

Effects of Ammonia on Acid-Base Transport by the B-Type Intercalated Cell

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Abstract. Ammonia, in addition to its role as a constituent of urinary net acid excretion, stimulates cortical collecting duct (CCD) net bicarbonate reabsorption. The current study sought to begin determining the cellular transport processes through which ammonia regulates bicarbonate reabsorption by testing whether ammonia stimulates B-type intercalated cell bicarbonate secretion, bicarbonate reabsorption, or both. The effects of ammonia on single CCD intercalated cells was studied by use of measurements of intracellular pH taken from *in vitro* microperfused CCD segments after luminal loading of the pH-sensitive fluorescent dye BCECF. These results showed, first, that ammonia inhibited B-cell unidirectional bicarbonate secretion and that this occurred despite no effect of ammonia on

apical $\text{Cl}^-/\text{HCO}_3^-$ exchange activity. Second, ammonia increased the contribution of a SCH28080-sensitive apical $\text{H}^+-\text{K}^+-\text{ATPase}$ to basal intracellular pH regulation and it stimulated basolateral $\text{Cl}^-/\text{HCO}_3^-$ exchange activity. Thus, ammonia activated both apical proton secretion and basolateral base exit, consistent with stimulation of unidirectional bicarbonate reabsorption. It was concluded that ammonia regulates CCD net bicarbonate reabsorption, at least in part, through the coordinated regulation of the separate processes of B-cell bicarbonate reabsorption and bicarbonate secretion. These effects do not reflect a general activation of ion transport but, instead, reflect coordinated and specific regulation of ion transport.

Ammonia plays a central role in acid-base homeostasis through a variety of mechanisms. Perhaps the most widely known mechanism is the relationship whereby renal ammonia metabolism results in new bicarbonate generation (reviewed in reference 1). Furthermore, renal ammonia production is stimulated by metabolic acidosis (2,3), resulting in substantial changes in bicarbonate formation attributable to ammonia metabolism. This relationship allows ammonia production and excretion to play a major role in acid-base homeostasis.

Ammonia also may mediate a second role through a possible role as an intrarenal signaling molecule that regulates collecting duct ion transport. In multiple species and throughout the collecting duct, ammonia stimulates proton secretion (typically measured as net bicarbonate reabsorption). In the rat, ammonia changes cortical collecting duct (CCD) bicarbonate transport from net bicarbonate secretion to net bicarbonate reabsorption (4). In the rabbit, ammonia substantially increases net bicarbonate reabsorption (5). This effect is concentration dependent and is independent of ammonia's effect on either intracellular pH or active sodium transport (5). In other portions of the rabbit collecting duct, increasing ammonia from 4 to 10 mM stimulates outer medullary collect duct proton secretion by

approximately 50% (6), and 6 mM ammonia stimulates inner medullary collecting duct proton secretion by approximately 250% (7). Thus, ammonia contributes to acid-base homeostasis by stimulating net bicarbonate reabsorption throughout the collecting duct and in multiple species.

The CCD B-type intercalated cell (B cell) plays an important role in renal acid-base transport because of its ability to both secrete and reabsorb luminal bicarbonate (8,9) and because it is the predominant intercalated cell in collecting ducts from the outer cortex (10). The purpose of the current study was to determine whether ammonia regulates CCD acid-base transport by inhibiting B cell-mediated bicarbonate secretion, stimulating B-cell-mediated proton secretion, or both. To do so, we examined the effect of ammonia on specific B-cell acid-base transporters. In the current studies, ammonia inhibited B-cell unidirectional bicarbonate secretion but did so without altering apical $\text{Cl}^-/\text{HCO}_3^-$ exchange activity. In addition, ammonia stimulated both apical $\text{H}^+-\text{K}^+-\text{ATPase}$ and basolateral $\text{Cl}^-/\text{HCO}_3^-$ exchange activity, which indicates that ammonia stimulated B-cell unidirectional bicarbonate reabsorption. Thus, ammonia regulates net bicarbonate transport through the coordinated and specific regulation of both bicarbonate reabsorption and bicarbonate secretion.

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Materials and Methods

Microperfusion

We used standard *in vitro* microperfusion techniques using female New Zealand white rabbits (1.5 to 2 kg) (11,12). The solutions used were artificial solutions and, unless otherwise mentioned, contained 119.2 mM NaCl, 3 mM KCl, 25 mM NaHCO_3 , 2 mM KH_2PO_4 , 1 mM Na-acetate, 1.2 mM CaCl_2 , 1 mM MgSO_4 , 5 mM alanine, and 8.3 mM

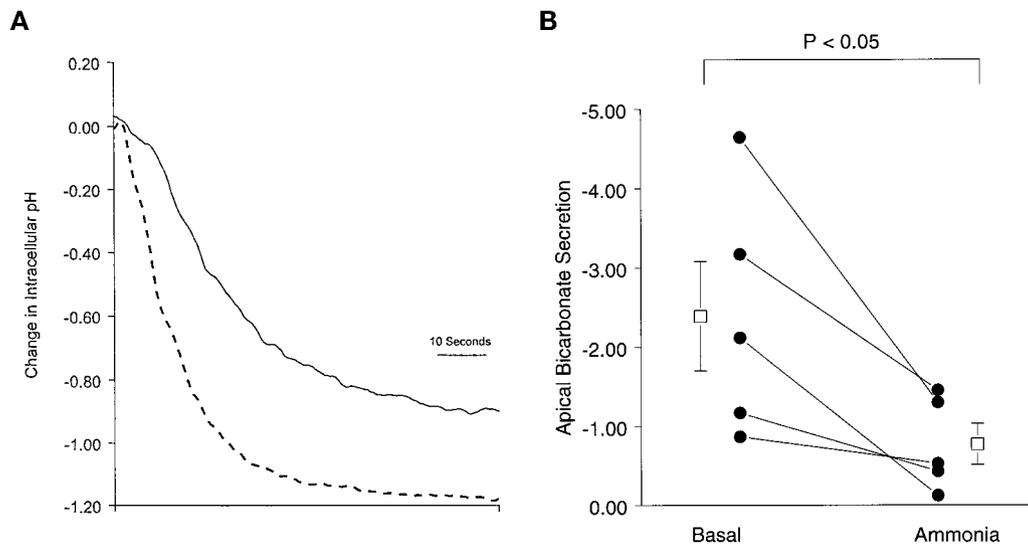


Figure 1. Effect of ammonia on B-cell unidirectional bicarbonate secretion. B cell unidirectional bicarbonate secretion was measured as the rate of intracellular acidification due to apical bicarbonate secretion after peritubular Cl^- removal. (A) Representative experiment demonstrating the intracellular acidification induced by peritubular chloride removal. --- , basal; — , ammonia. (B) Summary of results of all experiments, reported in pH units per minute. Lines connect measurements in the same cell.

glucose. The solutions had osmolality adjusted to 290 ± 7 mOsm/kg H_2O with NaCl. All solutions were gassed with 95% $\text{O}_2/5\%$ CO_2 . Ammonium chloride (10 mM) substituted for sodium chloride in both the luminal and peritubular solutions when ammonia was used. Some studies used an approximately 1.5-ml bath chamber that was thermostatically controlled to 37°C in which the peritubular solution was exchanged continuously at 0.3 ml/min. Other studies used a low-volume, laminar flow bath chamber to which preheated, continuously bubbled solutions were delivered at approximately 6 ml/min. Thirty min was allowed before making initial measurements and between experimental periods.

Intracellular pH

Intracellular pH was measured by use of techniques that we have described previously in detail (5,11,13,14). Briefly, we loaded intercalated cells with the fluorescence, pH-sensitive dye, BCECF, by adding BCECF-AM (15 μM) to the luminal solution for approximately 5 min (14). An approximately 15- μm diameter region was excited at 490 and 440 nm and emission measured at 530 nm. The 490/440 nm ratio was calibrated to intracellular pH at the end of the experiment by use of the high potassium-nigericin technique (14).

$\text{Cl}^-/\text{HCO}_3^-$ Exchange Activity

$\text{Cl}^-/\text{HCO}_3^-$ exchange activity was measured as the rate of intracellular alkalinization after luminal (to measure apical $\text{Cl}^-/\text{HCO}_3^-$ exchange activity) or peritubular (to measure basolateral $\text{Cl}^-/\text{HCO}_3^-$ exchange activity) Cl^- removal. We calculated $\text{Cl}^-/\text{HCO}_3^-$ exchange activity using least-squares linear regression during the initial 6 to 10 s of alkalinization during which alkalinization was linear.

Unidirectional Bicarbonate Secretion

Unidirectional bicarbonate secretion was measured by use of techniques that we have described previously in detail (10,15). Briefly, unidirectional bicarbonate reabsorption is inhibited by removing peritubular chloride (16,17). The initial B-cell response to peritubular

chloride removal is intracellular alkalinization as a result of reversal of basolateral $\text{Cl}^-/\text{HCO}_3^-$ exchange (10), followed by intracellular acidification (18) that results from apical bicarbonate secretion via the B-cell apical $\text{Cl}^-/\text{HCO}_3^-$ exchanger (10,11). The rate of intracellular acidification, when measured at basal intracellular pH, indicates the rate of apical bicarbonate secretion (10,15).

Chemicals

BCECF-AM was obtained from Molecular Probes, Inc. (Eugene, OR). SCH28080 was the kind gift of Dr. James Kaminski (Schering Co., Bloomfield, NJ). All other chemicals were from Sigma Chemical Co. (St. Louis, MO).

Statistical Analyses

Results are presented as mean \pm SEM. The data were analyzed by use of paired *t* test and ANOVA, as appropriate, and $P < 0.05$ was used as evidence of statistical significance.

Results

Unidirectional Bicarbonate Secretion

The B cell plays an important role in acid-base physiology through physiologically regulated bicarbonate secretion. In

Table 1. Effect of ammonia on B-cell apical bicarbonate secretion

Ammonia	Initial pH _i	Apical Bicarbonate Secretion (pH Units/Min ⁻¹)
Absent (<i>n</i> = 5)	7.41 \pm 0.20	-2.39 \pm 0.69
Present (<i>n</i> = 5)	7.39 \pm 0.11	-0.77 \pm 0.26 ^a

^a $P < 0.05$.

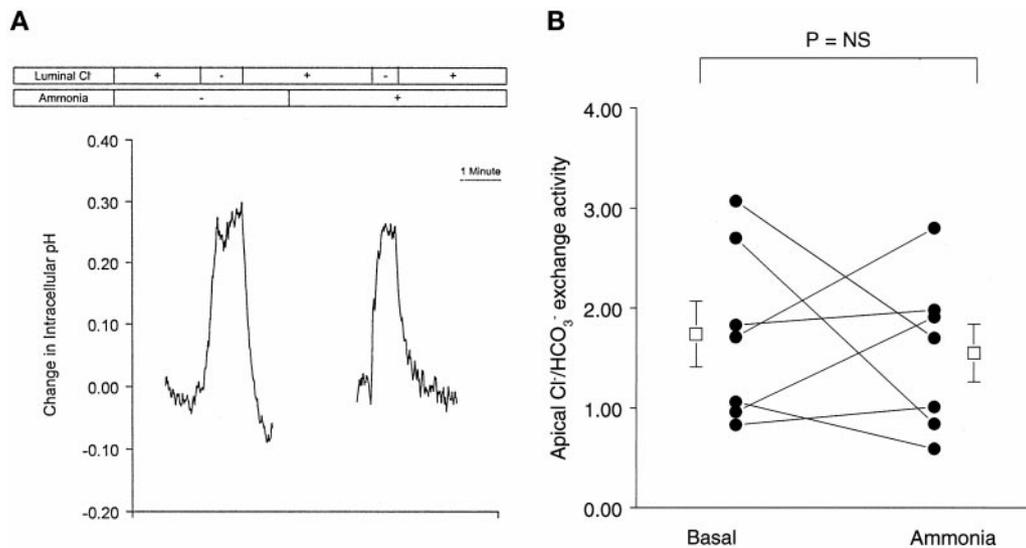


Figure 2. Effect of ammonia on B-cell apical Cl⁻/HCO₃⁻ exchange activity. Apical Cl⁻/HCO₃⁻ exchange activity was measured as the rate of intracellular alkalinization resulting from luminal Cl⁻ removal in the absence and the presence of ammonia. (A) A representative experiment. A 30-min equilibration period was allowed after addition of ammonia. (B) Summary of results of all experiments, reported in pH units per minute. Lines connect measurements in the same cell.

several models, bicarbonate secretion has been the primary mode of CCD bicarbonate transport regulated by physiologic stimuli (15,19,20). Accordingly, we tested whether inhibition of B-cell unidirectional bicarbonate secretion mediates, at least in part, ammonia’s stimulation of CCD net bicarbonate reabsorption.

To measure unidirectional B-cell-mediated bicarbonate secretion, we removed peritubular chloride, an obligate anion for apical proton secretion and luminal bicarbonate reabsorption (21). Peritubular chloride removal stimulates apical bicarbonate secretion mediated by apical Cl⁻/HCO₃⁻ exchange (11), resulting in intracellular acidification. Thus, the rate of intracellular acidification indicates the rate of B-cell bicarbonate secretion (15) (Figure 1, Table 1). In the absence of ammonia, apical bicarbonate secretion caused intracellular acidification averaging -2.39 ± 0.69 pH units/min ($n = 5$). In the presence of ammonia, bicarbonate secretion induced intracellular acidification averaging -0.77 ± 0.26 pH units/min ($n = 5$). Ammonia significantly inhibited unidirectional bicarbonate secretion ($P < 0.05$ versus in the absence of ammonia, $n = 5$). These changes are unlikely to be due to time-dependent changes; there are no time-dependent changes in B-cell bicarbonate

secretion when this technique is used (15). Thus, ammonia seems to inhibit B-cell unidirectional bicarbonate secretion.

B-cell bicarbonate secretion requires anion transport via an apical Cl⁻/HCO₃⁻ exchanger and a basolateral Cl⁻ channel (9,22). Stimuli regulating bicarbonate secretion may do so through effects on either of these transporters (13,23,24). The next set of studies tested whether ammonia inhibited bicarbonate secretion by inhibiting apical Cl⁻/HCO₃⁻ exchange activity (Figure 2, Table 2). In the absence of ammonia, apical Cl⁻/HCO₃⁻ exchange activity averaged 1.74 ± 0.33 pH units per minute ($n = 7$). In the presence of ammonia apical Cl⁻/HCO₃⁻ exchange activity averaged 1.55 ± 0.29 pH units/min ($n = 7$). Ammonia did not significantly alter B-cell apical Cl⁻/HCO₃⁻ exchange activity ($P = NS$, $n = 7$). These results indicate that ammonia inhibits B-cell unidirectional bicarbonate secretion without altering apical Cl⁻/HCO₃⁻ exchange activity, which suggests that ammonia may regulate bicarbonate secretion through the regulation of basolateral transport processes.

Effect of Ammonia on B-Cell Unidirectional Bicarbonate Reabsorption

The B cell possesses both an apical H⁺-K⁺-ATPase (25) and a basolateral Cl⁻/HCO₃⁻ exchanger (10), which suggests that it can, under appropriate conditions, reabsorb luminal bicarbonate. Ammonia stimulates H⁺-K⁺-ATPase-mediated CCD bicarbonate reabsorption (5), raising the possibility that ammonia stimulates B-cell-mediated bicarbonate reabsorption. To test this possibility, we examined the effect of ammonia on the B-cell apical H⁺-K⁺-ATPase.

To do so, we examined whether ammonia alters the contribution of apical H⁺-K⁺-ATPase to intracellular pH regulation (Figure 3 [SCH28080 with Amm], Table 3). In the presence of

Table 2. Effect of ammonia on B-cell apical Cl⁻/HCO₃⁻ exchange activity

Ammonia	Initial pH _i	Intracellular Alkalinization (pH Units/Min ⁻¹)
Absent ($n = 7$)	7.41 ± 0.09	1.74 ± 0.33
Present ($n = 7$)	7.30 ± 0.07	1.55 ± 0.29

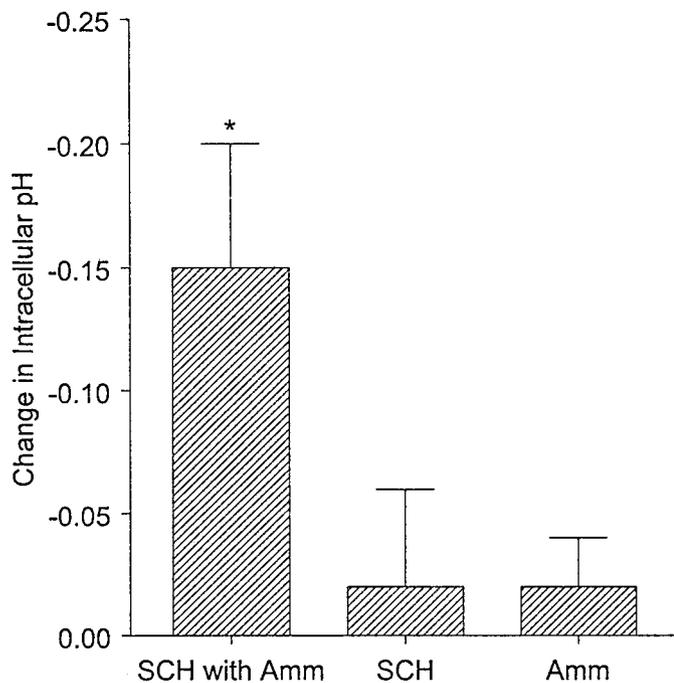


Figure 3. Effect of ammonia on B-cell SCH28080-sensitive intracellular pH. Shown are the changes in intracellular pH after addition of luminal SCH28080 in the presence of ammonia (SCH with Amm), after addition of luminal SCH28080 in the absence of ammonia (SCH), and in the continued presence of ammonia without adding luminal SCH28080 (Amm). *, $P < 0.05$.

ammonia, addition of the H^+-K^+ -ATPase inhibitor SCH28080 (10 μ m) to the luminal solution acidified the B cell significantly, decreasing intracellular pH by -0.15 ± 0.05 pH units ($P < 0.05$, $n = 5$).

Intracellular acidification after addition of luminal SCH28080 in the presence of ammonia could represent either inhibition of proton transport by SCH28080 that is independent of ammonia, intracellular acidification induced by ammonia that is independent of SCH28080, or SCH28080-mediated inhibition of ammonia-stimulated apical proton secretion. The next series of studies sought to differentiate between these possibilities.

First, we examined whether the intracellular acidification observed after addition of luminal SCH28080 occurred in the absence of ammonia (Figure 3 [SCH], Table 3). Addition of SCH28080 (10 μ m) to the luminal solution in the absence of

ammonia did not alter B-cell intracellular pH significantly ($\Delta = -0.02 \pm 0.04$ pH units; $P = NS$ versus 0; $n = 12$). Similar results occurred in previous studies (25). Thus, the effect of luminal SCH28080 on B-cell intracellular pH cannot be explained by SCH28080-mediated inhibition of ammonia-independent proton secretion.

Next, we examined whether the continued presence of ammonia, in the absence of luminal SCH28080, would explain these results. An identical protocol was used, with ammonia present throughout the experiment, and a mock perfusate change was made without addition of SCH28080 to the luminal fluid between the first and second set of intracellular pH measurements (Figure 3 [Amm], Table 3). No significant change in intracellular pH occurred in the continued presence of ammonia if SCH28080 was added to the luminal fluid ($\Delta = -0.02 \pm 0.02$ pH units; $P = NS$; $n = 4$). These results are consistent with our previously reported results (5). Thus, the effect of luminal SCH28080 on B-cell intracellular pH in the presence of ammonia is not mediated by the continued presence of ammonia.

These results indicate that ammonia increases the contribution of an apical H^+-K^+ -ATPase to B-cell intracellular pH regulation. Because ammonia stimulates SCH28080-sensitive CCD net bicarbonate reabsorption (5), ammonia may stimulate CCD net bicarbonate reabsorption, at least in part, by stimulating B-cell-mediated unidirectional bicarbonate reabsorption.

To confirm that ammonia stimulates B-cell unidirectional bicarbonate reabsorption, we studied whether ammonia stimulated basolateral Cl^-/HCO_3^- exchange activity (Figure 4, Table 4). In the absence of ammonia, basolateral Cl^-/HCO_3^- exchange activity averaged 4.06 ± 0.40 pH units/ min^{-1} , and in the presence of ammonia, it averaged 5.17 ± 0.24 pH units/ min^{-1} ($n = 5$ for both). Ammonia significantly stimulated B-cell basolateral Cl^-/HCO_3^- exchange activity ($P < 0.05$, $n = 5$). Thus, ammonia increases both apical H^+-K^+ -ATPase and basolateral Cl^-/HCO_3^- exchange activity. Ammonia seems to stimulate B-cell unidirectional bicarbonate reabsorption.

Effect of Intracellular pH

Ammonia acidifies the B cell (5), which raises the possibility that the effects observed in the current study are mediated indirectly by changes in intracellular pH. However, in each experimental protocol, there was no correlation between intracellular pH and transporter activity ($P = NS$ by ANOVA). Thus, the effect of ammonia on B-cell proton and bicarbonate transporter activity seems to be independent of ammonia's effects on B-cell intracellular pH.

Table 3. Effect of ammonia and/or SCH28080 on B-cell intracellular pH^a

Ammonia	SCH28080	<i>n</i>	Initial pH _i	Final pH _i	Change
Present	Present	5	7.12 ± 0.12	6.97 ± 0.02	-0.15 ± 0.05 ^b
Absent	Present	12	7.17 ± 0.09	7.15 ± 0.08	-0.02 ± 0.04
Present	Absent	4	6.98 ± 0.05	6.97 ± 0.05	-0.02 ± 0.02

^a Cortical collecting ducts were incubated in the presence of absence of ammonia for 30 min, then initial pH_i was measured. SCH28080, if present, then was added to the luminal fluid, and after a 30-min incubation final pH_i was measured.

^b $P < 0.05$ versus 0.

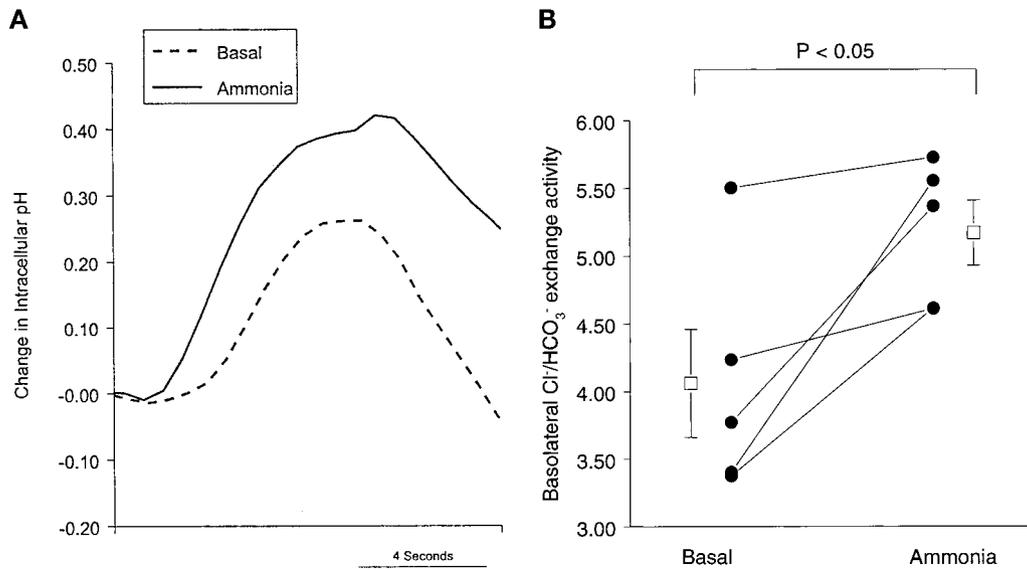


Figure 4. Effect of ammonia on B-cell basolateral Cl⁻/HCO₃⁻ exchange activity. Basolateral Cl⁻/HCO₃⁻ exchange activity was measured as the rate of intracellular alkalinization after peritubular Cl⁻ removal in the absence of ammonia and then again after a 30-min equilibration with ammonia. (A) A representative experiment. (B) Summary of results of all experiments, reported in pH units per minute. Lines connect measurements in the same cell.

Discussion

The current study examined the effects of ammonia on collecting duct net bicarbonate transport by examining the separate processes of bicarbonate reabsorption and bicarbonate secretion in a single cell, the B cell. Ammonia inhibits B-cell unidirectional bicarbonate secretion, and it does so without altering apical Cl⁻/HCO₃⁻ exchange activity. Simultaneously, ammonia stimulates apical H⁺-K⁺-ATPase and basolateral Cl⁻/HCO₃⁻ exchange activity in the B cell, which indicates that it increases B-cell-mediated unidirectional bicarbonate reabsorption. Thus, ammonia can serve as a potential intrarenal signaling molecule with specific and coordinated effects on B-cell bicarbonate transport.

A major physiologic function of the B cell is bicarbonate secretion, which mediates recovery from metabolic alkalosis (8,26,27). A major mechanism by which physiologic stimuli regulate CCD net bicarbonate transport is by regulating B-cell unidirectional bicarbonate secretion (15-17,19,20). The current study adds to these previous studies by showing that B-cell

unidirectional bicarbonate secretion is regulated by the physiologic stimulus ammonia.

Bicarbonate secretion requires both apical Cl⁻/HCO₃⁻ exchange activity and a basolateral chloride channel. The current study shows that ammonia inhibits B-cell unidirectional bicarbonate secretion without inhibiting apical Cl⁻/HCO₃⁻ exchange activity. This suggests that ammonia may inhibit the B-cell basolateral chloride channel. Whether these effects of ammonia reflect changes in the open probability of this channel, endocytic removal of the chloride channel from the plasma membrane, or other changes in its conductance characteristics cannot be determined by the current study. Further studies will be necessary to address these different possibilities.

Although the B cell generally has been modeled to secrete bicarbonate, the B cell also possesses the transporters for unidirectional bicarbonate reabsorption, namely apical proton and basolateral base transport (10,25,28). Ammonia stimulates both apical proton secretion and basolateral bicarbonate exit, and the B-cell apical proton transporter stimulated by ammonia, H⁺-K⁺-ATPase, is the same transporter stimulated when assessing the entire CCD (5). Thus, it is likely that ammonia stimulates B-cell-mediated proton and potassium transport and that this contributes to the regulation of transport in the entire CCD.

Two common conditions that stimulate renal ammoniogenesis are metabolic acidosis and hypokalemia. It is interesting that *in vivo* metabolic acidosis stimulates the B-cell apical H⁺-K⁺-ATPase (28), whereas *in vitro* metabolic acidosis has only minimal effects on H⁺-K⁺-ATPase (29). One potential explanation is that *in vivo* metabolic acidosis increases renal ammonia production and accumulation and that it is ammonia,

Table 4. Effect of ammonia on B-cell basolateral Cl⁻/HCO₃⁻ exchange activity

Ammonia	Initial pH _i	Basolateral Cl ⁻ /HCO ₃ ⁻ Exchange Activity (pH Units/Min ⁻¹)
Absent (n = 6)	7.42 ± 0.16	4.06 ± 0.40
Present (n = 6)	7.41 ± 0.09	5.18 ± 0.24 ^a

^a P < 0.05.

not the changes in extracellular pH, that stimulate B-cell apical H^+K^+ -ATPase. Further studies are necessary to identify the specific roles of extracellular acidosis and ammonia in the regulation of CCD acid-base transport. A second major stimulus for ammoniogenesis is hypokalemia. Ammonia may contribute to recovery from hypokalemia by inhibiting CCD potassium secretion (30), at least in part by stimulating unidirectional potassium reabsorption (30). Ammonia's stimulation of unidirectional potassium reabsorption is likely to be related to activation of H^+K^+ -ATPase in both the entire CCD (5) and in the B cell (current study).

Although SCH28080 may cause intracellular ATP depletion in some cells (31), this is unlikely to explain the observed effects in the current study. First, the concentration required to demonstrate this effect was 20-fold greater (31) than that used in the current study. Moreover, if SCH28080 were mediating its effects in the CCD through alterations in intracellular ATP availability, then SCH28080 also should inhibit CCD H^+ -ATPase-mediated proton secretion. However, SCH28080 has no effect on H^+ -ATPase-mediated proton secretion in CCD intercalated cells (25,32), and the effect of SCH28080 on net proton secretion can be differentiated easily from inhibition of H^+ -ATPase (29). Thus, SCH28080 is unlikely to inhibit B-cell apical proton secretion by decreasing intracellular ATP availability. Instead, SCH28080 is a highly potent inhibitor of H^+K^+ -ATPase, particularly $HK\alpha_1$, with near-complete inhibition at concentrations substantially lower than those used in the current study (33,34). Thus, SCH28080 seems to be altering both CCD acid-base transport and B-cell intracellular pH regulation by inhibition of an apical H^+K^+ -ATPase and not through generalized effects on intracellular ATP availability.

The mechanism by which ammonia stimulates B-cell basolateral Cl^-/HCO_3^- exchange activity is unclear. One possibility is that ammonia directly stimulates transport activity, similar to the effect of ammonia on the Cl^-/HCO_3^- exchange isoform, AE2 (35). Changes in intracellular pH are unlikely to explain the increased activity. Ammonia either acidifies CCD intercalated cells (5) or causes minimal changes in intracellular pH (current study), which would be expected to either inhibit or not alter anion exchanger activity, respectively. This expected effect of intracellular pH on anion exchanger activity contrasts with the stimulation induced by ammonia in the current study. In addition, there was no significant correlation between intracellular pH and basolateral Cl^-/HCO_3^- exchange activity in the current studies. The lack of relationship between intracellular pH and basolateral Cl^-/HCO_3^- exchange activity is not due intracellular pH being below the range that stimulates Cl^-/HCO_3^- exchange activity. AE1 is stimulated over a broad range of intracellular pH, whereas AE2 is stimulated only above pH 7.3 (36). As shown in Table 2, this was the intracellular pH range in the studies that examined ammonia's effect on basolateral Cl^-/HCO_3^- exchange activity. More studies will be necessary to identify the mechanism(s) through which ammonia regulates B-cell basolateral Cl^-/HCO_3^- exchange activity.

An additional result of the current studies was confirmation that the B-cell apical and basolateral Cl^-/HCO_3^- exchangers

are differentially regulated by ammonia. These results are similar to previous studies that suggested that isoproterenol differentially regulates apical and basolateral Cl^-/HCO_3^- exchange activity (37). The differential regulation of these transporters is consistent with the apical and basolateral Cl^-/HCO_3^- exchangers representing different anion exchanger isoforms. In particular, antibodies to the anion exchanger AE4 localize to proteins present on the B-cell apical but not the basolateral plasma membrane (38). Thus, the B-cell apical and basolateral Cl^-/HCO_3^- exchangers both are immunologically distinct and are differentially regulated, which suggests that they could mediate different functional roles.

The current studies used millimolar ammonia concentrations. Ammonia is produced by the proximal tubule and is concentrated into the renal interstitium by the loop of Henle, yielding levels that average 4 to 6 mM in the late distal tubule (39). These levels are increased significantly by both metabolic acidosis (1,39) and hypokalemia (40–42). The exact interstitial ammonia concentrations that are present in the kidney are unclear. Although it is tempting to assume that renal cortical interstitial ammonia can be determined by the measurement of renal venous ammonia concentration, these calculations rely on NH_3 being in diffusion equilibrium between the renal interstitium and the renal vein, which is known to be untrue (43). Because ammonia secretion is believed to occur via NH_3 diffusion, interstitial $[NH_3]$ almost certainly is greater than intraluminal $[NH_3]$. When measurements of intraluminal $[NH_3]$ in metabolic acidosis (39) and measured values of renal cortical pCO_2 (44) are used and under the assumption that cortical interstitial $[HCO_3^-]$ is similar to systemic $[HCO_3^-]$, an estimate of interstitial ammonia is approximately 1.7 mM. The exact interstitial ammonia concentration is unknown but is likely to be substantially greater, to mediate high rates of net ammonia secretion that occur in the cortex (39).

In summary, the current study identified several important facets in renal physiology. First, ammonia inhibits B-cell unidirectional bicarbonate secretion, and this effect is independent of apical Cl^-/HCO_3^- exchange activity, which suggests that ammonia regulates net bicarbonate secretion through regulation of the basolateral chloride channel. Next, the B-cell apical H^+K^+ -ATPase and basolateral Cl^-/HCO_3^- exchanger can be regulated in parallel by a single stimulus, ammonia, which demonstrates that the B cell can contribute to urinary acidification and luminal bicarbonate reabsorption. Thus, ammonia increases CCD net bicarbonate reabsorption through coordinated effects on the separate processes of unidirectional bicarbonate reabsorption and secretion. These findings contribute importantly to our understanding of the mechanisms through which ammonia may serve as an intrarenal signaling molecule that regulates CCD ion transport.

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References

- Knepper MA: NH_4^+ transport in the kidney. *Kidney Int Suppl* 40: S95–S102, 1991
- Nagami GT, Sonu CM, Kurokawa K: Ammonia production by isolated mouse proximal tubules perfused in vitro: Effect of metabolic acidosis. *J Clin Invest* 78: 124–129, 1986
- Nonoguchi H, Uchida S, Shiigai T, Endou H: Effect of chronic metabolic acidosis on ammonia production from L-glutamine in microdissected rat nephron segments. *Pflügers Arch* 403: 229–235, 1985
- Knepper MA, Good DW, Burg MB: Ammonia and bicarbonate transport by rat cortical collecting ducts perfused in vitro. *Am J Physiol* 249: F870–F877, 1985
- Frank AE, Wingo CS, Weiner ID: Effects of ammonia on bicarbonate transport in the cortical collecting duct. *Am J Physiol* 278: F219–F226, 2000
- Flessner MF, Wall SM, Knepper MA: Ammonium and bicarbonate transport in rat outer medullary collecting ducts. *Am J Physiol* 262: F1–F7, 1992
- Wall SM: NH_4^+ augments net acid-secretion by a ouabain-sensitive mechanism in isolated-perfused inner medullary collecting ducts. *Am J Physiol* 270: F432–F439, 1996
- Schuster VL: Bicarbonate reabsorption and secretion in the cortical and outer medullary collecting tubule. *Semin Nephrol* 10: 139–147, 1990
- Schuster VL: Physiology and cell biology update: Control mechanisms for bicarbonate secretion. *Am J Kidney Dis* 13: 348–352, 1989
- Weiner ID, Weill AE, New AR: Distribution of $\text{Cl}^-/\text{HCO}_3^-$ exchange and intercalated cells in the rabbit cortical collecting duct. *Am J Physiol* 267: F952–F964, 1994
- Weiner ID, Hamm LL: Regulation of intracellular pH in the rabbit cortical collecting tubule. *J Clin Invest* 85: 274–281, 1990
- Weiner ID, Hamm LL: Regulation of $\text{Cl}^-/\text{HCO}_3^-$ exchange in the rabbit cortical collecting tubule. *J Clin Invest* 87: 1553–1558, 1991
- Milton AE, Weiner ID: Regulation of B-type intercalated cell apical anion exchange activity by $\text{CO}_2/\text{HCO}_3^-$. *Am J Physiol* 274: F1086–F1094, 1998
- Weiner ID, Hamm LL: Use of fluorescent dye BCECF to measure intracellular pH in cortical collecting tubule. *Am J Physiol* 256: F957–F964, 1989
- Weiner ID, New AR, Milton AE, Tisher CC: Regulation of luminal alkalization and acidification in the cortical collecting duct by angiotensin II. *Am J Physiol* 269: F730–F738, 1995
- Garcia-Austt J, Good DW, Burg MB, Knepper MA: Deoxycorticosterone-stimulated bicarbonate secretion in rabbit cortical collecting ducts: Effects of luminal chloride removal and in vivo acid loading. *Am J Physiol* 249: F205–F212, 1985
- Hamm LL, Hering-Smith KS, Vehaskari VM: Control of bicarbonate transport in collecting tubules from normal and remnant kidneys. *Am J Physiol* 256: F680–F687, 1989
- Schwartz GJ, Barasch J, Al-Awqati Q: Plasticity of functional epithelial polarity. *Nature* 318: 368–371, 1985
- Schuster VL: Cyclic adenosine monophosphate-stimulated anion transport in rabbit cortical collecting duct: Kinetics, stoichiometry, and conductive pathways. *J Clin Invest* 78: 1621–1630, 1986
- Hayashi M, Yamaji Y, Iyori M, Kitajima W, Saruta T: The effects of isoproterenol on intracellular pH of the intercalated cells in the rabbit cortical collecting ducts. *J Clin Invest* 87: 1153–1157, 1991
- Stone DK, Kokko JP, Jacobson HR: Anion dependence of rabbit medullary collecting duct acidification. *J Clin Invest* 71: 1505–1508, 1983
- Schuster VL, Stokes JB: Chloride transport by the cortical and outer medullary collecting duct. *Am J Physiol* 253: F203–F212, 1987
- Matsuzaki K, Schuster VL, Stokes JB: Reduction in sensitivity to Cl^- channel blockers by $\text{HCO}_3^-/\text{CO}_2$ in rabbit cortical collecting duct. *Am J Physiol* 257: C102–C109, 1989
- Matsuzaki K, Stokes JB, Schuster VL: Stimulation of Cl^- self exchange by intracellular HCO_3^- in rabbit cortical collecting duct. *Am J Physiol* 257: C94–C101, 1989
- Weiner ID, Milton AE: H^+/K^+ -ATPase in rabbit cortical collecting duct B-type intercalated cell. *Am J Physiol* 270: F518–F530, 1996
- Galla JH, Gifford JD, Luke RG, Rome L: Adaptations to chloride-depletion alkalosis. *Am J Physiol* 261: R771–R781, 1991
- Gifford JD, Sharkins K, Work J, Luke RG, Galla JH: Total CO_2 transport in rat cortical collecting duct in chloride-depletion alkalosis. *Am J Physiol* 258: F848–F853, 1990
- Silver RB, Mennitt PA, Satlin LM: Stimulation of apical H-K-ATPase in intercalated cells of cortical collecting duct with chronic metabolic acidosis. *Am J Physiol* 270: F539–F547, 1996
- Tsuruoka S, Schwartz GJ: Adaptation of rabbit cortical collecting duct HCO_3^- transport to metabolic acidosis in vitro. *J Clin Invest* 97: 1076–1084, 1996
- Hamm LL, Gillespie C, Klahr S: NH_4Cl inhibition of transport in the rabbit cortical collecting tubule. *Am J Physiol* 248: F631–F637, 1985
- Codina J, Cardwell J, Gitomer JJ, Cui Y, Kone BC, DuBose TD Jr: SCH-28080 depletes intracellular ATP selectively in mIMCD-3 cells. *Am J Physiol Cell Physiol* 279: C1319–C1326, 2000
- Milton AE, Weiner ID: Intracellular pH regulation in the rabbit cortical collecting duct A-type intercalated cell. *Am J Physiol* 273: F340–F347, 1997
- Beil W, Hackbarth I, Sewing KF: Mechanism of gastric antisecretory effect of SCH 28080. *Br J Pharmacol* 88: 19–23, 1986
- Beil W, Staar U, Sewing KF: SCH 28080 is a more selective inhibitor than SCH 32651 at the K^+ site of gastric K^+/H^+ -ATPase. *Eur J Pharmacol* 139: 349–352, 1987
- Humphreys BD, Chernova MN, Jiang L, Zhang Y, Alper SL: NH_4Cl activates AE2 anion exchanger in *Xenopus* oocytes at acidic pH. *Am J Physiol* 272: C1232–C1240, 1997
- Jiang L, Stuart-Tilley A, Parkash J, Alper SL: pH_i and serum regulate AE2-mediated $\text{Cl}^-/\text{HCO}_3^-$ exchange in CHOP cells of defined transient transfection status. *Am J Physiol* 267: C845–C856, 1994
- Weiner ID, Weill AE: Distribution and regulation of $\text{Cl}^-/\text{HCO}_3^-$ exchange in rabbit cortical collecting ducts [Abstract]. *J Am Soc Nephrol* 3: 791, 1992
- Tsugenezawa H, Kobayashi K, Iyori M, Araki T, Kim DK, Monkawa T, Kanai Y, Endou H, Hayashi M: Apical anion exchanger of β -intercalated cells is a new member of the bicarbonate transporter superfamily [Abstract]. *J Am Soc Nephrol* 11: 10A, 2000
- Wilcox CS, Granges F, Kirk G, Gordon D, Giebisch, G: Effects of saline infusion on titratable acid generation and ammonia secretion. *Am J Physiol* 247: F506–F519, 1984
- Wall SM, Davis BS, Hassell KA, Mehta P, Park SJ: In rat tIMCD, NH_4^+ uptake by the Na^+ , K^+ -ATPase is critical to net

- acid secretion during chronic hypokalemia. *Am J Physiol* 277: F866–F874, 1999
41. Tannen RL, McGill J: Influence of potassium on renal ammonia production. *Am J Physiol* 231: 1178–1184, 1976
 42. Tannen RL: Relationship of renal ammonia production and potassium homeostasis. *Kidney Int* 11: 453–465, 1977
 43. DuBose TD Jr, Good DW, Hamm LL, Wall SM: Ammonium transport in the kidney: New physiological concepts and their clinical implications. *J Am Soc Nephrol* 1: 1193–1203, 1991
 44. DuBose TD Jr, Pucacco LR, Seldin DW, Carter NW: Direct determination of PCO₂ in the rat renal cortex. *J Clin Invest* 62: 338–348, 1978

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