More actors in ammonia absorption by the thick ascending limb

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AMMONIUM IS THE MAIN REGULATED component of urinary net acid excretion.1 Its renal production and excretion rise in response to an acid load, thereby allowing the kidney to appropriately increase net acid excretion. Most of the ammonia2 produced in the proximal tubule and entering the loop of Henle is reabsorbed upstream to the distal tubule. It is then secreted either into the collecting duct to allow excretion of ammonia in urine or into the long-loop thin descending limbs (11) (Fig. 1). The thick ascending limb (TAL) is therefore a tubular segment absorbing ammonia interposed between two ammonia-secret- ing segments. It has been assumed that reabsorption of ammonia in the TAL is an important step in the process of ammonia secretion since it allows, under most circumstances, accumulation of ammonia in the medullary interstitium according to a countercurrent multiplication phenomenon, which creates a driving force for ammonia secretion in the collecting duct system.

The corticopapillary ammonium gradient, present in control animals on a regular diet, is enhanced during chronic metabolic acidosis and water deprivation and almost abolished in base- line conditions. The corticopapillary ammonium gradient is probably responsible for the progressive decline in renal ammonia excretion that is a feature of chronic kidney disease and, especially, chronic renal failure (7).

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The corticopapillary ammonium gradient, present in control animals on a regular diet, is enhanced during chronic metabolic acidosis and water deprivation and almost abolished in base-loaded animals and during treatment by furosemide (25, 30). Therefore, this gradient displays some plasticity, being maximal when ammonium secretion in the collecting duct must be enhanced (metabolic acidosis) or ammonium excreted at a high concentration (water deprivation), and minimal when ammonium secretion in the collecting duct must be lowered (base loading) or the ability to concentrate urine is lost.

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The concentration of ammonia in the tubular fluid at the bend of the loop of Henle ranges from 2.5 to 14.5 mM in control animals and increases to 9.4–24.2 mM in metabolic acidosis (5–7, 10, 15); at the beginning of the distal tubule and under control conditions, the luminal ammonia concentration ranges from 1.2 to 2.2 mM (31, 35). The medullary portion of the TAL is therefore surrounded by fluids containing, at the least, millimolar concentrations of ammonium. More than 10 years ago, Good et al. (12, 13, 16, 18, 34) demonstrated that ammonium is reabsorbed in the TAL of the loop of Henle against a concentration gradient for NH3 and NH4+. The TAL also reabsorbs bicarbonate, resulting in an acidification of the luminal fluid and a lowering of the luminal NH3 concentration.

In addition, the apical membrane of the TAL cells is highly impermeable to NH3 (18, 34). It was then concluded that NH4+ rather than NH3 is transported by the TAL of the loop of Henle, that this transport is necessarily active, and that the low permeability to NH3 of the apical membrane is helpful to prevent the backdiffusion of NH3 into the lumen and the dissipation of the gradient.

Although some NH4+ is reabsorbed paracellularly, given the lumen-positive transcellular potential difference existing in this segment, the bulk of ammonium is reabsorbed across the transcellular pathway. Studies in apical membrane vesicles prepared from medullary TAL cells demonstrated a direct competition between NH4+ and K+ for their transport by the Na-K-2Cl cotransporter NKCC2 (BSC1) (19). Although NH4+ can also permeate the apical membrane through barium-sensi-
The basolateral step of ammonium reabsorption has long been not well understood, despite the finding that amiloride at high concentration (1 mM), when applied to the basolateral, but not the apical, membrane, partly inhibits ammonium transport (33). Actually, the permeability of the basolateral membrane is of the same order of magnitude as that of the apical membrane, but, given its much larger surface, a measurable transport of NH$_3$ can occur across the basolateral membrane (29). In addition, the basolateral membrane also displays a measurable permeability to NH$_4^+$. In the last decade, two basolateral transporters have been proposed to play a role in the basolateral exit of NH$_3$/NH$_4^+$ out of the TAL cells.

Contrasting with the electrogenic, SITS-sensitive, Na-HCO$_3^-$ cotransporter identified in the cortical TAL (20), the basolateral electroneutral Na-HCO$_3^-$ cotransporter NBCn1 (Slc4a7, GenBank accession no. AF070475), initially cloned from the rat smooth muscle cell (9), has subsequently been identified in the basolateral membrane of the rat medullary TAL cells (3, 32). Being electroneutral, NBCn1 acts as a base-loader, driving sodium and bicarbonate into the cell, the latter being able to react with the NH$_4^+$ ions present within the cell, thereby decreasing the intracellular concentration of NH$_4^+$, increasing the NH$_3$ concentration, and favoring the diffusion of NH$_3$ across the basolateral membrane. More recently, NBCn1 has also been localized in the TAL and medullary collecting duct cells in the mouse (2).

The NBCn1 protein abundance in the kidney and its activity in the TAL cells are significantly increased during chronic metabolic acidosis induced by NH$_4$Cl loading, with free or controlled water intake (21). Of potential interest, NBCn1 protein abundance was increased $\sim$10 times under the first condition (without control of water intake) and only 2.6 times under the second one. NBCn1 has been functionally localized in the basolateral membrane of the medullary TAL cells (23); using in vitro micropерfused medullary TALs from control and acid-loaded rats, Odgaard et al. (23) were able to demonstrate a 2.6-fold increase in basolateral, Na-dependent HCO$_3^-$ influx in tubules harvested from acid-loaded animals, quite consistent with an upregulation of NBCn1 under this condition. Recently, Lee et al. (22) reported that the NBCn1 protein abundance in ST-1 cells, a cell line exhibiting several features of the medullary TAL cells, also increased after 24 h of incubation in an acid medium.

This property of NBCn1 to be upregulated by a systemic acid load is not restricted to the kidney. It is shared by the NBCn1 protein expressed in various regions of the brain (26). This effect seems to be related, at least in part, to a higher mRNA abundance (26). NBCn1 has also been found expressed in osteoclasts, but not in osteoblasts or osteocytes, and localized to the ruffled border facing the trabeculae, where its expression increases during metabolic acidosis, a condition known to elicit an osteoclast-dependent increase in bone resorption (28). The inhibition of NBCn1 in osteoclasts leads to a decrease in bone resorption in vitro, and it has been assumed that NBCn1 could be important for removing the bicarbonate/carbonate released during osteoclast-dependent osteolysis and sustaining the action of osteoclasts.

However, although its expression is controlled by the acid-base status, the exact role, if any, of NBCn1 in ammonium reabsorption by the TAL and ammonium excretion by the kidney is still unclear. In fact, the evidence that the lack of
NBCn1 in the TAL cells is responsible for a decrease in transepithelial ammonium absorption, in ammonium accumulation in the medulla, and in ammonium excretion in final urine is lacking.

Recent findings have unraveled the important role of another molecule, the Na\(^+\)/H\(^+\) exchanger NHE4 (Slc9a4, GenBank accession no NM_173098). Using highly purified basolateral membrane vesicles from medullary TAL cells, Blanchard et al. (1) were able to demonstrate that the basolateral Na\(^+\)/H\(^+\) exchange could operate as a Na\(^+\)/NH\(_4\)\(^+\)/H\(^+\) exchange. This exchange is inhibited by amiloride derivatives and operates even in the absence of any NH\(_3\) gradient. Finally, the basolateral Na\(^+\)/H\(^+\) exchange exhibits a half-maximal intracellular H\(^+\) activation value of 6.58, which is remarkably close to the intracellular intracellular pH value of medullary TAL cells when they absorb ammonia.

The basolateral membrane of the medullary TAL cells expresses not only one but two distinct sodium-hydrogen ion exchangers. Indeed, Chambrey et al. (8) clearly demonstrated that, besides NHE1, the basolateral membrane of the TAL cells expresses a second isoform of the Na\(^+\)/H\(^+\) exchangers, namely, NHE4 (8). NHE4 has a remarkably low sensitivity to amiloride derivatives: in purified basolateral vesicles, the IC\(_{50}\) value for ethyl isopropyl amiloride has been calculated to 2.5 \(\mu\)M, contrasting with the high sensitivity of NHE1 (IC\(_{50}\) = 11 nM) (8). NHE1 and NHE4 also differ from each other by their respective sensitivity to intracellular pH: the apparent pK\(_a\) values of NHE1 and NHE4, the values of internal pH yielding half-maximal activation of the transporter, have been calculated to 6.75 and 6.21, respectively (8, 24). Consequently, when the medullary TAL cells are not in the presence of extracellular ammonium and have an intracellular pH of approximately 6.5 (34), NHE4 is probably almost inactive. However, when the medullary TAL cells absorb ammonium, their intracellular pH, at \(\sim 6.5-6.6\) (34), is expected to activate NHE4.

NHE4-null mice are viable and fertile and grow normally under regular conditions. However, when on a regular laboratory diet, they display a compensated, hyperchloremic metabolic acidosis together with an inappropriate renal response (4). Actually, the renal net acid excretion is similar to that of normal mice, revealing the inability to excrete more acid as would normally be expected in metabolic acidosis. Noteworthy, the urinary pH of NHE4-null mice is measurably lower than that of control mice, by \(\sim 0.3\) pH units. This suggests, on the one hand, that the lack of NHE4 does not impair the mechanisms of hydrogen ion secretion in the collecting duct and, on the other hand, that the lower urinary pH could be a

Fig. 2. Experimental evidence that Na\(^+\)/H\(^+\) exchanger NHE4 is required for ammonia transport in the TAL of the loop of Henle. A: altered renal response to an oral acid load in mice lacking NHE4. B: evidence for a defect in creating the corticopapillary gradient of ammonia in the absence of NHE4. C: mice lacking NHE4 have a defect in the reabsorption of ammonia in the medullary TAL. Figure is modeled after Ref. 4.

Fig. 3. Current model of transcellular ammonia transport in the TAL of the loop of Henle. Ammonium enters the cell mainly by substituting for potassium on the Na\(^+\)/K\(^+\)-2Cl\(^-\) cotransporter NKCC2. Due to the very low permeability of the apical membrane to NH\(_3\), the entrance of NH\(_4\)\(^+\) to the cell results in a decrease in cytosolic pH (pH\(_i\)). At half of the basolateral exit of ammonium is accounted for by NHE4 operating as a Na\(^+\)/NH\(_4\)\(^+\) exchanger. It is possible that the basolateral electroneutral Na-HCO\(_3\) cotransporter NBCn1 plays also a role in the basolateral exit of ammonia in favoring the dissociation of NH\(_4\)\(^+\) into NH\(_3\) and H\(^+\) and creating a favorable chemical gradient for the diffusion of NH\(_3\) across the basolateral membrane.
means to partly sustain ammonium secretion across the collecting duct epithelium. Despite this favorable pH gradient for ammonium secretion, that NHE4-null mice do fail to excrete the required amount of acid to correct their metabolic acidosis suggests that their ability to normally accumulate ammonium in the medullary interstitium is already altered.

When challenged with an additional acid load for 1 wk, NHE4-null mice display a worsening of their metabolic acidosis whereas control littermate mice recover from acidosis (4). The most striking difference between normal and NHE4-null mice is the inability of the latter to properly increase their urinary ammonium excretion and, hence, their urinary net acid excretion (Fig. 2). Good et al. (15) underlined the importance of medullary ammonia accumulation, and hence of ammonia absorption in the loop of Henle, as a primary determinant of ammonia secretion in the inner medullary collecting duct in vivo. In agreement, NHE4-null mice have partially lost the ability to normally increase ammonia concentration in the outer and the inner medulla (Fig. 2). The reason is probably that the medullary TAL cells reabsorb significantly less ammonia when NHE4 is absent than when it is present and that NHE4 gene expression and protein activity are positively regulated during ammonia nor the corticopapillary ammonia gradient is compromised.

Very little is known regarding the factors that control NHE4 expression and activity. However, the sequence of the NHE4 gene has been characterized when it is present and that NHE4 gene expression and protein activity are positively regulated during metabolic acidosis (Fig. 2). Of note, neither the absorption of ammonia nor the corticopapillary ammonia gradient is completely abolished in the absence of NHE4, indicating that at least two different transporters are involved in the basolateral exit of ammonia out of the medullary TAL cell.

The data recently obtained on the role of NHE4 in the medullary TAL help to build a more comprehensive model of transcellular ammonia absorption in this segment (Fig. 3). Since the apical membrane of medullary TAL cells has a very low permeability to NH3, it is clear that ammonia enters this cell in the form of NH4+ (18, 34). By contrast, it was still unclear whether ammonia leaves the cell as NH4+ or as the gas NH3 (together with a parallel transport of hydrogen ions or in exchange with hydroxyl ions). Several data support the view that NHE4 actually transports NH4+ First, as already mentioned, in basolateral membrane vesicle studies, an outwardly directed NH4+/H+ gradient-stimulates Na influx in the absence of a transmembrane NH gradient (1). Second, the inhibition of NHE1 activity in the basolateral membrane of medullary TAL cells by the amiloride derivative EIPA does not alter transepithelial ammonia reabsorption, suggesting that NHE1 does not play a direct role in the process of ammonia absorption and, therefore, that basolateral ammonia transport is not (only) the result of the parallel transport of hydrogen ions and NH3. However, NHE4 does not account for the entirety of ammonia exit out of the medullary TAL cells (4). It remains possible that the NHE4-independent transport is accounted for by the coupling of NH3 diffusion and secondary active, basolateral, HCO3− entrance to the cell, conceivably via NBCn1. In the absence of a specific inhibitor for this latter transporter, this hypothesis could only be tested in NBCn1-null mice.

Very little is known regarding the factors that control NHE4 expression and activity. That, under chronic metabolic acidosis, the activity of NHE4 is increased in proportion to its mRNA abundance is consistent with an acidosis-induced enhanced gene expression. However, the sequence of the NHE4 promoter has not yet been analyzed, and no information is available regarding the factors that could act as transactivators. It is also unknown whether NHE4 activity could change in response to acute metabolic disturbances, for example, those affecting its intracellular traffic. Given that NHE4 is required for the accumulation of ammonia in the renal medulla, its activity/expression might be enhanced during water deprivation and decreased during water loading, but the evidence that it is actually the case is lacking.

All types of distal renal tubular acidosis (RTA) are associated with a decrease in urinary ammonium excretion, which could result from a defect in ammonium transport in any place in the distal nephron. The absence of NHE4 is responsible for a unique phenotype associating a defect in urinary ammonium excretion together with a low urinary pH, whereas distal RTA is usually characterized by a high urinary pH. To our knowledge, a phenotype of RTA consistent with a defect in medullary ammonia accumulation has not yet been described in patients with a normal glomerular filtration rate.

Finally, the involvement of Na+/H+ exchanger(s) in transepithelial ammonia transport has also been described in fishes, which need to excrete ammonia against a defavorable concentration gradient. Actually the giant mudskipper Periophthalmonodon schlosseri can survive in 100 mM NH4Cl for more than 1 wk because it can excrete ammonia against large inward NH3 and NH4+ gradients (17), a process that is inhibited by amiloride (27). The secretion of ammonia by the branchial epithelial cells likely involves Na+/H+ exchanger(s) located in the apical membrane. Therefore, the involvement of Na+/H+ exchanger(s) might be a general process when ammonia needs to be secreted against a defavorable gradient between two compartments with similar pH.

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