but their quantitative contribution is probably less. A system ASC glutamine transporter is present, at least in the renal cortex, and may contribute to glutamine transport under basal conditions (76). The basolateral y^+L exchange transport system, which is formed by y^+-LAT1 (SLC7A7) and 4F2hc subunits, is present in the basolateral plasma membrane of proximal tubule cells (17, 127) and mediates Na^+-dependent glutamine uptake and cationic amino acid efflux. Metabolic acidosis decreases y^+-LAT1 expression, but does not alter 4F2hc protein expression (107). If this results in decreased transport activity, it would be predicted to decrease y^+L-mediated glutamine uptake, and thus would not contribute to increased glutamine uptake in the proximal tubule. However, this would decrease y^+L-mediated arginine basolateral export, which could increase arginine-dependent ammoniagenesis.

Because the initial enzymatic step in ammoniagenesis involves the inner mitochondrial enzyme, PDG, glutamine transport into mitochondria is a critical step. Conditions associated with increased ammoniagenesis and with increased urinary ammonia excretion, such as metabolic acidosis, hypokalemia, and high-protein diets, stimulate mitochondrial glutamine uptake (1, 3, 46). Mitochondrial glutamine uptake involves a specific transporter-mediated mechanism (1, 46, 133, 151), which may have an apparent molecular weight of 41.5 kDa (71). The specific molecular identity of this transporter, however, has not been identified.

Regulation of Ammoniagenesis

Multiple conditions stimulate renal ammoniagenesis. Both acute and chronic metabolic acidosis stimulate ammoniagenesis (159); this involves increases in the PDG pathway. Although acute respiratory acidosis stimulates ammoniagenesis, the effect of chronic respiratory acidosis is unclear as different studies have found either no change or increased ammoniagenesis (158). Chronic metabolic acidosis is also associated with increases in PDG, GDH, and PEPCK expression and activity (159), all of which contribute to increased ammoniagenesis. Because changes in extracellular cell pH cause parallel changes in mitochondrial pH, these changes are generally thought to be an important mechanism in the regulation of ammoniagenesis (117, 135). In addition, a separate, pH-independent mechanism involving a small, nondialyzable factor may also regulate ammoniagenesis (5). Many pH sensors, including GPR4, InsR-RR, Pyk2, Erb1/2, pH sensitive ion channels, such as acid-sensing ion channel (ASIC), Tandem of P domains in a Weak Inward rectifying K^+ channel (TWIK)-related acid-sensitive K^+ channel (TASK), and Rat Outer Medullary K^+ (ROMK), and bicarbonate-stimulated adenylyl cyclase, have been identified over the past few years (21). Studies examining whether any of these directly regulate renal ammoniagenesis have not been reported to date.

Chronic hypokalemia increases ammoniagenesis through mechanisms involving increased enzyme expression and activity similar to those identified for chronic metabolic acidosis. Acute changes in extracellular K^+ also regulate proximal tubule ammoniagenesis; these effects are directly mediated by extracellular K^+ and, because they can be observed within 45 min of altering extracellular K^+, likely do not require changes in protein expression (113). How hypokalemia signals the proximal tubule to increase ammoniagenesis is incompletely understood. *In vivo* studies, measuring intracellular pH with ^{31}P-NMR, have shown hypokalemia causes intracellular acidification, which could explain the stimulation of ammoniagenesis (2). However, *in vitro* studies demonstrated that hypokalemia induces only transient intracellular acidification, maximal at approximately 27 min, that reverts to no change in intracellular pH by 8 h (8). Moreover, dietary K^+ deficiency increases both urinary ammonia excretion and expression of ammoniagenic enzymes prior to detectable changes in extracellular K^+ concentration, suggesting that factors other than extracellular K^+ concentration regulate ammoniagenesis in response to K^+ deficiency (67). In particular, in view of evidence that changes in dietary K^+ intake alter renal K^+ excretion prior to detectable changes in serum K^+ (67) and that gastrointestinal tract-derived factors can regulate renal K^+ excretion (91), it is intriguing to speculate that intestinal tract-derived factors might regulate renal ammonia excretion in response to changes in K^+ intake.

Because hypokalemia-induced increases in ammonia excretion often induces an acid-base disorder, metabolic alkalosis, rather than protecting against extracellular pH changes, other physiologic benefits of changes in ammonia metabolism have been considered. One possibility is that ammonia regulates renal potassium transport, that is, that it serves as a signaling molecule regulating K^+ transport. Studies in both humans and rodents have shown that stimulating ammoniagenesis, by administering exogenous glutamine, simultaneously increases urinary ammonia excretion and decreases potassium excretion, and that the time course of changes are similar (72, 134, 160). Because changes in K^+ excretion occur despite no significant change in extracellular K^+ concentration, these findings suggest changes in ammonia metabolism and/or transport can regulate renal K^+ transport. These effects on K^+ transport have been localized to sites distal to the late micropuncturable tubule, suggesting regulation of collecting duct K^+ transport (72). *In vitro* microperfusion studies show ammonia decreases both net and unidirectional K^+ secretion in the collecting duct (54), and that ammonia stimulates H^+-K^+-ATPase-mediated H^+ secretion (43). These effects of ammonia on H^+-K^+-ATPase occur independent of changes in intracellular pH and involve microtubule formation, intracellular calcium, and SNARE-protein-mediated vesicular trafficking (41-43). Thus, changes in proximal tubule ammoniagenesis in response to hypokalemia may facilitate changes in intrarenal ammonia concentrations, which then function as signaling molecules to regulate collecting duct K^+ transport and net renal K^+ excretion.

Ammoniagenesis is also modulated through mechanisms independent of acid base and potassium. These include tricarboxylic acid cycle intermediates, prostaglandin F2 alpha, insulin, growth hormone, angiotensin II (Ang II), corticosteroids, aldosterone, and tubular flow rate (159). Ang II likely mediates an important role regulating ammoniagenesis, with evidence that both luminal and peritubular Ang II stimulate ammonia production rates, but

that more of the ammonia that is produced enters the lumen with luminal Ang II while more exits across the basolateral membrane with peritubular Ang II (114).

Ammonia Transport

Proximal tubule

Ammonia produced in the proximal tubule is secreted preferentially into the tubule lumen, although there is some transport across the basolateral plasma membrane. Preferential apical secretion dominates due to multiple factors, including luminal acidification which binds to secreted NH_3, forming NH_4^+ and decreasing luminal NH_3 concentration, thereby maintaining the gradient for NH_3secretion, and apical NHE-3-mediated Na^+/NH_4^+ exchange (112,149). An apical Ba^{+2}-sensitive K^+ channel and a diffusive NH_3-transport component also appear to be involved in ammonia secretion (57, 149). H^+ secretion via H^+-ATPase as well as by NHE-3 titrates this secreted $NH_3$to form NH_4^+. Figure 7 summarizes the model of our current understanding of proximal tubule ammonia secretion.

The proximal tubule also can reabsorb luminal ammonia; this appears to occur primarily in the late proximal tubule (56). The proximal tubule expresses glutamine synthetase, which catalyzes the reaction of NH_4^+ with glutamate to form glutamine (23). Metabolic acidosis converts late proximal tubule ammonia transport from net reabsorption to net secretion (56). The molecular mechanism underlying this conversion includes, at least in part, decreased glutamine synthetase-mediated reaction of NH_4^+ with glutamate to form glutamine (29,129). Because this glutamine synthetase-accelerated reaction consumes NH_4^+ decreased glutamine synthetase activity results in increased net ammonia production.

Loop of Henle

The thick ascending limb of the loop of Henle (TAL) is an important site for reabsorbing luminal ammonia. The apical Na^+-K^+-$2Cl^-$ cotransporter, NKCC2, transports NH_4^+ at the K^+-binding site, and is the primary protein responsible for ammonia reabsorption (10). The relatively low permeability of the TAL apical plasma membrane to NH_3 relative to its permeability to NH_4^+ is critical in preventing back leak of NH_3(78). Ammonia exit across the basolateral plasma membrane involves basolateral Na^+/NH_4^+ exchange mediated by NHE-4 (19). Although NHE-1 is also present in the TAL basolateral plasma membrane (27), pharmacologic inhibition studies do not support a significant role for NHE-1 in TAL ammonia reabsorption (19). A second mechanism of basolateral ammonia transport may involve dissociation of cytosolic NH_4^+ to NH_3 and H^+, with basolateral NH_3 exit and buffering of the intracellular H^+ load by basolateral bicarbonate uptake mediated by the NBCn1 cotransporter (87, 94, 125). Finally, there is a Cl^--dependent NH_4^+-transport mechanism, likely in the basolateral membrane and likely to be mediated by one of the isoforms of the KCC family (9). Figure 8 summarizes this model of TAL ammonia reabsorption.

Some of the ammonia absorbed by the medullary thick ascending limb (mTAL) of the loop of Henle undergoes recycling into the descending thin limb of the loop of Henle (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 (39). The molecular mechanisms of DTL NH_3 and NH_4^+ transport have not been well characterized to date.

Ammonia reabsorption by the thick ascending limb of the loop of Henle has two important consequences. First, passive ammonia secretion into the thin descending limb of the loop of Henle causes development of axial ammonia concentration gradient in the medullary interstitium that parallels the hypertonicity gradient. Second, ammonia absorption by the mTAL results in ammonia delivery to the distal tubule accounting for only approximately 20% to 40% of final urinary ammonia content (34, 55). Consequently, urinary ammonia excretion requires a major component of ammonia secretion at sites distal to the loop of Henle, that is, distal convolute tubule, connecting segment and the collecting duct.

Distal convoluted tubule and connecting segment

A small component of ammonia secretion occurs in the regions of the distal tubule prior to the collecting duct, that is, the DCT and CNT. Micropuncture studies in the rat kidney have generally shown net ammonia secretion between the early and late portions of the micropuncturable distal tubule, accounting for approximately 10% to 15% of total urinary ammonia excretion under basal conditions (147, 185). The specific mechanism of ammonia transport in these segments has not been specifically determined, but in view of multiple similarities with the collecting duct in the expression of H^+ and ammonia transporters it is likely that similar mechanisms are utilized.

Collecting duct

Ammonia secretion by the collecting duct appears to account for the majority, 60% to 80%, of urinary ammonia content. Mechanistically, collecting duct ammonia secretion involves parallel NH_3 and H^+ secretion, and there is no significant component of transepithelial NH_4^+ transport (34, 83). H^+ secretion likely involves both H^+-ATPase- and H^+-K^+-ATPase-mediated transport. Excellent reviews of H^+-ATPase and H^+-K^+-ATPase are available for the interested reader (53, 165). Our current model of collecting duct ammonia transport involves the integrated action of a large number of proteins (Fig. 9). These include proteins involved in basolateral ammonia uptake (Rhbg, Rhcg, and Na^+-K^+-ATPase), cytosolic H^+ production (carbonic anhydrase II), apical ammonia transport (Rhcg), apical H^+ secretion (H^+-ATPase, H^+-K^+-ATPase), and basolateral HCO_3^- transport (Cl^-/HCO_3^- exchange).

NH_3 movement across collecting duct cell apical and basolateral membranes appears to involve both diffusion- and transporter-mediated NH_3 transport. The transporter-mediated component of NH_3 is Na^+ and K^+ independent and is electroneutral (62, 63). Studies using cultured collecting duct cells show that transporter-mediated NH_3 movement is the predominant route of ammonia transport across both the apical and basolateral plasma membranes at the ammonia concentrations present in the cortex and outer medulla (62, 63).

The Rh glycoprotein, Rhcg, mediates a critical role in renal ammonia excretion. Rhcg is an ammonia-specific transporter, with no identifiable affinity for solutes other than ammonia and its methyl-derivative, methylammonia (13, 102, 106, 108). Rhcg is expressed in the DCT, CNT, initial collecting tubule (ICT), CCD, OMCD, and IMCD (35, 58, 167). Rhcg is expressed both in intercalated cells and in principal cells, DCT cells and CNT cells in these

tubule segments, with the exception that it is not expressed in non-intercalated cells in the IMCD or in the B-type intercalated cell (58, 167). In the human kidney, Rhcg is expressed in the same distribution, with the exception that principal cells lack Rhcg expression (58). Although originally Rhcg expression was only detected in the apical plasma membrane, more recent studies have shown both apical and basolateral Rhcg expression (20, 58, 80, 141, 142).

A variety of studies, using mice with intact Rhcg expression and with global and cell-specific *Rhcg* gene deletions, have shown Rhcg's essential role in renal ammonia excretion. Metabolic acidosis stimulates Rhcg expression in the cortex and the outer medulla, and it induces changes in Rhcg's subcellular distribution, with decreased relative intracellular expression and increased expression in the apical plasma membrane (92, 141, 142). Gene-deletion studies, utilizing both global and collecting duct-specific Rhcg deletion, decrease basal urinary ammonia excretion, and impair the normal increase in urinary ammonia excretion in response to metabolic acidosis (16, 92); Rhcg deletion only from intercalated cells does not alter net basal ammonia excretion, but does significantly inhibit ammonia excretion in response to metabolic acidosis (93).

Studies using *in vitro* microperfused collecting duct segments have also shown that Rhcg has a critical role in ammonia secretion. These studies examined cortical and OMCD segments from mice with chronic metabolic acidosis and found global Rhcg deletion decreases apical NH_3 permeability by 60% to 70% (16). The mechanism of the remaining apical NH_3 permeability was not determined, but studies using cultured collecting duct cells have shown that apical ammonia permeability involves components of both transporter-mediated, likely via Rhcg, and diffusive NH_3 movement (63).

Rhbg is another member of the Rh glycoprotein family which, like Rhcg, is an ammonia-specific transporter. The majority of studies examining the molecular form of ammonia transported by Rhbg have identified NH_3 transport (101, 102, 194), but other studies have identified NH_4^+ transport (120-122). Rhbg is expressed in the same cells as Rhcg, with the significant difference that Rhbg has only basolateral expression (128, 167). Whether Rhbg expression is necessary for renal ammonia excretion has been the subject of two separate studies. An initial report using mice with global Rhbg deletion did not find altered ammonia excretion either under basal conditions or after acid loading (26). In contrast, a more recent study using a different model of acid loading, producing greater stimulation of renal ammonia excretion, found that intercalated cell-specific Rhbg deletion inhibited ammonia excretion (15).

A second mechanism of basolateral ammonia uptake involves basolateral Na^+-K^+-ATPase. In the IMCD, basolateral Na^+-K^+-ATPase has been shown to contribute to basolateral NH_4^+ uptake (169, 174). Intracellular NH_4^+ dissociates to H^+ and NH_3, which are then secreted across the apical plasma membrane. However, in the CCD basolateral Na^+-K^+-ATPase does not contribute to ammonia secretion (84), and its role in the OMCD has not been reported.

Carbonic anhydrase has several important roles in collecting duct ammonia secretion. First, cytosolic carbonic anhydrase activity is necessary for ammonia secretion, probably through

a role in supplying cytosolic H^+ for secretion (170). Second, the presence or absence of luminal carbonic anhydrase activity, mediated by apical carbonic anhydrase IV, has an important impact on the rate of collecting duct ammonia secretion. In the absence of luminal carbonic anhydrase activity, the luminal [H^+] increases above equilibrium levels due to delayed dissociation of H_2CO_3; this is termed a luminal "disequilibrium pH." Because the $H^+ + NH_3 \leftrightarrow NH_4^+$ reaction occurs rapidly, this shifts the ammonia buffer reaction toward NH_4^+ decreasing the luminal NH_3 concentration and thereby increasing the transepithelial gradient for NH_3 secretion. The importance of this is underscored by experiments showing that addition of carbonic anhydrase to the luminal fluid reduces ammonia secretion (85). Detailed studies of the collecting duct have demonstrated a luminal disequilibrium pH in the rat in the inner stripe of the OMCD and the terminal IMCD (40, 172), and in the rabbit in the CCD (84, 156) and outer stripe of OMCD (155), but not in the inner stripe of OMCD (155).

Specific proteins involved in renal ammonia metabolism

Phosphate-dependent glutaminase

PDG is the initial enzymatic step in renal ammoniagenesis. It is located in mitochondria and catalyzes the reaction, L-glutamine + $H_2O \rightarrow$ L-glutamate$^-$ + NH_4^+. In the kidney, PDG activity has been localized to the proximal straight and convoluted tubules (32, 77). However, enzymatic assays have also identified PDG activity in many other sites, including the descending thin limb of the loop of Henle (DTL), mTAL, DCT, and throughout the collecting duct (32,77,190). Whether activity in these sites is stimulated by conditions associated with increased ammonia excretion is unclear. Some studies have found that metabolic acidosis increases PDG activity in the DTL, mTAL in the outer stripe, and DCT (77), while others have found no change in activity in sites other than the proximal tubule (190).

PDG is also present in many extrarenal tissues, including liver and brain. In humans, the gene for the kidney-type isoform has 19 exons, and gives rise to at least two transcripts, a KGA mRNA resulting from joining exons 1-14 and 16-19 and GAC mRNA product which uses only exons 1-15 (104). A separate gene gives rise to an LGA isoform. The KGA protein is ubiquitously expressed, including the renal proximal tubule. The GAC isoform is expressed primarily in the heart and pancreas, at least based on mRNA expression, and the LGA isoform is widely expressed, including liver, brain, pancreas and breast cancer (104). The KGA isoform is increased in response to metabolic acidosis and appears to be the source of the majority of renal PDG activity.

Metabolic acidosis increases proximal tubule PDG activity; these increases derive from multiple mechanisms. There is increased protein expression, and this appears due to increased mRNA expression (162, 163). Increased PDG mRNA results from stabilization of the mRNA product, and does not involve increased transcription rates (69). A direct repeat of an 8-nt AU sequence functions as a pH-response element, and is both necessary and sufficient to generate pH-response gene stabilization. This response element binds multiple RNA-binding proteins, including ζ-crystallin, AU-factor 1 and HuR. Acidosis also causes an endoplasmic reticulum stress response that causes formation of cytoplasmic stress granules, ζ-crystallin undergoes transient movement to stress granules and simultaneously HuR is

translocated from the nucleus to the cytoplasm (70). A second regulatory mechanism likely involves changes in intramitochondrial glutamate. Glutamate is a competitive inhibitor of PDG (143), and decreases in intramitochondrial glutamate concentration, as can occur during metabolic acidosis, result in increased PDG activity (4).

Glutamate dehydrogenase

GDH is a mitochondrial enzyme that catalyzes the reaction, L-glutamate^{-} + H_2O + NAD^+ (or $NADP^+$) → α-KG^{-2}+ NH_4^+ + NADH (or NADPH) + H^+. Two GDH isoforms exist, and are products of two different genes; GLUD1 is widely expressed, whereas GLUD2 appears to be a neural and testicular specific isoform (105,144). GDH activity is high in liver, brain, kidney, heart, pancreas, ovaries, and testis in addition to the kidney. Brain and testicular GDH reflects products of GLUD1 and GLUD2, whereas in most other tissues, including the kidney, GDH is a product of the GLUD1 mRNA.

Metabolic acidosis stimulates renal GDH activity (189), both by altering its affinity for glutamate (4) and by increasing protein (33) and mRNA (GLUD1) expression (31). GDH activity is increased in part through metabolic acidosis-induced decreases in intramitochondrial α-KG concentration (143). This decrease in α-KG both accelerates GDH activity, by relieving α-KG-mediated competitive inhibition of the enzymatic reaction, and it inhibits the reverse reaction (136, 137). The increased mRNA expression appears related to increased mRNA stability, not transcription (75). Four AU-rich elements are present in the 3′-UTR, bind ζ-cystallin and appear to confer pH-responsive stabilization of GDH mRNA (138).

Phosphoenolpyruvate carboxykinase

Renal PEPCK is a cytosolic enzyme and is the product of the *PCK1* gene. In the kidney, as in extrarenal sites, including liver, adipose tissue, and small intestine, PEPCK is a key enzyme in gluconeogenesis through its role in conversion of oxaloacetate into PEP and HCO_3^-. It also mediates an important role in the renal response to metabolic acidosis (24) by contributing to renal bicarbonate synthesis and by promoting ammoniagenesis by degrading α-KG, an end product of glutamine degradation. The adaptive increase in PEPCK activity and protein expression results from increased protein synthesis and mRNA expression (31). Increased PEPCK mRNA expression appears to result both from increased gene transcription and from mRNA stabilization (65,109). Acidosis-induced increases in gene transcription appear to involve binding to the P3(II) and CRE-1 elements in the PCK1 promoter (31). The 3'-nontranslated region of the PEPCK mRNA also contains an instability element that facilitates its rapid turnover and contributes to the regulation of *PEPCK* gene expression (90). Metabolic acidosis also stimulates *PEPCK* gene expression through p38 MAPK phosphorylation of ATF-2, which binds to the CRE-1 element and either recruits or interacts with additional transcription factors that activate gene transcription (31).

γ-GT

γ-GT accounts for phosphate-independent glutaminase activity identified in many early enzymatic studies of renal ammoniagenesis. Its quantitative role, however, in ammoniagenesis remains unclear. γ-GT is primarily expressed in the proximal straight

tubule (32), and micropuncture studies suggest that glutamine is completely reabsorbed by the PCT, suggesting PST ammoniagenesis via γ-GT is unlikely to contribute significantly to renal ammoniagenesis. Studies examining the late proximal convoluted tubule have shown that γ-GT activity contributes only a minor component of total ammoniagenesis (150), and chronic metabolic acidosis does not alter γ-GT activity (32). However, although filtered glutamine is essentially entirely reabsorbed prior to reaching the downstream γ-GT-containing proximal tubule segments, there is evidence that glutamine may back flux into the lumen and support ammonia production via this enzyme (146, 182).

NHE-3

The majority of current evidence suggests that the apical Na^+/H^+ exchanger, NHE-3, mediates a substantial component of proximal tubule ammonia secretion, through substitution of NH_4^+ for H^+, resulting in functional Na^+/NH_4^+ exchange activity. This data include evidence that proximal tubule brush border membrane vesicles exhibit NH_4^+/Na^+ exchange activity (82), that combining a low luminal Na^+ concentration with Na^+/H^+ exchange inhibitor, amiloride, decreases ammonia secretion (111) and that the Na^+/H^+ exchange inhibitor, 5-(N-Ethyl-N-isopropyl)amiloride (EIPA), blunts ammonia secretion when alternative secretory pathways are blocked (149). Although NHE-3 is the predominant apical Na^+/H^+ exchanger in the proximal tubule, direct studies examining the effect of proximal tubule-specific NHE-3 deletion are necessary to confirm its central role in Na^+/NH_4^+ exchange activity. In particular, some studies suggest that facilitated NH_3 transport in parallel with H^+ secretion is a primary mechanism of proximal tubule ammonia secretion (148) and that a Ba^{2+}-sensitive transporter, most likely an apical K^+ channel, also contributes to NH_4^+ secretion (149).

Nonetheless, NHE-3 appears to be important in the regulation of proximal tubule ammonia transport. In response to chronic metabolic acidosis, changes in extracellular potassium and Ang II, changes in NHE-3 expression, and activity parallel changes in ammonia secretion (6, 37, 117). In both the S2 and S3 segments, chronic metabolic acidosis increases AT1 receptor-mediated stimulation of NHE-3 (115,116,118). Other studies show that increased endothelin-1 expression with subsequent activation of the endothelin-B receptor mediates an important role in increasing NHE-3 expression and renal ammonia excretion in metabolic acidosis (88).

Apical NHE-3 is also present in the TAL (7). However, since this transporter secretes NH_4^+ and the TAL reabsorbs NH_4^+ NHE-3 is unlikely to mediate an important role in TAL ammonia transport.

Potassium channels

Although molecular K^+ and NH_4^+ have different molecular weights and atomic radii (243 pm for K^+ and 137 pm for NH_4^+), hydration in aqueous solutions alters these characteristics, such that they have nearly identical hydrodynamic radii (180). When examined, almost every K^+ transporter examined also transports NH_4^+; in general, the relative rate of NH_4^+ transport is 10% to 20% of that observed for K^+ (177). The primary evidence that K^+ channels contribute to proximal tubule ammonia transport comes from *in vitro*

microperfusion studies showing that barium, a nonspecific K^+ channel inhibitor, can inhibit proximal tubule ammonia transport (149). Multiple K^+ channels are present in the apical membrane of the proximal tubule, including KCNA10, KCNQ1/KCNE1, and TWIK-1; which of these mediate ammonia transport is not currently known.

In the TAL, apical K^+ channels are present and can contribute to luminal NH_4^+ uptake when apical Na^+-K^+-$2Cl^-$ cotransport is inhibited. Quantitatively, they may mediate 14% to 30% of luminal NH_4^+ uptake, with the exact proportion dependent on luminal total ammonia concentration and on the specific assay used to quantify apical NH_4^+ transport (175). However, NKCC2 inhibitors almost completely inhibit TAL transepithelial ammonia reabsorption (51, 175), suggesting that apical K^+ channels are unlikely to mediate a quantitatively important role in TAL ammonia reabsorption.

Na^+-K^+-$2Cl^-$ cotransport

The Na^+-K^+-$2Cl^-$-cotransporters are widely expressed proteins that transport Na^+, K^+ and2 Cl^- ions in an electroneutral manner across plasma membranes. Two isoforms have been identified, NKCC-1 (SLC12A1) and NKCC-2 (SLC12A2). They have distinct expression patterns and differing roles in renal ammonia transport.

NKCC1 is a widely expressed isoform that in the kidney is present in the basolateral membrane of A-type intercalated cells in the outer and IMCD cells (45). However, addition of bumetanide, an NKCC1/NKCC2 inhibitor, to the peritubular solution does not alter OMCD ammonia secretion (170). In the IMCD, NH_4^+ and K^+ compete for a common binding site on NKCC1, but inhibiting NKCC1 does not alter peritubular NH_4^+ uptake significantly (169). Thus, NKCC1 appears unlikely to mediate a substantial role in renal ammonia secretion.

NKCC-2 is a kidney-specific isoform expressed selectively in the apical plasma membrane of the TAL. It is the major mechanism of ammonia reabsorption across the TAL apical membrane through coupled NH_4^+ Na^+, and 2 Cl^- transport (48, 175). Competition of luminal NH_4^+ with K^+ for the K^+-transport site has several significant implications. Most importantly, changes in luminal K^+ in hypokalemia and hyperkalemia alter net NH_4^+ transport and thus medullary interstitial ammonia concentration, which likely alters collecting duct ammonia secretion and thus net renal ammonia excretion. Transport of NH_4^+ at the K^+-binding site may also contribute to TAL NaCl transport, particularly in forms of Bartter's syndrome where abnormal ROMK expression prevents K^+ recycling (181). Metabolic acidosis increases TAL ammonia reabsorption (47) through transcriptionally mediated increases in NKCC2 expression (11) due to metabolic acidosis-induced elevation in systemic glucocorticoids (12).

Na^+-K^+-ATPase

Na^+-K^+-ATPase is a P-type ATPase present in the basolateral plasma membrane of renal epithelial cells, and its expression is greatest in the mTAL of the loop of Henle, with lesser expression in the cortical thick ascending limb, DCT, CCD, MCD, and the proximal tubule (74). NH_4^+ competes with K^+ at the K^+-binding site of Na^+-K^+-ATPase, enabling net Na^+-NH_4^+ exchange (86, 173). However, the relative affinities of Na^+-K^+-ATPase for NH_4^+

approximately 11 mmol/L, and K^+, approximately 1.9 mmol/L, have important implications for Na^+-K^+-ATPase-mediated NH_4^+ transport (86). In the cortex, interstitial ammonia concentrations are less than 1 mmol/L, suggesting NH_4^+ is unlikely to be transported to a significant extent by basolateral Na^+-K^+-ATPase. In addition, even in the presence of high concentrations of peritubular ammonia, Na^+-K^+-ATPase inhibitors do alter CCD ammonia secretion (84). 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 (86, 173). In hypokalemia, the decreased interstitial K^+ concentration, by facilitating increased NH_4^+ uptake by Na^+-K^+-ATPase, enables increased rates of NH_4^+ secretion despite an absence of changes in Na^+-K^+-ATPase expression (171).

H^+-K^+-ATPase

H^+-K^+-ATPase proteins are other members of the P-type ATPase family that transport NH_4^+. Potassium deficiency increases expression of the $HK\alpha_1$ and $HK\alpha_2$ isoforms of H^+-K^+-ATPase (53). This has been postulated to mediate increased NH_4^+ secretion via binding of NH_4^+ to and transport at the H^+-binding site (119). Other studies, however, have suggested that NH_4^+ binds to and is transported at the K^+-binding site (44). Additional studies are needed to define more clearly the specific role(s) of H^+-K^+-ATPase in collecting duct ammonia secretion.

In addition to being a substrate for H^+-K^+-ATPase transport, ammonia also appears to regulate H^+-K^+-ATPase H^+ secretion. In studies examining *in vitro* microperfused CCD segments, ammonia causes concentration-dependent stimulation of net H^+-K^+-ATPase, but not H^+-ATPase-mediated, H^+ secretion (43). In the OMCD, ammonia may stimulate net H^+ secretion by as much as approximately 50% (40). Ammonia's stimulation of CCD H^+-K^+-ATPase is independent of changes in intracellular pH and involves changes in intracellular calcium, microtubules and SNARE protein-mediated vesicle fusion (41-43). This stimulation of H^+-K^+-ATPase activity, by increasing unidirectional K^+ reabsorption, likely contributes to ammonia's ability to regulate CCD net K^+ secretion (54).

$K^+/NH_4^+(H^+)$ exchange

An electroneutral, Ba^{+2}- and verapamil-inhibitable apical K^+/NH_4^+ (H^+) activity is present in the apical membrane of the TAL (10). 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 transepithelial TAL ammonia transport (51, 175) suggests that K^+ for NH_4^+ exchange activity may not mediate a major role in transepithelial TAL ammonia reabsorption.

Aquaporins

The aquaporins belong to an extended family of proteins that facilitate water transport. Because both H_2O and NH_3 have similar molecular sizes and charge distribution, several studies have examined the role of aquaporins in NH_3 transport. Importantly, many, but not all aquaporins transport ammonia (110).

AQP1 was the first aquaporin shown to transport ammonia. Studies using AQP1 expression in Xenopus oocytes demonstrated AQP1 expression increases NH_3 transport (110,123). However, not all studies have confirmed NH_3 transport by AQP1 (66). AQP1 is present in the descending thin limb of the loop of Henle, suggesting it may contribute to ammonia permeability in this segment. It is also present in both the apical and basolateral plasma membranes in the proximal tubule; whether it contributes to proximal tubule, ammonia transport has not been determined experimentally, but it is certainly ideally located to mediate a component of the Ba^{+2}- and NHE-3-independent apical NH_3 secretion.

AQP3 is present in the basolateral plasma membrane of collecting duct principal cells and transports NH_3, when expressed in the Xenopus oocytes, transports NH_3 (66). Whether AQP3 contributes to renal principal cell basolateral NH_3 transport has not been determined.

AQP8 is expressed in intracellular sites in the proximal tubule, CCD and OMCD in the kidney, but not in the plasma membrane (38). AQP8's specific intracellular site in mammalian cells has not been determined, but it localizes to the inner mitochondrial membrane when expressed in yeast (154). AQP8's role in renal ammonia metabolism is unclear. Genetic deletion slightly decreases hepatic and increases renal ammonia concentrations, but does not alter systemic pH, serum chloride concentration, urine ammonia concentration or urine pH either under basal conditions or in response to acid loading (191, 192). Thus, AQP8 does not appear to mediate a major role in renal ammonia transport.

Carbonic anhydrase

Carbonic anhydrase, in addition to its role in bicarbonate reabsorption, also contributes to ammonia secretion. Studies examining isolated perfused OMCD segments have shown that carbonic anhydrase inhibition, presumably through effects on CA II, block ammonia secretion (170).

The membrane-bound isoform, CA IV, likely contributes to the regulation of both bicarbonate reabsorption and ammonia secretion. Collecting duct CA IV, although functioning to increase bicarbonate reabsorption, likely decreases collecting duct ammonia secretion by preventing a luminal disequilibrium pH. Apical CA IV expression has been demonstrated in the rabbit CCD-type A intercalated cell, in the rabbit OMCD and IMCD (140), and in the human CCD and OMCD (99), but has not been found in the rat collecting duct (22). This pattern is consistent with the evidence of luminal disequilibrium pH in the rabbit CCD and OMCD outer stripe segments (155, 156). Whether collecting duct CA IV expression is regulated by physiologic stimuli has not been rigorously examined. Studies examining tissue homogenates have identified that metabolic acidosis increases CA IV mRNA expression (164,188). This observation is consistent with the observation of basolateral CA IV expression in the proximal tubule, where it may contribute to bicarbonate reabsorption (139).

Rh glycoproteins

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, 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 (103, 131, 183, 184). Because studies in human (176) and mouse (unpublished observations) kidneys have found no evidence of renal RhAG expression, RhAG/Rhag appears unlikely to contribute to renal ammonia metabolism.

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 (61, 64, 98, 128, 167, 178). The kidneys express basolateral Rhbg immunoreactivity in the DCT, CNT, ICT, CCD, OMCD, and IMCD (128, 167). In general, both intercalated and principal cells express Rhbg, with greater expression in intercalated cells than in principal cells. The exceptions to this rule are the IMCD, where only intercalated cells express Rhbg, and the B-type intercalated cell, which does not express Rhbg detectable with immuno-histochemistry (167). Rhbg's basolateral expression appears due to basolateral stabilization through specific interactions of its cytoplasmic carboxy-terminus with ankyrin-G (100). Although, the human kidney expresses high amounts of RhBG mRNA (98), a recent study using a variety of antibodies did not identify detectable RhBG protein expression (20).

RhBG/Rhbg transports ammonia and the ammonia analogue, methylammonia, but does not appear to have other known substrates. Different studies have reached different conclusions regarding the exact ammonia species, NH_3 or NH_4^+ transported. Most studies have shown that Rhbg mediates electroneutral, Na^+- and K^+-independent, NH_3 transport (101, 102, 194), while other studies identified electrogenic NH_4^+ transport (122). Recent studies also indicated that Rhbg might transport both NH_3 and NH_4^+ (121). In all of these studies, the affinity for ammonia was approximately 2 to 4 mmol/L. The explanation underlying the seemingly differing molecular substrates transported in different studies is not evident at present. From a physiologic perspective, both electroneutral NH_3 transport and electrogenic NH_4^+ transport result in net basolateral ammonia uptake in renal collecting duct cells.

Rhbg's specific role in renal ammonia metabolism has been the subject of several studies. In the mouse, one study showed increased mRNA expression with metabolic acidosis, but the effect on Rhbg protein expression was not reported (28). In another study, genetic deletion of pendrin, an apical Cl^-/HCO^{3-} exchanger present in the B-type intercalated cell and non-A, non-B intercalated cell, decreased Rhbg expression (81). Since pendrin deletion increases urine acidification, the decreased Rhbg expression may have been a compensatory mechanism to maintain normal ammonia excretion. Genetic deletion of Rhcg from acid-loaded mice increased Rhbg expression, suggesting increased Rhbg protein expression may contribute to ammonia excretion under these conditions (92, 93).

Two studies have used mice with genetic deletion of Rhbg to determine Rhbg's role in renal ammonia transport. In the first, mice with global Rhbg deletion had normal basal acid-base

parameters, normal responses to acid loading with addition of NH_4Cl to the drinking water, and normal basolateral NH_3 and NH_4^+ transport in microperfused CCD segments (26). Studies examining mice with intercalated cell-specific Rhbg deletion, however, came to different conclusions. Mice with intercalated cell-specific Rhbg deletion that were acid loaded by addition of HCl to food had impaired ammonia excretion in response to acid loading (15). Moreover, although basal ammonia excretion was normal, adaptive responses to Rhbg deletion were identified that appeared to compensate for Rhbg's absence, indicating a role for Rhbg in basal ammonia excretion (15).

RhCG/Rhcg—RhCG/Rhcg is expressed in multiple ammonia transporting/metabolizing organs, including kidneys, CNS, testes, lung, liver, and throughout the gastrointestinal tract (61, 64, 97, 178). In the kidney, Rhcg expression parallels Rhbg's expression, that is, in the DCT, CNT, ICT, CCD, OMCD, and IMCD (35, 167). Intercalated cell expression exceeds principal cell expression in the DCT, CNT, ICT, CCD, and OMCD, and in the IMCD Rhcg expression has only been detected in intercalated cells (35, 167).

Rhcg's subcellular location differs from the exclusive basolateral location observed for Rhbg. In the human, rat, and mouse kidney, Rhcg immunoreactivity is both apical and basolateral (20, 58, 80, 141, 142). Immunogold electron microscopy has shown Rhcg immunoreactivity in the apical plasma membrane, basolateral plasma membrane and, additionally, in sub-apical vesicles (142). Basolateral Rhcg expression is approximately 20% of total cellular expression, at least in the rat OMCD in the inner stripe (80, 142). In the mouse there are substantial strain-specific variations in the extent of basolateral Rhcg expression, and these differences correlate with differing ability to excrete ammonia in response to acid loading (80, 179).

Multiple studies have addressed the molecular ammonia species transported by RhCG/Rhcg. Most studies examining heterologous expression of Rhcg have shown that Rhcg mediates electroneutral NH_3 transport (102,194), while others have reported NH_4^+ transport (13) or both NH_3 and NH_4^+ transport (personal communication, T Caner and NL Nakhoul). Studies examining purified Rhcg expression in liposomes have found that Rhcg transports NH_3, not NH_4^+ (52,108), and studies examining apical membrane NH_3 and NH_4^+ permeability using *in vitro* microperfused collecting duct segments have shown that Rhcg deletion decreases NH_3 permeability, and does not change NH_4^+ permeability (16).

Rhcg's expression parallels ammonia excretion in several conditions. Chronic metabolic acidosis significantly increases Rhcg protein expression in both the OMCD and the IMCD, but not in the cortex (141). These changes appear to occur through posttranscriptional mechanisms, as Rhcg steady-state mRNA expression does not significantly change (141). In response to reduced renal mass, where there is increased single nephron ammonia secretion, apical Rhcg expression increases in the CCD A-type intercalated cell, CCD principal cell, OMCD intercalated cell and OMCD principal cell, and basolateral expression increases in the CCD principal cell, OMCD intercalated cell, and the OMCD principal cell (79). In ischemia-reperfusion injury, where there is decreased renal ammonia excretion despite intact interstitial ammonia concentrations, there is selective damage to Rhcg-expressing OMCD intercalated cells, with induction of apoptosis and cellular extrusion (59). Cyclosporine A

nephropathy is associated with decreased Rhcg expression, which likely contributes to impaired ammonia excretion and to development of metabolic acidosis (96). Lastly, hypokalemia, which increases ammonia excretion despite development of metabolic alkalosis in many species, increases Rhcg expression significantly (60).

At least two distinct mechanisms contribute to increased Rhcg expression in these models. First, there is increased Rhcg protein expression without increased Rhcg mRNA expression, suggesting regulation through posttranscriptional mechanisms (142). Second, there are changes in Rhcg's subcellular location. Under basal conditions, Rhcg is located in both the apical and basolateral plasma membrane and in subapical sites in both principal cells and intercalated cells. In response to chronic metabolic acidosis, particularly in the intercalated cell, apical plasma membrane expression increases and cytoplasmic expression decreases (142). Importantly, the relative magnitude of these two regulatory mechanisms differs in different renal epithelial cells; a change in the subcellular distribution is the predominant response in the OMCD intercalated cell and increased protein expression is the predominant mechanism in the OMCD principal cell (142).

Basolateral plasma membrane Rhcg expression increases in chronic metabolic acidosis, hypokalemia, and reduced renal mass (60, 79, 142). This increase does not appear to involve redistribution from cytoplasmic sites to the basolateral plasma membrane (142).

Most recently, genetic deletion studies have confirmed the key role of Rhcg in renal ammonia excretion. Global Rhcg deletion decreases basal ammonia excretion and impairs urinary ammonia excretion in response to metabolic acidosis (16). Collecting duct-specific Rhcg deletion produced identical findings, demonstrating that these effects of Rhcg deletion reflected impaired collecting duct ammonia secretion and were not indirectly mediated through mechanisms involving extrarenal Rhcg expression (92). Moreover, Rhcg deletion decreases transepithelial ammonia permeability in perfused collecting duct segments and decreases apical membrane NH_3 permeability (16).

Nonglycosylated Rh proteins—The nonglycosylated Rh proteins, RhD and RhCE in humans and Rh30 in nonhumans, are present in erythrocytes in a multimeric complex with RhAG. RhCE appears to neither transport ammonia or its analogue, methylammonia, nor alter transport by RhAG (183). Structural models suggest that the arrangement of key amino acids is sufficiently different in RhCE and RhD that they either do not transport ammonia or that they do so by mechanisms different from those used by the glycosylated Rh glycoproteins and their bacterial orthologs (30).

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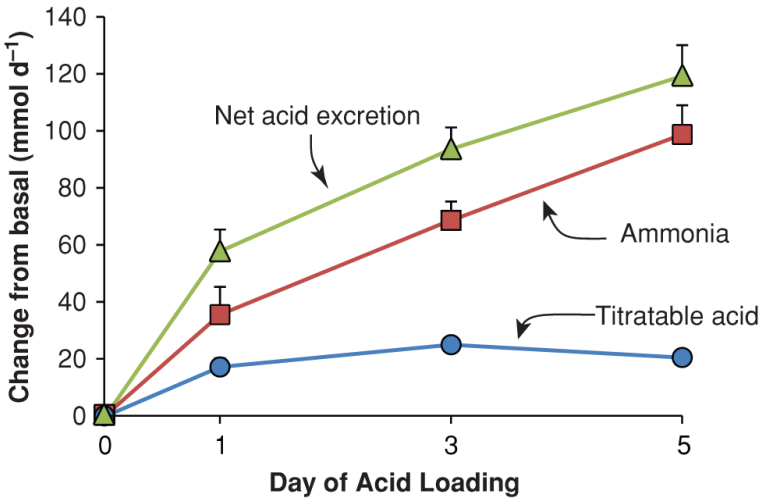

Figure 1.

Relative responses of titratable acid and ammonia excretion in the response to metabolic acidosis. Normal human volunteers were acid loaded with approximately 2 mmol/kg/d of ammonium chloride and changes in urinary ammonia and titratable acid excretion were quantified. Data recalculated, with permission, from reference (36).

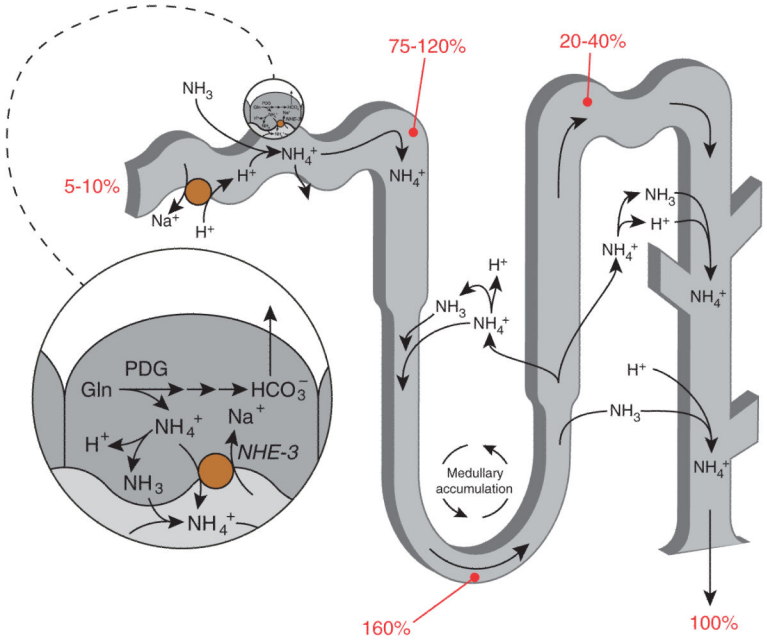

Figure 2.

Summary of renal ammonia metabolism. The proximal tubule produces ammonia, as NH_4^+, from glutamine. NH_4^+ is then secreted preferentially into the luminal fluid, primarily by NHE-3, and, in addition, there is a component of NH_3 secretion. Ammonia is then reabsorbed in the thick ascending limb, resulting in ammonia delivery to the distal nephron accounting for approximately 20% to 40% of final urinary ammonia. The remaining approximately 60% to 80% of urinary ammonia is secreted in the collecting duct through parallel NH_3 and H^+ transport. Numbers in red indicate the proportion of total urinary ammonia present at the indicated sites under baseline conditions. Figure modified from reference (180) with permission.

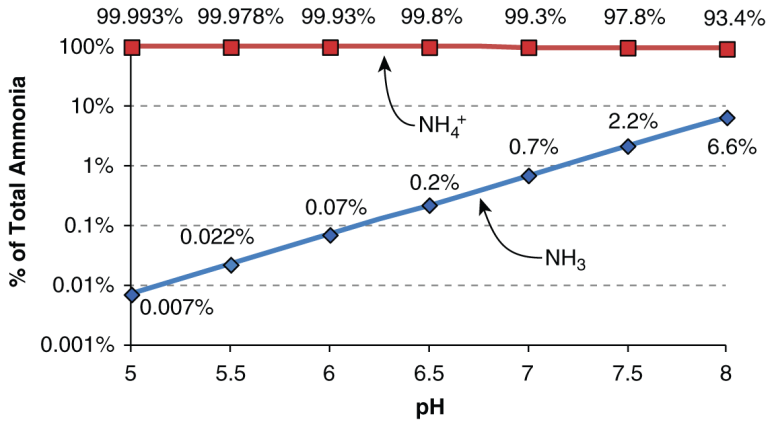

Figure 3.

Relative changes in NH_3 and NH_4^+ in solution as a function of solution pH. NH_3 and NH_4^+ contributions to total ammonia were determined from the buffer reaction, $NH_3 + H^+ \leftrightarrow NH_4^+$. A pKa′ of 9.15 was used for calculations. Amounts shown are proportion of total ammonia present as NH_3 and as NH_4^+. Note that the Y-axis is log transformed.

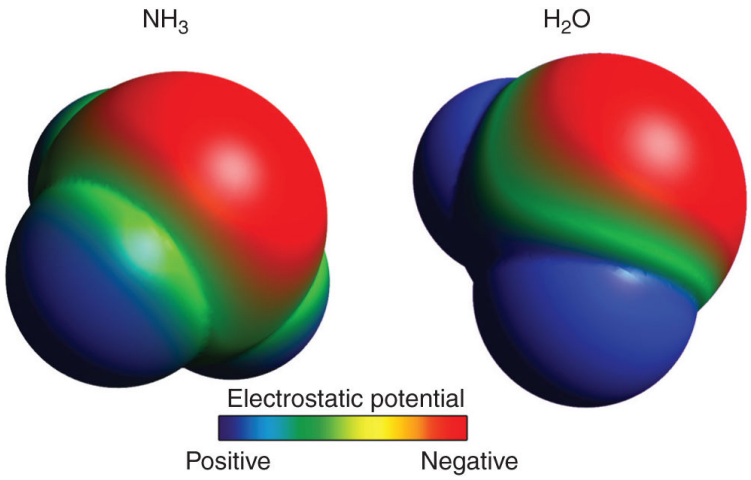

Figure 4.

Space-filling model of NH_3 showing molecular polarity. Space-filling model was created using Avogadro, v1.0.3, software. Surface is pseudocolored to demonstrate surface charge characteristics (red—negative, blue—positive). A similar process was used to generate space-filling model of H_2O. Molecules are not drawn to scale.

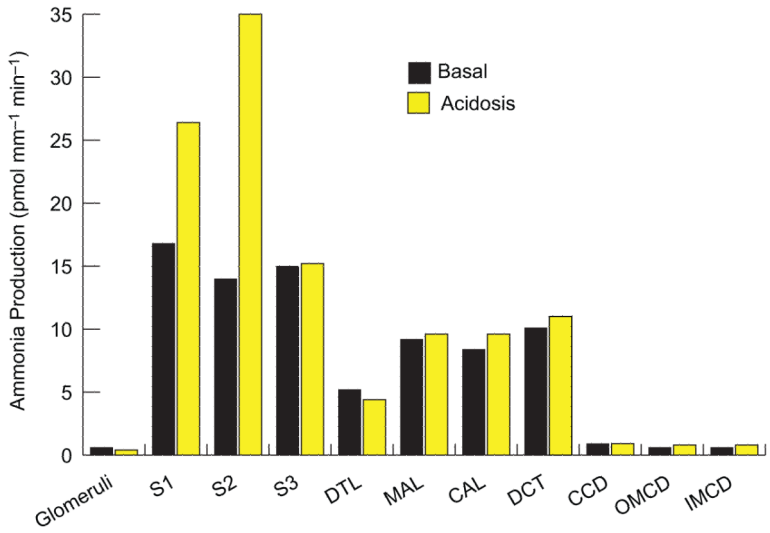

Figure 5.

Ammonia production in various renal segments. Ammonia production rates in different renal components measured in microdissected tubule segments from rats on control diets and after inducing metabolic acidosis. All segments produced ammonia. Metabolic acidosis increases total renal ammoniagenesis, but only through increased production in proximal tubule segments (S1, S2, and S3). Rates calculated from measured ammonia production rates and mean length per segment as described in (49). Size of pie graph is proportional to total renal ammoniagenesis rates. Abbreviations: DTL, descending thin limb of Henle's loop; MAL, medullary thick ascending limb of Henle's loop; CAL, cortical thick ascending limb of Henle's lop; DCT, distal convoluted tubule; CCD, cortical collecting duct; OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct.

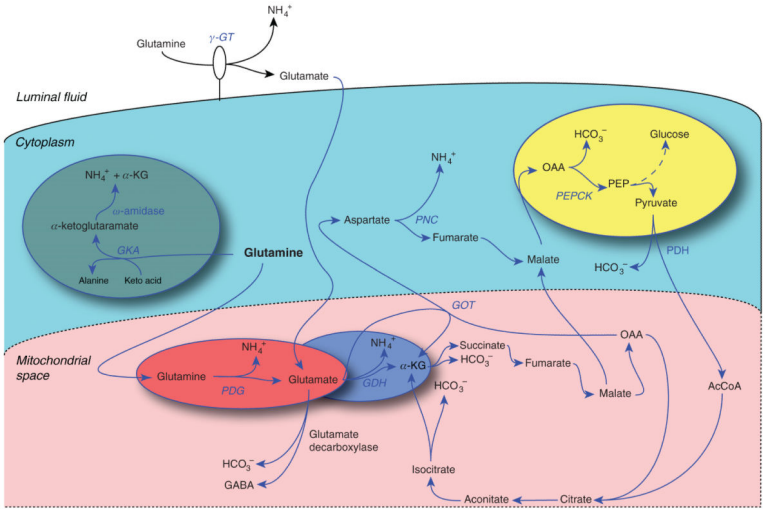

Figure 6.

Mechanisms of ammoniagenesis. Multiple pathways for enzymatic ammonia production originating from glutamine metabolism are present in the proximal tubule. Glutamine metabolism through phosphate-dependent glutaminase (PDG) and glutamate dehydrogenase (GDH) and involving phosphoenolpyruvate carboxykinase (PEPCK) is the quantitatively most significant component of renal ammoniagenesis and the primary pathway stimulated in response to metabolic acidosis.

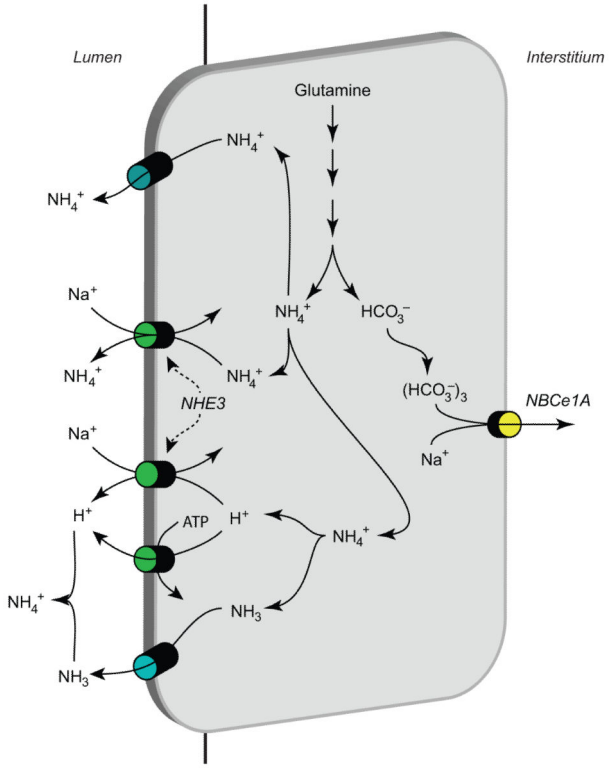

Figure 7.

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^+/NH_4^+ exchange via NHE-3, parallel NH_3 secretion and NHE-3-mediated Na^+/H^+ exchange, and a Ba^{2+}-sensitive NH_4^+ conductance likely mediated by apical K^+ channels. Secreted NH_3 is titrated to NH_4^+ by reaction with H^+, which is secreted either by NHE-3 or by apical H^+-ATPase. HCO_3^- is produced in equimolar amounts as NH_4^+ in the process of ammoniagenesis, and is primarily transported across the basolateral plasma membrane by NBCe1. Minor components of basolateral NH_4^+ uptake via Na^+-K^+-ATPase and by basolateral K^+ channels are not shown. Figure modified from reference (180) with permission.

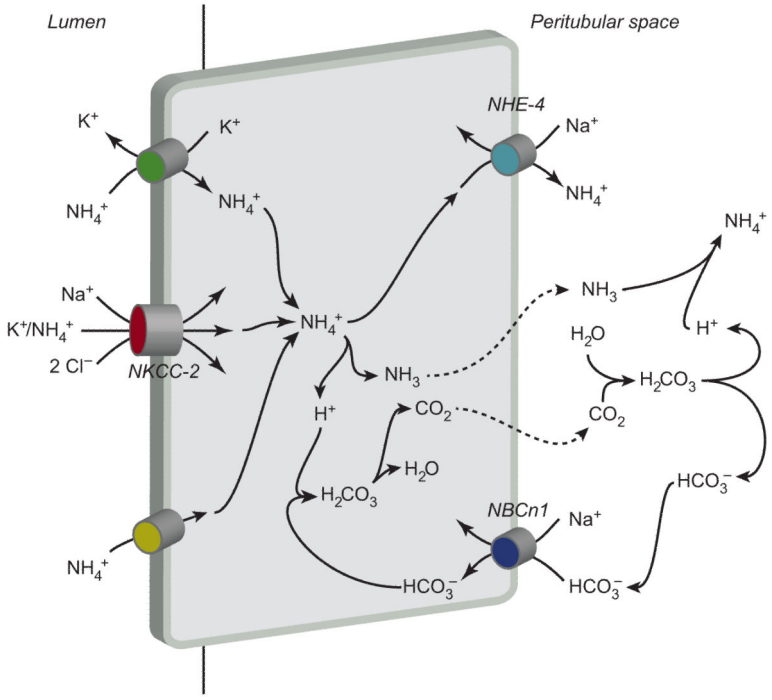

Figure 8.

Ammonia transport in the thick ascending limb (TAL). The primary mechanism of ammonia reabsorption in the TAL is via substitution of NH_4^+ for K^+ and transport by Na^+-K^+-$2Cl^-$ cotransporter 2 (NKCC-2). Electroneutral K^+/NH_4^+ exchange and conductive K^+transport are also present, but quantitatively less significant components of apical K^+ transport. Diffusive NH_3 transport across the apical plasma membrane is present, but not quantitatively significant. Cytosolic NH_4^+ can exit via basolateral NHE-4. A second mechanism of basolateral NH_4^+ exit may involve dissociation to NH_3 and H^+, with NH_3 exit via an uncharacterized, presumably diffusive, mechanism, and buffering of intracellular H^+ released via NBCn1-mediated HCO^{3-} entry. Figure modified from reference (180) with permission.

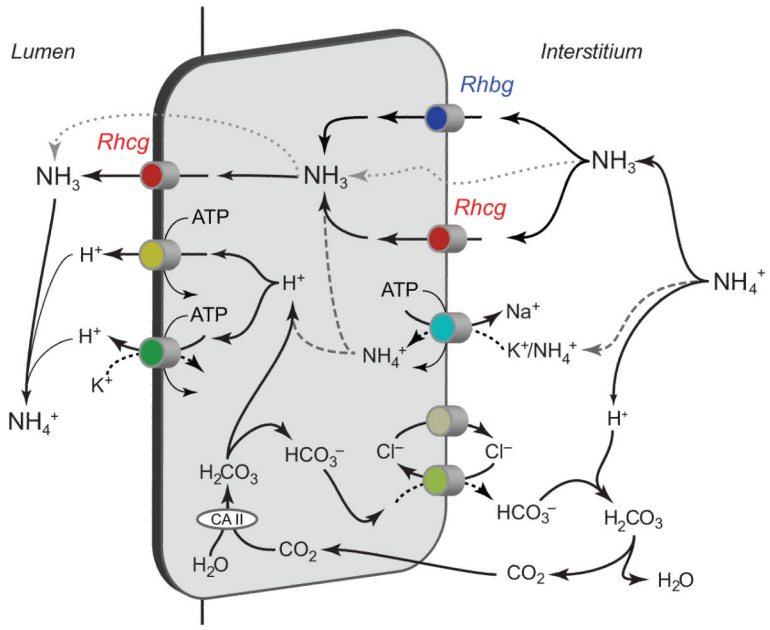

Figure 9.

Ammonia transport in the collecting duct. Interstitial NH_4^+ is in equilibrium with NH_3 and H^+. NH_3 is transported across the basolateral membrane, predominantly by Rhcg, but also possibly partly by Rhbg. In the inner medullary collecting duct, basolateral Na^+-K^+-ATPase transports NH_4^+. Intracellular NH_3 is secreted across the apical membrane by apical Rhcg. H^+-ATPase and H^+-K^+-ATPase secrete H^+, which combines with luminal NH_3 to form NH_4^+ which is "trapped" in the lumen. Intracellular H^+ is generated by CA II-accelerated CO_2 hydration that forms carbonic acid, which dissociates to H^+ and HCO_3^-. Basolateral Cl^-/HCO_3^- exchange transports HCO_3^- across the basolateral membrane; HCO_3^- combines with H^+ released from NH_4^+ to form carbonic acid, which dissociates to CO_2 and water. This CO_2 can recycle into the cell, supplying the CO_2 used for cytosolic H^+ production. The net result is NH_4^+ transport from the peritubular space into the luminal fluid. Figure modified from reference (180) with permission.