J. Membrane Biol. 159, 253–262 (1997)

The Journal of
Membrane
Biology

Intracellular Acidification Induces Cl/HCO_3 Exchange Activity in the Basolateral Membrane of β-intercalated Cells of the Rabbit Cortical Collecting Duct

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Received: 11 January 1994/Revised: 13 June 1997

Abstract. High speed video imaging microscopy and the pH-sensitive fluorophore2′,7′,-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) were used to examine acid-base functions of beta-intercalated cells of the rabbit cortical collecting duct. The presence of intercalated cells was established and the properties of apical and basolateral acid-base transporters assessed by monitoring cell pH during acid loading and luminal and basolateral ion substitutions. We showed that treatment of beta-intercalated cells with ammonium chloride (20 mM) induced a profound decrease of their intracellular pH from 6.98 ± 5.93 ± 0.08. pH recovery occurred after different lag periods ranging between 2 to 15 min (0.22 ± 0.04 dpH/dt). We demonstrated that this pH recovery mechanism was independent of basolateral Na^+ and apical HCO_3^- and K^+. It was also not affected by apical and basolateral addition of NEM, by basolateral DIDS and by apical application of the H-KATPase inhibitor SCH28080. The process of pH recovery was however, critically dependent on basolateral HCO_3^-. These results are best explained by acid-induced insertion and/or activation of chloride-bicarbonate exchangers that are functional properties with their apical analogues.

Key words: Intercalated cells — Cortical collecting tubule — Acidification — Cl^-/HCO_3^- exchange — Plasticity of transport — Polarity

Introduction

The renal cortical collecting duct exhibits both structural and functional heterogeneity [2, 8, 10, 13, 16, 20–23, 25, 31] and plays an important role in Na^+, K^+, Cl^-, and HCO_3^- transport. Recent evidence has shown that the two main cell types lining this nephron segment display different functions. Principal cells mediate Na^+ reabsorption and K^+ secretion [26] whereas intercalated cells (IC) take part in HCO_3^- and Cl^- transport [14, 21]. By means of flux studies, intracellular pH measurements and immunohistochemical studies, the intercalated cells can be further divided into several subclasses based on their specific transport characteristics. The alpha-IC possesses an H^+ pump on the apical surface in addition to a Cl^--HCO_3^- exchanger and a Cl^- conductance in the basolateral membrane. The beta-IC has an apical Cl^--HCO_3^- exchanger and a basolateral H^+ pump and Cl^- conductance [8, 21]. The Cl^-/HCO_3^- exchanger is responsible for net HCO_3^- secretion that can be observed at this nephron site.

Recently several investigators have identified intercalated cells that have Cl/HCO_3^- exchanger activity on both the apical and basolateral cell membranes [7, 36]. Our studies were confined to β cells in which basolateral Cl/HCO_3^- exchange in the basolateral membrane was absent in control conditions. Distinct differences have been noted with respect to the two bicarbonate Cl^- exchangers in the apical and basolateral membrane of intercalated cells. The basolateral anion exchanger in the alpha-cells is sensitive to stilbenes and shares immunohistological characteristic with band 3 protein. In contrast, the apical Cl^-/HCO_3^- exchanger in beta-intercalated cells is insensitive to stilbene inhibitors and has been shown by many [1, 12, 21] but not all [30] investigators to lack the immunological properties of the anion transporter in alpha-intercalated cells.

Significant plasticity of intercalated cell function, reflected by changes in the activity of apical and basolateral acid-base transporters, has been observed and

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Table 1. List of solutions

	Solution 1	Solution 2	Solution 3	Solution 4	Solution 5	Solution 6	Solution 7
NaCl	145	—	—	—	—	120	
NMDG	—	140	120	—	—	—	140
$CaCl_2$	1.0	1.0	1.0	—	1.0	1.0	1.0
$MgSO_4$	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Glucose	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Alanine	4.0	4.0	4.0	4.0	4.0	4.0	4.0
KH_2PO_4	2.0	2.0	2.0	—	2.0	2.0	2.0
KCl	3.0	3.0	3.0	—	3.0	3.0	3.0
NH_4Cl	—	—	20.0	—	—	—	20.0
K Gluconate	—	—	—	3.0	—	—	—
Na Gluconate	—	—	—	140.0	—	—	
Ca Gluconate	—	—	—	4.0	—	—	
HEPES	32.0	32.0	32.0	—	—	—	
$NaHCO_3$	—	—	—	25.0	25.0	25.0	25.0

All concentrations are expressed in millimoles per liter.
Solutions 4–7 were continuously bubbled with 95% O_2/5% CO_2. Solution 6 and 7 were gassed with 95% O_2/5% CO_2.
All stock solutions were brought to volume and the osmolarity adjusted to 300 mOsm.

shown to be related to the acid-base status [10, 18, 21, 24, 25, 29]. Of particular relevance are stimulation of Cl^-/HCO_3^- exchange during induction of alkalosis, elevation of luminal Cl^-, and increased mineralocorticoid levels [21]. Cl^-/HCO_3^- exchange activity diminished in an acidified environment [18] at a time when increased H^+-secretion could be demonstrated in alpha-intercalated cells [13].

To examine the functional properties of apical and basolateral acid-base transporters in beta-intercalated cells during acute acidosis we have used excitation ratio high-speed-video imaging microscopy to monitor intracellular pH. We found that acute acid loading by the ammonium pulse technique [3] induced a novel Cl^--sensitive bicarbonate extrusion mechanism in the basolateral membrane of beta intercalated cells.

Materials and Methods

Microperfusion Studies of Rabbit Cortical Collecting Tubule

Female New Zealand white rabbits were used and maintained on standard diet with tap water until use in the experiments. The animals were sacrificed by an overdose of intravenous sodium pentobarbital. The left kidney was removed and 1 mm thick tissue slices were taken from the cortex. Individual cortical collecting ducts were dissected in bicarbonate-free media at 4°C. The isolated tubule fragments were then transferred to a thermostatically controlled chamber (37°C), containing HCO_3-free Ringer solution (solution 1) and mounted on the stage of an inverted microscope (Nikon Diaphot). The tubules were then cannulated and perfused using techniques previously described [4, 9]. The bath solution was exchanged at a rate of 10 to 12 ml/min, resulting in a complete fluid exchange 5 times/sec. The luminal perfusion was maintained at rates $\geqslant$ 10 nl/min using a gravity system.

Solutions

The composition of the solutions used is given in Table 1. N-ethylmaleimide (NEM), nigericin and 4,4′-diisothiocyanostilbene-2, 2′-disulfonate (DIDS) were purchased from Sigma Chemical, St. Louis, MO. The H-K inhibitor Schering 28080 was a generous gift of Schering-Plough. The pH of all solutions used in this study was 7.40 at 37°C unless otherwise noted. In NH_3/NH_4 solutions NH_4Cl replaced NaCl mole for mole. The high K^+ Nigericin solution contained 10 μM Nigericin and (in mM): 105 KCl, 1.0 $CaCl_2$, 1.0 $MgSO_4$, 2 NaH_2PO_4, 10.2 dextrose, and 32 Hepes. pH was adjusted with HCl or N-methyl-D-glucamine. All solutions were equilibrated with air unless otherwise noted and adjusted to an osmolality of 300–310 mOsm/kg.

Intracellular pH Measurements and Cell Identification

pH_i was measured using the fluorescent pH-sensitive due, BCECF, using a ratiometric technique previously described in our laboratory. We used the techniques of Weiner et al. [33] to load intercalated cells with BCECF by adding the acetoxymethyl ester, 2′,7′-bis(carboxyethyl)-carboxyfluorescein (BCECF-AM) (Molecular Probes, Eugene, OR) (10 μM) to the luminal perfusate for 5–10 min. This procedure results in specific uptake of dye and conversion to BCECF, the pH-sensitive moiety of the dye, by intercalated but not principal cells. Following dye loading the perfusion solution was switched to a dye-free solution before pH_i monitoring commenced. The loading process was monitored by periodic dye fluorescence intensity measurements using a high speed video imaging system (Georgia Instruments, Atlanta, Georgia).

Following the initial period of dye loading, pH_i was measured continually by methods previously described [9]. Briefly, the excitation light (490 nm/440 nm respectively) was delivered to the tubule from a 150 W Xenon light source coupled to two high speed monochromators set at a bandwidth of 2 nm. A specially designed bifurcated quartz fiber optic bundle allowed for even field illumination. Emitted fluorescence was collected at 535 nm using an intensified CCD camera. The resultant ratio (490/440 nm) was simultaneously presented graphically on the computer and television monitor and images acquired

every 30 sec. To limit possible interference between two BCECF-loaded adjacent cells, only cells surrounded by non loaded ''principal'' cells were investigated. Cells above and below the plane of focus were examined prior to the start of the experiment to eliminate out of focus fluorescence.

Nigericin Calibration

We concluded each experiment with a single point calibration, using the Nigericin/high K^+ technique to obtain a I_{490}/I_{440} ratio at pH_i 7.0. We divided all I_{490}/I_{440} ratios from an experiment by the I_{490}/I_{440} ratio at pH 7.00 to yield normalized ratios $(I_{490}/I_{440})/(I_{490}/I_{440})$ pH = 7.00 of pH_i between 5.5 and 8.5 at 0.5 pH unit increments. The relationship is described by a pH titration curve of the form $I_{490}/I_{440}/(I_{490}/I_{440})$ pH = $7.00 = 1 + a\ X[(10^{(pH\text{-}pK)}/1 + 10^{(pH\text{-}pK)} - (10^{7.0\text{-}pK})/1 + 10^{(7.0\text{-}pK)})]$. Using a nonlinear least squares method we then found the best fit for a scaling factor and pK based on 200 cells from 40 tubules harvested from 8 rabbits.

Identification of β-Cells

To distinguish β-cells from alpha intercalated cells we used the classical approach of Cl^- removal from the bath and lumen of the tubule [34, 35]. Bath Cl^- removal resulted in an acidification of the β cell under study whereas luminal Cl^- removal caused a rapid and reversible alkalinization of the cell.

Results

β-Intercalated Cell pH_i Behavior in HCO_3/CO_2 Free Solutions

The first set of experiments examined the response of intercalated cells in bicarbonate free buffer to an acute acid load induced by a basolateral NH_4 pulse. Figure 1*A* shows a typical experiment. The control cell pH_i was in the range of 7.5 and declined after the NH_4 pulse was discontinued. When Na^+ was removed from the bath, acidification was followed by a gradual pH_i recovery over a period of 10 min. Subsequent addition of Na^+ to the bath lead to a rapid return of cell pH to control levels. This pH recovery is consistent with the known presence of Na-H exchange in the basolateral membrane of beta-intercalated cells [20, 33]. As shown in Table 2*A*, mean values of control cell pH were 7.50 ± 0.09 ($n = 20$), acidification following NH_4 pulsing reduced pH to 7.20 ± 0.06 ($n = 20$), and the pH change during the initial phase of pH_i recovery was dpH/dt: 240 ± 20 × 10^{-4} pH units/min ($n = 20$). To study further the mechanisms of pH recovery following an acid load in the absence of Na^+, another series of experiments was carried out to evaluate active electrogenic H^+ extrusion, a mechanism present in the basolateral membrane of beta-intercalated cells [21]. Figure 1*B* summarizes the results of an experiment in which the H^+-transport inhibitor NEM was tested. First, it should be noted that addition of this inhibitor resulted in an immediate decrease of cell pH_i.

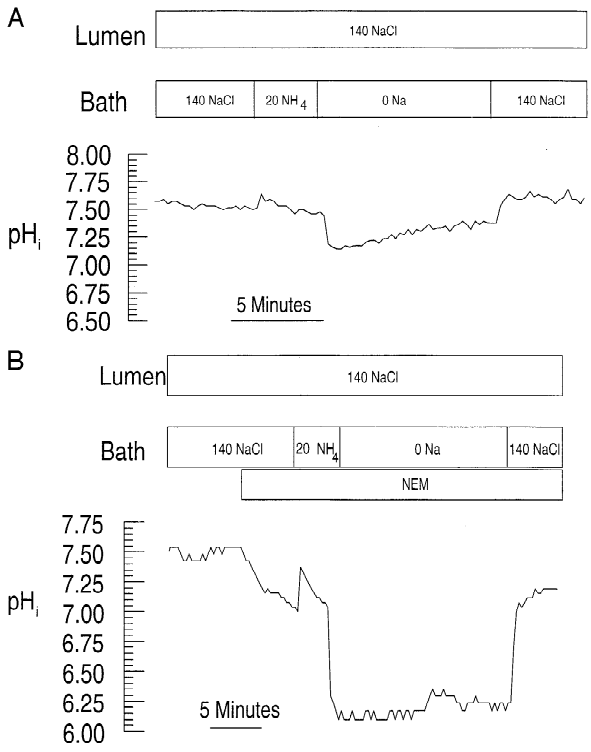

Fig. 1. pH recovery of intercalated cells after an acid load in the absence of HCO_3/CO_2 buffer. (*A*) The tubules were perfused with HCO_3-free solutions throughout the experiment (sol 1–3). The basolateral ammonium pulse (sol 3) induced a small acidification. pH_i returned within 10 min to the prepulse value when perfused in Na-free solution (sol 2). (*B*) The conditions are the same as in *A* except that 0.5 mM NEM was added to the bath before the NH_4 pulse.

Second, withdrawal of ammonium in the presence of NEM initiated a further dramatic acidification. Finally, pH recovery was completely suppressed until reintroduction of sodium in the peritubular milieu. Table 2*B* summarizes results observed in this series of experiments. Luminal NEM had no effect on cell pH (*data not shown*).

We conclude from these studies that in the absence of bicarbonate at least two components, Na-H exchange and electrogenic H^+-extrusion in the basolateral membrane, participate in cell pH recovery of this subpopulation of β-intercalated cells.

β-Intercalated Cell pH_i Behavior in HCO_3/CO_2 Containing Solutions

Experiments carried out in the presence of bicarbonate enabled us to characterize β-intercalated cells further. It has been shown previously that removal of luminal Cl^- results in significant alkalinization as a consequence of inactivation of Cl^-/HCO_3^- exchange in the apical membrane [33, 34]. Inspection of the results summarized in Fig. 2*A* and *B* and Table 3 confirm the presence of apical

Table 2. pH recovery in the absence of bicarbonate

A. Effect of NH_4 on pH recovery in the absence of bicarbonate

*N**	Control pH_i	$+NH_4$ pH_i	dpH/dT $\times 10^{-4}$ pH units/min
6 (20)	7.50 ± 0.09	7.20 ± 0.06	240 ± 20

B. Effect of NEM and 0 Na on pH recovery in the absence of bicarbonate

*N**	Control pH_i	+NEM pH_i	$+NH_4$ pH_i	dpH/dT $\times 10^{-4}$ pH units/min
5 (15)	7.49 ± 0.09	7.00 ± 0.12	5.91 ± 0.24	26 ± 10

* The *N* value represents the number of animals used in the individual studies; the number in brackets is the number of individual cells that were used in the study.

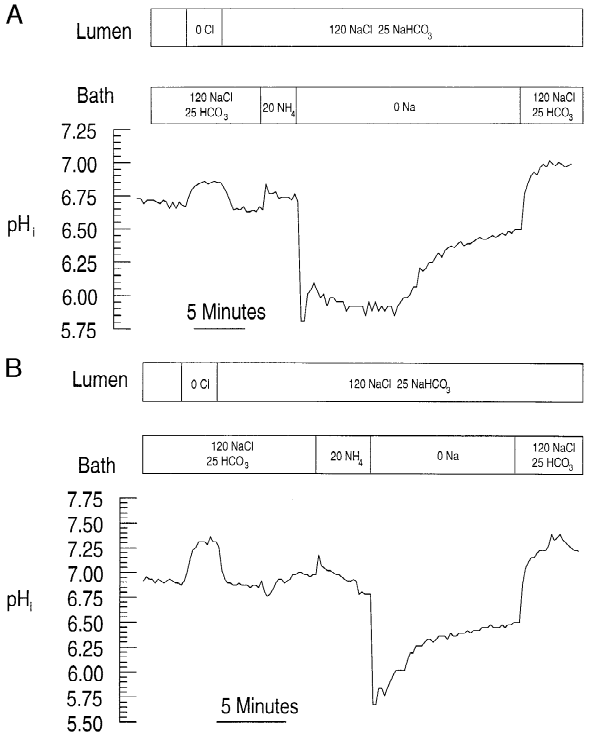

Fig. 2. pH_i recovery of a β IC after an acid load in the presence of HCO_3/CO_2 in the bath. (*A*) The tubules were bathed and perfused with HCO_3^--Ringer. The alkalinization induced by the removal of chloride from the lumen indicated this cell was a β IC. An ammonium pulse was used to acidify the cells. In the absence of peritubular sodium (sol 2) a pH_i recovery occurred after a 10-min lag period. (*B*) In this cell the lag period of the pH_i recovery was much shorter and pH_i recovery began soon after the cell pH fell.

Cl^-/HCO_3^- exchange as evidenced by the significant alkalinization after removal of Cl^- from the lumen. Also consistent with the presence of an apical Cl^-/HCO_3^- exchanger are lower control cell pH values (*see* Table 3). The results summarized in Figs. 2*A* and *B* are examples of pH_i responses following acidification in the absence of Na^+ in the bath. It can be seen that significant pH-recovery occurs following NH_4 withdrawal. Comparing the pH recovery rates in Fig. 1*A* with those shown in Figs. 2*A* and *B* we note that the presence of HCO_3^- accelerates cell alkalinization. We observed that the rate of alkalinization in the presence of HCO_3^- (*see* Table 3, and Figs. 1*A* and 2*A* and *B*) accelerated the rate of recovery from the acid load in all experiments ($n = 14$), however the time course of pH_i recovery differed in individual experiments. In one group of tubules, an example of which is shown in Fig. 2*A*, pH_i remained low for a time period of 10 to 15 min before partial recovery commenced. In another group of experiments, as illustrated in Fig. 2*B*, pH_i recovery began almost immediately after the sharp fall in pH_i following NH_4 removal.

To investigate further the properties of the Na^+-independent pH recovery mechanism, the effect of NEM, a known inhibitor of H^+-ATPase, was tested. The results of a representative experiment are shown in Fig. 3. It is apparent that NEM in the bath did not block the pH recovery following cell acidification ($n = 14$). NEM added to the lumen perfusate was also ineffective in modifying cell pH recovery (*data not shown*, $n = 14$). In the next series of experiments we examined the potential role of apical HCO_3^- in the pH_i recovery process. In particular, we wished to examine whether HCO_3^- entry from lumen to cell via the Cl^-/HCO_3^- exchanger, along a favorable chemical gradient, could be responsible for pH_i recovery after acid loading. Such a process would be favored by the very low intracellular pH that results from the NH_4 pulse protocol.

To examine this possibility, experiments were conducted in the nominal absence of apical HCO_3^-. As shown in Fig. 4, apical HCO_3^- withdrawal induced a small acidification, a finding consistent with HCO_3^- efflux from cell to lumen along a favorable chemical gra-

Table 3. pH recovery in the presence of bicarbonate

A. Effect of NH_4 on pH recovery in the presence of bicarbonate

*N**	Control pH_i	+NH_4 pH_i	dpH/dT $\times 10^{-4}$ pH units/min
5 (15)	6.98 ± 0.09	5.95 ± 0.06	305 ± 25

B. Effect of NEM and 0 Na on pH recovery in the presence of bicarbonate

*N**	Control pH_i	+NEM pH_i Lumen	+NEM pH_i Bath	+NH_4 pH_i	dpH/dT $\times 10^{-4}$ pH units/min
5 (14)	6.96 ± 0.09	6.93 ± 0.12	—	5.91 ± 0.24	299 ± 35
5 (14)	6.92 ± 0.11	—	6.92 ± 0.11	5.94 ± 0.24	310 ± 35

* The *N* value represents the number of animals used in the individual studies; the number in brackets is the number of individual cells that were used in the study.

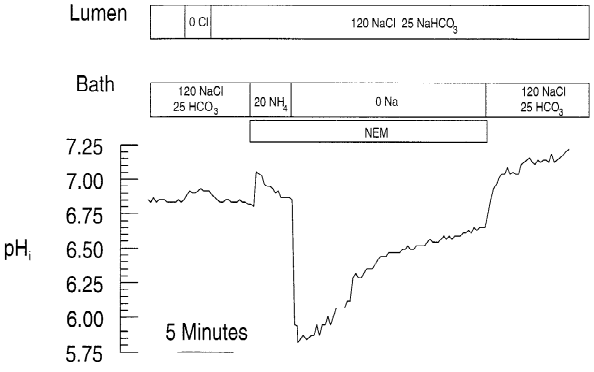

Fig. 3. Effect of NEM on the pH_i recovery mechanism of β IC after an acid load. Bicarbonate was present in both lumen and bath perfusates (sol 6). The addition of 0.5 mM NEM to the bath did not affect the pH_i recovery from an acid load.

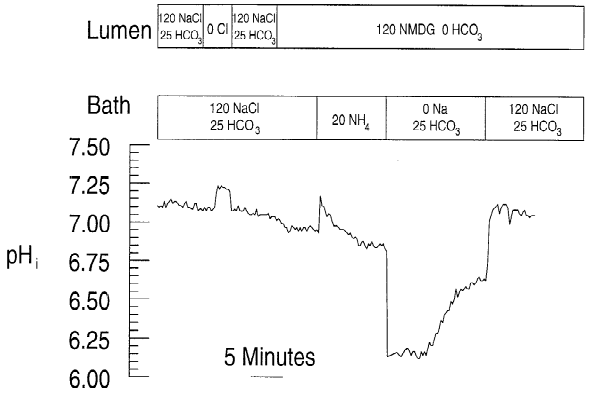

Fig. 4. Effect of removal of apical HCO_3 on the pH_i recovery of β IC after an acid load. Bicarbonate was initially present in lumen and bath. A β IC was identified as evidenced by cell alkalinization following removal of luminal chloride (sol 4). HCO_3 was removed from the lumen and decreased pH_i (sol 1). pH recovery after an acid load was not effected.

dient. Importantly however, a dramatic pH recovery was still observed following the ammonium induced acid loading. We conclude from these experiments that the pH recovery mechanism is independent of apical HCO_3, and cannot be explained by HCO_3^- transport from lumen to cell.

However, recovery of cell pH is strongly dependent on bicarbonate in the basolateral fluid. Whereas pH_i recovery occurred in bicarbonate-containing solutions despite the absence of Na^+ and in the presence of NEM in the bath, it was totally blocked when under the same conditions bicarbonate was absent (compare Fig. 1*B* with Fig. 3). The next series of experiments was designed to clarify the role of HCO_3^- transport in the pH_i recovery process. These experiments were carried out by comparing the response of cell pH to the removal of Cl^- from the bath in control and acid loaded cells.

Induction of a Cl/HCO_3 Exchanger in the Basolateral Membrane of β IC

To examine the possibility that a basolateral Cl^-/HCO_3^- exchange mechanism mediates the NEM-insensitive pH_i recovery, we tested the effects of removal of Cl^- from the bath in control and acid loaded intercalated cells. Figure 5 shows the results of a representative experiment, Table 4 summarizes this series of data. Before the ammonium pulse, basolateral Cl^- removal induced significant acidification of the cell, 0.43 ± 0.03 pH units (*see* Fig. 5 and Table 4). In control conditions removal of Cl^- from the lumen induced a significant alkalinization of the cytoplasm (mean change of pH_i; 0.21 ± 0.01, $n = 14$), a finding consistent with inhibition of apical Cl^-/HCO_3^- exchange. Our results confirm those of

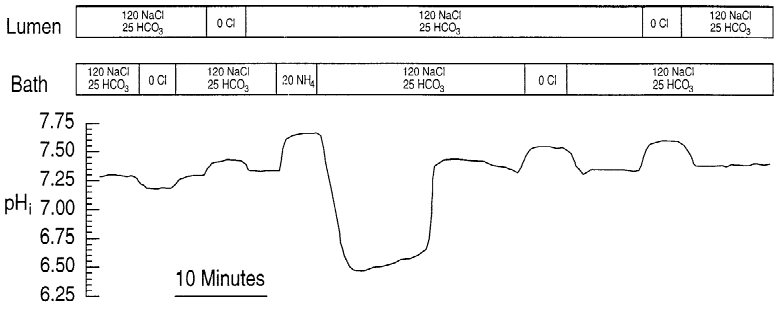

Fig. 5. Induction of a basolateral Cl/HCO_3 exchanger activity in β-IC. Basolateral Cl^- removal before acid-loading induced a small cell acidification, whereas removal of Cl^- (sol 4) from the lumen alkalinized the cell. Following the acid pulse removal of Cl^- from the lumen again alkalinized the cell. In contrast, removal of Cl^- from the bath now alkalinized the cell.

Table 4. Effects of Cl^- removal from lumen and bath

A. Effect of deletion of Cl^- from the lumen and bath on pH recovery in the absence of bicarbonate from both lumen and bath

*N**	Control	Lumen		dpH/dT	$+NH_4$	dpH/dT
		0 Cl^-	$+Cl^-$	$\times 10^{-4}$ pH units/min		$\times 10^{-4}$ pH units/min
	pH_i	pH_i	pH_i		pH_i	
7 (14)	7.25 ± 0.07	7.46 ± 0.01	7.08 ± 0.07	275 ± 22	6.04 ± 0.07	315 ± 27

* The *N* value represents the number of animals used in the individual studies; the number in brackets is the number of individual cells that were used in the study.

Weiner and Hamm who observed similar cell pH changes during removal of Cl^- from either lumen or bath or both [34, 35]. Figure 5 and Table 4 also show the data of cell pH following removal of Cl^- from lumen and bath *after* acidification. Following an ammonium pulse and after pH_i restoration, luminal Cl^- removal again alkalinized the cell demonstrating that the observed pH change was similar to that observed prior to acidification. However, removal of Cl^- from the bath now alkalinized the cell. The mean pH change observed was 0.29 ± 0.13 pH units ($n = 5$). Whereas the difference between cell pH values between pre- and post-acid loading conditions following basolateral Cl^- removal was 0.46 ± 0.13 pH units, the corresponding cell pH changes following luminal Cl^- deletion was 0.02 pH units; thus, there were significant changes of cell pH after removal of Cl^- from the bath solution, whereas cell pH changes were virtually identical following Cl^- removal from the lumen. Time-control experiments in the presence of HCO_3^- *without* acidification showed no significant modification of the basolateral response. Chloride removal from the bath induced identical changes in cell pH with acidification, at the beginning, and end of the 30-min time period.

We propose the following interpretation of these data. Removal of bath Cl^- is expected to lower cell Cl^- as Cl^- moves from cell to basolateral fluid through Cl^- channels in this membrane. As a consequence, acceleration of apical Cl^-/HCO_3^- exchange by a more favorable electrochemical gradient for Cl^- ions lowers cell pH_i. In contrast, removal of Cl^- from the lumen should impede HCO_3^- extrusion and thus induce alkalinization.

Addition of NEM to either the bath or lumen perfusate under these conditions also failed to influence the Cl^- induced pH_i response (*data not shown* ($n = 12$)). To examine the possible role of apical H-K exchange in the pH recovery, several maneuvers were employed. These included changes in luminal K^+ as well as addition of the K-H inhibitor SCH28080 during the period following the acid load. Increasing lumen K^+ (5–20 mM) also failed to stimulate pH_i recovery (pH_i change at $K^+ = 5$ mM: 0.24 ± 0.04 pH units ($n = 9$), pH change at $K^+ = 20$ mM: 0.25 ± 0.02 pH units ($n = 9$). In a separate series of experiments we observed that reducing lumen K^+ to 0 mM, or addition of 150 μM SCH28080, did not inhibit pH_i recovery (pH change at $K^+ = 5$ mM: 0.28 ± 0.05 × 10^{-4} pH units/min ($n = 5$), at $K^+ = 0$ mM: 0.25 ± 0.03 × 10^{-4} pH units/min ($n = 5$). pH_i recovery was also unchanged following exposure to SCH28080 (0.23 ± 0.04 × 10^{-4} pH units/min ($n = 5$). It appears from these results that cell acidification did not activate apical K-H exchange.

In additional experiments the response to DIDS of the newly activated Cl^-/HCO_3^- exchanger was also investigated. The experiment presented in Fig. 6 shows that the processes of pH restoration following ammonium withdrawal as well as the responses of cell pH to removal of Cl^-, both in the lumen and the bath, are not affected by 100 μM DIDS. pH increased by 0.25 ± 0.02 pH units, ($n = 5$) in the presence of DIDS, a value not significantly different from that measured in its absence (0.024 ± 0.03 pH units $P < 0.01$). Thus, insensitivity to stilbenes is shared by the Cl^-/HCO_3^- exchanger in the

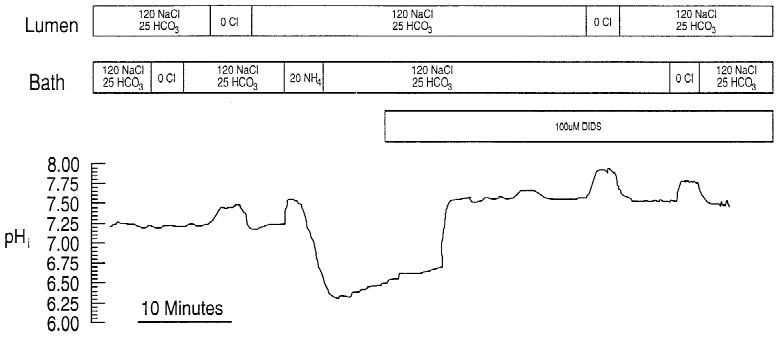

Fig. 6. Effect of DIDS (100 μM) on basolateral Cl/HCO_3 exchange. Note that the response to removal of Cl^- from the bath was unchanged in the presence of DIDS.

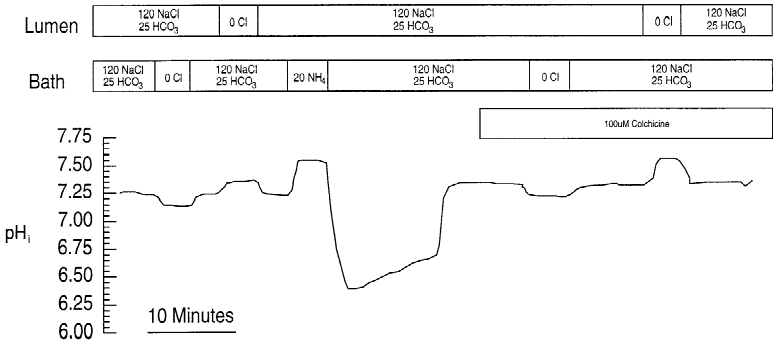

Fig. 7. Effects of pretreatment with 100 μM chochicine the microtubule and microfilament inhibitor prior to acid load. Please note that the cell no longer expresses an alkalinization following Cl^- removal (sol 4).

basolateral membrane of beta-intercalated cells [7, 19, 27].

Effects of Microtubule and Microfilament Inhibitors

To further investigate the possibility involvement of the cytoskeleton and of vesicular trafficking, the microtubule and microfilament inhibitors cholchicine (10 μM) or Cytocholasin B (100 μM) were applied to the tubule prior to the Cl^--removal and readdition step. Figure 7 illustrates the effects of removal of luminal Cl^- on pH_i in the absence and presence of Cytocholasin B. When Cl^- was removed from the bath prior to treatment cell acidification was observed. Following acidification, in the presence of microtubule or microfilament inhibitors cholchicine (or Cytocholasin B), removal of luminal Cl^- led to alkalinization. However, when bath Cl^- was now removed, the cell acidified to a level comparable to that prior to the acidification. The results of this study are summarized in Table 5, and indicate that the disruption of the cytoskeleton leads to failure of expression of the Cl^-/HCO_3^- exchanger in the basolateral membrane.

Discussion

The main finding of the present studies was the apparent activation of a Cl^-/HCO_3^- exchanger in the basolateral membrane of beta-intercalated cells of the rabbit cortical collecting tubule. At least three acid-base-related transport mechanisms are present in the basolateral membrane of beta-intercalated cells: electrogenic H^+ extrusion, Na-H exchange and a Cl^- conductance [8, 11, 15, 21, 24, 33, 34]. However, a new transport mechanism appears in the basolateral membrane following acute acidification and participates in the regulation of cell pH. Figure 8 is a schematic presentation of the distribution of acid-base related transport mechanisms in control conditions (*A*), during recovery from acidosis (*B*), and in post-acidotic conditions (*C*).

Two lines of evidence support the view that acidosis activates Cl^-/HCO_3^- exchange in the basolateral membrane of beta-intercalated cells. First, pH_i recovery following acute acid loading persists, and can be shown to be dependent on the presence of bicarbonate at a time when the activity of other acid-extrusion mechanisms has been excluded. The presence of electroneutral Cl^-/HCO_3^- exchange would allow bicarbonate ions to enter beta-intercalated cells in exchange for Cl^- as the steep fall in cell pH enhances the trans-membrane concentration gradient for bicarbonate. Second, the observation of cell alkalinization that follows removal of Cl^- from the bath supports the hypothesis that a new anion exchanger, not present in control conditions, has been activated by acidosis. The manipulation of bath Cl^- prior to acidification did not induce cell alkalinization.

An intriguing finding is the observation that the newly activated Cl^-/HCO_3^- exchanger was insensitive to

Table 5. Effects of microtubule/microfilament inhibitors

A. Effect of microtubule/microfilament inhibitors on basolateral Cl^- dependent acidification

*N**	Control	$0Cl^-$		Control +Cholchicine (10 μM)	$0Cl^-$ +Cholchicine (10 μM)	
	pH_i	Lumen pH_i	Bath pH_i	pH_i	Lumen pH_i	Bath pH_i
5 (12)	7.24 ± 0.04	7.42 ± 0.03	7.03 ± 0.04	7.25 ± 0.05	7.46 ± 0.17	7.19 ± 0.12
				+Cytocholasin B (100 μM)	+Cytocholasin B (100 μM)	
5 (11)	7.22 ± 0.06	7.46 ± 0.05	7.02 ± 0.08	7.21 ± 0.08	7.44 ± 0.12	7.18 ±0.14

* The *N* value represents the number of animals used in the individual studies; the number in brackets is the number of individual cells that were used in the study.

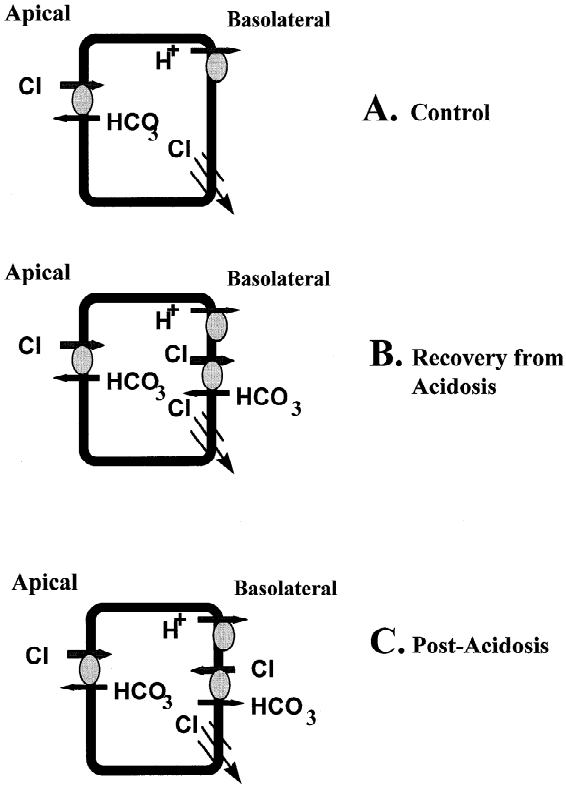

Fig. 8. Cell models of beta-intercalated cells prior and following cell acidification. (*A*) Control conditions. (*B*) Bicarbonate enters the cell by the apical exchanger. (*C*) Activity of the anion exchanger following restoration of cell pH after acidification.

disulfonic stilbenes. This behavior is similar to that of the apical Cl^-/HCO_3^- exchanger but differs sharply from the analogous basolateral form of the transporter in alpha intercalated cells [7, 19, 27, 32]. Could the observed cell alkalinization following acid loading occur as a consequence of the activation of parallel conductances of Cl^- and HCO_3^-? Were this the case, lowering chloride in the bath would reduce the cell-negative potential and decrease the driving force for possible bicarbonate exit by anion channels. Recent careful experiments have shown that clamping of the membrane potential across the basolateral membrane does not alter the response of cell pH_i to the removal of chloride in the bath [36]. Accordingly, the observed cell alkalinization is best explained by activation of Cl/HCO_3^- exchangers and our results can be interpreted that acidification leads to basolateral insertion of new anion exchangers or activation of those already present. The observation that the cholchicine inhibits the chloride-dependent postacidotic alkalinization supports as a possible mechanism the insertion into the basolateral membrane of new-anion exchangers from a cytoplasmic vesicular pool of transporters.

The appearance of new anion transporters in the basolateral membrane of beta intercalated cells extends the examples of plasticity of acid-base transporters in the pool of alpha and beta-intercalated cells. Cellular remodeling of acid-base transporters [18, 21, 24, 30] occurs during neonatal development [17], and following alterations in acid-base status. For instance, systemic acidosis increases and alkalosis decreases the intensity of the staining pattern of apical H^+-ATPase in alpha-intercalated cells [32]. Furthermore, careful morphological investigations during metabolic alkalosis have demonstrated the appearance of a submembrane population of basolateral vesicles in beta intercalated cells, consistent with an increase in the delivery of vacuolar H^+-ATPase for insertion at this site.

Additional evidence also suggests significant cell remodeling of bicarbonate-secreting cells [18]. Such studies have shown that both acid feeding as well as incubation of cortical collecting ducts in an acid medium for several hours leads to an increase in apical endocytosis in alpha-intercalated cells suggesting insertion of additional H^+-transporters. Importantly, these morphological alterations were associated with reduced cell binding to peanut agglutinin (PNA), a reflection of diminished beta-intercalated cell activity [18]. These studies in perfused tubules of the rabbit collecting duct are consistent with changes in the distribution and polarity of bicarbonate

transporters. Such processes are thought to participate in the shift from bicarbonate reabsorption (H^+ secretion) to bicarbonate secretion during transition from acidosis to alkalosis. It is likely that cell pH changes play an important role in such remodeling of intercalated cell polarity. Changes in intracellular pH could also be involved in modulating cell polarity in neonatal collecting ducts. Relevant observations are experiments showing that the maturation process of alpha-and-beta-intercalated cells can be significantly modulated by systemic acid-base changes [17, 25]. Intercalated cells develop gradually after birth and the relative distribution between alpha and beta cells takes several weeks to develop fully. Maternal alkalosis can be shown to shift the relationship between these two subpopulations in favor of beta-intercalated cells. Acidosis had the opposite effect [17, 21, 25].

The situation is complicated by findings that some cortical collecting duct cells have the potential of expressing properties of both alpha and beta-intercalated cells. Of particular relevance and underscoring the variable distribution of bicarbonate transporters are recent observations that cells of the rabbit cortical collecting duct exhibit Cl^-/HCO_3^- exchange in *both* apical and basolateral membranes [7, 36]. In a recent careful study of the distribution of Cl/HCO_3^- exchangers along the outer and inner medullary collecting ducts, a strikingly large number of β-intercalated cells with both apical and basolateral Cl/HCO_3^- exchangers was noted in tubules harvested from rabbits in control acid base conditions. This work led to the description of yet another intercalated cell type, the γ cell. Our studies focused on the classical β cell which lack basolateral Cl/HCO_3^- exchange in control acid-base conditions.

Our observations extend the concept of plasticity of acid-base transporters in intercalated cells by demonstrating the appearance of basolateral Cl^-/HCO_3^- exchange in beta-intercalated cells following acidification of the cytoplasm. Consistent with such activation of basolateral Cl^-/HCO_3^- exchangers was our observation of pH_i restoration in acidosis depended upon the presence of bicarbonate in the bath, but was not attenuated by deletion of HCO_3^- from the lumen. Recovery from cell acidosis was independent of Na^+-H^+,K^+-H^+ exchange and of H^+-ATPase activity. If apical anion exchange had been involved in the response to acidosis the reduction in cell Cl^- following deletion of Cl^- from the bath should have further acidified the cell, yet cell alkalinization was observed.

Several studies have addressed the problem of the regulation of the apical Cl^-/HCO_3^- exchanger in beta-intercalated cells. This anion exchanger is insensitive to stilbene inhibition [21] despite the fact that apical membranes cross-react with antibodies to the cytoplasmic domain of band 3 [30]. It has also been reported that the apical Cl^-/HCO_3^- exchanger does not participate in cell pH regulation as evidenced by its failure to respond to changes in cell pH that were induced by modulating basolateral HCO_3^- and pCO_2 [33–35]. Accordingly, it is safe to conclude that basolateral and not apical Cl^-/HCO_3^- exchange restores cell pH following acid loading. The mechanism by which acidosis activates basolateral Cl^-/HCO_3^- exchange needs further explanation. Several examples of targeting, insertion and activation of renal transporters involve the cytoskeleton [5, 6]. Possibly, changes in acid-base balance could also modify the insertion and subsequent activation of Cl^-/HCO_3^- exchangers by altering the microenvironment at the lipid cytoplasmic interface [29]. The prolonged presence of the Cl^-/HCO_3^- exchanger following restoration of cell pH_i after acidosis is consistent with but does not prove stimulation of vesicular delivery of anion exchangers to the basolateral membrane. It is presently not known how long the newly recruited transporters remain within the basolateral membrane. Were they to continue to be active continuously they could, as suggested by Weiner et al. [36], act as a sensor for bicarbonate and thereby play a role in the regulation of bicarbonate secretion.

In summary, our studies demonstrate that beta-intercalated cells respond to acidosis with activation of basolateral Cl^-/HCO_3^- exchange, a process normally limited to the domain of the apical membrane in the β cell population of our study. These observations further support the notion of plasticity of acid-base transport polarity in intercalated cells of the cortical collecting tubule.

We thank NIKON for technical support, and Drs. Robert Berliner and Gerhard Malnic for their comments on the manuscript. This work was supported by National Institutes of Health grant DK-17433 (G.G., J.G.) and the Connecticut Innovations Cooperative Research and Development grant (J.G.). Parts of this work have been presented in abstract form in the *Journal of American Society for Nephrology* (1991)2:706.

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