Mineralocorticoids and Acidosis Regulate H^+/HCO_3^- Transport of Intercalated Cells

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Abstract

The effects of acidosis and mineralocorticoids on cellular H^+/HCO_3^- transport mechanisms were examined in intercalated cells of the outer stripe of outer medullary collecting duct (OMCDo) from rabbit. Intracellular pH (pHi) of intercalated cells was monitored by fluorescence ratio imaging using 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF). pHi recovered from an acid load at $2.8\pm0.5 \times 10^{-3}$ pHU/s in the absence of ambient Na^+. This pHi recovery rate was similar in chronic acidosis induced by NH_4Cl loading, but it was enhanced (+111%) by treatment with deoxycorticosterone acetate (DOCA). In a DOCA-treated group, luminal 10 μM SCH28080 and 0.1 mM omeprazole, H^+-K^+-ATPase inhibitors, did not change the pHi recovery rate, while luminal 0.5 mM *N*-ethylmaleimide blocked the rate by 68%. DOCA, but not acidosis, increased (~40%) initial pHi response to bath HCO_3^- or Cl^- reduction in Na^+-free condition. After an acid load in the absence of Na^+ and HCO_3^-, pHi response to basolateral Na^+ addition was stimulated (+66%) by acidosis, but not by DOCA. Our results suggest that (*a*) mineralocorticoids stimulate H^+/HCO_3^- transport mechanisms involved in transepithelial H^+ secretion, i.e., a luminal NEM-sensitive H^+ pump and basolateral Na^+-independent Cl^--HCO_3^- exchange; and (*b*) acidosis enhances the activity of basolateral Na^+-H^+ exchange that may be responsible for pHi regulation. (*J. Clin. Invest.* 1992. 89:1388–1394.) Key words: NEM-sensitive H^+ pump • Na^+-independent Cl^--HCO_3^- exchange • Na^+-H^+ exchange • cell pH • fluorescence ratio imaging

Introduction

Elimination of metabolically produced acids is a major function of the kidney. Recent studies have demonstrated that the outer stripe of outer medullary collecting duct (OMCDo)[1] plays an important role in renal acid secretion (1–3). Most intercalated cells are type A cells in this segment (4, 5), and an H^+ pump located in the luminal membrane of this cell type is regarded as the mechanism of luminal H^+ secretion (1, 5). In a previous study we found functional evidence for the presence of a luminal *N*-ethylmaleimide (NEM)-sensitive H^+ pump in intercalated cells of OMCDo (6). A similar H^+ pump was identified in the inner stripe of OMCD (OMCDi) (7, 8).

Luminal H^+ secretion leads to HCO_3^- production inside the cell. The generated HCO_3^- must exit the cell to maintain a constant intracellular pH (pHi). Our previous study showed two mechanisms of basolateral HCO_3^- (or related base) transport in intercalated cells of OMCDo: Na^+-independent Cl^--HCO_3^- exchange, and Na^+-HCO_3^- cotransport, which are responsible for ~ 70% and ~ 30% of basolateral HCO_3^- transport, respectively (9). In the renal collecting duct, previous studies have demonstrated basolateral Na^+-independent Cl^--HCO_3^- antiport (10–13) and basolateral Na^+-HCO_3^- symport (11). Because the amount of HCO_3^- generation is proportional to that of luminal H^+ secretion, changes in the luminal H^+ secretion rate would affect basolateral HCO_3^- transport. Besides HCO_3^- transport, we also found the existence of basolateral Na^+-H^+ exchange, whose function was attributed to pHi regulation (6).

Numerous factors influence urinary acidification of the collecting duct. Above all, the effects of changes in systemic acid base and mineralocorticoids are the subjects of main interest. Madsen and Tisher (14, 15) found evidence for a morphological adaptation of OMCD intercalated cells to acidosis. In contrast, in vitro microperfusion studies indicated that acidosis and alkalosis did not alter the HCO_3^- reabsorption rate in OMCD (3, 16, 17). It also remains controversial whether the activity of NEM-sensitive ATPase is stimulated (18) or not (19) during acidosis. On the other hand, mineralocorticoids have been shown to enhance the HCO_3^- reabsorption rate in OMCD (3, 20), and conversely, adrenalectomy reduces the rate in OMCD (20). The activity of NEM-sensitive ATPase increased with aldosterone (21, 22) and decreased with adrenalectomy in OMCD (23). These results suggested that mineralocorticoids modulate H^+/HCO_3^- transport in this segment.

In the present study, we examined the influences of acidosis and mineralocorticoids on cellular H^+/HCO_3^- transport mechanisms of OMCDo intercalated cells, i.e., luminal NEM-sensitive H^+ pump, basolateral Na^+-independent Cl^--HCO_3^- exchange, basolateral Na^+-H^+ exchange, and basolateral Na^+-HCO_3^- exchange.

Methods

Treatment of animals. Female Japanese white rabbits (2.0–2.5 kg) were fed a standard rabbit chow. They were divided into three groups. Control group rabbits had free access to water. The second group of rabbits was given a drinking water containing 50 mM NH_4Cl and gavaged with 20 ml of 1 M NH_4Cl once daily for 3–4 d. The third group of rabbits

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1. *Abbreviations used in this paper:* ANOVA, analysis of variance; BCECF, 2',7'-bis(carboxyethyl)-5(6)-carbofluorescein; BCECF-AM, acetoxymethyl derivative of BCECF; B_B, CO_2-HCO_3^- buffering capacity; B_I, non-CO_2-HCO_3^- buffering capacity; DOCA, deoxycorticosterone acetate; NEM, *N*-ethylmaleimide; NMDG, *N*-methyl-D-glucamine; OMCD, outer medullary collecting duct; $OMCD_i$, inner stripe of OMCD; $OMCD_o$, outer stripe of OMCD; pHi, intracellular pH.

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was treated similar to the control group, except that they were injected daily with deoxycorticosterone acetate (DOCA) (Wako Chemical, Osaka, Japan), intramuscularly at a dose of 2 mg/kg body wt for 5–7 d.

Just before the rabbits were anesthetized with pentobarbital, samples of arterial blood were collected from the central ear artery. Blood gases and electrolytes were analyzed by a blood–gas analyzer (178 pH; Corning Medical and Scientific, Medfield, MA) and an electrolyte analyzer (Model 6; NOVA Biomedical, Waltham, MA), respectively. After removal of the left kidney, urine samples were collected from the bladder, and urine pH was measured by a glass pH electrode.

In vitro microperfusion. Isolated rabbit OMCDo was perfused in vitro, as described previously (6, 24). Briefly, kidneys were cut in coronal slices. Segments of OMCDo (300–600 μm in length) were dissected and perfused in a thermoregulated bath of 150-μl vol. The bath was placed on the stage of an inverted epifluorescence microscope (IMT-2; Olympus, Tokyo, Japan). The luminal flow rate was > 10 nl/min. The bath solution (preheated at 37°C) was exchanged at ~ 10 ml/min by gravity. Composition of the bath fluid was changed by rotating a four-way valve placed near the bath. At a 10-ml/min bath exchange rate, new fluid replaced old fluid by > 90% within 1 s. The solutions used in the present study are shown in Table I. The osmolalities of the solutions were adjusted to 290±2 mosmol/kg H_2O. The HCO_3^--containing and HCO_3^--free solutions were bubbled with 5% CO_2–95% O_2, and 100% O_2 gases, respectively.

Intracellular pH measurement. pHi of intercalated cells was measured with 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) using fluorescence ratio imaging microscopy, as reported previously (6, 9). Intercalated cells were morphologically distinguished from principal cells. This cell type has numerous cytoplasm organelles, with a circular outline in en face view, and a circular outline bulged into lumen in a lateral view (6, 25). pHi was measured in cells at the side of tubules in this study. After an equilibration period of 20–30 min, the tubular lumen was perfused with a control solution (solution 1) containing 20 μM of the acetoxymethyl derivative of BCECF (BCECF-AM) (Molecular Probes Inc., Junction City, OR) for 20–30 min. With the luminal dye loading, fluorescence intensity is much higher in intercalated cells than in principal cells (6, 26). This maneuver is favorable for pHi measurement of intercalated cells because the interference from neighboring principal cells is small (6).

For pHi measurement, tubule cells were viewed with a 40× objective (DPlan Apo; Olympus). Tubules were excited at 490±10 and 440±10 nm, alternately, and emission fluorescence was measured at 535±6 nm. Two images (490- and 440-nm excitation) were taken in 1 s with a silicon-intensified target (SIT) camera (C2400-08H; Hamamatsu Photonics Corp., Hamamatsu, Japan), and they were stored in an image processor (ARGUS 100; Hamamatsu Photonics). After the background signal was subtracted, the 490-nm excitation image was divided by the 440-nm excitation image on a television monitor (Trinitron PVM-1371Q; SONY, Tokyo, Japan). A rectangular measuring area was set on the center of the cells to detect pHi.

To convert 490/440-nm excitation ratio to pHi, in situ calibration curves were obtained using the nigericin high-K^+ method (27). The calibration solution contained (mM) 105 KCl, 30 phosphate, 1.2 $MgSO_4$, 20 Hepes, 40 *N*-methyl-D-glucamine (NMDG), 5.5 glucose, 6 alanine, and 1.0 $CaCl_2$, as well as 10 μM nigericin (Sigma Chemical Co., St. Louis, MO). Solution pH was adjusted to 7 different pH levels (5.8–8.2) by KOH or HCl. A linear conversion formula (490/440-nm ratios = a + b × pHi) was made separately to obtain pHi in three pH ranges (5.8–6.6, 6.6–7.8, and 7.8–8.2).

Buffering capacity. Non-CO_2-HCO_3^- (i.e., intrinsic) buffering capacity (B_I) was determined by a NH_3/NH_4^+ prepulse in the absence of extracellular Na^+ and HCO_3^- (28). pHi reduction induced by NH_3/NH_4^+ removal (solution 8 to 7) was measured, and B_I (mmol/liter per pHU) was calculated using the following formula,

$$B_I = \Delta[NH_4^+]i/\Delta pHi \quad (1)$$

$$[NH_4^+]i = [NH_3]i \times 10^{(pKa-pHi)} \quad (2)$$

where $[NH_4^+]i$ and $[NH_3]i$ are, respectively, intracellular NH_4^+ and NH_3 concentrations just before NH_3/NH_4^+ withdrawal, ΔpHi is the magnitude of the decrease in pHi upon acid load. $[NH_3]i$ was assumed to be equal to the extracellular NH_3 concentration. A pKa value of 9.1 was used. CO_2-HCO_3^- buffering capacity (B_B) was estimated from the formula (28),

$$B_B = 2.3\,[HCO_3^-]i \quad (3)$$

Total buffering capacity was calculated as the sum of B_I and B_B.

Protocols. All experiments were started 5–10 min after the dye removal. In the first series of experiments, pHi recovery from cell acidification was observed in the absence of ambient Na^+. At the removal of luminal BCECF-AM, both luminal and bath fluids were changed to Na^+-free solutions (solution 1 to 2). An intracellular acid load was

Table I. Composition of Solutions

	1 Control	2 ONa	3 ONa-NH_4	4 ONa-2.5 HCO_3	5 OCl	6 OCl-2.5 HCO_3	7 ONa-OCl	8 $OHCO_3$	9 $OHCO_3$-ONa	10 $OHCO_3$-ONa-NH_4
Na^+	145				143	143		135		
K^+	5	5	5	5	5	5	5	5	5	5
NH_4^+			20							20
$NMDG^+$		120	100	142.5			120		135	115
$Choline^+$		25	25	2.5			25			
Ca^{2+}	1	1	1	1	3.5	3.5	3.5	1	1	1
Mg^{2+}	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Cl^-	126	126	126	148.5				141	141	141
HCO_3^-	25	25	25	2.5	25	2.5	25			
$H_2PO_4^-$	1	1	1	1	1	1	1	1	1	1
SO_4^-	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Hepes-Tris								20	20	20
$Gluconate^-$					129	151.5	131			
Glucose	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Alanine	6	6	6	6	6	6	6	6	6	6
pH	7.4	7.4	7.4	6.4	7.4	6.4	7.4	7.4	7.4	7.4

Units are mM.

Table II. Acid-Base and Electrolyte Data

Treatment	n	Blood					Urine
		pH	P_{CO_2}	TCO_2	Na	K	pH
			(mmHg)	*(meq/liter)*	*(meq/liter)*	*(meq/liter)*	
Control	14	7.39±0.01	38.2±1.4	24.1±1.1	142±1	5.2±0.2	8.17±0.09
Acidosis	12	7.29±0.03§	28.6±1.9§	15.7±1.6§	144±2	5.3±0.2	5.56±0.18§
DOCA	14	7.42±0.02	39.8±1.6	27.2±0.9*	142±1	4.3±0.1‡	7.59±0.21*

Values are means±SE; *n*, number of rabbits. Acidosis; rabbits were given 20 ml of 1 M NH_4Cl daily by gavage and drinking water containing 50 mM NH_4Cl for 3–4 d. DOCA; DOCA and injected intramuscularly at 2 mg/kg body wt per d for 5–7 d. * $P < 0.05$, ‡ $P < 0.01$, and § $P < 0.001$ by ANOVA, compared with controls.

induced by a NH_3/NH_4^+ prepulse technique (28). After tubule cells were bathed with 20 mM NH_3/NH_4^+ for 3 min, NH_3/NH_4^+ was eliminated from the bath. The time courses of pHi were monitored at 5-s intervals. Because an initial pHi recovery was linear in most cases, the rate of pHi change (dpHi/dt) was determined from data points of the first 20-s response. dpHi/dt was expressed as a change in pH unit/1,000 s (pH U/s × 10^3). In one protocol, pHi was measured every 60 s to observe a long time course of pHi recovery. When the effect of NEM (Wako Chemical), an inhibitor of H^+ ATPase, was tested, 0.5 mM NEM was added to the lumen 10 min before experiments. SCH28080 (kindly provided by Schering Corp. Kenilworth, NJ) and omeprazole (Yoshitomi Pharmaceutical Industries Ltd., Osaka, Japan), inhibitors of gastric H^+-K^+-ATPase (8, 29–31), were applied at 10 μM and 0.1 mM, respectively, to the tubule lumen 20 min before experiments.

To evaluate the activity of basolateral Na^+-independent Cl^--HCO_3^- exchange, changes of pHi after lowering bath HCO_3^- or bath Cl^- removal were observed in the nominal absence of Na^+. Basolateral Na^+-HCO_3^- cotransport activity was determined by monitoring pHi after bath HCO_3^- reduction or Na^+ removal in the absence of Cl^-. In this protocol, tubules were bathed and perfused with Cl^--free solution for 30–40 min before experiments. Because the responses of pHi in these protocols were more prompt than those of pHi recovery from an acid load, pHi was monitored at 2.5 s, and dpHi/dt was calculated using the initial 10-s response. The activity of basolateral Na^+-H^+ exchange was examined in the absence of HCO_3^- to eliminate the contribution of HCO_3^- transport mechanisms. After an acid load in Na^+-free condition, 135 mM Na^+ was added to the bath, and the increase in dpHi/dt in response to Na^+ addition was determined. pHi was measured at 5 s, and dpHi/dt was obtained from the initial 20-s response.

Statistics. The results are expressed as means±SE. Analysis of variance (ANOVA) was used to determine statistical significance. Values of $P < 0.05$ were considered significant.

Results

Table II shows systemic acid base and electrolyte data of rabbits in three different conditions. Blood pH, P_{CO_2}, TCO_2, and urine pH of the acidosis group were significantly lower than those of the control group. In the DOCA-treated group, TCO_2 was higher and serum K^+ and urine pH were lower than those of the control. Chronic treatment with mineralocorticoids is known as an established procedure to make a K-deprived animal model. In the present study, serum K decreased significantly in rabbits with 5–7 d of DOCA treatment. However, mean serum K level in DOCA group was still > 4 meq/liter.

Luminal H^+ secretion. Fig. 1 *A* depicts a typical time course of pHi recovery from cell acidification by a NH_3/NH_4^+ prepulse in the control condition. pHi was monitored at 60-s intervals in the absence of Na^+. After NH_3/NH_4^+ removal (solution 3 to 2), pHi fell from ~ 7.1 to ~ 6.4. Then pHi recovered continuously until it returned to a stable value in ~ 10 min. To examine early responses, pHi was measured at 5 s in the same condition. Examples are shown in Fig. 1 *B*. pHi recovered from an intracellular acid load at an initial rate of 3.9×10^{-3} pHU/s in the control, and 8.0×10^{-3} pHU/s in the DOCA-treated conditions.

These experiments were performed in control, acidosis, and DOCA groups, and the results are summarized in Table III and Fig. 2. Initial pHi (lowest pHi) did not significantly differ among the three groups. In control cells, pHi recovered at $2.8 \pm 0.5 \times 10^{-3}$ pHU/s. Acidosis had no effect on dpHi/dt. In contrast, DOCA treatment significantly increased dpHi/dt, by 111%. Total buffer capacity (millimole/liter per pH units) was 44.1±1.9 (n = 16 cells) in control, 45.5±2.2 (n = 17 cells) in acidosis, and 48.2±2.1 (n = 17 cells) in DOCA-treated intercalated cells. These values were not significantly different. It has been shown that mineralocorticoids do not increase the cell volume of intercalated cells (32). Consequently, the difference in dpHi/dt in these experiments reflects the difference in single cell H^+ flux. Our results suggest that DOCA, but not acidosis, stimulates H^+ secretion of intercalated cells.

To clarify the mechanisms of luminal H^+ secretion, the effects of three ATPase inhibitors on pHi recovery were tested (Table III, Fig. 2). Luminal NEM decreased dpHi/dt by 61% in controls, suggesting that luminal NEM-sensitive H^+ pump is the major mechanism of H^+ secretion. Luminal application of

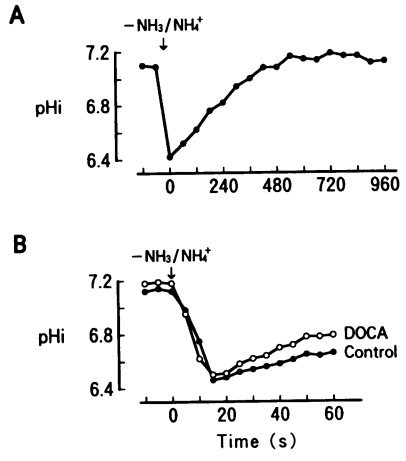

Figure 1. Time course of pHi recovery from intracellular acid loading. In the absence of ambient Na^+, 20 mM NH_3/NH_4^+ was removed from the bath (solution 3 to 2) at time −15 s (*A*) or at time zero (*B*). (*A*) Whole time course of pHi recovery monitored at 60 s in the control condition; (*B*) early phase of pHi recovery monitored at 5 s in the control (—●—) and DOCA-treated (—○—) conditions.

Table III. pHi Recovery in Response to Acid Loading

	Control	Acidosis	SCH28080	NEM
Initial pHi	6.40±0.06	6.50±0.07	6.52±0.06	6.47±0.06
dpHi/dt	2.8±0.5	3.5±0.8	3.0±0.5	1.1±0.3*
n	38/8	28.7	33/7	26/6

DOCA	DOCA + SCH28080	DOCA + omeprazole	DOCA + NEM
6.52±0.05	6.49±0.06	6.43±0.04	6.55±0.05
5.9±0.9‡	5.1±0.6‡	6.5±0.7‡	1.9±0.6
37/7	35/7	28/6	38/7

Values are means±SE; *n*, number of cells/tubules. For intracellular acid loading, 20 mM NH_3/NH_4^+ was eliminated from bath solution in the absence of Na^+ (solution 3 to 2). SCH28080, omeprazole, and NEM were given from the lumen at 10 μM, 0.1 mM, and 0.5 mM, respectively. dpHi/dt, rates of pHi change (pHU/s × 10^3); * $P < 0.05$ and ‡ $P < 0.01$ by ANOVA compared with controls.

H^+-K^+-ATPase inhibitors, SCH28080 and omeprazole, did not change dpHi/dt significantly in DOCA-treated cells. In the presence of 0.5 mM luminal NEM, dpHi/dt was significantly inhibited by 68% in the DOCA group. These results suggest that DOCA enhances H^+ secretion by increasing the activity of a NEM-sensitive H^+ pump.

Basolateral Na^+-independent Cl^--HCO_3^- exchange. The activity of Na^+-independent Cl^--HCO_3^- exchange was examined by decreasing bath HCO_3^- or Cl^- concentration in a Na^+-free condition. When bath HCO_3^- was lowered from 25 to 2.5 mM (solution 2 to 4), pHi decreased quickly to a stable value in ~ 20 s. Fig. 3 *A* shows typical pHi responses in the control and DOCA-treated conditions. Table IV and Fig. 4 *A* represent a summary of this protocol in control, acidosis, and DOCA groups. Initial pH was not significantly different in three groups. A reduction in bath HCO_3^- decreased pHi at an initial rate of $-14.2\pm0.9 \times 10^{-3}$ pHU/s in the control condition. Acidosis did not alter dpHi/dt, whereas DOCA treatment augmented dpHi/dt significantly, by 43%.

As shown in Fig. 3 *B*, pHi rose rapidly in response to basolateral Cl^- removal (solution 2 to 7). The results of this protocol are summarized in Table IV and Fig. 4 *B*. Resting pHi was similar in these three conditions. dpHi/dt was $21.7\pm2.1 \times 10^{-3}$ pHU/s in the control. Again, acidosis caused no change in dpHi/dt, and DOCA increased dpHi/dt by 36%. These results suggest that DOCA, but not acidosis, stimulates basolateral Na^+-independent Cl^-/HCO_3^- antiport.

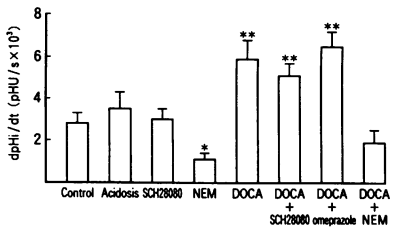

Figure 2. Rates of pHi recovery (*d*pHi/*dt*) from intracellular acidification. Cells were acidified by a NH_3/NH_4^+ prepulse technique in the absence of ambient Na^+. In some protocols, tubules were pretreated with 10 μM SCH28080 or 0.1 mM omeprazole for 20 min, or with 0.5 mM NEM for 10 min. Each bar shows means±SE of 26–38 cells from six to eight tubules. *$P < 0.05$ and **$P < 0.01$ by ANOVA, compared with control.

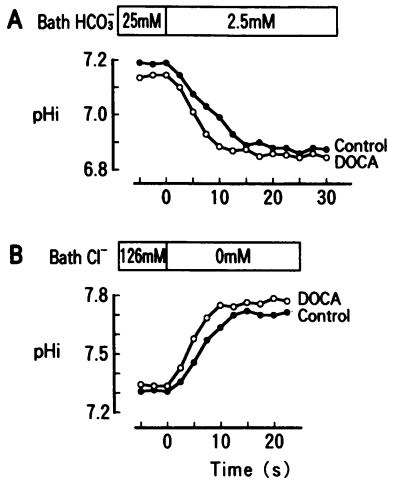

Figure 3. Time course of pHi in response to lowering basolateral HCO_3^- concentration (*A*) or basolateral Cl^- removal (*B*) in the control (—●—) and DOCA-treated (—○—) cells. (*A*) Bath HCO_3^- was reduced from 25 to 2.5 mM at constant P_{CO_2} at time zero in the absence of ambient Na^+ (solution 2 to 4). (*B*) Bath Cl^- was eliminated from the bath (replaced by gluconate) at time zero in the absence of Na^+ (solution 2 to 7).

Basolateral Na^+-H^+ exchange. We examined whether acidosis and DOCA alter the basolateral Na^+-H^+ exchange activity. Experiments were performed in the nominal absence of HCO_3^-. After an acid load in Na^+-free condition (solution 10 to 9), 135 mM Na^+ was added to the bath (solution 9 to 8). Fig. 5 *A* shows examples of this protocol in the control and acidosis conditions. The pHi recoveries were accelerated after addition of bath Na^+. These results are summarized in Table V and Fig. 5 *B*. An increase in dpHi/dt (ΔdpHi/dt) induced by basolateral Na^+ addition was $6.8\pm0.9 \times 10^{-3}$ pHU/s in the control condition. Acidosis increased ΔdpHi/dt by 66%, whereas DOCA had no significant effect on ΔdpHi/dt, suggesting that acidosis, but not DOCA, stimulates basolateral Na^+-H^+ antiport.

Basolateral Na^+-HCO_3^- cotransport. We previously reported functional evidence for the presence of basolateral Na^+-HCO_3^- cotransport in intercalated cells of OMCDo (9). To test this transport activity, bath HCO_3^- or Na^+ was reduced in the absence of ambient Cl^-. Both maneuvers decreased pHi to a steady-state value in 15–30 s. These results are summarized in Table VI and Fig. 6. By lowering bath HCO_3^- from 25 to 2.5 mM, pHi fell at $6.0\pm0.7 \times 10^{-3}$ pHU/s in controls. Both acidosis and DOCA did not change the rate significantly (Fig. 6 *A*). Bath Na^+ removal decreased pHi at $5.1\pm0.6 \times 10^{-3}$ pHU/s in controls. Again, acidosis and DOCA had no effect on the rate (Fig. 6 *B*).

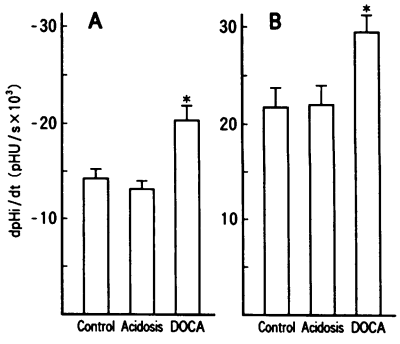

Figure 4. Rates of pHi change (dpHi/dt) by lowering bath HCO_3^- (*A*) or bath Cl^- removal (*B*). (*A*) Bath HCO_3^- was reduced from 25 to 2.5 mM at constant P_{CO_2} in the absence of extracellular Na^+. Each bar represents means±SE of 42–53 cells from 10–15 tubules. (*B*) Bath Cl^- was totally replaced by gluconate in the absence of ambient Na^+. Each bar represents means±SE of 27–30 cells from seven tubules. *$P < 0.01$ by ANOVA, compared with control.

Table IV. Effects of Lowering Bath HCO_3^- or Cl^- on pHi in the Absence of Na^+

	Control	Acidosis	DOCA
HCO_3^- reduction			
Initial pHi	7.20±0.03	7.23±0.04	7.16±0.03
dpHi/dt	−14.2±0.9	−12.9±1.0	−20.3±1.6*
n	53/15	49/11	42/10
Cl^- removal			
Initial pHi	7.14±0.03	7.12±0.07	7.19±0.05
dpHi/dt	21.7±2.1	22.1±2.2	29.5±1.9*
n	30/7	27/7	27/7

Values are means±SE; *n*, number of cells/tubules. HCO_3^- reduction, bath HCO_3^- concentration was lowered from 25 to 2.5 mM (solution 2 to 4). Cl^- removal, bath Cl^- was totally replaced by gluconate (solution 2 to 7). dpHi/dt, rates of change in pHi (pHU/s × 10^3); * $P < 0.01$ by ANOVA compared with controls.

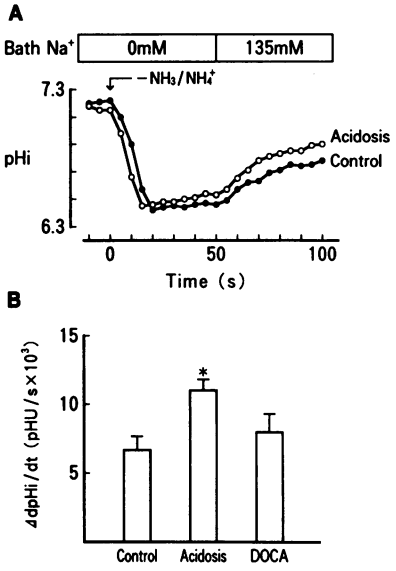

Figure 5. (*A*) Time course of pHi in response to basolateral addition of Na^+ in control (—●—) and acidosis (—○—). In the absence of Na^+ and HCO_3^-, 20 mM NH_3/NH_4^+ was eliminated at time zero (solution 10 to 9). 50 s later, 135 mM Na^+ was added to the bath (solution 9 to 8). (*B*) Increases in pHi recovery rate (ΔdpHi/dt) after Na^+ addition to the bath. Each bar represents means±SE of 26–28 cells from 6–7 tubules. *$P < 0.01$ by ANOVA, compared with control.

Discussion

Luminal H^+ transport in intercalated cells. In the absence of ambient Na^+, luminal NEM decreased the initial pHi recovery from cell acidification by 61% (Fig. 2). We previously observed that basolateral application of NEM did not change the rate in OMCDo intercalated cells (6). These findings suggested that cell H^+ secretion is mainly due to a luminal NEM-sensitive H^+ pump. A similar H^+ pump has been shown in OMCDo (1) and OMCDi (7, 8).

In the presence of luminal NEM, pHi still recovered from an acid load, at a small but significant rate (Fig. 2). Similar observations were described in S_3 proximal tubule (33), OMCDo intercalated cells (6), and OMCDi (8). Recently the presence of H^+-K^+-ATPase, another H^+ pump, has been suggested in OMCDi (29–31). This ATPase was insensitive to ouabain (29, 30) but was inhibited by vanadate (29, 30), and, more specifically, also by omeprazole (29–31) and SCH28080 (8, 30). In this study, we tested the effects of omeprazole and SCH28080 on the pHi recovery. These compounds resulted in no significant change in dpHi/dt in both control and DOCA-treated conditions (Fig. 2). A similar finding has been recently reported in OMCDi by Hays and Alpern (8). We failed to show the evidence that H^+-K^+-ATPase contributes to luminal H^+ secretion. H^+-K^+-ATPase may serve H^+ secretion and K^+ reabsorption during K^+ depletion (29, 31). This ATPase activity might be so low in our experimental condition (i.e., in the absence of apparent K^+ depletion) that we were unable to detect its function.

The nature of cell H^+ secretion in the presence of luminal NEM is not clear at present. It is possible that the activity of NEM-sensitive ATPase (presumably H^+ ATPase) is only partially inhibited by NEM. Alternatively, another Na^+-independent H^+ extrusion mechanism might be present in the luminal or basolateral membrane. These possibilities were not examined further in this study.

Effect of acidosis on H^+/HCO_3^- transport. Acidosis did not change the pHi recovery rate from cell acidification in the absence of Na^+ (Fig. 2). Because the buffer capacity was similar in the control and acidosis conditions, our result suggested that luminal H^+ secretion is not activated by acidosis. With regard to basolateral transport, it was previously shown that Na^+-independent Cl^--HCO_3^- antiport and Na^+-HCO_3^- symport are HCO_3^- (or related base) exit processes in intercalated cells of

Table V. Effect of Addition of Bath Na^+ on pHi Recovery

	Control	Acidosis	DOCA
Initial pH	6.34±0.05	6.38±0.04	6.39±0.05
ΔdpHi/dt	6.8±0.9	11.3±0.9*	8.1±1.2
n	28/7	28/6	26/6

Values are means±SE; *n*, number of cells/tubules. 20 mM NH_3/NH_4^+ was removed from the bath in the absence of Na^+ and HCO_3^- (solution 10 to 9). After 50 s Na^+ was added to the bath at 135 mM (solution 9 to 8). Initial pHi, pHi just before Na^+ addition; ΔpHi/dt, an increase in pHi recovery rate caused by basolateral Na^+ addition. * $P < 0.01$ by ANOVA compared with controls.

Table VI. Effects of Lowering Bath HCO_3^- or Na^+ on pHi in the Absence of Cl^-

	Control	Acidosis	DOCA
HCO_3^- reduction			
initial pHi	7.51±0.03	7.46±0.04	7.50±0.03
dpHi/dt	6.0±0.7	5.7±0.6	6.8±0.7
n	25/6	22/5	21/5
Na^+ removal			
initial pHi	7.45±0.03	7.44±0.04	7.49±0.04
dpHi/dt	5.1±0.6	4.9±0.7	4.2±0.6
n	21/5	20/5	20/5

Values are means±SE; *n*, number of cells/tubules. HCO_3^- reduction, bath HCO_3^- was decreased from 25 to 2.5 mM (solution 5 to 6). Na^+ removal, bath Na^+ was totally replaced by *N*-methyl-D-glucamine and choline (solution 5 to 7). dpHi/dt, rates of pHi change (pHU/s × 10^3).

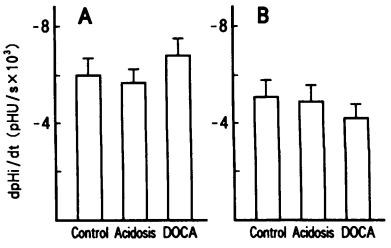

Figure 6. Rates of pHi change (dpHi/dt) by lowering bath HCO_3^- (*A*) or bath Na^+ removal (*B*). (*A*) Bath HCO_3^- was decreased from 25 to 2.5 mM at constant P_{CO_2} in Cl^--free condition (solution 5 to 6). Each bar shows means±SE of 21–25 cells from five to six tubules. (*B*) Total bath Na^+ was removed in Cl^--free condition (solution 5 to 7). Each bar shows means±SE of 20–21 cells from five tubules.

OMCDo (9). These transport activities were also unchanged in acidosis (Figs. 4, 6). Thus, acidosis may not stimulate H^+/HCO_3^- transport mechanisms that are thought to be associated with transepithelial H^+ secretion. This view is consistent with the finding that acidosis had no significant effect on HCO_3^- reabsorption rate in microperfused rabbit OMCDo (3) and OMCDi (16, 17). However, in intercalated cells of OMCD from both acute and chronic acidosis rats, Madsen and Tisher (14, 15) observed an increase in the surface density of the apical membrane and a depletion of the tubulovesicular structures (possibly containing a H^+ pump) in the apical region, using electron microscopy. Their findings suggested that a morphological adaptation occurs during acidosis. Biochemical measurements of NEM-sensitive ATPase activity showed conflicting results. Garg and Narang (18) observed an augmentation of this ATPase activity in response to acidosis in rat OMCD, whereas Sabatini et al. (19) failed to find such an augmentation in a similar condition in rat OMCD. The reason for these discrepancies is presently unclear. It might be partly due to the difference in the duration and the magnitude of acidosis, and to the species difference (rabbit vs. rat).

We previously showed the presence of basolateral Na^+-H^+ exchange in intercalated cells of OMCDo (6). This exchange has also been found in the cortical collecting tubule (CCT) (10, 34) and OMCDi (12). The primary role of basolateral Na^+-H^+ exchange in the collecting tubule is thought to be pHi regulation (6, 10, 12, 34). In the present study, we are making the first report that acidosis enhances basolateral Na^+-H^+ exchange activity of collecting duct cells (Fig. 5).

Effect of mineralocorticoids on H^+/HCO_3^- transport. In the absence of Na^+, an initial pHi recovery rate after cell acidification increased (+111%) with DOCA treatment (Fig. 2). DOCA did not change the buffering capacity. A morphological study found that mineralocorticoids did not increase the cell volume of intercalated cells (32). These data suggest that DOCA enhances H^+ extrusion in intercalated cells of OMCDo. NEM decreased dpHi/dt by 68%, but SCH28080 and omeprazole did not change dpHi/dt in DOCA-treated cells (Fig. 2). These results suggest that mineralocorticoids stimulate a NEM-sensitive H^+ pump rather than H^+-K^+-ATPase. Consistent with this observation, biochemical studies have demonstrated that aldosterone significantly increases the activity of NEM-sensitive ATPase in OMCD (21, 22).

DOCA also activated basolateral Na^+-independent Cl^--HCO_3^- antiport. Thus mineralocorticoids simultaneously stimulate the major mechanism of luminal H^+ secretion (NEM-sensitive H^+ pump) and basolateral HCO_3^- exit (Na^+-independent Cl^-/HCO_3^- exchange) in intercalated cells of OMCDo. This result is consistent with the increment of HCO_3^- reabsorption by DOCA treatment in rabbit OMCDo (3). Hays and Alpern (35) have recently reported a similar mineralocorticoid effect on cellular H^+/HCO_3^- transport of OMCDi in a preliminary form.

DOCA as well as acidosis did not alter the activity of basolateral Na^+-HCO_3^- symport (Fig. 6). This is in contrast to the results that acidosis activated basolateral Na^+-H^+ antiport (Fig. 5), and DOCA activated both luminal H^+ pump (Fig. 2) and basolateral Na^+-independent Cl^--HCO_3^- antiport (Fig. 4). This symport might be regulated by other factors besides acidosis and mineralocorticoids, or might play some other roles besides epithelial H^+ transport and pHi regulation.

Regulation of cellular H^+/HCO_3^- transport mechanisms of intercalated cells. Intercalated cells of OMCDo secrete H^+ into tubule lumen via a luminal H^+ pump (1, 4, 6), and the generated HCO_3^- exits mainly through Na^+-independent Cl^--HCO_3^- exchange (9). DOCA stimulated both of these two transports simultaneously (Fig. 4). These results suggest that mineralocorticoids specifically regulate transepithelial H^+ secretion. Simultaneous enhancement of apical H^+ secretion and basolateral HCO_3^- exit would allow an increase of transepithelial H^+ secretion without changing pHi.

Na^+-H^+ exchanger is present in a large variety of cell types, and one of its main roles is maintenance of normal pHi (28, 36). In renal epithelial cells, the localization of this exchanger depends on their functions. For example, Na^+-H^+ antiporter is present in the luminal membrane of proximal tubules to reabsorb luminal HCO_3^- (37–41). The antiporter also exists in the basolateral membrane of proximal tubules (42–44) and collecting ducts (6, 10, 12, 34). Its function is speculative, but regarded as pHi regulation.

The present study showed for the first time that acidosis stimulates basolateral Na^+-H^+ exchange (Fig. 5). This finding is consistent with the thesis that basolateral Na^+-H^+ exchange works for pHi regulation, because cell H^+ load would be increased through passive leakage during acidosis. Many studies of proximal tubules revealed that acidosis increases the transport rate of luminal Na^+-H^+ exchange (39–41). In contrast, examination of basolateral Na^+-H^+ exchange was lacking. Our finding, that acidosis stimulates basolateral Na^+-H^+ exchange, suggests that acidosis is a common trigger for an increase in both luminal and basolateral Na^+-H^+ exchange activity. Horie et al. (45) have reported in cultured proximal tubule cells that incubation with an acid medium enhances Na^+-H^+ antiport, indicating that a low pH per se increases its activity. However, localization of the antiport was not examined in their study. Krapf et al. (46) have recently showed an increase of Na^+-H^+ antiporter mRNA levels of renal cortex in metabolic acidosis by Northern blot analysis. Again, it is not clear whether the increased mRNA levels are associated with luminal or basolateral Na^+/H^+ antiporter. Further studies are necessary to understand the cellular and molecular mechanisms of the regulation of luminal and basolateral Na^+-H^+ antiporters.

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