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Renal Handling of NH_3/NH_4^+: Recent Concepts

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Abstract

To be appropriately excreted in urine, NH_4^+, the major component of urinary acid excretion, must be synthesized by proximal tubular cells, secreted into the proximal tubular fluid, reabsorbed by the medullary thick ascending limb (MTAL) to be accumulated in the medullary interstitium, and finally secreted in medullary collecting ducts. Several targets have been identified to account at the gene expression level for the adaptation of renal NH_4^+ synthesis and transport in response to a chronic acid load. These targets are the key enzymes of ammoniagenesis (mitochondrial glutaminase and glutamate dehydrogenase) and gluconeogenesis (phosphoenolpyruvate carboxykinase) and the Na^+/H^+(NH_4^+) exchanger NHE_3 in the proximal tubule, the apical Na^+-K^+(NH_4^+)-$2Cl^-$ cotransporter of the MTAL, the basolateral Na^+-K^+(NH_4^+)-$2Cl^-$ cotransporter, and likely the epithelial Rh B and C glycoproteins in the collecting ducts. An acid pH per se appears to be a major factor in the control of the expression of these genes during metabolic acidosis probably through activation of pH sensors. Glucocorticoids may also act in concert with an acid pH to coordinate the adaptation of various tubular cell types. The present review focuses on some new aspects of NH_3/NH_4^+ transport and of regulations of gene expression that have recently emerged.

Introduction

Ammonium ion NH_4^+ urinary excretion is one of the main components by which the kidney regulates systemic acid-base balance. Indeed, NH_4^+ represents two third and ~90% of the acids excreted in the urine under normal conditions and in response to chronic metabolic acidosis, respectively, in mammals. Ammonium is metabolically produced by proximal tubular cells, and part of this production is secreted within the tubular fluid. NH_4^+ then reaches the thick ascending limb (TAL) of Henle's loop where 40–80% of the amount delivered by the proximal tubule are reabsorbed. Absorption of NH_4^+ and NH_3 by the medullary TAL (MTAL) without water creates tran-

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sepithelial concentration differences of these chemical species, which provides the energy for countercurrent exchanges between the various tubular segments arranged in parallel in the renal medulla. This leads to the accumulation of total ammonia (the sum of NH_4^+ and NH_3) in the medullary interstitium. This medullary accumulation of total ammonia favors its secretion into the tubular fluid of adjacent medullary collecting ducts, which is necessary to the excretion of appropriate amounts of NH_4^+ in final urine. Thus virtually 100% of the amount of NH_4^+ excreted in urine comes from the fraction of NH_4^+ synthesized by proximal tubular cells that is absorbed by the MTAL. Diffusion of NH_3 coupled to H^+ transport and trapping as NH_4^+ in acidic compartments is an important mechanism of transepithelial ammonium transport but NH_4^+ is also transported as such at almost all of the various steps mentioned above. The transport mechanisms of this particular NH_4^+ renal pathway have been previously comprehensively reviewed [1–3] and will be only summarized here. Most importantly, NH_4^+ synthesis and transport by the various tubular segments are highly regulated by the acid-base status and the present review will focus on some aspects of transport and regulation that have emerged recently.

Proximal Tubule

The bulk of renal ammoniagenesis comes from the proximal tubule which is the site of adaptation in response to variations in the acid-base status. The increased ability of the proximal tubule to synthesize NH_4^+ from glutamine during metabolic acidosis is due to the acidosis-induced induction of three key enzymes: the mitochondrial glutaminase (GA) and glutamate dehydrogenase which convert glutamine to glutamate and glutamate and α-ketoglutarate, respectively, and the cytosolic phosphoenolpyruvate carboxykinase which is the rate-limiting enzyme in the process of conversion of the α-ketoglutarate to glucose. These metabolic pathways generate two NH_4^+ and two bicarbonate per one glutamine molecule. Recent studies by Curthoys et al. [4] have addressed the mechanisms of the stimulation of GA expression during metabolic acidosis. The induction of GA mRNA in the renal cortex resulted from an augmented mRNA stability. Direct effects of an acid pH were demonstrated in as much as in vitro incubation of cultured renal epithelial cells in an acid medium reproduced the effects of metabolic acidosis on GA mRNA stability. Studies have shown that the increased GA mRNA stability resulted from an acid pH-induced stimulation of the binding of ζ-crystallin/NADPH:quinone reductase to a pH response element present in the AU-rich 3′-untranslated region of the GA mRNA, which augments the stability of the GA mRNA. In addition to the direct effects of an acid pH summarized above, hormonal factors contribute to the stimulation by metabolic acidosis of ammoniagenesis in the proximal tubule. The circulating levels of glucocorticoids increase during metabolic acidosis [5], and glucocorticoids were shown to be necessary to a fully enhanced NH_4^+ urinary excretion in response to acute and chronic metabolic acidosis.

Glutamine catabolism in the proximal tubule generates NH_4^+ and also bicarbonate after complete catabolism of α-ketoglutarate to CO_2 and H_2O. NH_4^+ must leave the cell preferentially through the apical membrane and bicarbonate through the basolateral membrane to permit urinary acid excretion and to replace the bicarbonate that was consumed by the acid load, respectively. It is well known that bicarbonate leaves the proximal tubular cell exclusively through the basolateral membrane principally by the activity of the electrogenic Na^+-$(HCO_3^-)_3$ cotransporter. The transports of NH_4^+ and NH_3 through the apical and basolateral membranes are not completely defined. It is generally accepted that NH_3 diffuses preferentially through the apical membrane because NH_3 is trapped as NH_4^+ in the acid luminal fluid. NH_4^+ may also be transported as such through the apical Na^+/H^+ exchanger NHE3 that may function in a Na^+/NH_4^+ exchange mode, which accounted for the majority of NH_4^+ secretion by the mouse proximal tubule isolated and perfused in vitro [6]. In addition, NH_4^+ may be taken up from the peritubular space into the cell by a barium- and quinidine-sensitive K^+/NH_4^+ antiport mechanism [7]. The latter basolateral transport mechanism of NH_4^+, which recaptures ammonia that has left the cell in the wrong direction, favors luminal ammonia secretion in the proximal tubule. It is worth noting that, under normal acid-base conditions, ammonia is abundantly secreted in the initial part of the proximal tubule, where the tubular fluid is not yet importantly acidified, and that part of this secreted ammonia is reabsorbed along the rest of the proximal convoluted tubule where the tubular fluid is substantially acidified. During metabolic acidosis, ammonia secretion is enhanced and occurs along the entire length of the proximal convoluted tubule. These considerations point to a major role of NH_4^+ transport as compared with non ionic diffusion of NH3 in the process of ammonia secretion and in its regulation in the proximal tubule. In this respect, the activity and abundance of the

apical Na^+/H^+ exchanger NHE_3 is increased in the proximal brush border membrane during metabolic acidosis, which could be expected to contribute to the enhanced NH_4^+ secretion by the proximal tubule during this condition. In this connection, it was recently shown that ammonia production and secretion were higher in S3 proximal segments from acidotic than from normal mice only when angiotensin II was present in the luminal perfusion fluid and when apical Na^+/H^+ exchange was functionally active [8]. Thus, it appears that stimulation of ammonia secretion by the proximal tubule during metabolic acidosis largely depends on $Na^+/H^+(NH_4^+)$ exchange activity and on angiotensin II.

Thick Ascending Limb of Henle's Loop

Total ammonia is absorbed by the MTAL primarily as NH_4^+ by secondary active transporters. Absorption of NH_3 also occurs because NH_4^+ absorption lowers the luminal NH3 concentration due to a shift in the NH_4^+/NH_3 equilibrium and because the low permeability of the MTAL apical membrane to NH_3 prevents NH_3 from diffusing back into the lumen in appreciable amounts. Diffusion of NH_4^+ from lumen to peritubular space also takes place through the paracellular pathway as a consequence of the lumen positive transepithelial voltage of the MTAL. Transcellular transport has been estimated to account for at least 60–70% of the amount of total ammonia absorbed by the rat MTAL. MTAL NH_4^+ absorption is regulated by the acid-base status. Indeed, the ability of the MTAL isolated and perfused in vitro to absorb total ammonia is increased during chronic metabolic acidosis [9]. These observations pointed to the MTAL as a key segment of the nephron with respect to urinary NH_4^+ excretion in relation to regulation of acid-base balance by the kidney. The functional and molecular characteristics of the MTAL NH_4^+ carriers have been recently reviewed in detail [3] and will be only briefly summarized hereafter. The Na^+-$K^+(NH_4^+)$-$2Cl^-$ cotransporter BSC1/NKCC2 is the main apical NH_4^+ carrier and is responsible for 50–65% and an electroneutral $K^+/NH_4^+(H^+)$ antiport mechanism for the rest of the MTAL NH_4^+ luminal uptake. On the basolateral side of TAL cells, the Na^+/H^+ exchanger NHE_1 of the MTAL could importantly contribute to the cell-to-peritubular space NH_4^+ transport in two ways. First, NHE1 may function in a Na^+ out/NH_4^+ in exchange mode like other NHEs. Second, Na^+/H^+ exchange could be coupled to NH_3 diffusion from cell to peritubular space after dissociation of the NH_4^+, entered within the cell from the lumen, into NH_3 plus H^+. The respective parts of these two possibilities as well as the overall role of NHE_1 in MTAL transepithelial NH_4^+ transport have not been experimentally defined. As noted above, the ability of the MTAL isolated and perfused in vitro to absorb NH_4^+ increases during CMA [9], and this adaptation favors the renal elimination of an acid load. The sole mechanism that has been documented up to now to explain this MTAL adaptation is the regulation of expression of the Na^+-$K^+(NH_4^+)$-$2Cl^-$ cotransporter BSC1/NKCC2 by metabolic acidosis in the rat [10]. The abundance of BSC1/NKCC2 mRNA increased in the MTAL as soon as after 3 h of metabolic acidosis induced by peritoneal dialysis and the augmentation of BSC1/NKCC2 mRNA and protein persisted after 6 days of metabolic acidosis caused by NH_4Cl administration. At least two factors may account for the stimulating effect of chronic metabolic acidosis on BSC1/NKCC2 expression: the pH value of the surrounding environment and glucocorticoids. First, in vitro incubation of rat MTAL fragments in suspension in an acid medium strongly enhanced the BSC1/NKCC2 mRNA and protein abundance and cotransport activity [10]. Second, administration of the glucocorticoid dexamethasone to adrenalectomized rats stimulated BSC1/NKCC2 expression at the mRNA and protein levels [11]. Furthermore, in vitro application of dexamethasone to rat MTAL fragments enhanced BSC1 mRNA and protein abundance and cotransport activity, which required interactions with cAMP-dependent factors [11].

Medullary Collecting Ducts

Ammonium that has accumulated in the medullary interstitium as a result of NH_4^+/NH_3 absorption by the MTAL is secreted in outer and inner medullary collecting ducts. That ammonia secretion in cortical collecting ducts appreciably contributes to NH_4^+ excretion in urine is uncertain. The mechanism of NH_4^+ secretion in outer and inner medullary collecting ducts is not completely clear at present. Several lines of evidence suggest that transport of NH_4^+ from the peritubular interstitium into the cell may be an important first step in transepithelial NH_4^+ secretion. Then, following dissociation of NH_4^+ into NH_3 plus H^+ in the cytosol, which acidifies the cell and provides a source for apical H^+ secretion [11], NH_3 diffusion coupled to H^+ secretion within the tubular fluid would account for a major part, if not all, of the apical step. Whether NH_4^+ may be also directly transported through

the apical membrane is unknown at present. Two NH_4^+ carriers have been described in medullary tubular cells. First, the basolateral $Na^+/K^+(NH_4^+)$-ATPase pump has been demonstrated to mediate NH_4^+ secretion in the rat inner medullary collecting duct isolated and perfused in vitro [12]. The latter mechanism may be quantitatively noticeable in the inner medulla in which the NH_4^+ concentration is high. Second, the secretory Na^+-$K^+(NH_4^+)$-$2Cl^-$ cotransporter BSC2/NKCC1 has been immunolocalized to the basolateral membrane of α-intercalated cells of outer and initial inner medullary collecting ducts in the rat, and mainly to the basolateral membrane of cells of the terminal two-thirds of inner medullary collecting ducts in the mouse. BSC2/NKCC1 was recently reported to mediate K^+- and NH_4^+-dependent chloride secretion, and thus to be involved in transepithelial solute transport, by outer medullary collecting ducts from deoxycorticosterone pivalate-treated rats [13]. Finally, BSC2/NKCC1 was recently shown to be upregulated by chronic metabolic acidosis in collecting ducts of the rat: the abundance of the mRNA increased in cortical, outer, and inner medullary collecting ducts, but that of the protein was detected only in outer medullary collecting ducts [14]. In addition, upregulation of the apical vacuolar H^+-ATPase of α-intercalated cells is well documented during metabolic acidosis, which should contribute to enhance the ability of collecting ducts to secrete NH_4^+.

Rh B and C Glycoproteins

Recently, new ammonia carriers have been identified in the kidney of mammals, i.e. the Rh B and C glycoproteins (RhBG and RhCG). RhBG and RhCG are nonerythroid homologs of the blood group Rh proteins which share significant sequence homology with the methyl-ammonium permease/ammonium transporters (Mep/Amt) superfamily of primitive organisms. In the rat kidney, RhBG and RhCG are expressed in the basolateral and apical membranes, respectively, in the same cells of the distal nephron, i.e. all cells of the connecting tubule and intercalated cells of the cortical and medullary collecting ducts [15]. When expressed in *Xenopus laevis* oocytes, RhCG was clearly shown to be a NH_3 carrier [16] but, because NH_4Cl exposure induced inward currents in RhCG-expressing but not in control oocytes, it was suggested that RhCG also mediates electrogenic NH_4^+ transport [16]. It must be emphasized, however, that AmtB of *Escherichia coli* has been recently demonstrated to form a trimer of channel-containing proteins which specifically conduct uncharged NH_3, not NH_4^+ [17]. Thus, it is possible that, in the study cited above [16], exposure to NH_4Cl of RhCG-expressing oocytes simply activated an endogenous NH_4^+ conductance after entry of NH_3 within the oocytes (the intensity of NH_4^+-induced current was dependent on the concentration of NH_4Cl but not on the amount of injected mRNA). Whether RhBG and RhCG are involved in transepithelial ammonia transport and/or in ammonia accumulation within the cells to regulate the activity of other acid-base transporters in the distal nephron is not established at present.

In summary, urinary NH_4^+ excretion in amounts appropriate to the acid-base status requires that the various steps of the renal handling of NH_4^+ be coordinately regulated. Two major factors have been recognized at present to mediate increases in gene expression in response to metabolic acidosis: an acid pH and glucocorticoids. Whereas it is known that glucocorticoids regulate gene transcription via the glucocorticoid receptor, whether an acid pH acts through activation of a proton sensor located in the plasma membrane or within the cell remains to be determined.

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