Effects of ammonia on bicarbonate transport in the cortical collecting duct

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Frank, Amy E., Charles S. Wingo, and I. David Weiner. Effects of ammonia on bicarbonate transport in the cortical collecting duct. Am. J. Physiol. Renal Physiol. 278: F219–F226, 2000.—Both acidosis and hypokalemia stimulate renal ammoniagenesis, and both regulate urinary proton and potassium excretion. We hypothesized that ammonia might play an important role in this processing by stimulating H\textsuperscript{+}-K\textsuperscript{+}-ATPase-mediated ion transport. Rabbit cortical collecting ducts (CCD) were studied using in vitro microperfusion, bicarbonate reabsorption was measured using microcalorimetry, and intracellular pH (pHi) was measured using the fluorescent, pH-sensitive dye, 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF). Ammonia caused a concentration-dependent increase in net bicarbonate reabsorption that was inhibited by luminal addition of either of the H\textsuperscript{+}-K\textsuperscript{+}-ATPase inhibitors, Sch-28080 or ouabain. The stimulation of net bicarbonate reabsorption was not mediated through apical H\textsuperscript{+}-ATPase, basolateral Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, or luminal electronegativity. Although ammonia caused intracellular acidification, similar changes in pHi, induced by inhibiting basolateral Na\textsuperscript{+}/H\textsuperscript{+} exchange did not alter net bicarbonate reabsorption. We conclude that ammonia regulates CCD proton and potassium transport, at least in part, by stimulating apical H\textsuperscript{+}-K\textsuperscript{+}-ATPase.

proton-potassium-exchanging ATPase; kidney tubules; collecting ducts; hydrogen ion concentration; proton-transporting ATP synthase; rabbits

AMMONIA is a low-molecular-weight molecule that plays an important role in renal physiology. It is produced in the proximal tubule (12, 33, 34), concentrated in the renal interstitium by the loop of Henle (6, 15), and secreted into the luminal fluid in the collecting duct (6, 13, 14, 25). Ammonia is the primary component of net acid excretion under basal conditions, and is the primary mechanism by which the kidney increases net acid excretion in response to metabolic acidosis (27, 60).

Whether ammonia plays a role in acid-base homeostasis beyond its role as a urinary constituent is not clear. However, two common clinical conditions, metabolic acidosis and hypokalemia, are associated with increased ammoniagenesis, loop of Henle-mediated ammonia reabsorption, and interstitial ammonia concentrations (12, 23). Both conditions are also associated with increased urinary proton excretion and with decreased urinary potassium excretion (28, 37). At present, no unifying signaling pathway completely explains the association of both increased renal proton secretion and decreased renal potassium excretion with both metabolic acidosis and hypokalemia.

We hypothesized that ammonia might stimulate proton secretion and potassium reabsorption in the renal cortical collecting duct (CCD), a major site for acid-base transport and for potassium transport. We studied the effect of ammonia on acid-base transport, and we determined whether the stimulation observed was mediated through effects of ammonia on H\textsuperscript{+}-K\textsuperscript{+}-ATPase, H\textsuperscript{+}-ATPase, or both. Since ammonia affects both transepithelial voltage (16) and intracellular pH (pHi) (30, 49, 50), and both may regulate CCD acid-base transport, we examined whether ammonia regulates transport through either of these mechanisms. Our results show that ammonia stimulates CCD net bicarbonate reabsorption by increasing H\textsuperscript{+}-K\textsuperscript{+}-ATPase-mediated proton secretion and that this effect is not due to regulation of basolateral Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, transepithelial voltage, or pHi. Thus ammonia may play an important role as a signaling molecule that regulates CCD proton and potassium transport.

METHODS

Microperfusion. Standard in vitro microperfusion techniques utilizing female New Zealand White rabbits (1.5–2 kg) were used (30, 48–50, 53, 54, 57). The solutions used were artificial solutions and, unless otherwise mentioned, contained (in mM) 119.2 NaCl, 3 KCl, 25 NaHCO\textsubscript{3}, 2 KH\textsubscript{2}PO\textsubscript{4}, 1 sodium acetate, 1.2 CaCl\textsubscript{2}, 1 MgSO\textsubscript{4}, 5 alanine, and 8.3 glucose. The solutions were equilibrated with 5% CO\textsubscript{2}-95% O\textsubscript{2} and had osmolality adjusted to 290 ± 7 mosmol/kg H\textsubscript{2}O with NaCl. NH\textsubscript{4}Cl substituted for NaCl in both the luminal and peritubular solutions when ammonia was used. Chloride-free solutions substituted gluconate for chloride and increased total calcium to 4.0 mM to account for complexing with gluconate. Unless specified otherwise, the ammonia concentration was 10 mM. Most studies used an ~1.5 ml bath chamber that was thermostatically controlled to 37°C in which the peritubular solution was continuously exchanged at 0.3 ml/min. Some studies measuring pHi used a low-volume, laminar flow bath chamber to which preheated, continuously bubbled solutions were delivered at ~6 ml/min (49, 50, 54). At least 30 min was allowed prior to any measurements and between experimental periods.

Bicarbonate transport. Transepithelial bicarbonate transport was measured using standard techniques (2, 3, 57, 61). Briefly, perfused fluid was collected in a calibrated pipette of...
known volume. The total CO₂ (tCO₂) concentration, which is predominantly bicarbonate at physiological pH, was measured using either a picapnoimeter (WPI, Sarasota, FL) or a flow-through ultramicrofluorometer (WPI) (43). Similar results were obtained with each technique, and were combined for analysis. Net bicarbonate transport, \( J_{\text{CO}_2} \), was calculated using the formula \( J_{\text{CO}_2} = V_o \times (C_o - C_2)/L \), where \( V_o \) is the collected fluid rate in nanoliters per minute, \( C_o \) and \( C_2 \) are the tCO₂ contents in the perfusate (\( C_o \)) and collected fluid (\( C_2 \)), and \( L \) is the tubule length in millimeters. The luminal flow rate was maintained at ~4 nl/min by adjusting the hydrostatic perfusion pressure. Bicarbonate transport was measured two to four times during each time period and averaged to yield a single measurement.

Extracellular pH, \( pHi \), was measured using standard techniques (30, 31, 49–53). Briefly, intercalated cells were loaded with the fluorescent, pH-sensitive dye, 2,7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF), by adding the acetoxymethyl ester of BCECF (BCECF-AM), 15 µM, to the luminal solution for ~5 min (48). An ~15-µm diameter region was excited at 490 and 440 nm, and emission was measured at 530 nm. The 490/440 ratio was calibrated to pH, at the end of the experiment using the high-potassium-nigericin technique (48). An A-type intercalated cell (A cell) was identified as an intercalated cell without apical Cl⁻/HCO₃⁻ exchange activity, and a B-type intercalated cell (B cell) was identified as an intercalated cell with Cl⁻/HCO₃⁻ exchange activity, as we have previously described (30, 31, 49–53).

Intracellular pH, \( pHi \), was measured using standard techniques (30, 31, 49–53). Intracellular alkalization was measured using the fluorescent, pH-sensitive dye, 2,7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF), by adding the acetoxymethyl ester of BCECF (BCECF-AM), 15 µM, to the luminal solution for ~5 min (48). An ~15-µm diameter region was excited at 490 and 440 nm, and emission was measured at 530 nm. The 490/440 ratio was calibrated to pH, at the end of the experiment using the high-potassium-nigericin technique (48). An A-type intercalated cell (A cell) was identified as an intercalated cell without apical Cl⁻/HCO₃⁻ exchange activity, and a B-type intercalated cell (B cell) was identified as an intercalated cell with Cl⁻/HCO₃⁻ exchange activity, as we have previously described (30, 31, 49–53).

Chemicals. BCECF-AM was obtained from Molecular Probes (Eugene, OR), Sch-28080 was the kind gift of Dr. James Kaminski (Schering, Bloomfield, NJ). All other chemicals were from Sigma Chemical (St. Louis, MO).

Statistics. Results are presented as mean ± SE. The data were analyzed using paired Student's t-test and ANOVA, as appropriate, and \( P < 0.05 \) was used as evidence of statistical significance.

RESULTS

Net bicarbonate transport. Our first set of studies examined the effect of ammonia on CCD acid-base transport. Figure 1A summarizes the results of these studies. Net bicarbonate reabsorption averaged 10.7 ± 3.9 pmol·mm⁻¹·min⁻¹ in the absence of ammonia and 17.7 ± 3.3 pmol·mm⁻¹·min⁻¹ in the presence of ammonia (\( P < 0.05 \) vs. no ammonia, \( n = 6 \)). In time control studies, in which a sham change in the luminal and peritubular solutions was made between the basal and control period, net bicarbonate reabsorption averaged 8.5 ± 2.6 in the basal period, and was not significantly different in the control period, 7.4 ± 5.1 pmol·mm⁻¹·min⁻¹ \( P = \text{not significant (NS) vs. basal, } n = 3 \). Figure 1B summarizes these results. Thus the increase in net bicarbonate reabsorption seen in Fig. 1A is due to ammonia and cannot be ascribed to time-dependent changes. Finally, ammonia caused concentration-dependent stimulation of net bicarbonate reabsorption (\( P < 0.02 \) by ANOVA). Figure 1C summarizes these results. We conclude that ammonia causes concentration-dependent stimulation of CCD net bicarbonate reabsorption.

![Figure 1A: effect of ammonia on cortical collecting duct (CCD) net bicarbonate reabsorption. Net bicarbonate reabsorption was measured in the absence of ammonia, then ammonia, 10 mM, was added to both the luminal and peritubular solutions, and, following a 30-min equilibration, net bicarbonate reabsorption was measured again. B: time-dependent changes in CCD net bicarbonate reabsorption. Net bicarbonate reabsorption was measured under basal conditions, then following a sham change in the luminal and peritubular solutions. Lines connect measurements in an individual CCD. Rates are reported as pmol·mm⁻¹·min⁻¹. C: concentration-dependent effects of ammonia. Basal and ammonia-stimulated net bicarbonate reabsorption were measured as in A and B, except that ammonia concentration added was either 2 or 4 mM. Data for 0 and 10 mM are from A and B. NS, not significant.](http://ajprenal.physiology.org/Downloadedfrom)
Effect of proton pump inhibitors. Ammonia could increase net bicarbonate reabsorption through effects on apical H\textsuperscript{+}-K\textsuperscript{+}-ATPase, H\textsuperscript{+}-ATPase, or both. Because conditions associated with increased intrarenal ammonia concentrations, such as metabolic acidosis and hypokalemia, are associated with decreased urinary potassium excretion (28, 37), we tested whether ammonia might regulate net bicarbonate reabsorption by stimulating a potassium reabsorbing H\textsuperscript{+}-K\textsuperscript{+}-ATPase.

Figure 2 summarizes these results. When apical H\textsuperscript{+}-K\textsuperscript{+}-ATPase was inhibited with luminal Sch-28080 (10 \textsuperscript{-5} M), bicarbonate reabsorption averaged 12.3 ± 1.9 pmol \cdot mm\textsuperscript{-1} \cdot min\textsuperscript{-1} in the absence of ammonia, and was not increased by ammonia, 13.4 ± 2.4 pmol \cdot mm\textsuperscript{-1} \cdot min\textsuperscript{-1} (P = NS vs. basal, n = 5). Thus ammonia increases CCD net bicarbonate reabsorption through effects on a luminal Sch-28080-sensitive transporter, most likely an apical H\textsuperscript{+}-K\textsuperscript{+}-ATPase.

However, ouabain also inhibits Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, raising the possibility that the effects of ouabain might be due to access to the peritubular solution and inhibition of basolateral Na\textsuperscript{+}-K\textsuperscript{+}-ATPase. To test this possibility, we added ouabain to the peritubular solution at a concentration (10 \textsuperscript{-5} M) that should inhibit CCD Na\textsuperscript{+}-K\textsuperscript{+}-ATPase (11), but not H\textsuperscript{+}-K\textsuperscript{+}-ATPase (8, 26, 32, 59), and determined whether this would inhibit the effects of ammonia on net bicarbonate reabsorption. Figure 3B summarizes these experiments. With peritubular ouabain (10 \textsuperscript{-5} M) present, bicarbonate reabsorption averaged 5.6 ± 1.6 pmol \cdot mm\textsuperscript{-1} \cdot min\textsuperscript{-1} in the absence of ammonia and was significantly stimulated by ammonia, 11.9 ± 2.8 pmol \cdot mm\textsuperscript{-1} \cdot min\textsuperscript{-1} (P < 0.01 vs. absence of ammonia, n = 5). Inhibiting basolateral Na\textsuperscript{+}-K\textsuperscript{+}-ATPase does not alter the increase in net bicarbonate reabsorption caused by ammonia (P = NS by ANOVA).

We conclude that ammonia stimulates net bicarbonate reabsorption through effects that are independent of basolateral Na\textsuperscript{+}-K\textsuperscript{+}-ATPase and are sensitive to luminal ouabain.
H^+-ATPase is the primary CCD proton transporter stimulated by in vitro metabolic acidosis (45) and is the primary apical proton transporter that regulates pH_i in both the A cell and the B cell, the cells responsible for CCD acid-base transport (30, 51). The next protocol tested whether ammonia stimulates net proton secretion by activating H^+-ATPase. Figure 4 summarizes the results. In the presence of luminal bafilomycin A_1 (10^{-8} M), a potent H^+-ATPase inhibitor (55), net bicarbonate reabsorption averaged 6.5 ± 2.0 pmol·mm^{-1}·min^{-1} and was significantly increased by ammonia to 13.3 ± 2.8 pmol·mm^{-1}·min^{-1} (P < 0.05 vs. basal, n = 6). Luminal bafilomycin A_1 did not alter ammonia's stimulation of bicarbonate transport (P = NS by ANOVA). Thus ammonia stimulates bicarbonate reabsorption by stimulating a luminal Sch-28080- and ouabain-sensitive H^+-K^+-ATPase and not by stimulating a bafilomycin A_1-sensitive H^+-ATPase.

Effect of ammonia on transepithelial voltage Active sodium transport can regulate bicarbonate transport through changes in luminal electronegativity. However, this is unlikely to be the mechanism through which ammonia regulates CCD bicarbonate transport. In the current studies ammonia did not change CCD transepithelial voltage significantly (-6.0 ± 2.2 mV, basal; -4.7 ± 1.9 mV, with ammonia; P = NS, n = 5). Also, as reported above, inhibiting active sodium reabsorption with peritubular ouabain did not prevent ammonia from increasing net bicarbonate reabsorption (see Fig. 3B).

Effect of ammonia on pH_i. Because ammonia can alter pH_i and because acute pH_i changes are believed to regulate proton secretion, we next tested whether ammonia stimulated bicarbonate transport through changes in pH_i. To do so, we used a three-part experiment. First, we determined the effect of prolonged ammonia incubation on pH_i. Because CCD net bicarbonate transport is believed to be mediated by the intercalated cells, of which there are two major subtypes, the A cell and the B cell, and not by principal cells, we measured the effect of prolonged ammonia incubation on A cell and B cell pH_i. Then, we identified that inhibiting basolateral Na^+/H^+ exchange activity would cause similar changes in steady-state A cell and B cell pH_i as ammonia. Finally, we tested whether acidifying the A cell and B cell by inhibiting basolateral Na^+/H^+ exchange would cause changes in CCD bicarbonate transport similar to those caused by ammonia.

The first step was to determine the effect of prolonged ammonia incubation on pH_i. The addition of ammonia acutely alkalinized both the A cell and B cell (not shown), followed shortly thereafter by intracellular acid loading. As can be seen in Fig. 5, ammonia decreased A cell and B cell pH_i below initial pH_i within as little as 10 min. The acidification was maximal at 30 min, decreasing A cell pH_i from 7.48 ± 0.06 to 7.27 ± 0.05 (P < 0.001, n = 12) and B cell pH_i from 7.43 ± 0.03 to 7.11 ± 0.03 (P < 0.001, n = 14) and did not increase any further at 60 min (ΔpH_i = -0.05 ± 0.03 and -0.02 ± 0.02 vs. pH_i at 30 min, P = NS and P = NS, n = 4 and n = 4, for the A cell and B cell, respectively). These changes were ammonia dependent; both A cell and B cell pH_i were unchanged in the absence of ammonia (data not shown). Thus, in contrast to ammonia’s immediate effect to cause intracellular alkalinization, prolonged incubations can cause net intracellular acidification.

Second, we identified that inhibiting basolateral Na^+/H^+ exchange would cause similar changes in A cell and B cell pH_i, as did ammonia incubation. As Fig. 6A shows, adding the Na^+/H^+ exchange inhibitor, 5-(N-ethyl-N-isopropyl)-amiloride (EIPA), 10^{-6} M, to the peritubular solution decreased significantly both A cell and B cell pH_i (ΔpH_i = -0.17 ± 0.07 and -0.23 ± 0.07, P < 0.05 and P < 0.025, respectively). Inhibiting basolateral Na^+/H^+ exchange activity with peritubular EIPA acidified the A cell and the B cell to the same extent as ammonia.
Third, we tested whether acidifying cells with EIPA would have the same effect on CCD bicarbonate transport as did ammonia. These results are summarized in Fig. 6B. Under basal conditions, CCD reabsorbed bicarbonate at a rate averaging 4.7 ± 1.0 pmol·mm⁻¹·min⁻¹ (n = 5). Peritubular EIPA addition (10⁻⁶ M) did not alter net bicarbonate reabsorption significantly, 4.2 ± 1.1 pmol·mm⁻¹·min⁻¹ (P = NS, n = 5). Thus intracellular acidification is unlikely to be the primary mechanism through which ammonia increases H⁺-K⁺-ATPase-dependent bicarbonate reabsorption.

DISCUSSION

The current study identifies several new and important features regarding the role of ammonia in ion transport. First, ammonia causes concentration-dependent stimulation of CCD bicarbonate reabsorption through activation of H⁺-K⁺-ATPase-dependent proton secretion. Second, ammonia causes intracellular acidification, but this is not sufficient to explain how ammonia increases net bicarbonate reabsorption. Finally, these findings suggest that ammonia can function as an extracellular signaling molecule that regulates CCD proton and potassium transport.

The first major finding of the current study is that ammonia increases rabbit CCD net bicarbonate reabsorption. The range of ammonia concentrations chosen in this study parallel those present in the late distal tubule (23, 56) and thus are likely to be similar to those to which the CCD is exposed. The findings in this study may be applicable to both the cortical and medullary collecting duct. For example, ammonia increases bicarbonate reabsorption in both the rat CCD and inner medullary collecting duct (IMCD) (25, 46). Furthermore, differences between the effects of 4 and 10 mM ammonia were also seen in the inner stripe of the outer medullary collecting duct (14). Thus the current study is both consistent with and, more important, extends these previous studies in several ways.

First, ammonia specifically activates H⁺-K⁺-ATPase-mediated proton secretion. In the current study, two H⁺-K⁺-ATPase inhibitors, Sch-28080 and ouabain, each completely inhibited the effect of ammonia on net bicarbonate reabsorption, whereas the H⁺-ATPase inhibitor, bafilomycin A₂, had no significant effect. Thus ammonia’s effect on proton secretion does not reflect a general effect on apical proton transporters. Stimulation of an H⁺-K⁺-ATPase is consistent with the observation that ammonia increases CCD ⁸⁶Rb⁺ (a surrogate for K⁺) reabsorption (16). The lack of stimulation of an electrogenic H⁺-ATPase is consistent with the observation that ammonia does not alter transepithelial voltage independent of its effects on sodium reabsorption (16). An alternative, but unlikely, possibility is that NH₄⁺ serves as substrate for apical K⁺/NH₄⁺ exchange. Because NH₄⁺ is a weak acid, active NH₄⁺ secretion could mediate the increased rate of net bicarbonate reabsorption. However, ammonia secretion is mediated by NH₃ diffusion, not by NH₄⁺ secretion (17, 24), making apical K⁺/NH₄⁺ exchange unlikely to mediate a significant proportion of apical NH₄⁺ secretion, and thus bicarbonate reabsorption. Instead, ammonia appears to increase net bicarbonate reabsorption by stimulating electroneutral H⁺-K⁺-ATPase-mediated proton secretion and potassium reabsorption. Whether ammonia also inhibits CCD unidirectional bicarbonate secretion cannot be determined definitively at present. However, our preliminary studies suggest that ammonia, in addition to its effects on proton secretion and luminal bicarbonate reabsorption, may also inhibit B cell unidirectional bicarbonate secretion (47). Identifying all of ammonia’s cellular effects in the CCD will be an important field for future study.

Which H⁺-K⁺-ATPase isofrom ammonia activates cannot be determined definitively at present. The α-subunit of the H⁺-K⁺-ATPase α-β heterodimer mediates...
catalytic and transport activity, and it determines the inhibitor sensitivity of the holoenzyme (58, 59). Of the known renal H^+ - K^+- ATPase α-isofoms, HK_{α1} is Sch-28080 sensitive and ouabain insensitive (58, 59), whereas HK_{α2a} and HK_{α2b} are Sch-28080 insensitive and ouabain sensitive when expressed in heterologous expression systems (8, 26, 32). A renal HK_{α2c} was recently cloned (7); its sensitivity to Sch-28080 and ouabain remains to be determined. Thus the pharmacological characterization of the ammonia-stimulated H^+ - K^+- ATPase differs from the characteristics of HK_{α4}, HK_{α2a}, and HK_{α2b}. Whether these differences are due to unidentified regulatory molecules or whether ammonia stimulates a novel H^+ - K^+- ATPase α-isofom will be an important field for further study.

Although H^+ - K^+- ATPase is the primary proton transporter mediating the response to ammonia, it is not the primary CCD proton transporter in all conditions. Apical H^+ - K^+- ATPase did not significantly contribute to basal bicarbonate reabsorption in either the current study or another study (61). Similarly, it mediates a relatively small role in both A cell and B cell pH_i regulation, at least compared with H^+ - ATPase (30, 51). Finally, different conditions that activate CCD bicarbonate reabsorption appear to activate different apical proton transporters. For example, in vitro metabolic acidosis primarily activates bicarbonate reabsorption through activation of H^+ - ATPase (45), whereas in vitro respiratory acidosis increases bicarbonate reabsorption by activating H^+ - K^+- ATPase, without apparent effects on H^+ - ATPase (61). Understanding the cellular signaling pathways through which these different stimuli increase CCD bicarbonate reabsorption through differing proton transporters will be an important issue for future study.

Ammonia appears to stimulate CCD net bicarbonate reabsorption through effects that are unrelated to its effects on principal cell-mediated electrogenic sodium transport and transepithelial voltage. In the current study, inhibiting active sodium reabsorption with peritubular ouabain did not prevent ammonia from increasing net bicarbonate reabsorption, nor did ammonia significantly alter transepithelial voltage. In another study, peritubular ouabain did not alter rat CCD net bicarbonate reabsorption when ammonia was present (24). Another report found that ammonia inhibited CCD sodium reabsorption and decreased luminal electronegativity (16), effects which would be expected to decrease bicarbonate reabsorption and thus cannot mediate the stimulation seen in the current study. Why the current study and the study by Hamm et al. (16) found different effects of ammonia on luminal electronegativity is unclear. Also, why there was a tendency for basal bicarbonate reabsorption to be decreased in the presence of peritubular ouabain is unclear, but this may be related to inhibition of intercalated cell basolateral Na^+ - K^+- ATPase (39), with secondary effects on membrane potential or on intracellular sodium, potassium, or calcium. More important, multiple studies confirm that ammonia's effects on bicarbonate transport are not due to its effects on principal cell-mediated sodium transport.

Another important finding is that ammonia can acidify CCD intercalated cells. Although acute ammonia exposure caused acute intracellular alkalization, as seen in previous studies (30, 49, 51), this was followed by sustained acid loading that caused net intracellular acidification. Although ammonia acidified the B cell slightly more than the A cell, the functional significance of this difference is not clear, as ammonia's effects on bicarbonate transport appear unrelated to its effects on pH_i. The ability of ammonia to cause intracellular acidification is not unique to CCD intercalated cells. Similar results have been observed in central nervous system (CNS) neurons (5, 35), and intracellular acidification may mediate ammonia's inhibition of CCD sodium reabsorption (16). Why basolateral Na^+ / H^+ exchange (30, 49) did not mediate any apparent pH_i recovery is not clear, but this may be related to possible substitution of NH_4+ for H^+ on Na^+ / H^+ exchange (22) or inhibition of Na^+ / H^+ exchange by ammonia (42).

Ammonia could induce intracellular acidification through any of several possible mechanisms. Because ammonia exists in solution predominantly as NH_4^+, a weak acid, it is possible that NH_4^+ uptake causes the intracellular acidification. This is consistent with observations in the medullary thick ascending limb of the loop of Henle (21). Alternatively, ammonia might directly regulate proton or bicarbonate transporters. For example, ammonia increases AE2 activity independent of its effects on pH_i (19). Further studies will be necessary to differentiate between these possibilities.

Although ammonia acidified CCD intercalated cells, changes in pH_i may not be the primary mechanism through which ammonia, or other stimuli, regulate CCD bicarbonate transport. For example, metabolic acidosis (decreased extracellular bicarbonate) increases CCD net bicarbonate reabsorption (4) by stimulating H^+ - ATPase (45), whereas respiratory acidosis (increased extracellular Pco_2) has been reported either to not alter bicarbonate reabsorption (4) or to increase net bicarbonate reabsorption by stimulating H^+ - K^+- ATPase (61). Despite these differing effects on net bicarbonate reabsorption, metabolic acidosis and respiratory acidosis cause similar changes in pH_i (49, 50). Similarly, isoproterenol acidifies the B cell and did not alter A cell pH_i (18) yet decreases net bicarbonate reabsorption (41). Since there appears to be a dissociation between effects of experimental stimuli on pH_i and on net bicarbonate reabsorption, it appears unlikely that pH_i is the most immediate signal through which these stimuli, and, in particular, ammonia, regulate proton secretion and bicarbonate reabsorption. However, we cannot exclude the possibility that pH_i may have a more minor effect on CCD bicarbonate transport.

A final consideration is that ammonia may function as a signaling molecule that can regulate ion transport. Several studies have shown that the proximal tubule produces ammonia and the rate of production is physiologically regulated by at least two stimuli, metabolic acidosis and hypokalemia (23, 33), that also regulate
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