Ammonium carriers in medullary thick ascending limb

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Ammonium carriers in medullary thick ascending limb. Am J Physiol Renal Physiol 280: F1–F9, 2001.—Absorption of NH4+ by the medullary thick ascending limb (MTAL) is a key event in the renal handling of NH4+, leading to accumulation of NH4+/NH3 in the renal medulla, which favors NH4+ secretion in medullary collecting ducts and excretion in urine. The Na+/K+-2Cl– cotransporter (BSC1/NKCC2) ensures 50–65% of MTAL active luminal NH4+ uptake under basal conditions. Apical barium- and verapamil-sensitive K+/NH4+ antiport and amiloride-sensitive NH4+ conductance account for the rest of active luminal NH4+ transport. The presence of a K+/NH4+ antiport besides BSC1 allows NH4+ and NaCl absorption by MTAL to be independently regulated by vasopressin. At the basolateral step, the roles of NH3 diffusion coupled to Na+/H+ exchange or Na+/NH4+ exchange, which favors NH4+ absorption, and of Na+/K+/NH4+–ATPase, NH4+/Cl– cotransport, and NH4+ conductance, which oppose NH4+ absorption, have not been quantitatively defined. The increased ability of the MTAL to absorb NH4+ during chronic metabolic acidosis involves an increase in BSC1 expression, but fine regulation of MTAL NH4+ transport probably requires coordinated effects on various apical and basolateral MTAL carriers.

AMMONIUM, QUANTITATIVELY THE main component of the acids excreted in the urine, is metabolically produced by proximal tubular cells, and part of this production is secreted within the tubular fluid. NH4+ reaches the thick ascending limb (TAL) of Henle’s loop, where 40–80% of the amount delivered by the proximal tubule is reabsorbed. Absorption of NH4+ and NH3 by the medullary TAL (MTAL) without water creates trans-epithelial concentration differences in these chemical species, which provide 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 NH4+ and NH3) in the medullary interstitium, which favors NH4+ secretion into the adjacent medullary collecting ducts and excretion in final urine. This particular renal handling of total ammonia has been previously comprehensively reviewed (48).

Most importantly, MTAL NH4+ absorption is regulated. Micropuncture experiments have established that the NH4+ amount absorbed by the loop of Henle is increased during chronic metabolic acidosis (CMA) because the amount of NH4+ delivered by the proximal tubule is augmented and because of an adaptation of the TAL during this condition (21, 65, 68). As a matter of fact, the ability of the MTAL isolated and perfused in...
vitro to absorb total ammonia is increased during CMA (30). These observations pointed to the MTAL as a key segment of the nephron with respect to urinary NH$_4^+$ excretion as related to regulation of acid-base balance by the kidney.

Diffusion of NH$_4^+$ coupled to H$^+$ transport and trapping as NH$_4^+$ in acidic compartments is an important mechanism of transepithelial ammonium transport but cannot account for all of the amount transported at the various steps of the specialized NH$_4^+$ renal pathway; i.e., NH$_4^+$ must be transported as such by some cell types that express the appropriate carriers. First, it has been recognized that NH$_4^+$ can substitute for other ions in renal transport systems such as the Na$^+$/H$^+$ antiport in the apical membrane of the proximal tubule (47), amiloride-sensitive cation channel in the inner medullary collecting duct (52), and Na$^+$/K$^+$/2Cl$^-$ cotransporter and Na$^+$/K$^+$/-2Cl$^-$ cotransporter in the TAL (46). Then, other NH$_4^+$ carriers have been discovered that may perhaps be more specific for NH$_4^+$ absorption by the MTAL primarily as NH$_4^+$ by secondary active transporters. Absorption of NH$_3$ also occurs because NH$_4^+$ absorption lowers the luminal NH$_3$ concentration because of a shift in NH$_4^+/NH_3$ equilibrium and because the low permeability of the MTAL apical membrane to NH$_3$ (42) 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 positive luminal transepithelial voltage of the MTAL (48). In this review, we will focus on the functional and molecular properties of the NH$_4^+$ carriers present in MTAL cells and on the regulation of MTAL NH$_4^+$ transport.

**MTAL APICAL NH$_4^+$ CARRIERS**

NH$_4^+$ absorption by the MTAL occurs by active transcellular transport and passive paracellular diffusion driven by the lumen-positive transepithelial voltage (48). Transcellular transport has been estimated to account for at least 60–70% of the amount of total ammonia absorbed by the rat MTAL (28). A schematic representation of MTAL NH$_4^+$ carriers is depicted in Fig. 1.

The first transporter that was recognized as a NH$_4^+$ carrier in the MTAL is the apical Na$^+$/K$^+$/2Cl$^-$ cotransporter. That 10$^{-4}$ M furosemide eliminated total ammonia absorption by the rat MTAL isolated and perfused in vitro, in the pioneer work of Good et al. (31), suggested that NH$_4^+$ was carried by the furosemide- and bumetanide-sensitive Na$^+$/K$^+$/-2Cl$^-$ cotransporter. Subsequently, Kinne et al. (46), using plasma membrane vesicles prepared from rabbit MTAL cells, have demonstrated that NH$_4^+$ is accepted by the K$^+$ site of the Na$^+$/K$^+$/-2Cl$^-$ cotransporter with an affinity for NH$_4^+$ that is relatively high [Michaelis-Menten coefficient ($K_m$) = 1.9 mM] and similar to that for K$^+$ ($K_m = 0.3$ mM). The apical Na$^+$/K$^+$/-2Cl$^-$ cotransporter (BSC1 or NKCC2) was recently cloned from rat (24), mouse (39, 57), rabbit (60), and human (67) kidneys. BSC1 protein has been localized by antibodies at the apical membrane of the cortical and MTAL, as well as the macula densa (23, 41, 59). In the

![Fig. 1. Schematic model of cellular NH$_4^+$ transport pathways in medullary thick ascending limb of Henle’s loop (MTAL). Inhibitors are indicated in italics. Apical carriers are Na$^+$/NH$_4^+/K^+$-2Cl$^-$ cotransporter (BSC1/NKCC2), K$^+$/NH$_4^+/H^+$ antiporter, NH$_4^+$ (nonselective) conductance, and Na$^+$/H$^+$/NH$_4^+$ (Na$^+$/H$^+$ exchangers NHE3 and/or NHE2). Basolateral carriers are Na$^+$/K$^+$-ATPase, NH$_4^+/K^+$-Cl$^-$ cotransporter (KCC4 and possibly KCC1), NH$_4^+$ conductance, and Na$^+$/H$^+$/NH$_4^+$ antiporter (NHE1). BSC1 is responsible for the majority (50–65%) and K$^+$/NH$_4^+$ probably for most of the rest of cellular luminal NH$_4^+$ uptake; the quantitative relevance of the various basolateral NH$_4^+$ transport pathways is unknown at present.](http://ajprenal.physiology.org/
mouse, at least six transcripts of the BSC1 gene are expressed in TAL cells (39, 57). This results from the combination of two alternative splicing mechanisms, which give rise to mBSC1–9 A, B, and F and mBSC1–4 A, B, and F isoforms (57). The mBSC1–9 isoforms all express Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransport activity, but not the mBSC1–4 isoforms, which have a dominant negative function in the transport effected by the mBSC1–9 isoforms (63). Preliminary data indicate that, when expressed in *Xenopus laevis* oocytes, the mBSC1–4 isoforms encode a hypotonically activated Na\(^{+}\)-Cl\(^{-}\) cotransport activity that is K\(^{+}\) independent, furosemide sensitive, and inhibited by cAMP (62). Thus, because they are K\(^{+}\) independent, mBSC1–4 isoforms are not expected to transport NH\(_{4}\)\(^{+}\). When the mBSC1–9 isoforms are expressed in *X. laevis* oocytes, cAMP and inhibition of cAMP-dependent protein kinase (protein kinase A; PKA) have no effect on Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransport activity (63). However, when mBSC1–9 and mBSC1–4 isoforms are simultaneously expressed, the dominant negative effect of the mBSC1–4 isoforms is reversed by cAMP (63). These recent results are consistent with the previous report that vasopressin alters the mechanism of apical Cl\(^{-}\) entry from Na\(^{+}\)-Cl\(^{-}\) to Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransport in mouse MTAL isolated and perfused in vitro (72). However, it must be noted that, in the rat, a species in which rBSC1 transport activity appears to have an absolute requirement for K\(^{+}\) (8, 64), BSC1–4 isoforms have not been described in the TAL. Yet, rat MTAL Na\(^{+}\)-K\(^{+}\)(NH\(_{4}\)\(^{+}\))-2Cl\(^{-}\) cotransport activity is also directly activated by cAMP through activation of PKA (2). In addition, the F isoforms are expressed predominantly in the MTAL, the B isoforms in macula densa cells, and the A isoforms throughout the TAL (60). Preliminary data obtained in *X. laevis* oocytes indicate that there are large differences in ion (Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\)) affinities among the A, B, and F isoforms consistent with an increase in affinities from MTAL to cortical TAL (CTAL) (27). Differences in affinity for NH\(_{4}\)\(^{+}\) are unknown at present but seem likely because there are differences in affinity for K\(^{+}\). Finally, the promoter of the murine BSC1 gene contains several consensus sites for various transcription factors, which suggests that transcription of BSC1 is responsive to physiological stimuli (40). As will be discussed below, BSC1 expression is indeed regulated by numbers of physiological and pathophysiological conditions. The Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter is thus a major, but not the sole, apical carrier of NH\(_{4}\)\(^{+}\) in the MTAL. That luminal furosemide abolished total ammonia absorption by the isolated and perfused rat MTAL (31) must be explained by both a direct effect on the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter and indirect effects on other NH\(_{4}\) transport pathways through changes in transepithelial voltage and intracellular Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\) concentrations. Indeed, Garvin et al. (25) have shown that 10\(^{-}^4\) M furosemide reduced transepithelial total ammonia absorption by only ~60% in voltage-clamped rabbit MTAL. In the latter work, however, indirect effects on carriers other than the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter through changes in intracellular ion concentrations may also have contributed to this result. Furthermore, several studies, using MTALs isolated and perfused or in suspension and measuring intracellular pH, have concluded that ~50% of the cell acidification caused by abrupt application of NH\(_{4}\)\(^{+}\) was furosemide insensitive (4, 5, 42, 43, 76). In particular, it has been observed that ~30% of the cell acidification rate and 65% of the degree of fall in intracellular pH caused by luminal application of NH\(_{4}\)\(^{+}\) were furosemide insensitive in the rat MTAL (76). As a matter of fact, two NH\(_{4}\) carriers that were present in the apical membrane of the rat MTAL besides the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter were recently discovered: an NH\(_{4}\)\(^{+}\) conductance and a K\(^{+}\)/NH\(_{4}\)\(^{+}\)(H\(^{+}\)) antiport mechanism (4, 8). The presence of a 1 \(\mu\)M amiloride-sensitive NH\(_{4}\)\(^{+}\) conductance in the apical membrane of MTAL cells was demonstrated by the following observations (4, 8). Abrupt exposure of rat MTAL fragments in suspension to NH\(_{4}\)Cl acutely depolarized the cell membrane, as assessed with use of the voltage-sensitive fluorescent probe 3,3\’-dipropylthiacycarboyanine (4). This NH\(_{4}\)\(^{+}\)-induced cell membrane depolarization was abolished by 1 \(\mu\)M amiloride but not by 2 mM barium, which, by itself, depolarized the cell membrane by blocking K\(^{+}\) channels (4). This was in agreement with a previous patch-clamp study of the rat MTAL apical membrane, from which it was concluded that NH\(_{4}\)\(^{+}\) is not conducted by but rather inhibits the MTAL apical K\(^{+}\) channel (18); this was confirmed in a subsequent work by the same group (17). Other membrane vesicles (46) and electrophysiological studies (75) also concluded that NH\(_{4}\)\(^{+}\) is poorly transported by K\(^{+}\) channels in the MTAL, if at all. In addition, 1 \(\mu\)M amiloride significantly reduced by ~30% the NH\(_{4}\)\(^{+}\)-induced cell acidification even in the presence of 10 mM barium (4). Additionally, 10 \(\mu\)M amiloride alkalinized MTAL cells preincubated in the presence of NH\(_{4}\)Cl but not in ammonia-free medium, which thus occurred by suppression of a sustained component of NH\(_{4}\)\(^{+}\) entry within the cells (4). Furthermore, this 1 \(\mu\)M amiloride-sensitive conductive pathway was shown to be located in the apical membrane with use of a MTAL membrane vesicle preparation that had a high enrichment factor (~24) and yield (~33%) in amiloride membrane marker enzyme (8). In addition, patch-clamp experiments indicated the presence in mouse CTAL cells of an apical ~20-pS channel through which NH\(_{4}\)\(^{+}\), Na\(^{+}\), K\(^{+}\), and Ca\(^{2+}\) as well are conducted (permeability values (P) were \(P_{\text{NH}_4^+} > P_{\text{Na}_+} > P_{\text{K}_+} > P_{\text{Ca}^{2+}}\), with \(P_{\text{NH}_4^+}/P_{\text{Na}_+} = 1.7\) (Guinamard R and Teulon J, unpublished observations); however, the sensitivity of this mouse CTAL apical channel to amiloride has not yet been tested. We think that the MTAL apical NH\(_{4}\)\(^{+}\) channel is likely the amiloride-sensitive nonselective cation channel that was purified from the bovine renal medulla and detected close to the apical cell membrane of rat MTAL cells with specific polyclonal antibodies by immunohistochemistry (20, 52). The precise contribution of this nonselective cation channel to NH\(_{4}\)\(^{+}\) absorption by the MTAL is unknown at present because measurement of total ammonia absorption in the-pres-
presence of 1 μM amiloride has not yet been performed to our knowledge. Intracellular pH measurements in the isolated and perfused rat MTAL have shown that a small residual component of luminal NH$_4^+$-induced cell acidification persisted in the presence of luminal 0.1 mM furosemide plus 12 mM barium (76). This residual cell acidification may be interpreted as having resulted from NH$_4^+$ entry through the amiloride-sensitive channel, which may even have been minimized because of a possible barium-induced apical membrane depolarization.

After the major work by Kikeri et al. (42), several studies have confirmed that a barium-sensitive pathway was responsible for an important part of luminal NH$_4^+$ entry within MTAL cells (4, 5, 43, 76). As pointed out above, compelling observations indicated that this pathway was not a K$^+$ channel. In fact, the barium-sensitive component of luminal NH$_4^+$ uptake by MTAL cells has been shown to be mediated by a K$^+$/NH$_4^+$ (H$^+$)$^{-}$ antiport system (4, 8). From intracellular pH, variations in cell membrane potential difference, and potassium transport measurements in rat MTALs in suspension, evidence was provided for the first time that an electroneutral K$^+$/NH$_4^+$ (H$^+$)$^{-}$ antiport is present in MTAL cells (4). This transport system is sensitive to verapamil and high concentrations of barium, but not to quinidine, millimolar concentrations of amiloride, SCH-28080, or DIDS (4). The sensitivity to verapamil and barium and the electroneutrality of the K$^+$/H$^+$ antiport were also observed in rat MTAL membrane vesicles (8). In MTAL membrane vesicles, the apparent inhibition constant of verapamil was 55 μM. A remarkable property of this transport system is that it carries NH$_4^+$ much better than H$^+$ in exchange for K$^+$ at physiological concentrations of NH$_4^+$ and H$^+$ (4). Thus this transporter essentially functions in a K$^+$/NH$_4^+$ antiport mode under normal conditions, which normally exchanges intracellular K$^+$ (K$_i^+$) for luminal NH$_4^+$ (NH$_4^+_{out}$). It can, however, function in a K$^+$/H$^+$ exchange mode when NH$_4^+$ is absent or at very low concentrations, and K$^+$/H$^+$ exchange can operate in a reversed H$^+$/K$^+$ exchange mode when an outwardly directed H$^+$ concentration gradient is imposed to drive the exchange (8). The apical location of K$^+$/NH$_4^+$ (H$^+$)$^{-}$ exchange has been demonstrated in MTAL membrane vesicle preparations from two lines of evidence. First, K$^+$/H$^+$ exchange transport activity was linearly related to the enrichment factor of alkaline phosphatase (8), a marker enzyme of the MTAL apical membrane that closely follows the transport activity of the Na$^+$/K$^-$2Cl$^-$ cotransporter in MTAL membrane vesicles (11). Conversely, K$^+$/H$^+$ exchange transport activity was inversely related to the activity of basolateral Na$^+$/K$^-$ATPase (8). Second, functional interactions with the apical Na$^+$/H$^+$ exchanger NHE3, but not with basolateral NHE1, could be demonstrated on the basis of results obtained with HOE-694 (HOE-694 inhibits NHE1 but not NHE3 at the appropriate concentration) and high concentrations of amiloride (8). Thus the apical K$^+$/NH$_4^+$ antiport, driven by the outwardly directed K$^+$ concentration gradient, provides an efficient means of NH$_4^+$ uptake by MTAL cells, and its quantitative contribution to MTAL NH$_4^+$ absorption appears substantial. In rat MTAL membrane vesicles, $^{86}$Rb$^+$ uptake by the K$^+$/H$^+$ antiport was quantitatively similar to $^{22}$Na$^+$ uptake by the Na$^+$/H$^+$ antiport under similar conditions of pH gradient and extracellular cation concentration (8). Furthermore, it has been shown that ~40% of transcellular ammonia absorption are not attributable to Na$^+$/NH$_4^+$-2Cl$^-$ co-transport in the rabbit MTAL (25). In rat and mouse, barium-sensitive NH$_4^+$ transport amounted to ~30–60% of luminal NH$_4^+$ uptake by the isolated and perfused MTAL, as assessed by intracellular pH measurements in several studies (42, 43, 76). It is interesting to note that increasing the potassium concentration from 4 to 24 mM in both perfusate and bath strongly inhibited total ammonia absorption by the rat MTAL (28).

This was interpreted as resulting from a reduction in the active transcellular component of NH$_4^+$ transport, specifically from competition between K$^+$ and NH$_4^+$ on the Na$^+$/K$^+$/2Cl$^-$ cotransporter. However, competition between K$^+$ and NH$_4^+$ on the K$^+$/NH$_4^+$ (H$^+$)$^{-}$ antiport may also have occurred. Little is known at present about the regulation of the MTAL K$^+$/NH$_4^+$ (H$^+$)$^{-}$ antiport. This transport system is inhibited by arginine vasopressin (AVP) through cAMP-activated PKA (8). Phorbol esters, on the contrary, stimulate K$^+$/NH$_4^+$ (H$^+$)$^{-}$ exchange through activation of protein kinase C (PKC; Boulanger H and Bichara M, unpublished observations). In other renal and related tissues, K$^+$/NH$_4^+$ (H$^+$)$^{-}$ antiport systems have been described in turtle bladder epithelium (78), in the basolateral membrane of the proximal tubule (14), and in cultured opossum kidney (33) and IMCD-3 cells (6). In mammals, the K$^+$/H$^+$ antiport has also been described in ileum (16) and corneal (19) epithelia. Differences in apical vs. basolateral location and in sensitivity to inhibitors suggest that these various K$^+$/NH$_4^+$ (H$^+$)$^{-}$ antiport systems may represent isoforms belonging to the same family of membrane transporters, the molecular identity of which is unknown.

Finally, MTAL cells express the apical Na$^+$/H$^+$ exchangers NHE3 (1, 9, 69) and NHE2 (22, 73), which may operate in a Na$^+$/NH$_4^+$ exchange mode (47). However, luminal 1 mM amiloride had no effect on NH$_4^+$ absorption by the rat MTAL isolated and perfused in vitro, which led to the conclusion that Na$^+$/H$^+$ exchange is not important for NH$_4^+$ absorption in the MTAL (32). It must be pointed out, however, that 1 mM amiloride also inhibits the apical amiloride-sensitive conductance mentioned above. Inhibition by amiloride of both NH$_4^+$ secretion by Na$^+$/NH$_4^+$ exchange and NH$_4^+$ absorption by the conductance may have resulted in no net effect on MTAL NH$_4^+$ transport.

Thus the available data strongly suggest that BSC1 ensures the majority (50–65%) and K$^+$/NH$_4^+$ exchange the rest of the cellular luminal NH$_4^+$ uptake in the MTAL under basal conditions in vitro. The role of the apical amiloride-sensitive NH$_4^+$ conductance remains speculative, perhaps being simply to counterbalance NH$_4^+$ secretion by Na$^+$/NH$_4^+$ exchange.
MTAL BASOLATERAL NH₄⁺ CARRIERS

On the basolateral side of TAL cells, several transport systems have been recognized as being able to carry NH₄⁺.

First, NH₄⁺ may be accepted at the K⁺ site of Na⁺/K⁺-ATPase (46). However, the affinity of this enzyme for NH₄⁺ is rather low (46). In rat MTAL suspension, intracellular pH measurements have been performed in the presence of furosemide, barium, and amiloride added just before application of NH₄Cl. Under this experimental condition in which NH₄⁺ carriers other than Na⁺/K⁺-ATPase were acutely blocked, the NH₄⁺-induced cell acidification was abolished and a simple return toward the basal value was observed after the initial cell alkalinization due to NH₃ entry (4). When ouabain was added to the other inhibitors, the cell pH recovery rate after the initial cell alkalinization was only slightly reduced (Amlal and Bichara, unpublished observations). Thus Na⁺/K⁺-ATPase probably carries low amounts of NH₄⁺ in the MTAL under basal conditions.

Second, intracellular pH measurements have shown that NH₄⁺-Cl⁻ cotransport takes place in rat MTALs in suspension (5). NH₄⁺-Cl⁻ cotransport was sensitive to high concentrations of barium, furosemide, and bumetanide and insensitive to hydrochlorothiazide (5). It has been concluded that this transporter was a K⁺(NH₄⁺)-Cl⁻ cotransporter because the sensitivity to furosemide and bumetanide suggested its membership in the cation-chloride cotransporter family. In addition, that NH₄⁺ can be transported by some K⁺ carriers and that barium-sensitive electroneutral K⁺-Cl⁻ cotransport had been suggested as present in the basolateral membrane of the rabbit CTAL in a previous electrophysiological study (34) also indicated that NH₄⁺-Cl⁻ cotransport was accomplished by a basolateral K⁺(NH₄⁺)-Cl⁻ cotransporter (5). The role of NH₄⁺-Cl⁻ cotransport in transepithelial NH₄⁺ absorption is unknown, but, on the basis of estimated extracellular and intracellular concentrations of Cl⁻ and NH₄⁺, the net NH₄⁺Cl⁻ flux across the basolateral cell membrane is expected to be inwardly directed, driven by the peritubular space-to-cell Cl⁻ concentration gradient under normal conditions (5). Thus the net NH₄⁺Cl⁻ cotransport flux should not contribute, but be opposed to, cell-to-peritubular space NH₄⁺ transport. Conversely, the net KCl cotransport flux must be outwardly directed, driven by the cell-to-peritubular space K⁺ concentration gradient. The K⁺-Cl⁻ cotransporters, some isoforms of which were recently cloned, belong to the cation-chloride cotransporter family. The basolateral K⁺-Cl⁻ cotransporter of the TAL appears to be KCC4 [initially named KCC3 by Mount et al. (58) but changed to KCC4 in a NOTE ADDED IN PROOF in that study] (70). Whether KCC1 (26, 38, 71) is also functional in the MTAL is uncertain because its detection at the protein level in this nephron segment was not mentioned in a recent work (51). Functional studies of KCC4 expressed in X. laevis oocytes have shown that this carrier, like MTAL NH₄⁺-Cl⁻ cotransport and CTAL K⁺-Cl⁻ cotransport (5, 34), is sensitive to barium, besides furosemide, bumetanide, DIDS, R(+)-(2-n-butyl-6,7-dichloro-2-cyclopentyl-2,3-dihydro-1-oxo-1H-inden-5-yl)oxy acetic acid, and metolazone (55). Thus these observations strongly suggest that MTAL barium-sensitive NH₄⁺-Cl⁻ cotransport is accomplished by KCC4. One major property of the MTAL K⁺-Cl⁻ cotransporter, as well as of KCC4, is to be considerably activated by cell swelling (55, 58). Cell swelling may occur on exposure to a hypotonic medium but also after an increase in solute entry within the cell through the apical membrane (74). K⁺(NH₄⁺)-Cl⁻ cotransport could thus be involved in both cell volume regulation and transepithelial transport in the MTAL.

Third, a basolateral rhesicotic NH₄⁺ transport system has been described in the MTAL of the hamster, a species in which there are two types of MTAL cells (75). In the latter study, the cell membrane depolarization caused by abrupt basolateral application of 50 mM NH₄⁺ was inhibited by 10 mM barium or 10 μM amiloride to incomplete and variable extents depending on the hamster MTAL cell type (75). In addition, no appreciable Na⁺ conductance could be detected in the basolateral membrane of hamster MTAL cells (75). Thus the nature of the basolateral NH₄⁺ conductance was not defined in the latter work, but it was suggested that it was distinct from K⁺ and nonselective cation channels. In any case, NH₄⁺ should be transported inside the cell by this conductance because of the inside negative membrane potential.

Finally, the basolateral Na⁺/H⁺ exchanger NHE1 in the MTAL (9, 15) could contribute importantly to cell-to-peritubular space NH₄⁺ transport in two ways. First, NHE1 may function in a Na⁺/H⁺ exchange mode like other Na⁺/H⁺ exchangers (47). Second, Na⁺/H⁺ exchange could be coupled to NH₃ diffusion from cell to peritubular space after dissociation of the NH₄⁺ entered within the cell from the lumen into NH₃ plus H⁺. The respective areas of these two possibilities as well as the overall role of NHE1 in MTAL transepithelial NH₄⁺ transport have not been experimentally defined, to our knowledge.

Taken together, these considerations suggest that MTAL basolateral Na⁺/H⁺ (NH₄⁺) exchange is the best candidate for NH₄⁺ transport from cell to peritubular space because Na⁺/K⁺-ATPase, NH₄⁺-Cl⁻ cotransport, and basolateral NH₄⁺ conductance should carry NH₄⁺ in the wrong direction. Nevertheless, the latter transporters could have a role in the regulation of MTAL NH₄⁺ absorption. It is also possible that the basolateral step of NH₄⁺ absorption may be accounted for by an as yet unidentified carrier.

REGULATION OF NH₄⁺ ABSORPTION BY THE MTAL

Little is known about the acute regulation of MTAL NH₄⁺ transport. As pointed out in MTAL apical NH₄⁺ carriers, Na⁺-K⁺(NH₄⁺)-2Cl⁻ cotransport activity accounts for the majority of luminal NH₄⁺ uptake by the MTAL under basal experimental conditions. Hence it was anticipated that stimulation of MTAL NH₄⁺ ab-
sorption should occur in the presence of AVP because MTAL Na\(^+\)-K\(^+\)(NH\(_4\)^+)\(\text{HCO}_3^-\) cotransport and NaCl absorption are stimulated by this peptide hormone through activation of PKA in various species (2, 36, 37, 66). Yet, AVP had no effect on NH\(_4\)^+ absorption by the rat MTAL (29). That K\(^+\)/NH\(_4\)^+ antiport was shown to be inhibited by AVP and 8-bromoadenosine 3',5'-cyclic monophosphate through activation of PKA (8) provided an explanation for the lack of effect of AVP on MTAL NH\(_4\)^+ transport: stimulation of Na\(^+\)-K\(^+\)(NH\(_4\)^+)\(\text{HCO}_3^-\) cotransport and inhibition of K\(^+\)/NH\(_4\)^+ antiport by AVP may have resulted in no net effect on NH\(_4\)^+ absorption. It is interesting to note that ANG II regulates rBSC1 transport activity through 20-hydroxyeicosatetraenoic acid and PKC, not cAMP, in the rat MTAL (3). The physiologically relevant effect of ANG II is stimulation of rBSC1 through PKC activation (3). 20-Hydroxyeicosatetraenoic acid, the production of which is augmented by very low concentrations of ANG II, inhibits the cotransporter through unknown mechanisms. Because, as noted above, the K\(^+\)/NH\(_4\)^+ (H\(^+\)) antiport is also activated by PKC, ANG II is expected to stimulate both BSC1 and the K\(^+\)/NH\(_4\)^+ (H\(^+\)) antiport and should then enhance both NaCl and NH\(_4\)^+ absorption by the MTAL.

The ability of the MTAL isolated and perfused in vitro to absorb NH\(_4\)^+ increases during CMA (30), and this adaptation favors the renal elimination of an acid load. Studies have recently investigated the mechanisms of this MTAL response and found that the expression of rBSC1 mRNA and protein was enhanced during CMA, as assessed by quantitative RT-PCR and immunoblotting analysis of total RNA and crude membranes, respectively, from rat MTAL suspensions (10). One of the main findings of the latter work was that the abundance of rBSC1 mRNA increased in the MTAL as soon as after 3 h of metabolic acidosis induced by peritoneal dialysis. The increase in rBSC1 mRNA preceded that of rBSC1 protein, and the augmentation of both persisted after 6 days of CMA caused by NH\(_4\)Cl administration. That another study failed to detect an increased rBSC1 protein abundance during CMA by immunoblotting analysis is unexplained at present (44). This negative result may have resulted from changes in rBSC1 protein abundance in the whole tissue of the inner stripe of outer medulla below the detection limit of the method employed in the latter work, as stated by the authors themselves (44). At least two factors may account for the stimulating effect of CMA on rBSC1 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 stimulated rBSC1 mRNA and protein abundance and cotransport activity (10). The acid pH effect on cotransport activity was dependent on gene transcription and protein synthesis because stimulation was abolished by actinomycin D or cycloheximide (10). Second, adrenal glucocorticoid production increases during CMA (54, 61, 77), and in recent work (12) dexamethasone administration to adrenalectomized rats stimulated rBSC1 expression at the mRNA and protein levels. Furthermore, in vitro application of dexamethasone to rat MTAL fragments enhanced rBSC1 mRNA and protein abundance and cotransport activity (12). The latter effects required the presence of AVP or 8-bromoadenosine 3',5'-cyclic monophosphate in the incubation medium, which is a physiological condition because the MTAL is chronically subjected to the influences of several cAMP-generating peptide hormones such as AVP, calcitonin, and glucagon (56). Under the same experimental conditions, D-aldosterone had no effect on rBSC1 cotransport activity in vitro (12). Thus activation of the glucocorticoid receptor stimulated rBSC1 expression and activity through interactions with cAMP-dependent factors (12). Taken together, these observations indicate that, during CMA, the increased ability of the MTAL to absorb NH\(_4\)^+ results, at least in part, from stimulation of rBSC1 expression and transport activity by both an acid pH and glucocorticoids. These effects would be complementary to the known stimulation of NH\(_4\)^+ production by proximal tubular cells by an acid pH and glucocorticoids. It is interesting to note that preliminary results indicate that CMA also decreases the abundance of ROMK protein in the MTAL (13). It may be speculated that a decrease in luminal K\(^+\) recycling should favor NH\(_4\)^+ uptake by the Na\(^+\)-K\(^+\)(NH\(_4\)^+)\(\text{HCO}_3^-\) cotransporter and K\(^+\)/NH\(_4\)^+ antiport because competition between NH\(_4\)^+ and K\(^+\) on these carriers should give the advantage to NH\(_4\)^+.

In addition, CMA enhances Na\(^+\)/H\(^+\) exchanger NHE3 mRNA and protein abundance and transport activity in the MTAL, which explains the increased MTAL ability to absorb bicarbonate during CMA (50). Effects of CMA on the expression of other MTAL NH\(_4\)^+ carriers are unknown at present. rBSC1 expression was also shown to be upregulated by chronic saline loading (23, 44), NaHCO\(_3^-\) administration (44), restriction of water intake (45, 53), prolonged AVP administration (45), and in models of chronic renal failure (49) and congestive heart failure (53). The effects of the latter experimental conditions, except NaHCO\(_3^-\) administration, on MTAL NH\(_4\)^+ absorption are unknown. Consistent with upregulation of BSC1 by NaHCO\(_3^-\) loading, NaHCO\(_3^-\) induced metabolic alkalosis was associated with a paradoxical increase in the ability of the MTAL to absorb NH\(_4\)^+ (30). Because NaHCO\(_3^-\) administration as well as NaCl loading also caused an increased ability of the MTAL to absorb HCO\(_3^-\), it was concluded that high sodium intake was an important determinant of MTAL HCO\(_3^-\) and NH\(_4\)^+ transport capacity (30). This conclusion is consistent with stimulation of rBSC1 protein expression by chronic saline loading (23, 44), although the ability of the MTAL to absorb NH\(_4\)^+ after NaCl loading was not effectively measured (30). It is worth noting that during potassium depletion, a condition generally associated with metabolic alkalosis, rBSC1 expression and activity were strongly downregulated (7). It is thus possible that regulation of BSC1 expression during metabolic alkalosis depends on the experimental model employed to induce this condition. Also, it is interesting to note that NH\(_4\)^+ urinary excretion is well known to be increased during potassium depletion, which might...
imply an augmented NH$_4^+$ absorption by the MTAL despite downregulation of BSC1 expression (7). This consideration suggests that either BSC1 might be still able to carry important amounts of NH$_4^+$ or another MTAL NH$_4^+$ carrier such as the K$^+$/NH$_4^+$ antiport might be upregulated during potassium depletion.

In summary, MTAL cells express a relatively large number of apical and basolateral NH$_4^+$ carriers (Fig. 1). This complexity and the lack of specific inhibitors for many of these transporters have rendered difficult a precise functional analysis of the quantitative role of each of them in transepithelial MTAL NH$_4^+$ transport. Whereas the roles of apical BSC1, the K$^+$/NH$_4^+$ antiport, and NH$_4^+$ conductance in luminal MTAL NH$_4^+$ uptake are reasonably well defined, those of the basolateral NH$_4^+$ carriers cited above in basolateral MTAL NH$_4^+$ transport are still a matter of speculation. Yet, it is likely that fine regulation of MTAL NH$_4^+$ transport involves effects on several NH$_4^+$ carriers simultaneously. For instance, NH$_4^+$ absorption is dissociated from that of NaCl in the MTAL in the presence of AVP, probably because stimulation of BSC1 is counterbalanced by inhibition of the K$^+$/NH$_4^+$ antiport (8). Otherwise, the increased ability of the MTAL to absorb NH$_4^+$ during CMA is due, at least in part, to stimulation of BSC1 expression and transport activity (10). An acid pH and glucocorticoids interacting with cAMP-dependent factors ensure the effect of CMA on BSC1 expression and activity (10, 12). However, fine tuning of MTAL NH$_4^+$ absorption during CMA probably requires coordinated regulation of expression of other MTAL carriers. Discovering the molecular identity of the NH$_4^+$ carriers that were not yet cloned will certainly provide new insights into the mechanisms and regulation of MTAL NH$_4^+$ absorption.

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REFERENCES


61. Sajo IM, Goldstein MB, Sonnenberg H, Stonebaugh BJ, Wilson DR, and Halperin ML. Sites of ammonia addition to...


