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Emerging Features of Ammonia Metabolism and Transport in Acid-Base Balance

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Abstract

Ammonia metabolism has a critical role both in acid-base homeostasis and in other cellular functions. Kidneys have a central role in bicarbonate generation, which occurs through the process of net acid excretion; ammonia metabolism is the quantitatively greatest component of net acid excretion, both under basal conditions and in response to acid-base disturbances. Several recent studies have substantially advanced our understanding of the molecular mechanisms and regulation of ammonia metabolism. First, the previous paradigm that ammonia transport could be explained by passive NH₃ diffusion and NH₄⁺ trapping has been advanced by the recognition that specific transport of NH₃ and of NH₄⁺ by specific membrane proteins is critical to ammonia transport. Second, significant advances have been made in the understanding of the regulation of ammonia metabolism. Novel studies show that hyperkalemia directly inhibits ammonia metabolism, thereby leading to the metabolic acidosis present in Type IV RTA. Other studies show that the proximal tubule protein, NBCe1, and specifically the A variant, NBCe1-A, have a major role regulating renal ammonia metabolism. Thirdly, there are important sex differences in ammonia metabolism that involve structural and functional differences in the kidney. In this review we discuss these important aspects of the emerging features of ammonia metabolism and transport.

Keywords

ammonia; NBCe1; glutamine synthetase; lithium; chronic kidney disease; hepatic encephalopathy

Introduction

Renal ammonia¹ metabolism has a critical role in acid-base homeostasis and in extrarenal cellular functions. Ammonia excreted in the urine results in equimolar “new bicarbonate” formation. This new bicarbonate is quantitatively the greatest component of renal new bicarbonate generation, otherwise known as net acid excretion, and adaptive changes in

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ammonia excretion are the predominant component of changes in net acid excretion in response to acid-base disturbances. Although titratable acid excretion and organic anion excretion can contribute to net acid excretion and new bicarbonate generation, their quantitative contribution is less than that of ammonia. Moreover, kidneys generate more ammonia than they excrete in the urine; the remainder is added to the systemic circulation, and can, if hepatic ammonia metabolism is impaired, contribute to hyperammonemic-hepatic encephalopathy.

The purpose of this review is to discuss new and emerging features in our understanding of renal ammonia metabolism. We use the term “metabolism” to refer to all components of the renal handling of ammonia, including its generation, recycling, and transport. First, we review basic concepts of the renal generation of ammonia. Next, we discuss the evidence that the previous paradigm of ammonia transport, diffusive NH_3 movement coupled with NH_4^+ trapping, should be replaced by a new paradigm in which specific and regulated transport of NH_3 and of NH_4^+ by specific membrane proteins is the major component of ammonia transport. Then, we will discuss the evidence for a new component of ammonia metabolism, namely ammonia recycling. After that we review new findings regarding the regulation of ammonia metabolism. Specifically, we discuss new insights into Type IV RTA which suggest that hyperkalemia inhibits ammonia metabolism directly and thereby causes metabolic acidosis. We review new data showing that NBCe1 and its splice variant, NBCe1-A, have critical roles regulating ammonia metabolism and thus are critical components of the signaling pathway through which acid-base homeostasis is maintained. Finally, recent information identifies important sex differences in ammonia metabolism that involve differences in renal structure and differences in expression of critical proteins in ammonia metabolism. Because of space limitations, we are unable to review all pertinent literature regarding these important features of ammonia metabolism and we apologize to the authors of the work that we have not selected.

Brief review of ammonia metabolism

The renal handling of ammonia differs fundamentally from that of almost every other urinary solute. Other solutes are delivered to the kidney via the renal artery, and the combination of glomerular filtration and epithelial cell transport determines their urinary excretion. For these solutes, the renal vein content is less than the renal arterial content. In contrast, the kidneys generate ammonia and then differentially transport it either into the urine or the renal vein, where it enters the systemic circulation. Arterial ammonia delivery comprises only a small component of urinary ammonia. In fact, renal venous ammonia exceeds arterial ammonia (Figure 1), because the kidneys generate systemic ammonia.

Ammonia is produced by essentially all renal epithelial cells. However, the proximal tubule produces the majority of renal ammonia and is the only site where ammonia generation is altered in response to acid-base disturbances such as metabolic acidosis^(2, 3). Ammonia is generated from the metabolism of amino acids, predominantly that of glutamine. Selective

¹Ammonia exists in two molecular forms, NH_3 and NH_4^+ . When referring to the combination of both molecular forms, we use the term “ammonia” in this manuscript. When referring specifically to either one of these molecular forms we specifically state either NH_3 or NH_4^+ .

and regulated ammonia transport in multiple nephron and collecting duct segments then determine the final distribution of ammonia (Figure 2).

Proximal tubule glutamine metabolism begins with glutamine uptake. One component of glutamine uptake likely involves filtered glutamine reabsorption mediated by the apical plasma membrane neutral amino acid transporter, B⁰AT¹ (SLC6A19). This process does not appear to be regulated, at least at the level of either mRNA or protein expression^(4, 5). The second component involves peritubular glutamine uptake; this appears to occur via the basolateral protein, SN1 (SLC38A3), also known as SNAT3, a Na⁺ - and H⁺ -dependent glutamine transporter. Detectable SN1 protein expression under basal conditions is limited to distal portions of the proximal tubule, predominantly the proximal straight tubule in the outer medulla⁽⁶⁾. Luminal glutamine uptake may be sufficient to supply glutamine in the more proximal portions, but the nearly complete reabsorption of glutamine may limit its availability to more distal portions. Glutamine not used for ammonia generation or for other cellular processes can be transported across the basolateral plasma membrane in a process that appears to involve LAT2–4F2hc and YLAT1–4F2hc⁽⁷⁾. During metabolic acidosis, ammoniogenesis increases to an extent that the glutamine supplied from the complete reabsorption of filtered glutamine is not sufficient. Additional intracellular glutamine is supplied through increased peritubular uptake via SN1, and SN1 expression increases substantially and detectable expression extends more proximally into earlier portions of the proximal tubule^(6, 8).

Complete glutamine metabolism in the proximal tubule results in equimolar generation of NH₄⁺ and HCO₃⁻. First, glutamine is transported into mitochondria, where a specific glutaminase isoform (KGA)⁽⁹⁾ converts glutamine to glutamate with simultaneous release of NH₄⁺. Because this enzyme activity is activated by phosphate, it is often referred to as “phosphate-dependent glutaminase” (PDG). Glutamate dehydrogenase (GDH) converts glutamate to 2-oxoglutarate (also known as α-ketoglutarate), with release of a second NH₄⁺. The NH₄⁺ formed is preferentially secreted across the apical plasma membrane⁽¹⁰⁾. 2-Oxoglutarate is metabolized to malate, which is transported out of mitochondria via a malate-phosphate exchange activity. Cytoplasmic malate metabolism results in phosphoenolpyruvate (PEP) production; a key step involves the enzyme PEP carboxykinase (PEPCK). PEP is then either converted to pyruvate, where it enters the tricarboxylic acid cycle and its metabolism results in HCO₃⁻ formation or enters the renal gluconeogenic pathway, forming glucose and HCO₃⁻. Bicarbonate that is formed is transported across the basolateral plasma membrane where it enters peritubular capillaries and serves as new bicarbonate generation. The interested reader is directed to a more comprehensive review for additional information⁽⁷⁾.

In the thick ascending limb of the loop of Henle (TAL), luminal ammonia is reabsorbed. Ammonia reabsorption results in the amount of ammonia exiting the TAL being substantially less than the amount of ammonia present in the late proximal tubule and the amount present in the transition from the thin descending limb to the thick ascending limb, as least as determined in micropuncture studies of ammonia delivery to the “early distal tubule.”⁽¹¹⁾ This results in the development of a corticomedullary gradient for ammonia. Interstitial sulfatides reversibly bind ammonia and are necessary for normal ammonia

excretion⁽¹²⁾. TAL ammonia reabsorption also results in the ammonia delivery out of the TAL accounting for only a fraction, ~20–40%, of the ammonia excreted in the urine^(7, 11).

Ammonia secretion in more distal epithelial sites, presumably the connecting segment and collecting duct, accounts for the remaining 60–80% of ammonia excretion. Ammonia secretion in the collecting duct involves H^+ secretion that binds with NH_3 , forming NH_4^+ , decreases the luminal NH_3 concentration, and enables gradient driven NH_3 movement across the collecting duct epithelium.

Recent advances in understanding ammonia metabolism

Changing paradigm of ammonia transport.

A traditional model of ammonia transport involves the concepts of rapid “ NH_3 diffusion” and “ NH_4^+ trapping.” This model is based on the concept that NH_3 , because it is a small neutrally-charged molecule, rapidly diffuses across plasma membranes and that NH_4^+ , because it is a small cation, cannot cross plasma membranes. However, both aspects of this traditional model are now known to be incorrect.

In regard to NH_3 , the positively-charged hydrogens are asymmetrically distributed around the central nitrogen, and an unshared electron pair is also asymmetrically distributed. As a result, NH_3 has significant molecular polarity (Figure 3). Water (H_2O) shares a similar atomic form, with asymmetric hydrogens and unshared electron pairs surrounding the central atom. Because of this molecular polarity, NH_3 , just like H_2O , has limited permeability across plasma membranes.

The concept that NH_4^+ is not transported across plasma membranes, enabling “ NH_4^+ trapping,” also requires updating. Careful analysis has shown that NH_4^+ and K^+ ions have almost identical biophysical characteristics⁽¹³⁾. Moreover, and likely resulting from this molecular similarity, NH_4^+ is transported by essentially all K^+ transporters⁽¹³⁾. It is also conceivable that NH_4^+ has sufficient atomic similarity to hydronium (H_3O^+), the primary atomic form of H^+ in aqueous solutions, to enable NH_4^+ transport by at least some H^+ transporters. Thus, although NH_4^+ does have a low permeability across a protein-free lipid bilayer, both K^+ -transporters and specific H^+ transporters are able to transport NH_4^+ .

A number of studies have led to a new paradigm in which transport of NH_3 and of NH_4^+ by specific membrane proteins is critical to ammonia metabolism. These proteins have key roles in proximal tubule mitochondria and apical plasma membrane ammonia transport, in TAL ammonia reabsorption and in collecting duct ammonia secretion. Below, we present a brief summary of these findings.

Proximal tubule

Two key aspects of proximal tubule ammonia transport involve: (a) movement of ammonia out of mitochondria, where it is generated; (b) and preferential ammonia secretion across the apical plasma membrane. AQP8 is a member of the aquaporin family, and many, but not all, aquaporin family members also transport ammonia^(14, 15). Several studies have specifically shown AQP8 transports ammonia^(reviewed in 7). In the kidney, AQP8 is present in the

proximal tubule, CCD and OMCD⁽¹⁶⁾, and cell fractionation studies of cultured proximal tubule cells show it is present in the inner mitochondrial membrane⁽¹⁷⁾. Metabolic acidosis increases proximal tubule AQP8 expression and AQP8 deletion decreases proximal tubule ammonia secretion, both under basal conditions and in response to in vitro acidosis⁽¹⁷⁾. Thus, a number of lines of evidence suggest that AQP8 may facilitate mitochondrial ammonia movement and thereby have an important role in proximal tubule ammonia metabolism. However, there is some degree of controversy regarding the exact physiologic role of mitochondrial AQP8 expression, as AQP8 deletion in a mouse model does not detectably alter renal ammonia handling⁽¹⁸⁾.

A second component of proximal tubule ammonia metabolism that appears to involve transporter-mediated ammonia movement is the preferential apical secretion. Substantial evidence indicates that apical NHE3 ($\text{Na}^+ - \text{H}^+$ exchanger, isoform 3; SLC9A3) can transport NH_4^+ at the H^+ binding site, enabling apical Na^+ for NH_4^+ exchange and preferential apical ammonia secretion^(19, 20). Somewhat surprisingly, however, proximal tubule-specific NHE3 deletion did not alter renal ammonia excretion⁽²¹⁾. The atomic mechanism through which NH_4^+ substitutes for H^+ is unknown. One possible mechanism is that the actual molecule transported by NHE3 is hydronium (H_3O^+), which is present due to the nearly complete hydration of H^+ in aqueous solutions, and there may be sufficient atomic similarity between NH_4^+ and H_3O^+ to allow substitution of NH_4^+ for H_3O^+ by NHE3. There is also evidence that one or more apical K^+ channels can transport ammonia in the proximal tubule⁽²²⁾. Figure 4 summarizes this model of proximal tubule ammonia transport.

TAL

TAL ammonia reabsorption is a second site where ammonia transport by specific membrane proteins is critical for normal ammonia metabolism. The primary mechanism of luminal ammonia uptake involves the apical loop diuretic-sensitive protein, NKCC2 ($\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ cotransporter, isoform 2; SLC12A1)^(23, 24, 25). This appears to involve atomic mimicry whereby NH_4^+ substitutes for K^+ and is transported at the K^+ -binding site of NKCC2. In addition, a number of other apical NH_4^+ transporting proteins have been identified in the TAL⁽²⁶⁾. Their role is less clear in view of evidence that inhibiting NKCC2 essentially abolishes TAL ammonia reabsorption⁽²⁷⁾. Cytosolic ammonia then appears to exit the cell across the basolateral plasma membrane via NHE4 ($\text{Na}^+ - \text{H}^+$ exchanger, isoform 4; SLC9A4)⁽²⁸⁾. NHE4 appears to mediate Na^+ for NH_4^+ exchange, using the peritubular to cytosolic Na^+ gradient to drive NH_4^+ exit from the cell. A second basolateral ammonia transport mechanism appears to involve NBCn1 (SLC4A7)⁽²⁹⁾; this may reflect its uptake of HCO_3^- , which reacts with NH_4^+ , yielding NH_3 , which is then transported across the basolateral plasma membrane through an as yet uncharacterized mechanism.

Collecting duct

Studies over the past several years, particularly those evaluating Rhesus glycoproteins, have dramatically altered our understanding of collecting duct ammonia secretion. Rhesus (Rh) glycoproteins are mammalian orthologues of bacterial, plant and yeast ammonia transporter family members⁽³⁰⁾. Two Rh glycoproteins, Rh C Glycoprotein (Rhcg, SLC42A3) and Rh B

Glycoprotein (Rhbg, SLC42A2) are found in the kidney; Rh A glycoprotein (Rhag, SLC42A1) has only been found in erythrocytes and in erythroid-progenitor cells.

Extensive information indicates Rhcg has a key role in ammonia metabolism. Rhcg is present in both the apical and basolateral plasma membrane in both Type A intercalated cells and principal cells in the collecting duct, in only the apical plasma membrane in non-A, non-B intercalated cells and not at detectable levels in Type B intercalated cells in the mouse and rat kidney. The expression in the human kidney is identical, with the exception that the principal cell does not have detectable Rhcg expression. Rhcg is expressed in parallel with renal ammonia excretion in multiple conditions, including metabolic acidosis, hypokalemia, ischemia-reperfusion injury, cyclosporine nephropathy and reduced renal mass^(reviewed in 31). Genetic deletion inhibits both apical and basolateral plasma membrane ammonia transport.^(32, 33) Furthermore genetic deletion, either global or renal epithelial cell-specific, causes abnormal ammonia metabolism under basal conditions and in response to either metabolic acidosis or hypokalemia^(32, 34, 35, 36, 37). Both heterologous cell expression studies and gene deletion studies indicate that Rhcg transports ammonia only in the molecular form NH₃ (reviewed in 31). Rhcg's transport of NH₃, but not NH₄⁺, is critical for it to contribute to apical ammonia secretion. If Rhcg transported NH₄⁺ to any significant extent the electrochemical gradient for NH₄⁺ movement, which results from high luminal NH₄⁺ concentration and from intracellular electronegativity, would result in ammonia reabsorption, not the secretion that occurs in the collecting duct⁽³¹⁾.

Rhbg is the second Rhesus glycoprotein present in the collecting duct. It is present exclusively in the basolateral plasma membrane, and, similar to Rhcg, is found in intercalated cells, principal cells and CNT cells^(38, 39, 40). Although an initial study did not identify a role for Rhbg in ammonia metabolism⁽⁴¹⁾, subsequent studies showed that Rhbg was necessary for basal, acidosis-stimulated and hypokalemia-induced ammonia excretion^(42, 43). Rhbg appears to transport ammonia both in the form of NH₃ and NH₄⁺⁽⁴⁴⁾; transport of either molecular form results in basolateral ammonia uptake given the typical collecting duct gradients⁽³¹⁾.

Direct interaction of Rhcg with H⁺-ATPase

Collecting duct ammonia secretion, which accounts for the majority of ammonia excretion, involves parallel H⁺ and NH₃ secretion. Under almost all conditions, urine pH is sufficiently far from the pK_a' of the NH₃ + H⁺ ↔ NH₄⁺ reaction, ~9.15, that >99% of urinary ammonia is in the form of NH₄⁺. This necessitates tight coupling of collecting duct NH₃ and H⁺ secretion. Recent studies have addressed this coupling⁽⁴⁵⁾. First, Rhcg and H⁺-ATPase co-immunoprecipitate, indicating close, if not direct, physical interaction. Second, Rhcg deletion decreased H⁺-ATPase activity in perfused cortical collecting duct segments, and it decreased expression of the B1 and B2 H⁺-ATPase subunits. Finally, Rhcg over-expression increased both H⁺ secretion and B1-subunit of H⁺-ATPase mRNA expression. These findings, that Rhcg expression alters H⁺-ATPase expression and activity in parallel, provide important mechanistic information regarding the tight coupling of collecting duct NH₃ and H⁺ secretion.

Other collecting duct ammonia transporters

Several other proteins transport ammonia in the collecting duct. In the inner medullary collecting duct, where Rhesus glycoprotein expression is low, the basolateral $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ mediates basolateral NH_4^+ uptake that is essential for ammonia secretion⁽⁴⁶⁾; this appears to occur as a result of NH_4^+ binding and transport at the K^+ -binding site⁽⁴⁷⁾. Increased $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ -mediated ammonia uptake during hypokalemia may contribute to the increased ammonia excretion that occurs in this condition⁽⁴⁸⁾. However, $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ does not appear to contribute significantly to ammonia transport in the cortical collecting duct (CCD)⁽⁴⁹⁾, and its role in the outer medullary collecting duct is untested. In the CCD, another protein that may contribute to ammonia transport is the hyperpolarization-activated cyclic nucleotide-gated HCN2 channel⁽⁵⁰⁾, although the specific role of HCN2 in ammonia secretion has not been determined.

Critical role of renal ammonia recycling

A second important advance in our understanding of ammonia metabolism has been the realization that the kidneys, in addition to generating ammonia, also “recycle” ammonia. Specifically, the enzyme glutamine synthetase (GS) catalyzes the reaction of glutamate with NH_4^+ , which “regenerates” glutamine, the substrate from which NH_4^+ had been generated, and releases an H^+ . In other words, GS “reverses” the process of ammonia generation, which we term “ammonia recycling.”

Glutamine synthetase is expressed in distinct cell populations in the kidney, proximal tubule cells, distal convoluted tubule (DCT) cells and in collecting duct type A and Non A, non B intercalated cells⁽⁵¹⁾. Proximal tubule glutamine synthetase has an important role in ammonia generation. Metabolic acidosis decreases both whole kidney and proximal tubule glutamine synthetase expression^(52, 53, 54); this leads to less ammonia recycling and contributes to increasing net ammonia generation. Similarly in hypokalemia, which also increases ammonia generation and excretion, proximal tubule GS expression decreases⁽⁵¹⁾, which likely contributes to the increased net ammonia excretion observed. Studies examining mice with proximal tubule cell-specific GS deletion further support its role in ammonia metabolism. Under basal conditions, proximal tubule-specific GS deletion increased urinary ammonia excretion significantly and the response to metabolic acidosis was impaired. This occurred despite adaptive/compensatory changes in multiple components of ammonia generation that minimized the apparent role of GS under both basal conditions and following acid-loading⁽⁵⁴⁾.

Another condition in which GS likely has an important role is dietary protein restriction. Dietary protein metabolism is a critical determinant of endogenous acid production, and dietary protein restriction decreases the amount of net acid excretion needed to maintain acid-base homeostasis; failure of this adaptive response could lead to metabolic alkalosis with its associated complications.

Dietary protein restriction increases glutamine synthetase expression⁽⁵⁵⁾, which contributes to decreased ammonia excretion by enhancing proximal tubule ammonia recycling; proximal tubule-specific glutamine synthetase deletion blunts this response⁽⁵⁶⁾. Thus, increased

proximal tubule glutamine synthetase expression in response to dietary protein restriction appears to increase ammonia recycling, thereby contributing to the decrease in ammonia excretion, and, by regenerating glutamine, contributes to conservation of this essential amino acid.

As mentioned above, glutamine synthetase is also expressed in collecting duct Type A intercalated cells⁽⁵¹⁾. Its regulation in type A intercalated cells differs, at least under some circumstances, from that in the proximal tubule. Hypokalemia decreases proximal tubule glutamine synthetase expression, discussed above, while simultaneously increasing expression in type A intercalated cells in both the CCD and the OMCD⁽⁵¹⁾. It is likely that increased intercalated cell glutamine synthetase expression during hypokalemia increases ammonia recycling inside intercalated cells, thereby limiting the increase in intercalated cell ammonia secretion and net ammonia excretion that otherwise would occur from the enhanced proximal tubule ammoniogenesis and secretion. This likely functions to reduce the severity of the metabolic alkalosis that can result from hypokalemia.

Type IV renal tubular acidosis (RTA)

The most common RTA seen in clinical medicine is Type IV RTA. Type IV RTA is characterized by hyperkalemia in association with metabolic acidosis that is out of proportion to changes in glomerular filtration rate. Understanding the mechanism(s) whereby Type IV RTA develops is complicated by the robust renal capacity for K^+ excretion, which makes it difficult to create a model of chronic hyperkalemia without inhibiting adrenal hormone release or impairing collecting duct K^+ transport.

A recent study used a novel approach that obviated these restrictions. This study used mice with early distal convoluted tubule (DCT)-specific overexpression of constitutively active Ste20/SPS1-related proline-alanine-rich kinase (DCT-CA-SPAK), which causes chronic hyperkalemia.⁽⁵⁷⁾ Because these mice have normal renal function and because the early DCT is not a critical site for ammonia metabolism, they enabled direct examination of hyperkalemia's effect on ammonia metabolism.

DCT-CA-SPAK mice spontaneously develop hyperkalemia in association with metabolic acidosis and suppressed ammonia excretion⁽⁵⁸⁾. The renal acid-base disorder was specific to ammonia excretion; titratable acid excretion was unchanged. Similar to patients with Type IV RTA, urine pH was not elevated, indicating that the impaired ammonia excretion was not due directly to impaired urine acidification. Instead, the abnormal ammonia excretion correlated with decreased proximal tubule expression of the enzymes essential for ammonia generation, PDG and PEPCK, and overexpression of the ammonia-recycling enzyme, glutamine synthetase. In addition, there was decreased expression in the inner stripe of the outer medullary collecting duct of both the key collecting duct NH_3 transporter, Rhcg, and decreased apical polarization of H^+ -ATPase, suggesting decreased collecting duct ammonia secretory capacity. Correcting the hyperkalemia corrected the metabolic acidosis, increased ammonia excretion, and normalized ammoniagenic enzyme and Rhcg expression. Finally, inducing hyperkalemia in wild-type mice, using the epithelial sodium channel blocker, benzamil, had similar effects as did DCT-CA-SPAK. Overall, there was a remarkably strong correlation between serum K^+ and ammonia excretion (Figure 5). Thus, hyperkalemia

appears to directly cause metabolic acidosis, i.e., cause Type IV RTA, by decreasing proximal tubule ammonia generation and collecting duct ammonia transport, thereby leading to impaired ammonia excretion that causes metabolic acidosis.

An important consideration when using animal models is whether they accurately reflect human physiology and pathophysiology. The mice used in this model, DCT-CA-SPAK, were generated to study mechanisms underlying human Familial Hyperkalemic Hypertension (FHHt), also known as pseudohypoaldosteronism, type 2 (PHA2), or as Gordon's syndrome⁽⁵⁷⁾. FHHt patients have spontaneous hyperkalemia and metabolic acidosis despite normal glomerular filtration rate and intact adrenal function^(59, 60, 61). Similar to the DCT-CA-SPAK mice used for the rodent studies described in the previous paragraph, FHHt patients have decreased ammonia excretion and correcting their hyperkalemia increases ammonia excretion and corrects the metabolic acidosis^(62, 63, 64, 65, 66, 67). Thus, the findings in mice with DCT-CA-SPAK replicate those seen in FHHt patients.

Another human condition associated with hyperkalemia and spontaneous metabolic acidosis is isolated hypoaldosteronism. These patients have hyperkalemia, metabolic acidosis and impaired ammonia excretion, and correcting the hyperkalemia corrects the acidosis and normalizes ammonia excretion⁽⁶⁸⁾. Moreover, the relationship between plasma K^+ and urinary ammonia excretion⁽⁶⁸⁾ in patients with isolated hypoaldosteronism is nearly identical to that observed in mice⁽⁵⁸⁾.

Regulation by NBCe1

Understanding the molecular mechanisms through which extracellular stimuli alter proximal tubule ammonia generation is fundamental to understanding ammonia metabolism. Recent studies have shown that the basolateral plasma membrane protein, Na^+ -bicarbonate cotransporter, electrogenic, isoform 1 (NBCe1, SLC4A4), may have a central role in this process. Deletion of this protein in mice results in spontaneous and severe metabolic acidosis^(69, 70). NBCe1 is the primary basolateral HCO_3^- transporter involved in proximal tubule bicarbonate reabsorption. NBCe1 deletion causes metabolic acidosis, at least in part, because of impaired proximal tubule HCO_3^- reabsorption, i.e., proximal RTA. However, NBCe1 deletion also decreases ammonia excretion⁽⁶⁹⁾, a highly abnormal ammonia response, because metabolic acidosis normally increases ammonia excretion. There also is abnormally low expression of the proximal tubule enzymes essential for ammoniogenesis, including mitochondrial phosphate-dependent glutaminase (PDG) and cytoplasmic phosphoenolpyruvate carboxykinase (PEPCK), and increased expression of the ammonia-recycling protein, glutamine synthetase (GS). These findings indicate that NBCe1 is necessary for the normal effect of metabolic acidosis to stimulate renal ammonia metabolism and excretion.

NBCe1 has several splice variants that exhibit different cellular expression, different activity levels and different regulatory mechanisms^(71, 72, 73). Three variants, NBCe1-A, NBCe1-B and NBCe1-C, are present in both humans and in rodents, and two, NBCe1-D and NBCe1-E, have to date been identified only in rodents. Only two of these variants, NBCe1-A and NBCe1-B appear to be expressed in the kidney^(74, 75). NBCe1-A is the more highly expressed of the two, and is found exclusively in cortical proximal tubule segments, whereas

NBCe1-B appears to be present throughout the entire proximal tubule, albeit at lower levels⁽⁷⁴⁾.

Recent studies show that NBCe1-A has a key role regulating renal ammonia metabolism. NBCe1-A-specific gene deletion was generated using TALEN-based gene editing approaches⁽⁷⁶⁾. Deletion of the NBCe1-A gene caused severe spontaneous metabolic acidosis⁽⁷⁶⁾. Despite this metabolic acidosis, which normally causes a dramatic increase in ammonia excretion, absolute urinary ammonia concentration was unaltered. Additionally, NBCe1-A deletion almost completely blocked the ability to increase ammonia excretion after exogenous acid loading (Figure 6). Urine pH was more acidic in mice with NBCe1-A deletion than in wild-type controls under both basal conditions and during acid-loading, indicating that the abnormal ammonia excretion was not caused by a primary failure of urine acidification. Instead, NBCe1-A deletion altered the expression levels of multiple enzymes involved in net proximal tubule ammonia generation, such as PDG, PEPCK and GS, under both basal conditions and after exogenous acid loading. NBCe1-A deletion did not impair expression of key proteins involved in collecting duct ammonia secretion. Thus, NBCe1-A has a critical role in basal and acidosis-stimulated ammonia metabolism through the regulation of proximal tubule ammonia-metabolizing enzymes.

Role of NBCe1 regulating human ammonia metabolism

These findings in mice with NBCe1 and with NBCe1-A deletion parallel observations in patients with genetic forms of isolated proximal RTA, a condition in which the only known genetic causes are mutations in NBCe1^(71, 77). These patients also have spontaneous metabolic acidosis; despite this metabolic acidosis, which in normal individuals dramatically increases ammonia excretion, their ammonia excretion is unchanged^(78, 79). In other words, they fail to increase ammonia excretion appropriately in response to their spontaneous metabolic acidosis. Moreover, their ability to increase ammonia excretion when provided with an exogenous acid load is impaired significantly⁽⁷⁹⁾. Thus, NBCe1, both in people and in rodents, has a critical role in ammonia excretion that appears to result from an essential role in regulating proximal tubule ammonia metabolism.

Sex effects

Sex differences are well recognized as an important biological variable in many aspects of human biology. Recent studies show that there are important sex differences in renal ammonia metabolism⁽⁸⁰⁾. Normal female mice excreted essentially twice as much ammonia as did male mice. This difference in ammonia metabolism was associated with fundamental structural differences between the female and male kidney. In the female mouse kidney, proximal tubules account for a lower percentage of the renal cortical parenchyma compared with the male kidney, whereas collecting ducts account for a greater percentage of the renal parenchyma than in male kidneys. This difference was also associated with differences in several proteins involved in ammonia metabolism that were separate from the renal structural differences. Specifically, greater ammonia excretion in females was associated with greater expression of PEPCK, glutamine synthetase, NKCC2, Rhbg, and Rhcg than was observed in male mice, and these effects were distinct from the structural differences. Thus,

there are sex differences in basal ammonia metabolism that involve both renal structural differences and differences in expression of proteins involved in ammonia metabolism.

Effects of chronic kidney disease (CKD)

CKD, metabolic acidosis and abnormal ammonia metabolism are fundamentally intertwined. Increasing severity of CKD correlates with an increased incidence of metabolic acidosis⁽⁷⁾ and metabolic acidosis increases the risk of progressive CKD^(reviewed in 81). The metabolic acidosis that often occurs in CKD correlates with decreased ammonia excretion⁽⁸²⁾, and low ammonia excretion, independent of the severity of metabolic acidosis, correlates with worse clinical outcomes, including progressive CKD and death^(83, 84).

CKD causes low ammonia excretion at least in part because of decreased number of functioning nephron and collecting duct segments. As discussed above, ammonia excretion results from renal ammonia generation coupled with cell- and transporter-dependent ammonia transport into the urine. When the number of functioning nephron and collecting duct segments is decreased, there are adaptive increases in single nephron ammonia excretion, i.e., an increase in ammonia excretion adjusted for GFR^(85, 86, 87, 88, 89, 90), that can maintain sufficient total ammonia excretion, at least initially. This adaptive response involves hypertrophy of both intercalated cells and principal cells in the collecting duct and increased apical and basolateral plasma membrane Rhcg polarization⁽⁹¹⁾. Other studies indicate a parallel increase in apical polarization of H⁺-ATPase⁽⁹²⁾. With more progressive CKD, there may be a failure of the adaptive response. Studies in the Han:SPRD rat, used as a model of spontaneous progressive CKD, show significant decreases in SN1, PDG, PEPCK and Rhcg expression that correlate with decreased urinary ammonia excretion⁽⁹³⁾. Older studies have also shown decreased PDG activity⁽⁹⁰⁾ and have suggested that decreased collecting duct ammonia secretion occurs in CKD associated with metabolic acidosis⁽⁹⁴⁾.

Whether intrarenal ammonia contributes to progressive renal injury in patients with CKD and metabolic acidosis is an area that remains unsettled at present. Early studies suggested that increased renal interstitial ammonia, at least in the cortex, contributed to development of interstitial fibrosis through a complement-mediated mechanism^(95, 96). However, subsequent cell culture studies showed that hyperosmolality blocked ammonia-stimulated, complement-mediated immune injury⁽⁹⁷⁾, which may provide a protective mechanism against interstitial injury, at least in the medulla. In contrast, clinical studies show that low urine ammonia correlates with increased risk of progressive CKD^(98, 83, 84). To the extent that urinary ammonia parallels interstitial ammonia, this clinical observation is not consistent with the hypothesis that interstitial ammonia contributes to progressive renal injury. Whether this discrepancy results from urinary ammonia not paralleling interstitial ammonia concentration, at least in CKD, or because interstitial ammonia is not pathogenic is unclear at this time. In support of the latter possibility is the observation that interstitial sulfatides reversibly bind interstitial ammonia, thereby limiting its “free” concentration⁽¹²⁾. This observation suggests that in normal animals “free” interstitial ammonia levels, because interstitial ammonia is largely bound to sulfatides, do not rise to levels that cause complement activation. If this is the case and interstitial sulfatide abundance is not altered by

CKD, alternative mechanisms may mediate the progressive renal injury, possibly involving aldosterone and endothelin.

Summary and conclusion

Research findings over the past several years have substantially changed our understanding of renal ammonia handling. The previous paradigm of ammonia transport involving passive NH_3 diffusion and NH_4^+ trapping has been replaced with a model in which specific transport of each of the two molecular forms of ammonia, NH_3 and NH_4^+ , by specific membrane proteins is critical to normal ammonia metabolism. Type IV RTA appears to occur as a result of hyperkalemia impairing both proximal tubule ammoniogenesis and collecting duct ammonia transport mechanisms. The proximal tubule protein, NBCe1-A, is central to both basal and acidosis-stimulated ammonia generation. In addition, there are important sex differences in ammonia metabolism. Finally, the exact role of ammonia, specifically whether it is beneficial or detrimental, in CKD remains an area of important investigation, but more recent studies suggest that impaired ammonia metabolism contributes to progressive CKD.

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Figure 1. Renal vein ammonia concentration is higher than renal artery ammonia concentration, indicating systemic ammonia addition of renal origin.

Graph was constructed using measurements of arterial and renal vein ammonia concentrations in ten patients. Data from ⁽¹⁾. All patients had impaired liver function, which likely contributed to the mildly elevated arterial ammonia.

Figure 2. Model of renal ammonia transport.

The majority of renal ammonia is generated in the kidney in the proximal tubule. There it is preferentially secreted into the luminal fluid. Ammonia is reabsorbed by the TAL, causing development of a corticomedullary ammonia gradient, and then is secreted by the collecting duct.

Figure 3. Molecular model of NH₃ and H₂O.

NH₃ and H₂O are uncharged molecules, but because of asymmetric distributions of charged elements they have significant molecular polarity. Top panels show ball and stick models, showing asymmetric distribution of hydrogen nuclei around the central atom. Red arrow shows the molecular dipole moment. Bottom panels show space-filling models pseudocolored to show electrostatic charge distribution. Models are not drawn to scale. Models generated using Avogadro, v1.0.3 and v1.2.0.

Figure 4. Model of primary NH_3 and NH_4^+ transporters and their role in ammonia transport. Top panel shows the proximal tubule. AQP8 may have a critical role in ammonia exit from mitochondria, and apical NHE3 may have a critical role in the preferential apical ammonia secretion. Middle panel shows the TAL. Apical NKCC2 mediates secondarily active NH_4^+ uptake via binding and transport of NH_4^+ at the K^+ binding site. Cytosolic ammonia exits the cell across the basolateral plasma membrane in part via Na^+ for NH_4^+ exchange by NHE4. A “bicarbonate shuttle” pathway involving NBCn1 may also contribute to basolateral ammonia exit. Bottom panel shows a model of collecting duct ammonia transport. Basolateral Rhbg and Rhcg mediate peritubular NH_3 and NH_4^+ uptake. In the IMCD,

basolateral $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ also contributes to peritubular NH_4^+ uptake by binding and transport of NH_4^+ at the K^+ binding site. Apical Rhcg enables selective apical NH_3 secretion. NH_3 that is secreted is titrated to NH_4^+ by tightly coordinated H^+ secretion via $\text{H}^+ - \text{ATPase}$ and $\text{H}^+ - \text{K}^+ - \text{ATPase}$. In the IMCD, basolateral $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ also contributes to peritubular NH_4^+ uptake by binding and transport of NH_4^+ at the K^+ binding site.

Figure 5. Correlation between plasma K^+ and urine ammonia excretion.

Over a wide range of plasma K^+ measurements, there was a very strong correlation between plasma K^+ and urinary ammonia excretion. All measurements were made in animals with normal glomerular filtration and without altering adrenal hormone synthesis or action.

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A. Absolute urinary ammonia excretion

B. Body weight-adjusted urinary ammonia excretion

Figure 6. NBCe1-A deletion alters the correlation between serum bicarbonate and urine ammonia excretion under basal conditions and after acid loading.

NBCe1-A deletion results in a substantial decrease in basal serum bicarbonate without a change in basal ammonia excretion, it causes a greater decrease in plasma bicarbonate after acid loading, and it decreases the change in ammonia excretion in response to acid loading. These conclusions were similar whether assessing total ammonia excretion (Panel A) or using body weight-adjusted ammonia excretion (Panel B). Reprinted with permission from *J Am Soc Nephrol* 29:1182–1197, 2018.