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Research Article

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Chronic Metabolic Acidosis Causes an Adaptation in the Apical Membrane Na/H Antiporter and Basolateral Membrane $Na(HCO_3)_3$ Symporter in the Rat Proximal Convoluted Tubule

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Abstract

The effect of chronic dietary acid on the apical membrane Na/H antiporter and basolateral membrane $Na(HCO_3)_3$ symporter was examined in the in vivo microperfused rat proximal tubule. Transporter activity was assayed with the epifluorescent measurement of cell pH using the intracellular, pH-sensitive fluorescent dye, (2'7')-bis(carboxyethyl)-(5,6)-carboxyfluorescein (BCECF). BCECF was calibrated intracellularly, demonstrating similar pH-sensitivity of the dye in control and acidotic animals. In subsequent studies, lumen and peritubular capillaries were perfused to examine Na/H and $Na(HCO_3)_3$ transporter activity in the absence of contact with native fluid. The initial rate of change in cell pH (dpHi/dt) was 97, 50, and 44% faster in tubules from acidotic animals when peritubular $[HCO_3]$ was changed from 25 to 10 mM in the presence or absence of chloride, or peritubular [Na] was changed from 147 to 50 mM, respectively. dpHi/dt was 57% faster in tubules from acidotic animals when luminal [Na] was changed from 152 to 0 mM. Buffer capacities, measured using NH_3/NH_4^+ addition, were similar in the two groups. The results demonstrate that chronic metabolic acidosis causes an adaptation in the intrinsic properties of both the apical membrane Na/H antiporter and basolateral membrane $Na(HCO_3)_3$ symporter.

Introduction

Bicarbonate absorption in the rat proximal convoluted tubule is mediated on the apical membrane by a Na/H antiporter and a Na-independent, amiloride-insensitive mechanism, most likely a H-translocating ATPase (1–9). Base generated within the cell exits across the basolateral membrane via a $Na(HCO_3)_3$ symporter (10–20). Basolateral membrane Na-dependent and Na-independent Cl/HCO_3 exchangers have also been described (14, 21) but probably have little role in bicarbonate absorption (22, 23).

Previous studies have demonstrated that bicarbonate absorption in the proximal tubule is subject to regulation by a number of factors (24). In particular, acute decreases in peritubular pH stimulate, and acute increases inhibit bicarbonate absorption (25–27). Of great interest is the finding that chronic acid feeding stimulates bicarbonate absorptive capacity to a degree that is far greater than achieved by acute changes in blood pH (26, 28, 29).

Regulation of transcellular bicarbonate absorption involves regulation of both apical and basolateral membrane transport mechanisms. These mechanisms can be regulated in three ways: by alterating the driving forces across the transporters; by allosteric regulation of the transporters; and by chronic adaptations affecting either transporter characteristic or transporter number. Chronic adaptations of the Na/H antiporter have now been described in response to chronic metabolic and respiratory acidosis (30–36), chronic metabolic alkalosis (37), and conditions associated with increased glomerular filtration rate (38, 39) when transporter activity has been assayed in brush border membranes in vitro.

The purpose of the present studies was to examine the effect of chronic dietary acid on the intrinsic properties of the apical membrane Na/H antiporter and the basolateral membrane $Na(HCO_3)_3$ symporter in vivo. Animals fed acid chronically were compared to animals maintained on a control diet. Under identical luminal and peritubular conditions, apical and basolateral membrane transporter activity was assayed using the epifluorescent measurement of cell pH in in vivo microperfused proximal convoluted tubules. The results demonstrate that intrinsic stimulations have occurred in both the apical membrane Na/H antiporter and basolateral membrane $Na(HCO_3)_3$ symporter of chronically acidotic rats. From these studies we suggest that such adaptations in the apical and basolateral membrane transporters contribute to the enhanced capacity of the proximal tubule to reabsorb bicarbonate in chronic metabolic acidosis.

Methods

Animal groups

Experiments were performed using male Sprague-Dawley rats (Harlan Breeding Laboratories, Houston, TX) weighing 200–300 g. The animals were housed in individual metabolic cages so that they could be individually fed 18 g daily of a diet consisting of (in g/kg diet): casein, 180 g; cornstarch, 200 g; sucrose, 500 g; corn oil, 35 ml; peanut oil, 35 ml; $CaHPO_4$, 10 g; $MgSO_4$, 6 g; NaCl, 6 g; K_2HPO_4, 8.3 g; and vitamin fortification mixture (ICN Pharmaceuticals, Cleveland, OH), 10 g (28). All rats studied ate all of the diet daily. The rats were divided into two groups. The control rats (CON)[1] were fed the above diet for 3–6 d. The acidotic rats (CMA) were fed the same diet to which 20 mmol HCl/kg body wt was added daily for the 3–6 d the rats were on the diet. All animals were allowed water ad lib. The control rats gained an average of 16.0±4.0 g and the acid-fed rats 10.8±2.3 g (NS).

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1. *Abbreviations used in this paper:* BCECF, (2',7')-bis(carboxyethyl)-(5,6)-carboxyfluorescein; CMA, acidotic rats; CON, control rats; pHi, intracellular pH; SITS, 4-acetamido-4'-isothiocyano-stilbene-2,2' disulfonic acid.

In vivo microperfusion technique

The rats were prepared for in vivo microperfusion as previously described (1). Briefly, rats were anesthetized with an intraperitoneal injection of Inactin (100 mg/kg body wt with additional dose as needed) and placed on a heated table that maintained body temperature at 36.5–37.5°C. The right femoral artery was catherized for monitoring blood pressure and for obtaining blood samples (< 0.3 ml/d). The left jugular vein was catheterized for continuous infusion throughout the experiment. All animals received a maintenance infusion at 3 ml/h. The control animals were infused with a Ringer's bicarbonate solution containing in mM: NaCl, 105; $NaHCO_3$, 25; Na_2HPO_4, 4; KCl, 5; $MgSO_4$, 1; $CaCl_2$, 1.8; while the acidotic animals were infused with normal saline. The left kidney was exposed by a flank incision and immobilized in a lucite cup. The ureter was cannulated (PE-50) to ensure free drainage of urine. Proximal tubule transit time was measured with an intravenous injection of 0.02 ml of 10% Lissamine green dye, and only those kidneys with transit times of < 12 s were accepted for study.

Basolateral membrane studies. Capillary and luminal pipettes were placed using a Leitz dissecting microscope (Leitz Wetzlar, Rockleigh, NJ). Peritubular capillaries were perfused as previously described (12) with a 12–14-μm tip pipette designed to allow rapid changes between two perfusion fluids. After placement of the capillary pipette, the lumen of a proximal convoluted tubule was perfused at 40 nl/min using a thermally insulated microperfusion pump (Wolfgang Hampel, Berlin, FRG) with a 6–8-μm tip pipette as previously described (12). This pipette (loading pipette) contained the acetoxymethyl derivative of (2′7′)-bis(carboxyethyl)-(5,6)-carboxyfluorescein (BCECF). After the pipettes were perfusing the lumen and capillaries for 5–7 min, the dissecting microscope was moved out of position and a Leitz epifluorescence microscope (MPV Compact System; Leitz Wetzlar, Rockleigh, NJ) was moved into position.

Apical membrane studies. The peritubular capillary pipette was the same as described above. After loading the cells with BCECF for 6 min, the loading pipette was removed. A second pipette (6–8 μm tip) was placed in the lumen as previously described (3), allowing rapid changes between two luminal solutions. After all pipettes were placed the epifluorescent microscope was moved into place. In our system luminal and peritubular fluid changes are 80–90% complete in < 1 s.

The luminal and peritubular capillary perfusates are listed in Table I. All solutions were bubbled with 7% CO_2/93% O_2 at 37°C. In solutions 4 and 5 the Ca gluconate concentration was chosen to yield an ionized [Ca^{+2}] of 1.3–1.5 mM (21).

Analysis and calculations

Measurement of pHi. As previously described (12), fluorescence was measured alternately at 500 and 450 nm excitation (emission 530 nm) using an epifluorescence microscope with interference filters (Corion Corp., Holliston, MA). All results were corrected for background. The fluorescence excitation ratio (F_{500}/F_{450}) was calculated as the mean of two 500 nm excitation measurements divided by the 450 nm excitation measurement obtained between them. The results of our intracellular dye calibration (see Results) were used to convert fluorescence excitation ratios to an apparent pHi.

Rate of change in pHi. The initial rate of change in pHi was calculated as previously described (12). Briefly, during either a luminal or peritubular fluid change, fluorescence was followed with 500 nm excitation (pH-sensitive wavelength) on a chart recorder (Linseis Corp., Marina Del Rey, CA). The slope of a line drawn tangent to the initial deflection defines the initial rate of change of 500 nm fluorescence [$d(F_{500})/dt$]. We have previously demonstrated that fluorescence at 450 nm excitation is not measurably affected by pHi, and thus can be considered constant (12). Therefore, the rate of change in the fluorescence excitation ratio [$d(F_{500}/F_{450})/dt$] can be calculated using the formula:

$$d(F_{500}/F_{450})/dt = [d(F_{500})/dt]/F_{450} \quad (1)$$

where F_{450} represents the 450-nm excitation fluorescence corrected for background at the time of the fluid change (interpolated from the measurements before and after the fluid change). This rate of change in the fluorescent ratio was converted to a rate of change in pHi ($dpHi/dt$) by dividing by the slope of the in vivo calibration of BCECF [$d(F_{500}/F_{450})/dpHi$]. Tubules were perfused for 2–4 h after completion of surgery. There was no apparent time-dependent change in $dpHi/dt$ over the course of the experiment.

*Table I. Solutions**

	1	2	3	4	5	6	7	8	9	10
	mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*
NaCl	120	135	23	—	—	81	81	127	—	140
$NaHCO_3$	25	10	25	25	10	25	25	25	—	5
KCl	5	5	5	—	—	5	5	3	3	5
$MgSO_4$	1	1	1	1	1	1	1	1	1	1
Na_2HPO_4	1	1	1	1	1	1	1	—	—	1
K_2HPO_4	—	—	—	—	—	—	—	1	1	—
$CaCl_2$	1.8	1.8	1.8	—	—	1.8	1.8	1.8	1.8	1.8
Glucose	5	5	5	5	5	5	5	—	—	5
Alanine	5	5	5	5	5	5	5	—	—	5
Urea	5	5	5	5	5	5	5	5	5	5
Na gluconate	—	—	—	120	135	—	—	—	—	—
K gluconate	—	—	—	5	5	—	—	—	—	—
Ca $(gluconate)_2$	—	—	—	9.3	9.3	—	—	—	—	—
Choline CL	—	—	97	—	—	15	—	—	127	—
Choline HCO_3	—	—	—	—	—	—	—	—	25	—
NH_4Cl	—	—	—	—	—	—	15	—	—	—
Hepes	—	—	—	—	—	25	25	—	—	—
NaOH	—	—	—	—	—	22	22	—	—	—
pH	7.32	6.92	7.32	7.32	6.92	7.32	7.32	7.32	7.32	6.62

* All solutions bubbled with 7% CO_2/93% O_2 at 37°C.

Calibration of the pH-sensitive dye

Intracellular BCECF was calibrated in vivo in both control and acidotic rats as previously described (12). Tubules were perfused with well-buffered solutions containing 25 mM Hepes, 60 mM K_2HPO_4, and appropriate amounts of $NaHCO_3$ to give pHs ranging from 6.6 to 7.6. Before adding the $NaHCO_3$, the Hepes and K_2HPO_4 solutions were pH titrated at 37°C with NaOH. All solutions were brought to a final osmolality of 295–300 mosmol with raffinose. These solutions contained 120 meq/liter potassium, which was selected to estimate the approximate intracellular potassium activity (12) and 10 $\mu g/ml^3$ nigericin (a K/H antiporter). In this setting, pHi is predicted to equal extracellular pH. Cells were loaded with BCECF before exposure to nigericin and were then perfused with one of the above solutions in the capillary. Over ~ 5–10 min the fluorescence excitation ratio approached a steady value that was used as the result.

Intracellular buffer capacity

The intracellular buffer capacity was determined in tubules from both control and acidotic rats using the technique of rapid NH_3/NH_4^+ addition (40, 41). Both lumens and capillaries were perfused with solution 6 (Table I), which was pH titrated at 37°C to pH 7.32. Peritubular fluid was then suddenly changed to solution 7 (Table I), which differed from solution 6 by the substitution of 15 mM NH_4Cl for 15 mM choline chloride. The NH_3 in this solution rapidly enters the cell and combines with intracellular protons to form NH_4^+, leading to a rapid cell alkalinization. After fluorescence with 500 nm excitation, a rapid initial increase in fluorescence intensity (cell alkalinization) occurred, followed by a slow decrease in fluorescence intensity (cell pH defense and NH_4^+ diffusion). The initial deflection with 500 nm excitation was divided by the 450 nm value (interpolated from neighboring 450 nm measurements) to calculate the magnitude of change in the F_{500}/F_{450} ratio. In some tubules, the cell pH defense began during the initial deflection leading to an underestimate of the magnitude of the initial deflection. This was dealt with by extrapolating the 500 nm fluorescence curve back to the time of the fluid change, as previously described by Roos and Boron (41). The buffer capacity (β mmol/liter · pH U) is given by the formula:

$$\beta = \frac{[NH_4^+]i}{\Delta pHi} \quad (2)$$

where $[NH_4^+]i$ is the intracellular $[NH_4^+]$ after addition of NH_3/NH_4^+, and ΔpHi is the change in pHi upon addition of NH_3/NH_4^+. The initial pHi was calculated from the fluorescence excitation ratio just before the addition of NH_3/NH_4^+, and the final pHi was calculated from the fluorescence ratio at the peak of the spike. The difference between these two values was used as the ΔpHi. The intracellular concentration of NH_4^+ was calculated as:

$$[NH_4^+]i = [NH_4^+]_0 \cdot 10^{(pHo-pHi)} \quad (3)$$

where $[NH_4^+]_0$ is the $[NH_4^+]$ of the perfusate and pH_o is the pH of the perfusate. pHi was calculated from the fluorescence excitation ratio just after addition of NH_3/NH_4^+ (at the peak of the spike).

Statistics

The slopes and intercepts of the calibration curves were determined by linear regression analysis. All results are expressed as mean±SE. Statistical significance was assessed by the Student's t test for unpaired data.

Results

The purpose of the present studies was to examine the effect of chronic metabolic acidosis on the intrinsic properties of the apical membrane Na/H antiporter and the basolateral membrane $Na(HCO_3)_3$ symporter in the proximal convoluted tubule. Rats made acidotic by the daily addition of acid to their diet were compared to control rats maintained on the same diet without the addition of acid.

Arterial blood gases obtained before surgery, at the end of surgery, and at the end of the experimental day are shown in Table II. The rats maintained on the acid diet had a significantly lower plasma $[HCO_3]$ than did the rats maintained on the control diet. In both groups of animals, the plasma $[HCO_3]$ tended to drift down over the course of the day; however, it was significantly lower in the acidotic group throughout the course of the day.

Calibration of the pH-sensitive dye. To ensure that the dietary regimen did not affect the pH sensitivity of intracellular BCECF, an in vivo calibration of the dye was done in both groups. The data are shown in Fig. 1. Chronic acidosis had no effect on the in vivo calibration of BCECF, as neither the slopes nor the intercepts were significantly different ($P < 0.9$ and $P < 0.4$, respectively).

Effect of changing peritubular fluid $[HCO_3]$. Initial studies examined the effect of changing the peritubular fluid $[HCO_3]$ and pH on pHi. The lumen and capillaries were initially perfused with an ultrafiltrate-like solution containing 25 mM HCO_3 (solution 1, Table I). Under these conditions pHi was 7.11±0.03 in tubules in control and 7.13±0.02 in tubules in acidotic animals. The capillary perfusate was then changed to one in which 15 mM HCO_3 was replaced by 15 mM Cl (solution 2, Table I). Typical tracings are shown in Fig. 2. When peritubular $[HCO_3]$ was changed from 25 to 10 mM a rapid cell acidification was observed in both groups, reflected as a decrease in 500 nm excitation fluorescence intensity. Upon returning the peritubular fluid $[HCO_3]$ to 25 mM, pHi alkalinized back toward the initial pHi. It can be seen in Fig. 2 that the initial rate of change of 500 nm fluorescence was faster in the tubule in the acidotic animal.

The absolute values of the mean initial rates of change in pHi for all tubules are summarized in Fig. 3. In tubules in control rats, when the peritubular fluid $[HCO_3]$ was changed from 25 to 10 mM, pHi decreased at an initial rate of 3.7±0.4 pH U/min ($dpHi/dt$) (n = 13) to 6.85±0.03. Upon readdition of 25 mM HCO_3, pHi increased at an initial rate of 3.7±0.5

*Table II. Rat Arterial Blood Gases throughout the Experiment**

	Control animals	Acidotic animals
Before surgery		
pH	7.35±0.01	7.31±0.02
P_{CO_2}, mmHg	45.7±1.9	43.8±1.6
$[HCO_3^-]$, mM	25.3±0.6	22.0±0.8‡
End of surgery		
pH	7.38±0.01	7.33±0.01§
P_{CO_2}, mmHg	39.4±1.0	38.5±0.9
$[HCO_3^-]$, mM	23.3±0.8	20.4±0.8‖
End of experiment		
pH	7.39±0.01	7.37±0.01
P_{CO_2}, mmHg	36.6±0.6	32.0±1.5‖
$[HCO_3^-]$, mM	22.1±0.6	18.9±1.3‖

* Means±SE.
‡ $P < 0.005$ vs. control.
§ $P < 0.01$ vs. control.
‖ $P < 0.05$ vs. control.

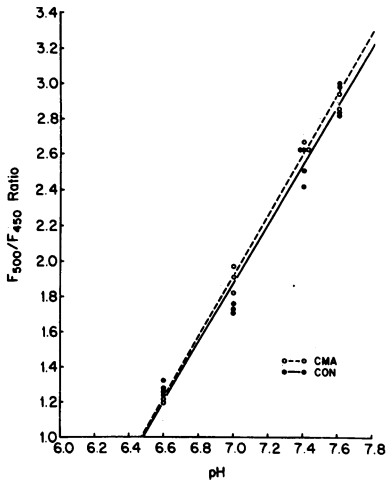

Figure 1. In vivo calibration of BCECF in control and chronic acidotic rats. F_{500}/F_{450} ratio is plotted as a function of pH. Solid circles and solid line are results from control animals; open circles and dash line results from chronically acidotic animals. The calibration was done using the nigericin technique (see Methods). The slopes are 1.69 and 1.72 (NS) and the *Y*-intercepts are −9.97 and −10.11 (NS) in tubules in control and acidotic animals, respectively.

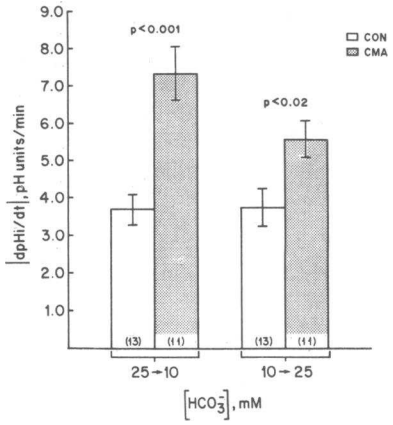

Figure 3. Initial rate of change in pHi in response to a peritubular fluid bicarbonate change. The absolute value of the initial rate of change in cell pH, ($|dpHi/dt|$), is plotted on the *y*-axis. Open bars represent results from tubules in control animals; stippled bars result from tubules in chronically acidotic animals. The left set of bars are the mean±SE $dpHi/dt$ when peritubular fluid $[HCO_3^-]$ was changed from 25 to 10 mM and the right set of bars the mean±SE $dpHi/dt$ when peritubular fluid $[HCO_3^-]$ was changed from 10 to 25 mM.

pH U/min to 7.16±0.03 (n = 13). When the same peritubular $[HCO_3]$ changes were made in tubules in acidotic rats, $dpHi/dt$'s were significantly faster [7.3±0.7 (n = 11) and 5.6±0.5 (n = 11) pH U/min, $P < 0.001$ and $P < 0.02$, respectively] compared to tubules in control rats. In tubules from acidotic animals pHi decreased from 7.13±0.02 to 6.82±0.02 and returned to 7.19±0.02 with the peritubular fluid changes. Thus, under identical luminal and peritubular fluid conditions, a change in the peritubular $[HCO_3]$ and pH resulted in a faster initial rate of change in pHi in tubules in acidotic rats, suggesting that an adaptation had occurred in a basolateral membrane bicarbonate transport mechanism in chronic acidosis.

In response to the peritubular fluid $[HCO_3]$ change in the above studies, steady-state pHi changed by 0.29±0.01 pH U in tubules in control rats (n = 11); in tubules in acidotic rats the change was 0.34±0.01 pH U (n = 13). Although the difference between these steady-state pH changes was small, it was statistically significant ($P < 0.01$). The fact that the magnitude of change in steady-state pHi was larger in tubules in acidotic rats supports the conclusion that an adaptation had occurred in a basolateral membrane bicarbonate exit step.

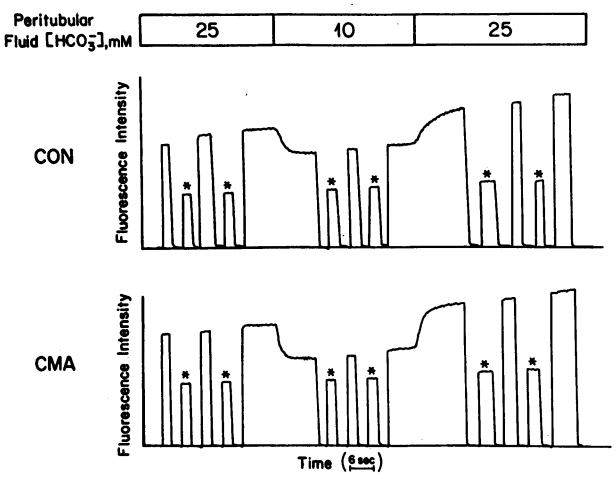

Figure 2. Typical tracing: response of cell pH to peritubular fluid $[HCO_3^-]$ change. Fluorescence intensity (*y*-axis) is plotted as a function of time (*x*-axis). Fluorescence intensity is measured alternately with 500 and 450 nm excitation, emission 530 nm. Asterisks are above bars measured with 450 nm excitation, the pH-insensitive wavelength. During a change in peritubular fluid composition fluorescence intensity with 500 nm excitation (pH-sensitive wavelength) is followed. A decrease in fluorescence intensity represents a cell acidification. The top panel is from a tubule in a control animal. The bottom panel is from a tubule in an acidotic animal.

Effect of changing peritubular fluid [Na]. To determine whether a Na-coupled bicarbonate transporter was involved in the apparent adaptation on the basolateral membrane, we performed similar studies in which peritubular fluid [Na] was changed from 147 to 50 mM. Choline was substituted for Na. The lumen was perfused with solution 1 (Table I) and the capillaries were perfused with solution 1 in the control and recovery periods and solution 3 in the experimental period (Table I). In the control periods with 147 mM Na in both the lumen and capillaries, pHi was 7.20±0.02 in tubules in control and 7.36±0.05 in tubules in acidotic animals. As shown in Fig. 4, in tubules in control animals when peritubular [Na] was changed from 147 to 50, the cells acidified at an initial rate of 3.4±0.4 pH U/min (n = 6) to 6.96±0.02. When peritubular [Na] was returned to 147 mM, the cells realkalinzed at an initial rate of 2.8±0.2 pH U/min (n = 7) to 7.19±0.02. Again, these rates were significantly faster in tubules in acidotic rats (4.9±0.2 [n = 8] and 4.3±0.5 [n = 9] pH U/min, $P < 0.003$ and $P < 0.03$, respectively). pHi decreased from 7.36±0.05 to 7.08±0.05 in the experimental period and returned to 7.37±0.07 in the recovery period. These data suggest that a Na-coupled bicarbonate transporter was involved in the adaptation on the basolateral membrane.

When peritubular [Na] was changed, the steady-state pHi changed by 0.24±0.02 pH U in tubules in control (n = 7) and 0.28±0.02 pH U in tubules in acidotic rats (n = 9). Again, the magnitude of the change in pHi was larger in tubules in acidotic rats, although in this series the two values were not statistically different (P = 0.07).

Dependence of the basolateral membrane adaptation on chloride. Both Cl-independent and -coupled Na/HCO_3 transport mechanisms have been identified on the basolateral membrane of the rat and rabbit proximal tubule (14, 21). The Cl-independent $Na(HCO_3)_3$ symporter appears to be the major basolateral membrane transport mechanism effecting bicar-

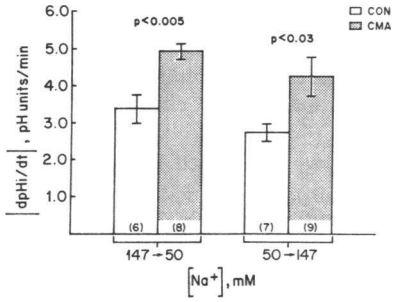

Figure 4. Initial rate of change in pHi in response to a peritubular fluid sodium change. *Y*-axis is as described for Fig. 3. Open bars represent results from tubules in control animals; stippled bars results from tubules in chronically acidotic animals. The left set of bars are the mean±SE dpHi/d*t* when peritubular [Na^+] was changed from 147 to 50 mM; right set of bars mean±SE dpHi/d*t* when [Na^+] was returned from 50 to 147 mM.

bonate absorption (22, 23). To ascertain whether the adaptation occurred in this Cl-independent mechanism, a peritubular fluid [HCO_3] change was made as described above, except that chloride was absent from both the luminal and peritubular fluids. Gluconate was substituted for chloride. Both the lumen and capillaries were initially perfused with solution 4 (Table I), which contained 25 mM HCO_3. Under these conditions pHi was 6.99±0.01 in tubules in control and 6.91±0.02 in tubules in acidotic animals. The capillary perfusate was then changed to one with 10 mM HCO_3 (solution 5, Table I). The data are shown in Fig. 5. In tubules in control rats, dpHi/d*t* was 2.8±0.4 (n = 13) and 2.2±0.2 (n = 13) pH U/min when peritubular [HCO_3] was changed from 25 to 10 (cell acidification) and returned to 25 mM (cell alkalinization), respectively. With these maneuvers, pHi decreased from 6.99±0.01 to 6.86±0.02 and returned to 7.01±0.02. Again, with identical changes in peritubular fluid [HCO_3] these initial rates were significantly faster in tubules in acidotic rats (4.2±0.4 [n = 8] and 3.2±0.4 [n = 8] pH U/min, $P < 0.03$ in both cases). In these tubules pHi decreased from 6.91±0.02 to 6.74±0.02 and returned to 6.92±0.03. Thus, the adaptation was still present in the absence of Cl from both the luminal and peritubular fluids, suggesting that the basolateral membrane adaptation in chronic acidosis involves the Cl-independent $Na(HCO_3)_3$ transporter.

In the absence of Cl, the peritubular fluid [HCO_3] change resulted in a steady-state pHi change of 0.14±0.01 pH U (n = 13) in tubules in control rats. Again, in tubules in acidotic rats, the magnitude of change was larger, 0.17±0.02 pH U (n = 8), although the values were not statistically different.

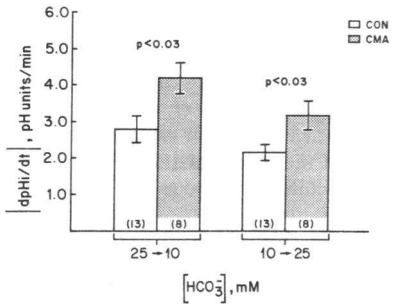

Figure 5. Initial rate of change in pHi in response to a peritubular fluid bicarbonate change in the absence of chloride. *Y*-axis as described for Fig. 3. Open bars represent results from tubules in control animals; stippled bars results from tubules in chronically acidotic animals. The left set of bars represent mean±SE dpHi/d*t* when peritubular [HCO_3^-] was changed from 25 to 10 mM; the right set of bars mean±SE dpHi/d*t* when [HCO_3^-] was returned from 10 to 25 mM.

Effect of changing luminal fluid [*Na*]. We have previously shown that the apical membrane amiloride-sensitive Na/H antiporter mediates ~ 65% of bicarbonate absorption (1). The next series of studies was designed to examine the activity of the apical membrane Na/H antiporter in control and chronically acidotic rats. For these studies the peritubular capillaries were perfused with a solution containing 5 mM HCO_3 and 1 mM 4-acetamido-4′-isothiocyano-stilbene-2,2′ disulfonic acid (SITS) (solution 10, Table I). We have previously shown that this protocol inhibits basolateral membrane bicarbonate movement without significantly alkalinizing cell pH, thus allowing changes in the rate of the apical membrane Na/H antiporter to change pHi (3). After loading the cells with BCECF, the lumen was initially perfused with a Na-containing solution without glucose and alanine (solution 8, Table I). Under these conditions pHi was 7.17±0.05 in tubules in control rats and 7.20±0.06 in tubules in acidotic rats. Then the luminal perfusate was rapidly changed to a Na-free solution (solution 9, Table I). We have previously shown that any effect on cell pH with this maneuver reflects a change in the rate of the amiloride-sensitive Na/H antiporter (1). The data are shown in Fig. 6. In tubules in control rats, when luminal [Na] was decreased from 152 to 0 mM, the cells acidified at a rate of 3.70±0.31 pH U/min (n = 8) and pHi decreased to 6.78±0.06. When Na was returned to the luminal perfusate, the cells realkalinized at a rate of 3.57±0.32 pH U/min (n = 8) and pHi increased to 7.18±0.04. In tubules in acidotic animals dpHi/d*t* was 5.81±0.57 (n = 8) (cell acidification) and 4.91±0.40 pH U/min (cell alkalinization) (n = 8) when luminal [Na] was decreased from 152 to 0 mM and returned to 152 mM ($P < 0.006$ and $P < 0.03$, respectively, compared to tubules from control animals). In the tubules from acidotic animals, pHi decreased from 7.20±0.06 to 6.75±0.05 and returned to 7.22±0.07 with the luminal fluid [Na] changes.

In tubules from control animals, the luminal fluid [Na] change resulted in a steady-state pHi change of 0.43±0.04 pH U (n = 9). The magnitude of this change was higher in tubules from acidotic rats (0.47±0.05 pH U), although the values are not statistically different.

Intracellular buffer capacity. In all of the above studies, the difference in the initial rate of change in pHi in the two groups of animals (dpHi/d*t*) was interpreted to reflect a difference in the initial change in rate of H/OH/HCO_3 movement across the apical or basolateral membranes. This interpretation is only

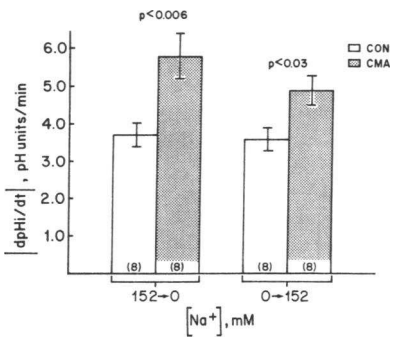

Figure 6. Initial rate of change in pHi in response to a luminal fluid sodium change. *Y*-axis as described for Fig. 3. Open bars represent results from tubules in control animals; stippled bars represent results from tubules in chronically acidotic animals. The left set of bars represent mean±SE dpHi/d*t* when luminal [Na^+] was changed from 152 to 0 mM; the right set of bars the mean±SE dpHi/d*t* when luminal [Na^+] was returned from 0 to 152 mM.

valid if the ability of the cells to defend cell pH, i.e., the buffer capacity, is the same in the two conditions. To determine this we measured intracellular buffer capacity using the technique of NH_3/NH_4^+ addition as previously described (40, 41). In these studies, both lumens and peritubular capillaries were initially perfused with solution 6 (Table I). Then the peritubular fluid was rapidly changed to one that was similar except that 15 mM NH_4Cl substituted for 15 mM choline chloride (solution 7, Table I). As described in Methods, buffer capacity was calculated from the initial pHi change. In tubules in control rats, the buffer capacity was 77±13 mmol/liter · pH U (n = 9) (Fig. 7). This value was similar to that obtained in tubules in acidotic rats, 74±9 mmol/liter · pH U (n = 13) ($P < 0.9$). Thus, the differences in dpHi/dt that were observed when luminal or peritubular fluid changes were made were due to true differences in transporter rates.

Discussion

The purpose of this study was to examine whether the intrinsic properties of the apical membrane Na/H antiporter and basolateral membrane $Na(HCO_3)_3$ symporter in the rat proximal convoluted tubule are altered in chronic metabolic acidosis.

The amiloride-sensitive Na/H antiporter on the apical membrane mediates about 65% of transepithelial bicarbonate absorption in the rat proximal convoluted tubule (1). In the present studies antiporter activity was assayed by following pHi while changing luminal [Na] in tubules from control and acidotic animals. A 57% faster initial rate of change in pHi was observed in tubules from acidotic animals, demonstrating a stimulatory adaptation in the antiporter in chronic metabolic acidosis.

Bicarbonate transport across the basolateral membrane has been demonstrated to occur by at least three possible transport mechanisms: a $Na(HCO_3)_3$ symporter (10–20), a Na-dependent Cl/HCO_3 exchanger, and a Na-independent Cl/HCO_3 exchanger (14, 21). As > 90% of basolateral membrane H-equivalent flux is dependent on the presence of sodium (12), it is unlikely that the Cl/HCO_3 exchanger plays a major role in bicarbonate transport. In the present studies if one compares dpHi/dt in response to a peritubular fluid [HCO_3] and pH change performed in the presence and absence of Cl (3.7 and 2.8 pH U/min, respectively), it can be seen that ~ 60–70% of basolateral membrane H-equivalent flux is Cl-independent, and thus mediated by the $Na(HCO_3)_3$ symporter. Similar results have been reported by Sasaki and co-workers (14). In addition, studies in the in vitro perfused proximal convoluted tubule have demonstrated that Cl removal from luminal and peritubular fluids has no effect on the rate of transcellular $NaHCO_3$ absorption (22, 23). Based on these findings, it appears that the $Na(HCO_3)_3$ symporter is the major mechanism for basolateral membrane bicarbonate efflux effecting transepithelial bicarbonate absorption.

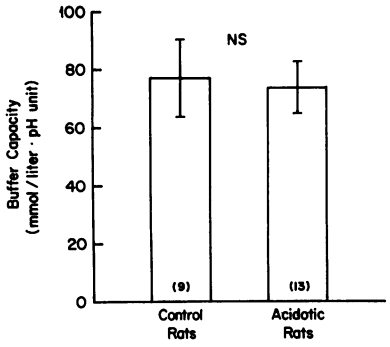

Figure 7. Buffer capacity (using NH_3/NH_4^+ addition). Buffer capacity is plotted on the y-axis.

When peritubular [HCO_3] was decreased from 25 to 10 mM, the change in cell pH occurred at a 97% faster initial rate in tubules in acidotic rats. A similar faster initial rate of change in cell pH was observed in response to a peritubular fluid [Na] change, demonstrating that the adaptation occurred in a Na-coupled transport mechanism. In addition, the observed increased initial rate of cell pH change in response to a peritubular fluid [HCO_3] change occurred in the absence of luminal and peritubular chloride, demonstrating that the effect was on a Cl-independent transporter.

Before concluding that these results represent adaptations in the Na/H antiporter and $Na(HCO_3)_3$ symporter, other possible explanations needed to be considered. First, it was possible that the pH-sensitivity of BCECF was different in cells of control and acidotic animals. If the dye sensitivity had a greater slope [$d(F_{500}/F_{450})/dpH$] in cells in acidotic animals, similar transporter rates would have led to more rapid changes in the fluorescent excitation ratio. Dye calibrations, using the nigericin technique, in tubules in control and acidotic animals found identical calibrations, and thus eliminated this concern.

A second possible concern was that the more rapid initial changes in cell pH in tubules in acidotic animals could be due to a lower buffer capacity in these tubules. If cells in acidotic animals were less able to defend their cell pH, i.e., had a lower buffer capacity, a given change in rate of H-equivalent flux could have a more rapid effect on cell pH. To address this problem, we measured intracellular buffer capacity using NH_3/NH_4^+ addition as described by Roos and Boron (41).[2] These studies yielded similar buffer capacities of ~ 75 mmol/liter · pH U, excluding differences in buffer capacity as an explanation for the enhanced dpHi/dt in tubules in acidotic rats.[3]

2. On theoretical grounds the buffer capacity is a constant value and should be independent of the method (i.e., NH_3/NH_4^+ addition vs removal). We observed a higher buffer capacity with NH_3/NH_4^+ removal as compared to addition in both groups. The values in control and acidotic rats were not statistically different ($P < 0.2$). There are two possible explanations for this observation. One is that the apical membrane Na^+/H^+ antiporter is transporting NH_4^+ out faster following NH_3/NH_4^+ removal than it transports NH_4^+ in following NH_3/NH_4^+ addition. Since pHi is more acidic following NH_3/NH_4^+ removal it is possible that allosteric regulation of the Na^+/H^+ antiporter (42) is responsible for the faster efflux than influx. The other possible explanation is that the measured buffer capacity is a function of pHi. Boron (40) has mathematically described the buffer capacity as a function of pHi, and found that lowering pHi (as occurs with NH_3/NH_4^+ removal) leads to a higher buffer capacity. Both of these possibilities would lead to an overestimate of the buffer capacity. However, as the purpose of these studies was to rule out a decrease in buffer capacity in cells in acidotic rats as the explanation of the faster dpHi/dt, we were more interested in the similarity of the values in the two groups, and not the absolute values.

3. Any differences in intracellular [NH_3] between the two groups should not affect our measurement of buffer capacity as adding NH_3 to the capillary should cause a similar increase in cell [NH_3] in both groups. Good and DuBose (46) have shown that in the late proximal tubule luminal [NH_3] was 3.1 μM in control and 4.8 μM in acidotic rats. If one assumes that this is approximately equal to the cell [NH_3],

Buffer capacity was measured without inhibiting any transport mechanisms. While concern exists as to whether membrane transporters are participating in cell pH defense in this setting, and thus leading to an overestimate of the true buffer capacity, this protocol was chosen because such transporters would also have contributed to the defense of cell pH during the experimental luminal or peritubular fluid composition changes. Thus, the protocol used should be a good estimate of the cells' ability to defend pH during the first few seconds of a peritubular fluid change.[4] Our results are similar to those reported by Yoshitomi, Burckhardt, and Fromter using a P_{CO_2} change (5 to 10 kPa) in the rat PCT (13), and those of Krapf et al. using NH_3/NH_4^+ removal in the rabbit PCT (16).

A third concern was that differences in cell composition ([Na], pH) or potential difference were responsible for the greater transporter rates in tubules in acidotic rats. Although we maintained identical luminal and peritubular composition, we could not assure identical cell composition. However, while differences in cell composition will affect steady-state transporter rate, they will not necessarily affect our assay for transporter activity, dpHi/dt (a measure of the initial rate of change in transporter rate in response to change in a driving force). The fact that vesicle studies have also found these adaptations, suggests that differences in cell composition are not responsible for our results.

Enhanced Na/H antiporter and $Na(HCO_3)_3$ symporter activity in metabolic acidosis could be expected to cause a larger change in steady-state pHi in tubules in acidotic animals in response to changes in luminal [Na] or peritubular [HCO_3] or [Na]. However, the steady-state pHi is determined by all of the H-transport mechanisms in the cell. It could be predicted that the initial rate of change of pHi (dpHi/dt) would be less sensitive to effects of other transporters, and therefore a more sensitive index of the adaptation than the change in steady-state pHi. This is what was observed. While changes in steady-state pHi were greater in acidotic animals in all series, differences were small, and inconsistently statistically significant.

In previous studies, we found that when luminal bicarbonate concentration was maintained constant, decreasing peritubular bicarbonate concentration stimulated the rate of bicarbonate absorption (26). However, Kunau and co-workers (28) found a much greater stimulation of bicarbonate absorption after chronic acid feeding than we would have predicted for the change in peritubular pH and [HCO_3], suggesting that chronic acid-feeding caused an adaptation in the proximal tubular cell. The present studies demonstrate that adaptations have occurred in the transport mechanisms involved in transepithelial bicarbonate absorption on both the apical and basolateral membranes. These studies are in agreement with a number of in vitro studies. Chronic acid feeding has been found in rats, rabbits, and dogs to cause a stimulation of Na/H antiporter activity as assayed in semipurified brush border membranes (31–35). Akiba et al (34) demonstrated that in vivo changes in blood pH lead to changes in $Na(HCO_3)_3$ transporter activity assayed in vitro (basolateral membrane vesicles). The present studies demonstrate that these adaptations are present in the intact tubule under physiologic conditions (normal intracellular and extracellular fluid composition).

The role of these adaptations in acid-base regulation is not presently clear. Stimulation of both the Na/H antiporter and $Na(HCO_3)_3$ symporter in metabolic acidosis should enhance bicarbonate reabsorptive capacity. The present studies demonstrate that chronic administration of large amounts of acid leads to only a slight decrease in plasma [HCO_3]. The small change in plasma [HCO_3] demonstrates the rat's capacity to excrete the acid load, and the sensitivity of the adaptations observed. In addition, the small change in plasma [HCO_3] means that the required increased net acid excretion after a chronic large acid load may occur in the presence of only slight decreases in plasma [HCO_3], and thus large filtered loads of HCO_3. In this setting, rates of proximal tubular HCO_3 absorption must be large to achieve minimal distal HCO_3 delivery.

In addition, changes in cell H transport rate can affect end proximal [HCO_3] even if filtered HCO_3 load is small (44). Whereas in tight epithelia, changes in transporter rate do not necessarily affect the limiting pH gradient that can be established, in leaky epithelia, such as the proximal tubule, this is not the case. In leaky epithelia, the maximal pH gradient is determined by the relative rates of the H-equivalent "pump" and "leak." In the late proximal tubule we have shown that the leak flux is 250% of the net flux (43), and thus changes in cell transport rate will be an important determinant of the pH in the late proximal tubule.

An additional role for these adaptations may be related to ammoniagenesis. Metabolism of glutamine in the proximal tubule leads to generation of NH_4^+ and base equivalents, which are believed to be metabolized to HCO_3. Previous studies have shown that the apical membrane Na/H antiporter can function as a Na^+/NH_4^+ exchanger (45, 46). Bicarbonate formed from glutamine metabolism would have to leave the cell on the basolateral membrane $Na(HCO_3)_3$ symporter. If this bicarbonate remained in the cell, cell pH would increase, which would inhibit ammoniagenesis. Good and DuBose have shown that the majority of increased ammonia secretion occurs in the early proximal tubule in acidosis (47). The adaptation observed in the present studies in the late proximal tubule may also occur in the early proximal tubule.

In the present studies, under identical luminal and peritubular fluid conditions (25 mM HCO_3), we did not find a consistent relationship between cell pH in tubules in control and acidotic animals. However, since there is a fair amount of variability in baseline cell pH, we cannot rule out missing a small difference in pHi between the groups. Nevertheless, it appears that with parallel adaptations in the Na/H antiporter, which extrudes acid, and $Na(HCO_3)_3$ symporter, which extrudes base, pHi is similar when luminal and peritubular fluids contain 25 mM HCO_3. While the adaptation in the Na/H antiporter in metabolic acidosis will defend cell pH in addition to increasing transepithelial bicarbonate absorption, the adaptation in the $Na(HCO_3)_3$ transporter will acidify the cell while enhancing bicarbonate absorption. The net result is no change in pHi with similar extracellular fluids. However, in acidotic animals when the native, more acid fluid is present in the lumen and capillaries, pHi will be lower.

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then the difference in cell [NH_3] = $\sim$ 1.7 μM. During the buffer capacity experiments we added 200 μM NH_3 to the peritubular fluid. Thus, the 1.7 μM difference in intracellular [NH_3] between the groups is small compared to the increase in cell [NH_3] when NH_3/NH_4^+ is added to the peritubular fluid.

4. In preliminary studies we have found that buffer capacity measured in the absence of sodium and chloride was slightly lower, 64±12 mmol/liter · pH U (unpublished observations).

In summary, the present studies demonstrate that chronic metabolic acidosis leads to intrinsic adaptations in both the apical membrane Na/H antiporter and basolateral membrane $Na(HCO_3)_3$ symporter in rat proximal tubules. These adaptations can be demonstrated in vivo in the absence of contact with native luminal and peritubular fluids. These adaptations contribute to the enhanced capacity for proximal tubular bicarbonate absorption observed in chronic acidosis, and may contribute physiologically to increased ammoniagenesis and a lower distal bicarbonate delivery.

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