

Adaptation of Rabbit Cortical Collecting Duct HCO_3^- Transport to Metabolic Acidosis In Vitro

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Abstract

Net HCO_3^- transport in the rabbit kidney cortical collecting duct (CCD) is mediated by simultaneous H^+ secretion and HCO_3^- secretion, most likely occurring in α - and β -intercalated cells (ICs), respectively. The polarity of net HCO_3^- transport is shifted from secretion to absorption after metabolic acidosis or acid incubation of the CCD. We investigated this adaptation by measuring net HCO_3^- flux before and after incubating CCDs 1 h at pH 6.8 followed by 2 h at pH 7.4. Acid incubation always reversed HCO_3^- flux from net secretion to absorption, whereas incubation for 3 h at pH 7.4 did not. Inhibition of α -IC function (bath Cl^- removal or DIDS, luminal bafilomycin) stimulated net HCO_3^- secretion by ~ 2 pmol/min per mm before acid incubation, whereas after incubation these agents inhibited net HCO_3^- absorption by ~ 5 pmol/min per mm. Inhibition of β -IC function (luminal Cl^- removal) inhibited HCO_3^- secretion by ~ 9 pmol/min per mm before incubation, whereas after incubation HCO_3^- absorption was stimulated by only ~ 3 pmol/min per mm. After acid incubation, luminal SCH28080 inhibited HCO_3^- absorption by only 5–15% vs the $\sim 90\%$ inhibitory effect of bafilomycin. In outer CCDs, which contain fewer α -ICs than midcortical segments, the reversal in polarity of HCO_3^- flux was blunted after acid incubation. We conclude that the CCD adapts to low pH in vitro by downregulating HCO_3^- secretion in β -ICs via decreased apical Cl^- /base exchange activity and upregulating HCO_3^- absorption in α -ICs via increased apical H^+ -ATPase and basolateral Cl^- /base exchange activities. Whether or not there is a reversal of IC polarity or recruitment of γ -ICs in this adaptation remains to be established. (*J. Clin. Invest.* 1996; 97: 1076–1084). Key words: acid–base equilibrium • ion transport • intercalated cell • kidney • H^+ -ATPase

Introduction

The cortical collecting duct (CCD)¹ plays an important role in the regulation of acid–base homeostasis. This nephron segment is capable of bicarbonate reabsorption and bicarbonate secretion, depending on the acid–base state of the animal (1–5). Each of these opposing transport processes is believed to be accomplished by a subtype of intercalated cell (5–10). Intercalated cells comprise about a third of all CCD cells; the majority cell type is the principal cell, which is not believed to be involved in acid–base homeostasis (1, 6, 7, 11). Reabsorption of HCO_3^- is believed to be accomplished by α -intercalated cells, which are endowed with an apical H^+ -ATPase and a basolateral Cl^- - HCO_3^- exchanger (5, 8–10, 12). Secretion of HCO_3^- is accomplished by β -intercalated cells, which have some form of H^+ pump in or near the basolateral membrane and a Cl^- - HCO_3^- exchanger in the apical membrane (5, 8, 9, 12–14). The function of other types of intercalated cells including “hybrid” and “ γ ” forms (9, 13, 15, 16) is not presently clear.

CCDs taken from control rabbits and perfused in vitro with solutions resembling ultrafiltrate of rabbit plasma generally secrete net HCO_3^- (3, 13, 17, 18). Acid feeding of the rabbit, however, usually results in net HCO_3^- absorption, equivalent to net H^+ secretion (2, 3, 5, 17, 18). The mechanisms underlying this change in direction of HCO_3^- flux are not well understood. We have recently developed a model of metabolic acidosis in vitro by which CCDs reverse their polarity of net HCO_3^- flux after 3 h of incubation at low pH (17). These studies revealed that in vitro exposure to an acid environment induced extensive remodeling of HCO_3^- -secreting β -intercalated cells, especially apical membrane components including the PNA binding sites and Cl^- - HCO_3^- exchanger (17). This remodeling was accomplished in β -cells by increased apical fluid-phase endocytosis, a marker heretofore associated primarily with α -intercalated cells (5, 19–21). Indeed, in response to acid exposure, CCDs showed decreased HCO_3^- secretion, a process which required de novo protein and RNA synthesis and integrity of cellular microfilaments (22). Functional changes in response to low pH were apparent in the β -intercalated cells but not in a limited study of α -intercalated cells (22).

It was the purpose of this work to study in more detail how net HCO_3^- transport of the mid-CCD responds to acid incubation in vitro. In this study we have focused primarily on α -intercalated cell function using inhibitors of α cell HCO_3^- transport to understand better the cellular mechanisms of adaptation to low pH in vitro. The data show that both α - and β -intercalated cells appear to adapt to low pH in vitro: β -intercalated cells decrease the secretion of HCO_3^- whereas α -intercalated cells increase their secretion of protons.

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1. Abbreviations used in this paper: CCD, cortical collecting duct; DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; OMCD, outer medullary collecting duct; SCH28080, 2-methyl-8-(phenylmethoxy)imidazo[1,2- α]pyridine-3-acetonitrile; Vte, transepithelial voltage.

Methods

Animals. Female New Zealand white rabbits weighing 2–3 kg and maintained on normal laboratory chow (Purina lab diet No. 5326; Purina Mills, Richmond, IN) plus free access to tap water were used in this study. The diet provides 0.25% Na, 1.2% K, 0.5% Cl, and 1.1% Ca. Animals were killed by intracardiac injection of 130 mg pentobarbital sodium after premedication with ketamine (44 mg/kg) and xylazine (5 mg/kg). Blood taken from the heart at the time of death yielded a mean pH of 7.39 ± 0.01 and an HCO_3^- concentration of 26.9 ± 0.6 mM ($n = 33$). The urine, obtained postmortem by bladder puncture, had a pH of 7.99 ± 0.11 ($n = 38$).

Tubule isolation. Kidneys were removed quickly and coronal slices were made. They were transferred to dishes with chilled solution for microdissection containing the following (in mM): 145 NaCl, 2.5 K_2HPO_4 , 2 CaCl_2 , 1.2 MgSO_4 , 5.5 D-glucose, 1 Na_3 citrate, 4 Na lactate and 6 L-alanine, pH 7.4, 290 ± 2 mosmol/kg (13, 17). CCDs were isolated by freehand dissection from the medullary rays of the midcortex using fine forceps under a stereomicroscope. The midcortical tubules used in this study did not contain arcades or the outermost part of the CCD, wherein γ -intercalated cells have been reported to be most common (15). The mean length averaged 1.2 ± 0.2 mm ($n = 33$). In one series, collecting ducts were obtained from the outer 1.5 mm of cortex, just distal to the last branch point (23) but clearly proximal to where mid-CCDs were microdissected. The length of these segments averaged 0.7 ± 0.1 mm ($n = 6$).

In vitro microperfusion. In vitro microperfusion developed by Burg (24) was used with modification (5, 17, 22). An isolated CCD was rapidly transferred to a 1.2-ml temperature-controlled chamber mounted on an inverted microscope (Diaphot; Nikon Instrument Corp., Madison, WI) and perfused and bathed at 37°C . The collecting end of the CCD was sealed into a holding pipet using Sylgard 184 (Dow Corning Corp., Midland, MI). Samples of tubular fluid were collected under water-saturated mineral oil by timed filling of a calibrated volumetric constriction pipette (12–14 nl). Three collections were made during each experimental period.

Bicarbonate transport. The concentration of total CO_2 (assumed to be equal to that of HCO_3^-) in perfusate (C_o) and collection fluid (C_L) were measured by microcalorimetry (Picapnotherm; Microanalytical Instrumentation, Mountain View, CA). Because there is no net water absorption in this segment (5, 25), the rate of HCO_3^- transport ($J_{\text{HCO}_3^-}$) was calculated as follows: $J_{\text{HCO}_3^-} = (C_o - C_L) (V_L/L)$, where V_L is the rate of collection of tubular fluid determined from the time (in minutes) required to fill the calibrated volumetric pipette, L is the tubular length (in mm), and J is in pmol/min per mm. When $J_{\text{HCO}_3^-} > 0$ there is net HCO_3^- absorption, and when $J_{\text{HCO}_3^-} < 0$ there is net HCO_3^- secretion. Net HCO_3^- absorption is considered to be net H^+ secretion, and these terms are used interchangeably. The effect of an experimental maneuver on baseline or control HCO_3^- transport was determined as the change in HCO_3^- flux or $\Delta J_{\text{HCO}_3^-}$. A positive $\Delta J_{\text{HCO}_3^-}$ indicates an increase in HCO_3^- absorptive flux, which can be mediated by increased H^+ secretion and/or decreased HCO_3^- secretion, whereas a negative $\Delta J_{\text{HCO}_3^-}$ indicates an increase in HCO_3^- secretory flux, which can be mediated by decreased H^+ secretion and/or increased HCO_3^- secretion.

The sensitivity of the Picapnotherm ranged between 9 and 18 counts per pmol tCO_2 , so that for samples of 13 nl there were 117–234 counts/mM tCO_2 . The coefficient of variation for a 20-mM standard measured in quadruplicate was $< 0.3\%$ (< 10 counts for a sample of 2,500 counts). This level of sensitivity allowed us to reliably detect HCO_3^- concentration differences of 1 mM between perfused and collected fluids.

Each solution was adjusted to pH 7.4 and 290 ± 2 mosmol/kg and continuously bubbled with 94% O_2 /6% CO_2 gas at 37°C (22, 26). The bath chamber and perfusate reservoir were suffused by the same gas continuously. The flow rate of bathing solution entering the specimen chamber was kept at 14 ml/h by a peristaltic pump. The perfusion rate was maintained at 1–3 nl/min.

Transepithelial voltage (Vte). Vte was measured using the perfusion pipette as an electrode. The voltage difference between calomel cells connected via 3 M KCl agar bridges to perfusing and bathing solutions was measured with a high impedance electrometer (World Precision Instruments, New Haven, CT).

After the initial equilibration, the study protocols were started. Collections of tubular fluid were initiated once the Vte had stabilized, and readings were recorded at the conclusion of each collection. Sample collections after each experimental maneuver did not commence until the Vte had stabilized (generally > 20 min).

Viability. Evidence for damaged cells and gross leak of perfusate were assessed by addition of 0.15 mg/ml FD & C green dye to all perfusates during the study (22).

Solutions. The composition of solutions used in this study are shown in Table I. Calcium concentration was raised in Cl-free solution to allow for additional complexing by gluconate (5, 17, 25). The concentration of chloride in Cl-free solution and potassium in K^+ -free solution were confirmed to be zero by electrode and flame photometer, respectively.

3-h incubation. In most protocols, $J_{\text{HCO}_3^-}$ was measured under control conditions, experimental conditions, and recovery before and after an acid incubation. A 3-h incubation at pH 7.4 served as a time control for the acid incubation. Because we (22) had previously shown a significant reduction in stimulated HCO_3^- secretion (accomplished by removing bath Cl^-) after 1 h at pH 6.8 followed by 2 h at pH 7.4, we used the same maneuver to examine net HCO_3^- -transport in the present studies. The rationale for dividing the 3-h incubation into a 1-h exposure to low pH followed by a 2-h recovery is based on the expectation that renal cell physiology, metabolism, and protein synthesis are better maintained at “normal” ambient pH, and that the adaptive process to respond to the acid stimulus would be facilitated at higher pH. The preincubation studies were generally completed within 3 h of death. After the 3-h incubation, the fluid collections and equilibrations were completed within 2 h, so that the total time of each experiment was usually 7–8 h.

The pH 7.40 ± 0.02 solution for incubation contained DME with 44 mM NaHCO_3 (GIBCO BRL, Gaithersburg, MD), Burg's solution, and HCO_3^- -free dissecting solution in a ratio of 3:5:1, respectively. The pH 6.80 ± 0.02 solution contained DME with no NaHCO_3 (GIBCO BRL.) Burg's solution, and dissection solution in a ratio of 3:2:4, respectively (17, 22). These solutions also contained 30 U/ml penicillin, 30 $\mu\text{g}/\text{ml}$ streptomycin (GIBCO BRL), and 3.3% FCS (GIBCO BRL).

Chemicals. Bafilomycin A1, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), and ouabain were purchased from Sigma Chemical Co. (St. Louis, MO). 2-methyl-8-(phenylmethoxy)imidazo[1,2- α]pyridine-3-acetonitrile (SCH28080) was kindly provided by Dr. Ted

Table I. Composition of Solutions

Solute	Burg's solution	Cl ⁻ free	10 Cl ⁻	K ⁺ free
NaCl	115	—	10	115
K_2HPO_4	2.5	2.5	1.5	—
CaCl_2	2.0	—	—	2.0
MgSO_4	1.2	1.2	1.2	1.2
Na lactate	4.0	4.0	4.0	4.0
Na_3 citrate	1.0	1.0	1.0	1.0
L-alanine	6.0	6.0	6.0	6.0
D-glucose	5.5	5.5	5.5	5.5
NaHCO_3	25	25	25	25
Na gluconate	—	110	100	—
Ca acetate	—	6.0	6.0	—
Na_2HPO_4	—	—	—	2.5

All concentrations are given in millimoles per liter.

Sybertz from Schering-Plough Research Institute (Kenilworth, NJ). Bafilomycin, SCH28080, and DIDS were dissolved in DMSO at 0.1% final concentration.

Statistics. Results are presented as mean \pm SE, where n is the number of tubules. Data from each tubule before incubation were compared with those from after the incubation in a paired t test. Comparisons of mid-CCDs to outer-CCDs were made by unpaired t test. $P < 0.05$ was regarded as significant. Statistical comparisons were performed using Number Cruncher Statistical System (Kaysville, UT).

Results

Acid incubation

We first performed two pilot studies to determine the minimum time required for a reversal of polarity of HCO_3^- flux. After a baseline net transport period, one CCD was exposed to pH 6.8 solutions in lumen and bath for 1 h, after which Burg's solution was restored to both sides of the tubule for repeat HCO_3^- transport studies. Two more cycles of low pH exposure and transport measurements were then performed. The net HCO_3^- flux was as follows: control, -4.49 ; 1 h, -4.46 ; 2 h, 1.20 ; and 3 h, 4.23 pmol/min per mm. After a baseline net transport period, a second CCD was exposed to pH 6.8 for 1 h followed by 1 h at pH 7.4, after which repeat net HCO_3^- flux was measured. Then, after another hour of incubation at pH 7.4, net HCO_3^- flux was determined again. The net HCO_3^- flux was as follows: control, -3.96 ; 2 h, -0.86 ; and 3 h, 3.51 pmol/min per mm.

Our preliminary studies showed that the secretion of net HCO_3^- flux reversed to approximately the same rate of absorption after 3 h of incubation at low pH or after 1 h of incubation at low pH followed by 2 h at pH 7.4, similar to what was previously reported by our group (17). As shown in Fig. 1 A, CCDs secreted HCO_3^- before incubation, and this secretory flux converted to about the same rate of HCO_3^- absorption after 1 h at pH 6.8 followed by 2 h at pH 7.4 (from -3.87 ± 0.23 to 4.10 ± 0.23 pmol/min per mm, $n = 29$, $P < 0.01$). The mean shift in net HCO_3^- flux ($\Delta J_{\text{HCO}_3^-} = 7.98 \pm 0.26$ pmol/min per mm, $P < 0.01$ vs 0). Concomitant with the increase in H^+ secretion was a decrease in electronegativity of V_{te} from -4.1 ± 0.2 to -3.6 ± 0.2 mV (mean change in voltage 0.4 ± 0.1 , $n = 29$, $P < 0.01$).

Time control. On the other hand, tubules that were incubated for 3 h at pH 7.4 (Fig. 1 B) showed no change in $J_{\text{HCO}_3^-}$, from -3.64 ± 0.18 to -3.57 ± 0.21 pmol/min per mm ($\Delta J_{\text{HCO}_3^-} = 0.07 \pm 0.10$ pmol/min per mm, $n = 4$, $P > 0.5$ vs 0). Nor was there a significant change in V_{te} (mean change -0.2 ± 0.1 mV, $P > 0.2$). Thus, there was no time-dependent decrease in HCO_3^- secretion, as previously observed by others (27), perhaps because of factors contained in the incubation medium or FCS that helped to maintain HCO_3^- secretion under the present experimental conditions.

Inhibition of α -intercalated cell function

To investigate the cellular mechanisms of this change, we examined the effects of several inhibitors of α -intercalated cell function to this adaptation. We hypothesized that if α -intercalated cells were stimulated to secrete protons after incubation at low pH, then inhibition of α cell function would have a larger effect after than before acid incubation.

Cl^- -free bath. Removal of basolateral Cl^- inhibits the activity of the basolateral Cl^- -base exchanger, thereby blocking HCO_3^- exit and luminal H^+ secretion from α -intercalated cells

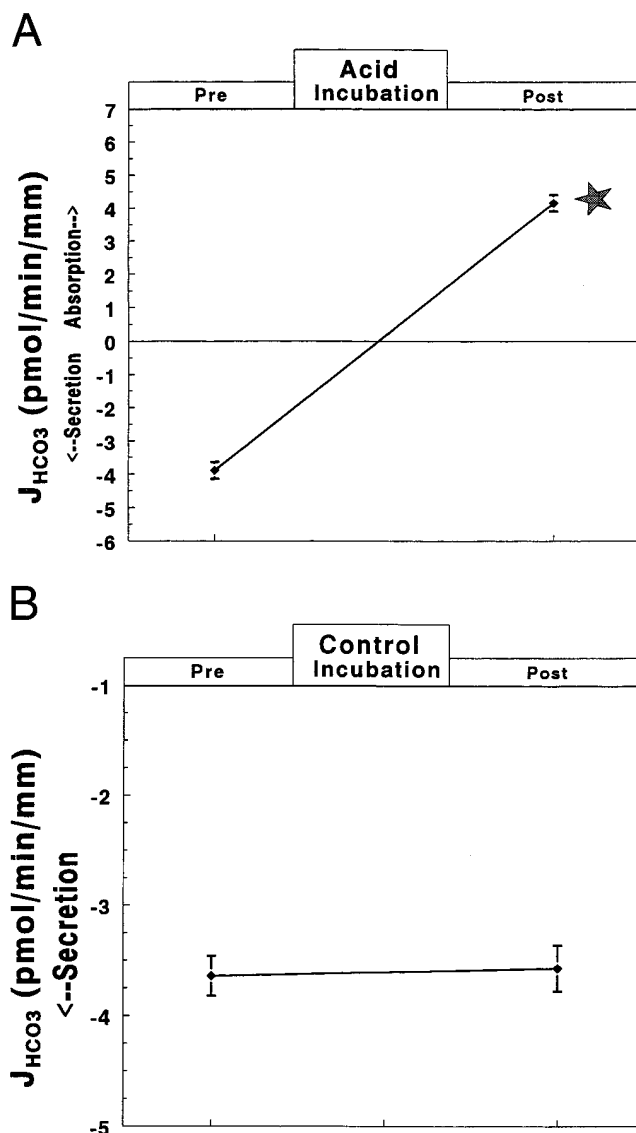


Figure 1. Mean \pm SE of net HCO_3^- flux ($J_{\text{HCO}_3^-}$, pmol/min per mm) before and after (A) acid incubation (1 h at pH 6.8 followed by 2 h at pH 7.4; $n = 29$ CCDs) or (B) control incubation (3 h at pH 7.4; $n = 4$ CCDs). Transport of HCO_3^- was obtained while perfusing and bathing each CCD in Burg's solution (25 mM HCO_3^- , pH 7.4). The star indicates $P < 0.01$.

(5, 10, 11, 28). This maneuver was performed in four CCDs to evaluate the magnitude of H^+ secretion (in the simultaneous presence of HCO_3^- secretion) before and then after acid incubation. Before incubation (Fig. 2), removal of Cl^- reversibly increased net HCO_3^- secretion from -3.88 ± 0.16 to -6.31 ± 0.13 and back to -3.90 ± 0.16 pmol/min per mm (mean $\Delta J_{\text{HCO}_3^-} = -2.42 \pm 0.09$ pmol/min per mm, $P < 0.01$ vs zero).

After acid incubation (see Fig. 2), net HCO_3^- flux was in the absorptive direction and was completely inhibited by removing basolateral Cl^- (from 4.31 ± 0.42 to -0.41 ± 0.03 pmol/min per mm, with mean $\Delta J_{\text{HCO}_3^-} = -4.72 \pm 0.44$ pmol/min per mm, $P < 0.01$ vs 0). Note that the inhibition in HCO_3^- flux after acid incubation was 2.30 pmol/min per mm more than that observed before incubation. The effect of removing basolateral Cl^- was completely reversible after acid incubation.

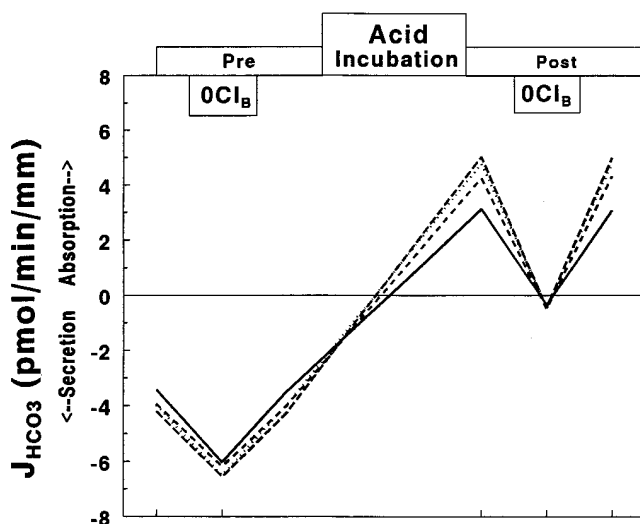


Figure 2. Effect of Cl^- -free bath on J_{HCO_3} in four individual tubules before (Pre) and after (Post) acid incubation. Unless noted in the boxes at the top of this and subsequent figures, J_{HCO_3} was measured using Burg's solution (25 mM HCO_3^-) in both perfusate and bath. Each line represents a separate tubule.

Basolateral DIDS. We used 50 μM DIDS as a direct inhibitor of the basolateral Cl^- -base exchanger of α -intercalated cells (10, 15, 16) in four CCDs (Fig. 3). Before acid incubation (when CCDs were secreting HCO_3^-), DIDS increased net HCO_3^- secretion from -3.43 ± 0.21 to -7.19 ± 0.66 pmol/min per mm (mean $\Delta J_{\text{HCO}_3} = -3.77 \pm 0.53$ pmol/min per mm, $P < 0.01$ vs 0).

After acid incubation, the resulting HCO_3^- absorption was completely inhibited by DIDS, and some small residual HCO_3^- secretion was uncovered (4.43 ± 0.35 to -1.81 ± 0.27 pmol/min per mm). The mean ΔJ_{HCO_3} was -6.24 ± 0.44 pmol/min per mm, 2.47 pmol more than that observed with DIDS before acid incubation ($P < 0.01$). The effect of DIDS was reversible, as shown previously (29), because after acid incubation all CCDs were absorbing net HCO_3^- (see Fig. 3), comparable to

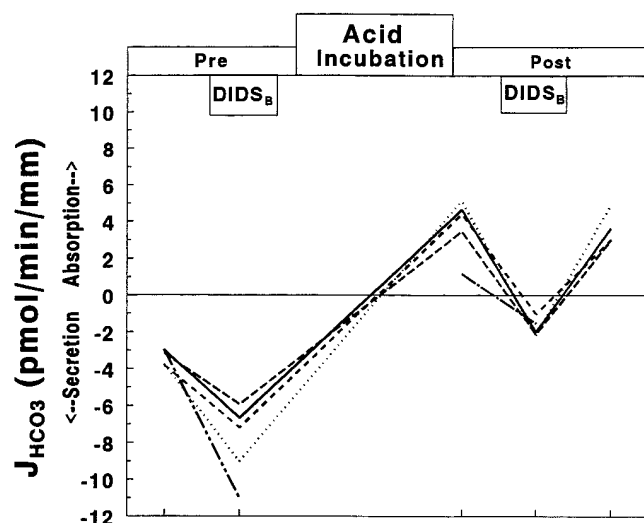


Figure 3. Effect of 50 μM DIDS added to the bath on J_{HCO_3} in five CCDs before (Pre) and after (Post) acid incubation.

what was observed in the Cl^- -free bath studies described above.

Luminal bafilomycin. We next examined the effect of 5 nM bafilomycin (30, 31) to inhibit the luminal H^+ -ATPase of α -intercalated cells (Fig. 4). Before the incubation, bafilomycin caused a small increase in net HCO_3^- secretion from -3.24 ± 0.33 to -4.31 ± 0.28 pmol/min per mm (mean $\Delta J_{\text{HCO}_3} = -1.07 \pm 0.34$, $n = 5$, $P < 0.05$). However, after the acid incubation there was net HCO_3^- absorption (4.09 ± 0.36 pmol/min per mm), and this was completely inhibited by luminal bafilomycin (0.23 ± 0.45 pmol/min per mm) with mean $\Delta J_{\text{HCO}_3} = -3.86 \pm 0.41$ pmol/min per mm, $n = 6$, $P < 0.01$. The effect of bafilomycin on net HCO_3^- transport was 2.79 pmol/min per mm larger after acid incubation compared with that observed before the incubation. Also, after acid incubation, when these CCDs were absorbing HCO_3^- , the effect of bafilomycin was completely reversible (recovery $J_{\text{HCO}_3} = 3.91 \pm 0.51$, not different from postincubation period before bafilomycin, $P > 0.2$), as shown previously (32).

As expected from the inhibition of electrogenic H^+ secretion, bafilomycin caused V_{te} to become more electronegative before incubation when net HCO_3^- was secreted (from -4.1 ± 0.5 to -4.8 ± 0.3 mV, $P < 0.05$), and after incubation when net H^+ was secreted (from -3.8 ± 0.3 to -4.9 ± 0.3 , $P < 0.01$). The mean change in V_{te} , ΔV_{te} , was 57% larger after acid incubation (mean $\Delta V_{\text{te}} = -0.7 \pm 0.2$ before and -1.1 ± 0.1 mV after incubation).

Bafilomycin was also tested in four CCDs incubated at pH 7.4 for 3 h (Fig. 5). Before the incubation, bafilomycin caused an increase in net HCO_3^- secretion of 2.09 ± 0.22 pmol/min per mm ($P < 0.01$), which was completely reversible (control, -3.64 ± 0.18 ; bafilomycin, -5.73 ± 0.1 ; recovery, -3.50 ± 0.20 pmol/min per mm). After incubation at pH 7.4, net HCO_3^- secretion was maintained at -3.57 ± 0.22 pmol/min per mm and was stimulated to the same extent by bafilomycin (-5.88 ± 0.14 pmol/min per mm). The mean increase in HCO_3^- secretion was 2.31 ± 0.15 pmol/min per mm, similar to the increase observed before incubation.

The effect of bafilomycin to increase the electronegativity of V_{te} was comparable before and after incubation at pH 7.4

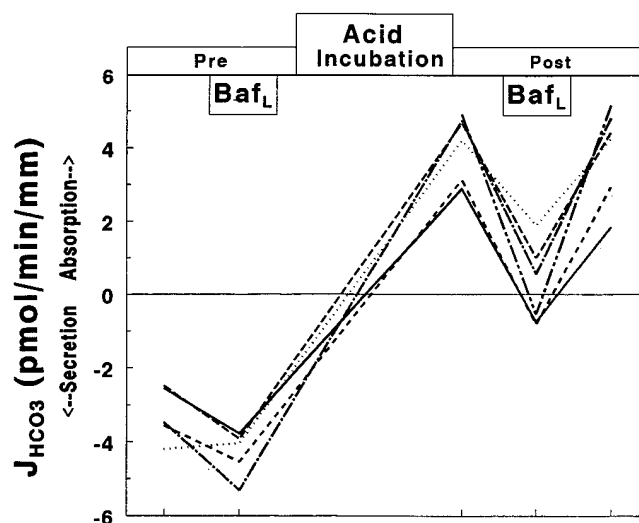


Figure 4. Effect in six CCDs of 5 nM luminal bafilomycin on J_{HCO_3} before (Pre) and after (Post) acid incubation.

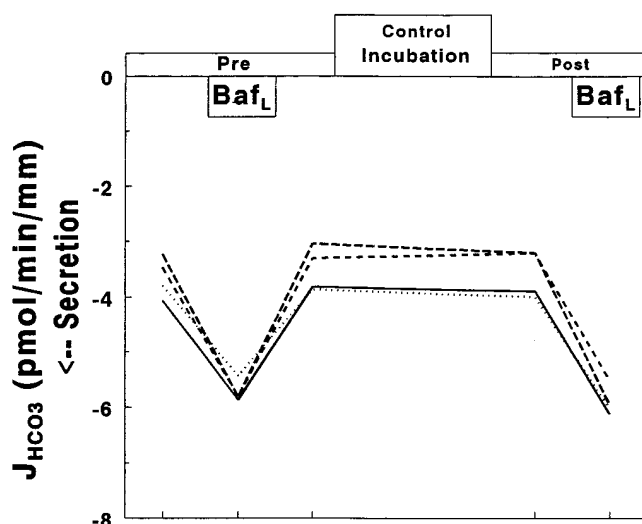


Figure 5. Effect in five CCDs of 5 nM luminal bafilomycin on J_{HCO_3} before (Pre) and after (Post) control incubation.

($\Delta V_{\text{te}} = -1.5 \pm 0.2$ before and -1.5 ± 0.2 mV after incubation). The effect of bafilomycin on V_{te} before pH 7.4 incubation was entirely reversible (control, -2.7 ± 0.1 ; bafilomycin, -4.2 ± 0.2 ; recovery, -2.6 ± 0.2 mV).

Luminal SCH28080. In view of the potential role of the H^+, K^+ -ATPase in mediating H^+ secretion in α -intercalated cells (31, 33, 34), the effect of 10 μM SCH28080, a specific inhibitor of this transporter, applied to the luminal solution, was examined after acid incubation (Fig. 6). The rate of net HCO_3^- absorption was inhibited only 5% by SCH28080 (incubation, 3.71 ± 0.26 ; SCH28080, 3.53 ± 0.22 ; $P < 0.05$). The effect was not completely reversible (recovery, 3.64 ± 0.24 ; $P < 0.05$ vs postincubation). The inhibitor had no significant effect on V_{te} (postincubation, -2.7 ± 0.4 ; SCH28080, -2.7 ± 0.4 ; recovery, -2.7 ± 0.4).

Because HCO_3^- absorption was only minimally inhibited in the CCD by SCH28080, it was possible that the sensitivity of the H^+, K^+ -ATPase in the CCD was different from that in outer medullary collecting duct (OMCD) or in gastric mucosa. We therefore tried a second approach: inhibiting the H^+, K^+ -

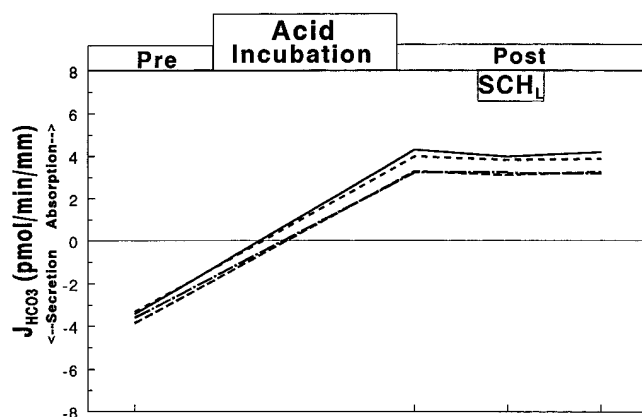


Figure 6. Effect in four CCDs of 10 μM luminal SCH28080 on J_{HCO_3} before (Pre) and after (Post) acid incubation.

ATPase not only by SCH28080, but also by removing luminal K^+ (34) (see Table I) and adding 0.1 mM ouabain to the solution bathing the tubule to inhibit any K^+ secretion by neighboring principal cells (Fig. 7). Still, the net HCO_3^- absorption observed after acid incubation was inhibited by only 10–15% (postincubation, 5.26 ± 0.45 ; SCH28080/0 K^+ /ouabain, 4.58 ± 0.3 pmol/min per mm; $P < 0.05$). The mean rate of H^+ secretion after SCH28080 was $89.4 \pm 1.3\%$ of that observed after acid incubation. The effect on J_{HCO_3} was reversible (recovery, 4.89 ± 0.38 pmol/min per mm, arithmetically lower but not different from the postincubation period before SCH28080; $P > 0.1$).

The use of ouabain, in addition to SCH28080, caused V_{te} to become electropositive, but this was not completely reversible (postincubation, -3.2 ± 0.3 ; SCH28080, $+1.0 \pm 0.1$ mV, $P < 0.01$; recovery, -1.5 ± 0.6 , $P < 0.05$). Presumably the small positive voltage reflects electrogenic H^+ secretion that was not inhibited by this maneuver (approximately of the same order of magnitude as that which was inhibited by bafilomycin after acid incubation [see above]).

Inhibition of β -intercalated cell function

The adaptation to metabolic acidosis might involve other acid-base transporting cells in addition to α -intercalated cells. Indeed, we have previously shown that acid incubation causes a decrease in maximally stimulated HCO_3^- secretion (accomplished by removing bath Cl^- to inhibit any H^+ secretion) (17, 22). In the present study we evaluated the effect of incubating CCDs for 1 h at pH 6.8 followed by 2 h at pH 7.4 on the rate of net HCO_3^- transport.

Cl^- -free lumen. We removed luminal Cl^- before and after acid incubation to determine the rate of Cl^- -dependent HCO_3^- secretion, a function of β -intercalated cells (13, 35–37). To avoid the high bath-to-lumen gradient, we reduced basolateral Cl^- to 10 mM (Table I). Before incubation when HCO_3^- secretion was inhibited (Fig. 8), a large H^+ secretory flux was uncovered (control, -4.92 ± 0.91 ; Cl^- -free lumen, 4.38 ± 0.50 pmol/min per mm). After acid incubation, net H^+ secretion was observed, and this was further stimulated by luminal Cl^- removal (postincubation, 3.54 ± 0.64 ; Cl^- -free lumen, 6.38 ± 0.69 ; recovery, 3.56 ± 0.68). The change in net HCO_3^- flux due to luminal Cl^- removal was more than three times larger before incubation ($\Delta J_{\text{HCO}_3} = 9.29 \pm 0.93$ before and 2.83 ± 0.46

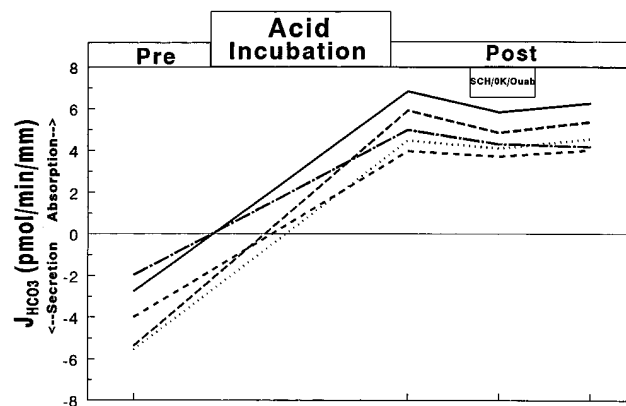


Figure 7. Effect in five CCDs of 5 μM luminal SCH28080 plus K^+ -free perfusate plus 0.1 mM ouabain added to the bath on J_{HCO_3} before (Pre) and after (Post) acid incubation.

pmol/min per mm after incubation, $P < 0.01$ vs before incubation). The increment observed after acid incubation was 6.46 pmol/min per mm smaller than before incubation, suggesting that the β -intercalated cells have downregulated HCO_3^- secretion in response to the low pH exposure. Also, the maximum rate of net HCO_3^- absorption (6.38 pmol/min per mm in Cl^- -free luminal solution) was achieved after, not before, acid incubation, indicating that, in addition to the decrease in HCO_3^- secretion, H^+ secretion had been stimulated.

Outer CCD

Because of the heterogeneity of the CCD, it is not possible to be unequivocally certain that regulation of HCO_3^- transport is occurring exclusively in α - or β -intercalated cells. This is a limitation of our net HCO_3^- flux analysis that cannot be readily addressed by cell pH experiments, morphological studies, or immunocytochemistry. Nevertheless, we surmised that another segment of the collecting duct, which has a different distribution and number of intercalated cell types, might respond differently to acid incubation and inhibition of α cell function. The outer CCD has few α -intercalated cells (16) and relatively more β and γ cells (15, 23). If there are fewer α -intercalated cells, then the magnitude of net HCO_3^- absorption resulting from acid incubation would be smaller than in mid-CCDs unless there is recruitment of other intercalated cell types. Also, removal of Cl^- from the bath would not likely have as large an effect as in mid-CCDs because there is little HCO_3^- absorption to inhibit.

Cl^- -free bath. Removal of basolateral Cl^- inhibits the activity of the Cl^- -base exchanger and thereby inhibits luminal H^+ secretion by α -intercalated cells (5, 10, 11, 28). This maneuver was performed in six outer CCDs to evaluate the magnitude of H^+ secretion before and then after acid incubation. Before incubation, CCDs (Fig. 9) secreted net HCO_3^- at a rate similar to that of mid-CCDs, and Cl^- removal increased this rate of HCO_3^- secretion from -3.94 ± 0.22 to -4.21 ± 0.19 pmol/min per mm, $P < 0.05$, $n = 6$). The mean $\Delta J_{\text{HCO}_3^-}$ was -0.28 ± 0.11 pmol/min per mm ($P < 0.01$ vs 0) and was only 11% of that observed in four mid-CCDs studied similarly ($P < 0.01$; see Fig. 2 and above).

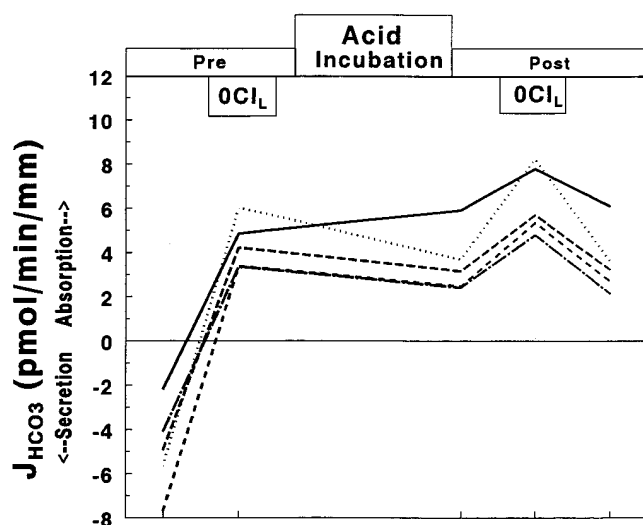


Figure 8. Effect in five CCDs of luminal Cl^- removal on $J_{\text{HCO}_3^-}$ before (Pre) and after (Post) acid incubation.

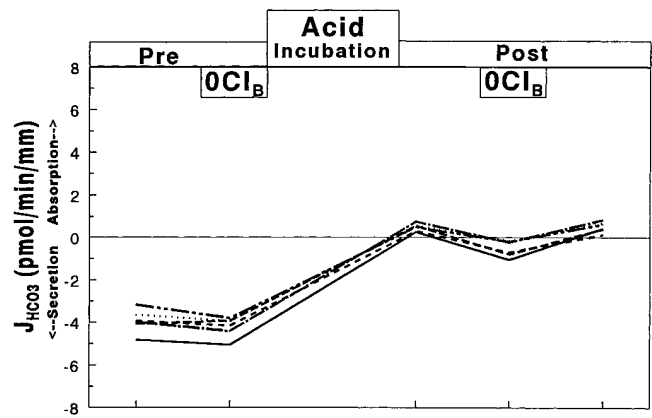


Figure 9. Effect of Cl^- -free bath on $J_{\text{HCO}_3^-}$ in six outer CCDs before (Pre) and after (Post) acid incubation.

After acid incubation (see Fig. 9), a small but significant net HCO_3^- absorptive flux was observed, and this was completely inhibited by removing basolateral Cl^- (from 0.51 ± 0.07 to -0.50 ± 0.15 pmol/min per mm, with mean $\Delta J_{\text{HCO}_3^-} = -0.94 \pm 0.15$ pmol/min per mm, $P < 0.01$ vs 0). The absorptive HCO_3^- flux after acid incubation was only 11% as large, and the $\Delta J_{\text{HCO}_3^-}$ after Cl^- removal was only 20% of that observed in four mid-CCDs studied similarly ($P < 0.01$ for both unpaired comparisons). As observed above, the removal of Cl^- was completely reversible (recovery, -0.54 ± 0.10 pmol/min per mm, see Fig. 9).

In keeping with the resulting net absorptive flux and as seen for mid-CCDs, the V_{te} fell significantly after acid incubation (preincubation, -4.4 ± 0.2 ; postincubation, -4.1 ± 0.2 mV; $P < 0.01$). This decrement of 0.3 mV was smaller than the 0.7 mV observed in the four mid-CCDs ($P < 0.05$).

Discussion

The kidney adapts to metabolic acidosis in part by increasing urinary acid excretion. Much of this adaptation occurs at the level of the collecting duct. The CCD is unique in that it is capable of changing the vectorial direction of HCO_3^- flux in vitro in response to acid-base perturbations in vivo (2–5). To characterize the adaptation of the CCD better, we have developed a model of metabolic acidosis in vitro (17). Normally, CCDs taken from control rabbits secrete net HCO_3^- , but, after 3 h of exposure to low pH in vitro, the polarity of HCO_3^- flux reverses to that of net HCO_3^- absorption (equivalent to net H^+ secretion) (17). Subsequent studies enabled us to show that inhibitors of protein or RNA synthesis or of cytoskeletal function impair the ability of the CCD to adapt to acidosis (22).

Our in vitro model has been validated by the findings of decreases in apical peanut agglutinin labeling and apical Cl^- - HCO_3^- exchange and reversal of polarity of net HCO_3^- flux that are comparable to those observed in CCDs taken from acid-fed rabbits in vivo (2, 5, 17, 22). In the present study, 29 of 29 CCDs exposed to 1 h at pH 6.8 followed by 2 h at pH 7.4 showed a reversal of polarity of net HCO_3^- flux from secretion to absorption (see Fig. 1 A).

Our recent studies have emphasized that HCO_3^- secretion, presumably by β -intercalated cells, is decreased by adapting CCDs (17, 22). The reduction in HCO_3^- secretion is accom-

plished in part by endocytotic removal of apical Cl^- - HCO_3^- exchangers (17). These studies confirm two previous ones in which acid loading in vivo caused primarily a decrease in HCO_3^- secretion, although a small increase in HCO_3^- absorption was also observed (18, 37).

Our previous data did not allow us to directly demonstrate adaptive changes in α -intercalated cell function (manifested by increased H^+ secretion or HCO_3^- absorption); however, CCDs from in vivo acid-treated animals have generally absorbed net HCO_3^- (2, 3, 5). On the other hand, in the OMCD of the outer stripe, which is comprised of only α -type (not β) intercalated cells, acidosis has not consistently stimulated H^+ secretion (28, 38, 39). Morphologically, in chronically acidotic animals, changes in H^+ -secreting cells have regularly been demonstrated: the cells become larger, with increased apical membrane surface area, decreased cytoplasmic tubulovesicles, increased basolateral membrane area, and redistribution to the apical and basolateral plasma membranes of H^+ -ATPase and band 3, respectively (40–42). Based on the morphologic and morphometric changes, it would be likely that these cells would secrete protons at higher rates.

The salient findings of the present study indicate that the α -intercalated cell does indeed modify its function in response to in vitro metabolic acidosis. Thus, we believe that this is the first functional demonstration that adaptation to acidosis in vitro is comprised of changes occurring in both H^+ -secreting and HCO_3^- -secreting cells of the CCD. Not only do β -intercalated cells decrease HCO_3^- secretion, but α -intercalated cells also increase H^+ secretion. This result can be examined in depth in Table II. Note that the tests of α cell function (Cl^- -free bath, basolateral DIDS, and luminal bafilomycin) elicit a much larger effect (increased by 2–3 pmol/min per mm) after acid incubation compared with before incubation. These results indicate that α cell function has been stimulated by the acid incubation in parallel with the reversal in polarity of net HCO_3^- flux. On the other hand, the test of β cell function (Cl^- -free perfusate) shows a much smaller effect (decreased by 6.5 pmol/min per mm) after acid incubation, indicating that β -intercalated cells have downregulated HCO_3^- secretion, as shown previously (17, 22, 37). And in outer CCDs, where few α cells are present, the effect of inhibiting α cell function after acid incubation was much smaller than in mid-CCDs (0.7 vs 2.3 pmol/min per mm) (see Table II).

The adaptive increase in H^+ secretion is accomplished predominantly via the vacuolar H^+ -ATPase rather than the P-type H^+ , K^+ -ATPase, because > 80% of resulting net H^+ secretion was inhibited by nanomolar concentrations of bafilomycin, a specific H^+ -ATPase inhibitor (30, 32, 43). Also, in two protocols using SCH28080, there was < 15% inhibition of HCO_3^- absorption in the acid incubated CCD (see Figs. 6 and 7 and Table II). SCH28080 alone inhibited HCO_3^- absorption by only 5%; we reasoned that secreted K^+ could have been competing with the inhibitor for the apical H^+ , K^+ -ATPase. In the second protocol, SCH28080 combined with basolateral ouabain to inhibit K^+ secretion by principal cells (44) plus elimination of luminal K^+ should have served to inhibit all apically oriented H^+ , K^+ -ATPase activity (31, 34); however, the inhibition averaged only 11%. Contrary to what has been observed in the OMCD taken from K^+ replete rabbits in which H^+ , K^+ -ATPase plays a major role in luminal acidification (31, 34), the adapted CCD appeared to depend more on luminal H^+ -ATPase activity; the bulk of net HCO_3^- absorption was in-

Table II. Effect of Maneuvers on $\Delta\text{J}_{\text{HCO}_3^-}$ before and after Incubation

Maneuver	Cell	Incubation	Pre	Post	Diff	Ratio
			(pmol/min/mm)	(pmol/min/mm)		
$-\text{Cl}^-_{\text{B}}$	α	Acid	-2.42	-4.72	-2.30*	1.95*
DIDS _B	α	Acid	-3.77	-6.24	-2.47*	1.66*
Baf _L	α	Acid	-1.07	-3.86	-2.76*	3.60*
Baf _L	α	Control	-2.09	-2.31	-0.22	1.11
SCH	α	Acid		-0.14		
SCH++	α	Acid		-0.68		
$-\text{Cl}^-_{\text{L}}$	β	Acid	+9.29	+2.83	-6.46*	0.30*

Values are means.

*Significantly different from 0 (Diff) or 1 (Ratio) ($P < 0.05$). Positive pre and post values denote increased HCO_3^- absorption or decreased HCO_3^- secretion and negative numbers denote decreased HCO_3^- absorption or increased HCO_3^- secretion. Each maneuver was performed before (Pre) and after (Post) a 3 h incubation and was generally reversible; the SCH++ protocol also involved the use of ouabain, which, in combination with SCH28080, was not completely reversible and therefore was not used before incubation. Acid incubation was 1 h at pH 6.8 plus 2 h at pH 7.4; control incubation was 3 h at pH 7.4. Baf, bafilomycin; SCH, SCH28080; SCH++, SCH plus 0 K^+ in lumen plus 0.1 mM bath ouabain; Diff, difference of post $\Delta\text{J}_{\text{HCO}_3^-}$ minus pre $\Delta\text{J}_{\text{HCO}_3^-}$; Ratio, ratio of post $\Delta\text{J}_{\text{HCO}_3^-}$ divided by pre $\Delta\text{J}_{\text{HCO}_3^-}$.

hibited by 5 nM bafilomycin, and relatively little (5–15%) was inhibited by SCH28080 (see Table II). In agreement with these findings, SCH28080 has been previously shown not to inhibit net HCO_3^- absorption in isolated perfused rat cortical collecting ducts (45).

Our results differ from Zhou and Wingo's studies (33) showing that an increase in HCO_3^- absorption after exposure to 10% CO_2 was totally inhibited by luminal 10- μM SCH28080, suggesting that the enhancement of HCO_3^- flux was mediated by an H^+ , K^+ -ATPase (33). To account for the differences between Zhou and Wingo's experiments and the present study, one must consider that the mechanism of acidosis was different, their CCDs were from deeper in the cortex, and the diet of the rabbits was different (ours were fed more than adequate amounts of K^+ [10–20 mEq/kg per d] and theirs were K^+ replete).

One might question whether luminal bafilomycin also permeates the cell to inhibit HCO_3^- secretion by β -intercalated cells. That appears not to be the case from the preincubation data and the control incubation studies, both of which showed that luminal bafilomycin actually stimulated HCO_3^- secretion (see Figs. 4 and 5 and Table II)². Also, there are experimental data from single-cell electrical measurements that show that *N*-ethylmaleimide, an inhibitor of H^+ -ATPase, depolarized the basolateral membrane potential of β -intercalated cells when applied from the basolateral but not from the luminal

2. In one CCD absorbing net HCO_3^- (after acid incubation), the addition of 5 nM bafilomycin to the bath did not inhibit acid secretion; if anything, there was a slight stimulation, perhaps because residual HCO_3^- secretion was inhibited: (postincubation, 3.51; bath bafilomycin, 3.98; recovery, 3.52 pmol/min per mm). The result of this experiment suggests that basolateral bafilomycin does not influence the luminal H^+ -ATPase of HCO_3^- -absorbing CCDs.

side (29). This would suggest the presence of a predominant H^+ -ATPase on the basolateral membranes of β -intercalated cells, but the inhibitor does not appear to permeate the cell and inhibit the H^+ -ATPase from the opposite membrane.

Whether or not this adaptation to low pH in vitro represents recruitment of preformed H^+ pumps from the apical cytoplasm via exocytosis (6, 21, 46) or de novo synthesis of one or more subunits of the proton pump cannot yet be determined. In previous studies of chronically acidotic rats, there was no increase in synthesis of H^+ pumps but rather a redistribution of preformed pumps from cytoplasm to apical membrane (42). A similar conclusion might be drawn from rabbits based on a recent immunolocalization study (41) that showed apical polarization of H^+ -ATPase and basolateral polarization of band 3 after 12 d of acid feeding compared with more cytoplasmic vesicular staining of both H^+ -ATPase and band 3 in control rabbits. Also, in response to acute cellular acidosis, there is fusion of cytoplasmic vesicles containing H^+ pumps with the apical membrane so as to increase net H^+ secretion in the CCD (46). Most of these studies would indicate that adaptation of α -intercalated cell would be rapid and rather independent of the cell's synthetic machinery. Indeed, early studies in isolated perfused CCDs initially showed a change in vectorial HCO_3^- transport ~ 20 h after an acid load (2), whereas others involved several days of acid loading in vivo (3, 5, 37). The present studies show an adaptation of both α - and β -intercalated cells after 3 h of incubation. Recently, we (22) have shown that the adaptation cannot be demonstrated after 2 h of acid incubation in vitro, and this has been confirmed again in two pilot experiments (see Results). Respiratory acidosis in vitro for 30 min stimulated HCO_3^- absorption by 252% in the CCD (47). Acidosis in vivo for 6 h or less in rabbits, however, did not cause obvious changes in CCD HCO_3^- transport (39). Alkalosis in vitro for 1 h had no effect on net HCO_3^- transport in isolated perfused rat CCDs (48).

A variety of studies have indicated that not all intercalated cells can be classified as α or β : some show characteristics of both cell types (9, 10, 15). Of greatest relevance to the present study is the finding of a subtype of intercalated cells with both apical and basolateral Cl^- - HCO_3^- exchangers; these cells were called γ cells and comprise $> 50\%$ of intercalated cells of the outer CCD (15). A more recent cell pH study, however, indicated that all CCD intercalated cells possessed basolateral Cl^- - HCO_3^- exchange activity (when the apical anion exchanger was concurrently inhibited) (16). β -intercalated cells had both apical and basolateral Cl^- -base exchangers, and both of these were insensitive to DIDS, whereas α -intercalated cells showed basolateral Cl^- -base exchange that was sensitive to DIDS (16). These investigators did not demonstrate the presence of γ cells. It is possible that not all β -intercalated cells secrete HCO_3^- and that this function might depend on the relative magnitudes of apical and basolateral anion exchange activities. Perhaps endocytotic retrieval of apical Cl^- - HCO_3^- exchangers, as previously demonstrated by our laboratory, results in sufficient diminishment of apical anion exchangers to substantially reduce HCO_3^- secretion by these adapted cells. Thus, the β or γ cell may be capable of regulating its transcellular HCO_3^- transport rate by controlling the activity of its apical Cl^- - HCO_3^- exchanger.

Whether or not γ cells can change into α -intercalated cells still remains an open question. Acid incubation of outer CCDs resulted in smaller, rather than larger, rates of HCO_3^- absorp-

tion compared with mid-CCDs, suggesting relatively little recruitment of γ cells under these conditions. Previous studies from our laboratory demonstrated that peanut agglutinin-labeled intercalated cells (presumably β -intercalated cells) became endocytotically active in response to in vivo metabolic acidosis: The lectin caps disappeared and net HCO_3^- transport converted from secretion to absorption (5). The evidence suggested a reversal of polarity of intercalated cells from β to α , but we were not able to demonstrate cells clearly in the midst of interconversion. Indeed, we later showed that endocytosis might not be a property of all intercalated cells (10); that is, not all H^+ -secreting cells regulate transport by endocytosis/exocytosis of H^+ pumps.

On the other hand, recent studies (49) in a clonal cell line of β -intercalated cells indicate that the density of plating influences the phenotype of such cells: At low density the cells have the β -intercalated cell phenotype and secrete HCO_3^- , but when plated at high density they assume an α -intercalated cell phenotype, showing basolateral band 3 and secreting protons. A specific extracellular matrix protein of 230 kD produced by the cells plated at high density was able to induce a similar reversal of epithelial polarity (49). Our data in the mid-CCD are certainly consistent with either an upregulation of H^+ secretion in existing α -intercalated cells plus a downregulation of HCO_3^- secretion in β -intercalated cells or with a recruitment of α cells from the pool of β/γ cells, with a reversal of polarity of these cells resulting in an increase in H^+ secretion and a decrease in HCO_3^- secretion. Studies to actually "catch" these intercalated cells in the process of interconversion should be able to resolve this important issue of renal acid-base physiology.

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