Regulation of Cl-/HCO3- exchange in the rabbit cortical collecting tubule.

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Research Article

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Regulation of Cl^-/HCO_3^- Exchange in the Rabbit Cortical Collecting Tubule

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Abstract

Cl^-/HCO_3^- exchange is present in all three cell types of the rabbit cortical collecting tubule, yet may mediate a different function in each cell type. The purpose of this study was to characterize further the location, function, and regulation of Cl^-/HCO_3^- exchange in two cell types using measurements of intracellular pH (pH_i). In the principal cell there was no evidence for apical Cl^-/HCO_3^- exchange, including no change in pH_i with increases in luminal HCO_3^-. The principal cell possesses a basolateral Cl^-/HCO_3^- exchanger that is inactive normally but stimulated by intracellular alkalosis. Decreased P_{CO_2} results in increased pH_i associated with activation of Cl^-/HCO_3^- exchange and partial recovery of pH_i.

In contrast, the β-intercalated cell possesses an apical Cl^-/HCO_3^- exchanger and alkalinizes with increases in luminal HCO_3^-. Also in contrast to the principal cell, the β-intercalated cell apical Cl^-/HCO_3^- exchanger does not appear to be involved in pH_i regulation and may be specifically modified for transcellular HCO_3^- transport.

In conclusion, the separate Cl^-/HCO_3^- exchangers in the principal cell and the β-intercalated cell not only have opposite polarity but are regulated differently. (*J. Clin. Invest.* 1991. 87:1553–1558.) Key words: acid-base • anion exchange • intracellular pH

Introduction

Intracellular pH (pH_i)[1] homeostasis is an integral function of most, if not all, cells (1). Specialized transporters exist for recovery from intracellular acidosis (Na^+/H^+ exchange [1–3] and Na^+-dependent Cl^-/HCO_3^- exchange [1]) and from intracellular alkalosis (Na^+-independent Cl^-/HCO_3^- exchange (4, 5) and $Na^+(HCO_3^-)_{n>1}$ cotransport [6]). These transporters are also involved in functions other than pH_i regulation. Examples include growth factor signal transduction (2, 3), cell volume regulation (7), and transepithelial solute flux (8–10).

The diversity of function for acid-base transporters is apparent in adjacent cell types in the rabbit cortical collecting tubule (CCT). The CCT is a heterogeneous tissue, composed of at least three cell types, principal cells and at least two types of intercalated cells (11–14). Each of these cells has a Na^+-independent Cl^-/HCO_3^- exchanger (15). Yet the role of this exchanger(s) may be different in each of these cells. The intercalated cells appear to mediate transepithelial HCO_3^- flux (16, 17) via H^+-ATPases (18) and Cl^-/HCO_3^- exchangers located on opposite plasma membranes (13–15, 19). The principal cell also appears to possess a Cl^-/HCO_3^- exchanger located on the basolateral membrane; however, this exchanger appears to be relatively inactive under baseline conditions (15). The factors that regulate the different Cl^-/HCO_3^- exchangers have not been defined.

Regulation of β-intercalated cell apical Cl^-/HCO_3^- exchange has been indirectly studied using measurements of Cl^- self-exchange and HCO_3^- secretion (20). Studies of Cl^- self-exchange take advantage of the observation that Cl^- self-exchange is an alternate mode of Cl^-/HCO_3^- exchangers and in the β-intercalated cell is 10–15 times that of Cl^-/HCO_3^- exchange (21–23). However, these transepithelial flux methods do not directly address the rate of the apical Cl^-/HCO_3^- exchanger.

Therefore these studies examine the acute regulation of Cl^-/HCO_3^- exchange in the rabbit CCT using measurement of pH_i. Principal cell and β-intercalated cell pH_i were separately measured in the in vitro microperfused CCT using the fluorescent, pH-sensitive dye 2',7'-bis-(2-carboxyethyl)-5(and-6)carboxyfluorescein (BCECF) (15, 24) to follow acute changes in HCO_3^- flux across plasma membranes.

Methods

Microperfusion. Cortical collecting tubules were perfused using standard techniques (25) as previously described (15, 26), with the following exceptions. In most experiments a low volume, laminar flow perfusion chamber was used. The peritubular bathing solution was exchanged at a rate of ~ 10 ml/min, resulting in a complete change of peritubular solution in ~ 3 s. Acid loading studies, however, were performed as previously described (15) in a 1-ml perfusion chamber. In these studies the peritubular fluid was exchanged at ~ 3 ml/min. Solution 1 was the initial peritubular and luminal solution except where specifically noted.

Solutions. Table I shows the components of the various solutions. Gluconate containing solutions had an increased total Ca^{+2} to compensate for the complexing of Ca^{+2} by gluconate. All chemicals were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise specified.

Fluorescent dyes. The acetoxymethyl ester of BCECF (BCECF-AM) was obtained from Molecular Probes, Inc., Eugene, OR, and maintained at −20°C as a 30-mM stock solution in DMSO. The stock solution was diluted with solution 1 to either 15 μM (for luminal loading) or 5 μM (for basolateral loading) on the day of an experiment. BCECF was loaded as previously described (15, 26). In brief, intercalated cells selectively concentrate luminal BCECF-AM while peritubular BCECF-AM is equally taken up by both principal and intercalated

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1. *Abbreviations used in this paper:* ANOVA, analysis of variance; BCECF, 2'7'-bis-(2-carboxyethyl)-5(and-6)carboxyfluorescein; BCECF-AM, acetoxymethyl ester of BCECF; CCT, cortical collecting tubule; DOCA, deoxycorticosterone acetate; pH_i, intracellular pH.

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*Table I. Solutions**

	1	2	3	4	5	6	7	8	9	10	11	12
NaCl	119.2		94.2	139.2		25	25	94.2				144.2
Choline chloride					139.2	94.2			119.2	99.2		
Sodium gluconate		119.2						25			94.2	
$NaHCO_3$	25	25	50	5		25	25	25			50	
NH_4Cl										20		
Choline bicarbonate					5				25	25		
KCl	3		3	3	2	2	97.2	3	2	2		3
Potassium gluconate		3									3	
Sodium acetate	1	1	2	1			1	1			1	1
Potassium acetate					1	1			1	1		
$CaCl_2$	1.2		1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2		1.2
Calcium gluconate		4.0						0.6			4.4	
KH_2PO_4	2	2	2	2	2	2	2	2	2	2	2	2
$MgSO_4$	1	1	1	1	1	1	1	1	1	1	1	1
Alanine	5	5	5	5	5	5	5	5	5	5	5	5
Glucose	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3

* All concentrations expressed in millimoles per liter. Osmolality adjusted to 285–295 mosmol/kg by addition of principal salt. All solutions continuously bubbled with 95% O_2/5% CO_2 unless specifically noted. 2% CO_2 solutions bubbled with 98% O_2/2% CO_2.

cells (26). As a result, luminal BCECF-AM loading was used to study intercalated cells. In all cases, intercalated cell type (α- or β-) was confirmed by peritubular Cl^- removal (15, 24). Principal cells were studied by loading first with luminal BCECF-AM, identifying an area of the tubule without intercalated cells, and then loading with peritubular BCECF-AM. In all experiments at least 5 min was allowed after loading BCECF-AM before measurement of pH_i.

Intracellular pH measurements. Fluorescence studies were performed on a microscope (Diaphot-TMD; Nikon Inc., Garden City, NY) modified for fluorescent use as previously described (15, 26). A Nikon Fluor-40, numerical aperture (n.a.) 1.30, oil immersion, and a Nikon Fluor-40, n.a. 0.85, objective were used interchangeably. An area of ~ 5-μm diameter was alternately excited at 500 nm and 450 nm. This field was generally positioned at the edge of the tubule in order to minimize fluorescence from cells above or below the plane of measurement. Use of a small excitation field centered at the edge of the tubule allowed measurement of the pH_i of either a single intercalated cell or portions of approximately one to four principal cells. Emission was measured at 530 nm. Cell pH_i was calibrated using the high K^+-Nigericin technique (27) as we have previously described (15, 26).

Cells were acid loaded by changing the peritubular solution to one containing 20 mM ammonium chloride (solution 10) for 5 min (1, 15). Changes in pH_i after acid loading are expressed as the difference in pH_i between 1 and 5 min after the solution change; the initial 1-min time point was chosen to match the nadir of pH_i after acid loading and to ensure complete solution change.

Statistics. Values are presented as mean±SEM. In general, statistical analysis used paired, two sided Student's t test. Analysis of variance (ANOVA) was used when appropriate and is noted in the text. Statistical significance is defined as at least $P < 0.05$. All pH_i results are reported as the mean of n tubules.

Results

Principal cell basolateral Cl^-/HCO_3^- exchange. The first set of experiments examined the hypothesis that intracellular alkalosis stimulates principal cell Cl^-/HCO_3^- exchange. A representative experiment of the effect of an acute decrease in P_{CO_2} is shown in Fig. 1. The acute decrease in P_{CO_2} resulted in a rapid intracellular alkalinization. (The increase in pH_i with a decrease in P_{CO_2} is less than expected if CO_2/HCO_3^- were the only buffer system active. This difference is probably due to the intrinsic [nonbicarbonate/CO_2] buffer capacity of the cell.) After the peak alkalinization, pH_i declined 0.07±0.02 pH U in the first 5 min ($P < 0.001$ vs. 0.00, $n = 12$). Removal of peritubular Cl^- (change to solution 2, bubbled with 2% CO_2) resulted in a reversal of the recovery and, in fact, a net alkalinization of 0.06±0.02 pH U after 5 min ($P < 0.005$ vs. in the presence of peritubular Cl^- by paired t test, $n = 6$).

This is in marked contrast to our previous study where the acute removal of peritubular Cl^- from solutions bubbled with 5% CO_2 (pH 7.4) resulted in no change in principal cell pH_i (15). One potential explanation is that this study used a laminar flow perfusion chamber, which enables the peritubular solutions to be changed much faster. The effect of acute peritubular Cl^- removal in the absence of a decrease in P_{CO_2} was therefore studied using the laminar flow chamber. Consistent with our previous findings, peritubular Cl^- removal resulted in no significant change in principal cell pH_i after 5 min (Δ = 0.02±0.06 pH U, P = NS, $n = 5$). Acute principal cell alkalin-

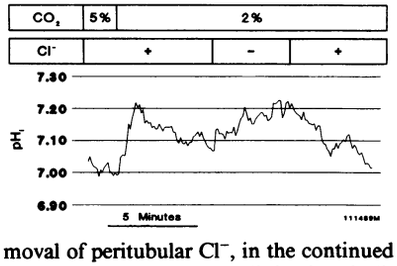

Figure 1. Effect of an acute decrease in peritubular P_{CO_2} on principal cell pH_i. The decrease in P_{CO_2} results in acute alkalinization. Principal cell pH_i then begins to recover towards baseline. Removal of peritubular Cl^-, in the continued presence of only 2% CO_2, results in intracellular alkalinization, indicative of reversal of Cl^-/HCO_3^- exchange. This alkalinization is reversible with return of Cl^- to the peritubular solution.

ization, as induced by an acute decrease in P_{CO_2}, results in activation of a Cl^--dependent, basolateral base exit process, i.e., Cl^-/HCO_3^- exchange.

The effect of an acute increase in peritubular HCO_3^- was investigated next. An increase in bath HCO_3^- (change in peritubular solution from 1 to 3) caused pH_i to slowly increase and peak at 0.13±0.03 pH U above baseline after 20 min. Return of peritubular HCO_3^- to 25 mM caused pH_i to decrease 0.09±0.02 pH U in the first 5 min ($n = 11$, $P < 0.001$). The Cl^- dependence of this recovery was examined next. After 30 min of high peritubular HCO_3^- (solution 3) the peritubular solution was changed to a 25 HCO_3^-, 0 Cl^- solution (solution 2). Instead of decreasing, pH_i actually increased 0.06±0.03 pH U in 5 min. The peritubular solution was then changed to a Cl^- containing, 25 mM HCO_3^- solution (No. 1). pH_i then decreased 0.22±0.03 pH U in 5 min ($P < 0.01$ vs. in the absence of Cl^-, $n = 5$). A 30-min exposure to a high HCO_3^- peritubular solution results in both a gradual alkalinization of principal cells and the activation of a basolateral, Cl^- dependent, base exit mechanism, i.e., Cl^-/HCO_3^- exchange.

Besides Cl^-/HCO_3^- exchange, other possible mechanisms of principal cell basolateral HCO_3^- transport, particularly $Na^+(HCO_3^-)_{n>1}$ cotransport, were investigated. For these experiments we measured the Na^+-dependence of the pH_i change in response to an acute decrease in peritubular HCO_3^- to 5 mM. Measurement of pH_i change was performed in a paired manner in each tubule and the sequence, in the presence versus in the absence of peritubular Na^+, was randomized. In the presence of Na^+ (change to solution 4), pH_i decreased 0.30±0.02 pH U after 5 min. In the absence of peritubular Na^+ (peritubular solution changed first to a Na^+-free, 25 mM HCO_3^- solution, solution 9, until pH_i stabilized, and then to a 0 Na^+, 5 mM HCO_3^- solution, 5), the change in pH_i was decreased by 30±9% to 0.21±0.03 pH U ($P < 0.05$ by paired t test vs. in the presence of Na^+, $n = 5$). These results suggest the presence of a basolateral, Na^+-linked acid-base transport mechanism. We next examined whether this was an electrogenic transport mechanism. The peritubular solution was first changed to a 50 Na^+, 5 K^+ solution (solution 6) for 5 min. This was done to allow subsequent changes in peritubular K^+ to be performed while keeping peritubular Na^+ constant. Principal cells were then acutely depolarized by increasing peritubular K^+ to ~ 100 mM (solution 7). This resulted in a reversible increase in pH_i measuring 0.11±0.03 pH U after 5 min ($P < 0.025$, $n = 5$). The principal cell appears to have a basolateral, Na^+-linked, electrogenic transporter, most likely $Na^+(HCO_3^-)_{n>1}$ cotransport.

Principal cell apical Cl^-/HCO_3^- exchange. A recent study has suggested the presence of apical principal cell Cl^-/HCO_3^- exchange (28). We, on the other hand, have shown that there is no change in principal cell pH_i with the acute removal of luminal Cl^- (15). Yet, acute Cl^- removal may not be sufficient to exclude Cl^-/HCO_3^- exchange under some circumstances (15, 29). Tubules were therefore bathed and perfused with Cl^- free solutions (solution 2) for 45 min to deplete principal cells of intracellular Cl^- and create a maximal gradient for Cl^- entry via Cl^-/HCO_3^- exchange. Acutely returning luminal Cl^- (change to solution 1) resulted in no significant change in principal cell pH_i after 5 min ($\Delta = -0.04\pm0.02$ pH U, $n = 8$, P = NS). The study suggesting apical principal cell Cl^-/HCO_3^- exchange used tubules from rabbits chronically treated with deoxycorticosterone acetate (DOCA) (28). Therefore, a set of rabbits was treated with DOCA, 5 mg/kg/d intramuscularly for 7–14 d. Tubules were bathed and perfused with Cl^--free solutions (solution 2) for at least 45 min. The acute addition of Cl^- to the perfusate (change to solution 1) resulted in no significant change in pH_i after 5 min ($\Delta = 0.00\pm0.01$ pH U, $n = 6$, P = NS). These experiments demonstrate no evidence for apical principal cell Cl^-/HCO_3^- exchange.

Direct apical entry of HCO_3^- was examined next by increasing apical HCO_3^- concentration. To control for changes in luminal Cl^- concentration (known to affect pH_i in cells with an apical Cl^-/HCO_3^- exchanger such as the β-intercalated cell [15]), the perfusate was changed initially to solution 8 containing 25 mM HCO_3^- but with a Cl^- concentration equal to that of the 50 mM HCO_3^- solution (No. 3). This resulted in no significant change in principal cell pH_i (data not shown). The perfusate was then changed to a 50 mM HCO_3^- solution (No. 3). This acute increase in luminal HCO_3^- had no effect on principal cell pH_i ($\Delta = -0.07\pm0.03$ after 5 min, $n = 4$, P = NS). These results therefore confirm that apical Cl^-/HCO_3^- is not present in the principal cell, either under baseline conditions or after chronic DOCA administration.

β-Intercalated cell apical Cl^-/HCO_3^- exchange. First, the response of the β-intercalated cell to an increase in luminal HCO_3^- was determined. A typical experiment is shown in Fig. 2. For the same reasons described for the principal cell the perfusate was changed initially to solution 8 containing 25 mM HCO_3^- but with a Cl^- concentration equal to that of the 50 mM HCO_3^- solution (No. 3). This resulted in no significant change in β-intercalated cell pH_i (data not shown). The perfusate was then changed to a 50 mM HCO_3^- solution (No. 3). β-intercalated cell pH_i increased rapidly ($\Delta = 0.18\pm0.04$ pH U after 5 min, $P < 0.05$, $n = 4$), peaked at 0.30±0.04 pH U above baseline after 20 min ($P < 0.005$, $n = 4$), and returned to baseline with return of luminal HCO_3^- to 25 mM. This is consistent with HCO_3^- entry via the apical Cl^-/HCO_3^- exchanger.

The next series of studies addressed the role of apical Cl^-/HCO_3^- exchange in β-intercalated cell pH_i regulation. A typical experiment is shown in Fig. 3. Similar to the principal cell, an acute decrease in P_{CO_2} resulted in acute alkalinization of the cell. However, despite the presence of Cl^- in both the peritubular and apical solutions, there was no significant recovery of pH_i towards baseline after 5 min ($\Delta = 0.01\pm0.01$ pH U, $n = 8$, P = NS). In three tubules, pH_i was followed for as much as 30 min without any significant recovery of pH_i (data not shown). The β-intercalated cell does not acutely recover from acute intracellular alkalinization induced by a decrease in P_{CO_2}, suggesting that apical Cl^-/HCO_3^- exchange is not stimulated by increases in pH_i.

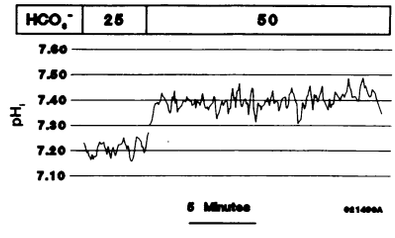

Figure 2. Effect of luminal HCO_3^- on β-intercalated cell pH_i. Luminal Cl^- is first decreased by 25 mM so that luminal HCO_3^- can be subsequently increased without a change in luminal Cl^- concentration (known to effect β-intercalated cell pH_i at low concentrations). Acutely increasing luminal HCO_3^- to 50 mM causes an acute intracellular alkalinization, consistent with apical HCO_3^- entry via the Cl^-/HCO_3^- exchanger. This alkalinization is reversible with return of luminal HCO_3^- to 25 mM.

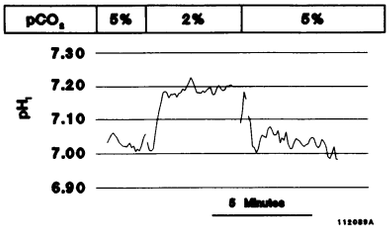

Figure 3. Effect of P_{CO_2} on β-intercalated cell pH_i. The decrease in CO_2 results in acute intracellular alkalinization. In contrast to the principal cell, there is no recovery of β-intercalated cell pH_i despite the presence of Cl^-.

The effect of an acute increase in peritubular HCO_3^- was then studied. As shown in Fig. 4, acutely increasing peritubular HCO_3^- from 25 to 50 mM (change peritubular solution from 1 to 3) resulted in intracellular alkalinization. After 5 min the average alkalinization was 0.10±0.03 pH U ($P < 0.025$, $n = 6$) and the mean maximal alkalinization was 0.15±0.04 pH U at 10 min ($P < 0.01$, $n = 6$). Normalizing peritubular HCO_3^- (change to solution 1) resulted in a return of pH_i to baseline.

We next studied the Cl^--dependence of the mechanism by which an increase in peritubular HCO_3^- effects β-intercalated cell pH_i. Cl^- was removed from both peritubular and luminal solutions in order to inhibit apical Cl^-/HCO_3^- exchange. A representative experiment is shown in Fig. 5. As previously shown, the removal of luminal Cl^- (change to solution 2) results in rapid alkalinization of the β-intercalated cell (15). Subsequent removal of peritubular Cl^- (change to solution 2) results in no acute change in β-intercalated cell pH_i. However, there occasionally was a slow fall in pH_i. A subsequent increase in peritubular HCO_3^- from 25 to 50 mEq/liter, still in the absence of Cl^- (change to solution 11), had no effect on pH_i. After 30 min the peritubular HCO_3^- was then changed back to 25 mEq/liter (change back to solution 2). Again, there was no significant change in pH_i due to the change in peritubular HCO_3^-. These results suggest that peritubular HCO_3^- alters β-intercalated cell pH_i via a Cl^--dependent mechanism.

The β-intercalated cell apical Cl^-/HCO_3^- exchanger can transport HCO_3^- either from cell-to-lumen or lumen-to-cell, depending on the HCO_3^- and Cl^- gradients across the apical membrane (15). In addition, Cl^-/HCO_3^- exchange in most, but not all, cells is inhibited by decreases in pH_i (5). To this extent, an acute decrease in the rate of Cl^-/HCO_3^- exchange as a result of acute intracellular acidosis might serve to effectively "load" a cell with HCO_3^- and increase the rate of recovery. Studies were therefore performed to determine if a decrease in the rate of turnover (normally HCO_3^- exit, Cl^- entry) of the β-intercalated cell apical Cl^-/HCO_3^- exchanger occurs in response to intracellular acidosis. β-intercalated cells were acid loaded by a 5-min exposure to a 20-mM NH_4Cl solution (No. 10). pH_i recovery was followed in a Na^+-free peritubular solution (No. 9) for 5 min and then the peritubular solution was changed to a Na^+-containing solution (No. 1). Experiments were performed in the presence and absence of luminal HCO_3^- (luminal solution changed to solution 12). As shown in Table II, peritubular Na^+ is the major determinant of β-intercalated cell pH_i recovery (via Na^+/H^+ exchange [15]). The presence or absence of luminal HCO_3^- had no independent effect on pH_i recovery. β-intercalated cell apical Cl^-/HCO_3^- exchange does not appear to participate in the response to acute intracellular acidosis.

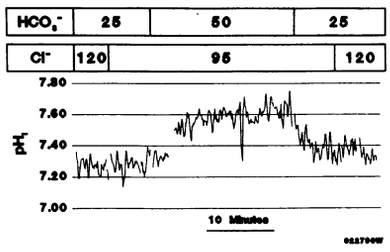

Figure 4. Effect of increasing peritubular HCO_3^- on β-intercalated cell pH_i. Increasing peritubular ("Bath") HCO_3^- from 25 to 50 mM causes alkalinization of the β-intercalated cell.

Discussion

These studies examine the regulation of principal cell and β-intercalated cell pH_i, particularly in response to alkalosis. Decreases in P_{CO_2} immediately alkalinize both cell types. Elevations in peritubular HCO_3^- also alkalinize both cell types; but, only the β-intercalated cell is alkalinized by an increase in luminal HCO_3^-. Both a decrease in P_{CO_2} and an increase in peritubular HCO_3^- stimulate the principal cell basolateral Cl^-/HCO_3^- exchanger. In contrast, the apical Cl^-/HCO_3^- exchanger of the β-intercalated cell does not appear to be acutely regulated by either alkali or acid loads. The β-intercalated cell does not regulate pH_i toward baseline after intracellular alkalinization induced by a decrease in P_{CO_2}; and removal of luminal HCO_3^- does not affect recovery from an acute intracellular acid load. Increases in peritubular HCO_3^- affect β-intercalated cell and principal cell pH_i through different mechanisms. The principal cell appears to have a basolateral $Na^+(HCO_3^-)_{n>1}$ cotransporter while increases in peritubular HCO_3^- alkalinize the β-intercalated cell via a Cl^--dependent mechanism.

The β-intercalated cell apical Cl^-/HCO_3^- exchanger has several unusual features. It does not acutely appear to regulate pH_i in response to acute intracellular alkalosis as induced by an acute decrease in P_{CO_2}, suggesting intracellular alkalosis does not stimulate it. Removal of luminal HCO_3^- does not affect β-intercalated cell pH_i recovery from acute intracellular acidosis, suggesting that β-intercalated cell Cl^-/HCO_3^- exchange is not inhibited by intracellular acidosis. It does not appear to be acutely regulated by pH_i, as it is neither stimulated by intracellular alkalosis nor inhibited by intracellular acidosis. Also, β-intercalated cell apical Cl^-/HCO_3^- exchange remains active to pH_i as low as 6.5 (15), while in most cells Cl^-/HCO_3^- exchange is inhibited by pH_i below ~ 7.1 (4, 5). Disulfonic stilbenes are inhibitors of Cl^-/HCO_3^- exchange in most cells, but do not

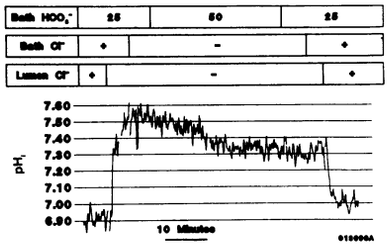

Figure 5. Effect of increased peritubular HCO_3^- on β-intercalated cell pH_i in the absence of Cl^-. Cl^- is removed, first from the perfusate (*Lumen*) and then from the peritubular solution (*Bath*). Removal of Cl^- from the perfusate results in rapid intracellular alkalinization due to reversal of the apical Cl^-/HCO_3^- exchanger. The subsequent removal of peritubular Cl^- does not affect pH_i. However, there is the development of a gradual decrease in pH_i after a more prolonged period of time. Neither acutely increasing peritubular HCO_3^- to 50 mEq/liter nor decreasing it back to 25 mEq/liter significantly alters β-intercalated cell pH_i. The return of luminal Cl^- results in pH_i returning to baseline.

Table II. Effect of Luminal HCO_3^- and Peritubular Na^+ on β-Intercalated Cell pH_i Recovery from an Acid Load

Peritubular Na^+	Luminal HCO_3^- Present*	Absent
Present	0.38±0.07 (n = 11)	0.45±0.07 (n = 7)‡
Absent	0.08±0.03 (n = 11)§	−0.04±0.06 (n = 7)‡§

* Data from (15).
‡ P = NS by both paired t test and by ANOVA (using unpaired data) versus in the presence of luminal HCO_3^-.
§ $P < 0.001$ by either paired t test or by ANOVA versus in the presence of peritubular Na^+.

appear to inhibit β-intercalated cell apical Cl^-/HCO_3^- exchange (22, 30). Nor do monoclonal antibodies to band 3 protein, the mammalian red blood cell anion exchanger, label the apical membrane of the β-intercalated cell (13, 14). This exchanger therefore appears to be distinct from the Cl^-/HCO_3^- exchanger in many other cell types and, in view of the loss of pH_i sensitivity, appears to be suited to mediate transcellular HCO_3^- flux.

The lack of acute effect of pH_i on Cl^-/HCO_3^- exchange may help explain several previous observations. Acute decreases in peritubular P_{CO_2} may not alter CCT HCO_3^- flux (31). This may be because acute respiratory alkalosis does not stimulate β-intercalated cell apical Cl^-/HCO_3^- exchange. In vitro variations in Cl^- and HCO_3^- gradients are known to alter CCT HCO_3^- secretion (8, 31, 32). Yet, chronic in vivo metabolic acidosis appears to decrease CCT HCO_3^- secretion in both normal (33) and DOCA-treated rabbits (32). Increases in peritubular P_{CO_2} alter CCT HCO_3^- transport via Ca^{+2}, calmodulin and microtubule-dependent mechanisms (34). Also, chronic in vitro acidosis causes both a decrease in the size of β-intercalated cell apical peanut lectin cap and a decrease in the pH_i change in response to Cl^- removal (35). The most likely mechanism for chronic regulation of HCO_3^- secretion appears to be insertion and removal of Cl^-/HCO_3^- exchangers from the apical membrane of the β-intercalated cell. This may be species specific, since in the rat increases in P_{CO_2} induce morphologic changes in only the α-intercalated cell and do not affect β-intercalated cell morphology (36). Together, these observations suggest that the apical Cl^-/HCO_3^- exchanger is regulated by insertion and removal of the transport protein and by lumen and cell Cl^- and HCO_3^- concentrations, but may not be allosterically regulated by pH_i.

Principal cell basolateral Cl^-/HCO_3^- exchange regulation appears to be very different from the β-intercalated cell. Increases in pH_i in response to either an acute increase in peritubular HCO_3^- or a decrease in P_{CO_2} stimulate principal cell Cl^-/HCO_3^- exchange. Furthermore, this exchange is relatively inactive at baseline pH_i, while the β-intercalated cell apical Cl^-/HCO_3^- exchanger is active under baseline conditions (20). The principal cell Cl^-/HCO_3^- exchanger appears to be specialized for pH_i regulation, specifically for recovery from intracellular alkalosis.

These studies also reveal differences in the mechanism by which an increase in peritubular HCO_3^- affects principal cell and β-intercalated cell pH_i. Our data and that of Wang and Kurtz (37) suggest that the principal cell has a Na^+-dependent, electrogenic base exit mechanism, most likely $Na^+(HCO_3^-)_{n>1}$ cotransport. This transporter is a major mechanism of HCO_3^- reabsorption in the proximal tubule (9, 38) and has been recognized in a wide variety of mammalian cells to mediate net cellular HCO_3^- extrusion (6, 39, 40). The exact role and regulation of this transporter in the principal cell is unclear at present, but it may mediate some of the changes in pH_i with changes in peritubular HCO_3^-.

Increases in peritubular HCO_3^- appear to alkalinize the β-intercalated cell by a different mechanism. In the absence of Cl^-, changes in peritubular HCO_3^- have no measurable effect on β-intercalated cell pH_i. The two major known Cl^- transporters in the β-intercalated cell are an apical Cl^-/HCO_3^- exchanger and a basolateral Cl^- channel. If elevations in peritubular HCO_3^- alkalinize the β-intercalated cell via effects on apical Cl^-/HCO_3^- exchange, then inhibition of apical Cl^-/HCO_3^- exchange would have to be postulated. However, increases in peritubular HCO_3^- cause an increased rate of HCO_3^- secretion by the CCT (31), suggesting that apical Cl^-/HCO_3^- exchange is stimulated, not inhibited. Regulation of the basolateral Cl^- channel may play a role in the regulation of apical Cl^-/HCO_3^- exchange (41–43). However, consideration of this does not clarify the Cl^--dependent mechanism by which an increase in peritubular HCO_3^- alkalinizes the β-intercalated cell. Another possibility is that there might be an additional, previously unrecognized Cl^--dependent acid-base transporter in the β-intercalated cell, e.g., basolateral Na^+-dependent or -independent Cl^-/HCO_3^- exchange. Na^+-dependent Cl^-/HCO_3^- exchange is unlikely since previous studies have revealed there is no significant amiloride insensitive recovery from an intracellular acid load (15). These results, however, are consistent with the possibility of a basolateral Na^+-independent Cl^-/HCO_3^- exchanger in the β-intercalated cell. The absence of an effect of basolateral Cl^- removal (in the absence of luminal Cl^-) lessens this possibility. Another possibility is that increases in peritubular pH stimulate basolateral H^+ extrusion (e.g., H^+-ATPase) and that Cl^- depletion inhibits this process; this would also explain the slow fall in pH_i with removal of all Cl^- (see Fig. 5).

These results provide additional evidence that the principal cell does not mediate transcellular HCO_3^- transport. Transcellular HCO_3^- transport requires both an apical and a basolateral acid-base transporter acting in series. Clearly the principal cell has several basolateral acid-base transporters, i.e., Na^+/H^+ exchange (15, 44), Na^+-independent Cl^-/HCO_3^- exchange (15) and an electrogenic, $Na^+(HCO_3^-)_{n>1}$ cotransporter. This study demonstrates no evidence for apical Cl^-/HCO_3^- exchange in the principal cell, either under baseline conditions or after chronic DOCA administration. Similarly, there is no evidence suggesting either an apical Na^+/H^+ exchanger (15, 44) or H^+-ATPase (18). In view of the lack of an apical acid or base transport mechanism transcellular HCO_3^- transport by the principal cell is unlikely.

These studies provide information on the expected changes in pH_i in the principal cell and the β-intercalated cell with both metabolic alkalosis and respiratory alkalosis in vivo. Respiratory alkalosis would be expected to initially alkalinize both cells, but with recovery of pH_i in only the principal cell. Increases in plasma bicarbonate with systemic metabolic alkalosis would result in alkalinization of both cell types; however, any increases in luminal HCO_3^- would only affect β-intercalated cell pH_i, not principal cell pH_i.

In summary, these studies demonstrate marked differences

in the regulation of Cl^-/HCO_3^- exchange in the principal cell and β-intercalated cell of the rabbit CCT. Neither acute intracellular alkalosis nor acidosis acutely regulates the apical Cl^-/HCO_3^- exchanger of the β-intercalated cell. This transporter appears to be specialized for transcellular HCO_3^- transport and not intracellular pH regulation. In the principal cell, intracellular alkalosis stimulates a relatively inactive basolateral Cl^-/HCO_3^- exchanger, suggesting that the role of the Cl^-/HCO_3^- exchanger is pH_i regulation. The Cl^-/HCO_3^- exchangers in the principal cell and the β-intercalated cell appear to differ not only in location and function, but also in acute regulation.

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