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Effect of chronic acid loading on rat renal basolateral membrane bicarbonate transport

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The effect of chronic acid loading on the activity of luminal membrane Na^+–H^+ exchange and basolateral membrane Na^+/HCO_3^- cotransport and Cl^-–HCO_3^- exchange was investigated using membrane vesicles isolated from rat renal cortex. Na^+–H exchange activity was increased approx. 50% in brush-border membranes isolated from acidemic compared to control kidneys. Na^+/HCO_3^- cotransport and Cl^--HCO_3^- exchange activity was increased approx. 45% and 100%, respectively, in basolateral membranes isolated from acidemic kidneys. The increased Na^+/HCO_3^- cotransport activity resulted from an increased apparent maximal rate of transport (V_{max}) with no change in affinity (K_m) for Na^+. In contrast to acid/base transport activities chronic acid loading had no effect on the activity of basolateral membrane Na^+/dicarboxylate cotransport. These results suggest proximal tubule cells coordinately increased luminal and basolateral membrane acid/base transport activities to accomodate an adaptive increase in the capacity for transcellular bicarbonate reabsorption.

Introduction

Toward restoration of a normal acid-base balance, the kidney adapts to chronic acid loading by increasing acid excretion. This renal compensatory response to chronic acidosis is effected by an increased capacity of proximal and distal tubules to secrete protons. Increased proximal tubular acidification facilitates a more complete reabsorption of filtered bicarbonate and increased distal tubular acidification generates 'new' bicarbonate to replace base equivalents lost in the immediate titration of the acid load.

The observed increase in the capacity of proximal tubules to reabsorb bicarbonate in response to chronic acid loading suggest a possible adaptive increase in the activities of those luminal and basolateral membrane acid/base transport mechanisms responsible for transcellular bicarbonate reabsorption [1–5]. At the luminal membrane an Na^+–H^+ exchange mediates the bulk of proximal tubular acidification by coupling the downhill influx of Na^+ to uphill efflux of H^+ [6–9]. In vitro assays of Na^+–H^+ exchange activity in brush-border membrane vesicles prepared from chronic acid-loaded and control animals demonstrate an acidosis-induced increase in exchange activity which may account in part for the increased bicarbonate reabsorptive capacity of proximal tubules [10–14]. More recently, this adaptive response to chronic acid loading has been verified in the in vivo microperfused rat proximal tubule preparation [15]. Kinetic analysis suggests the acidosis-induced increase in Na^+–H^+ exchange activity results from an increased number of Na^+–H^+ exchange molecules present in the luminal membrane and/or an increased rate of turnover for an individual transport event [10,12–14]. In addition to chronic acid loading, other physiological perturbations such as parathyroidectomy [11], K^+ depletion [16], glucocorticoid administration [17] and increased glomerula filtration rate [18–20] are reported to increase proximal tubule Na^+–H^+ exchange activity when assayed for in brush-border membrane vesicles.

At the basolateral membrane of the proximal tubule, the process of transcellular bicarbonate reabsorption is completed by net transfer of bicarbonate from cell to blood. Both an Na^+/HCO_3^- cotransport [21–26] and a Cl^-–HCO_3^- exchange [27–30] mechanism have been identified as basolateral membrane bicarbonate efflux

Abbreviations: TMA, tetramethylammonium; Hepes, *N*-2-hydroxyethylpiperazine-*N*-ethanesulfonic acid; Mes, *N*-morpholinoethanesulfonic acid; DIDS, 4,4-diisothiocyanostilbene-2,2-disulfonic acid.

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pathways; however, recent evidence suggests the former may be quantitatively more significant [28]. Similar to the increase in luminal membrane Na^+-H^+ exchange activity, the proximal tubule may also increase basolateral membrane bicarbonate transport to facilitate a greater bicarbonate reabsorptive capacity observed in response to chronic acid loading. Furthermore, the coordinate upregulation of both luminal and basolateral membrane acid/base transport activity may serve to maintain cell pH while accomodating increased levels of transcellular bicarbonate reabsorption.

In this report, the increased capacity of proximal tubules to transport bicarbonate in response to chronic acid loading was investigated at the cell membrane level by assessing the activities of acid/base transport pathways in luminal and basolateral membrane vesicles. The specific activities of brush-border membrane Na^+-H^+ exchange and basolateral membrane $Cl^-HCO_3^-$ exchange and Na^+/HCO_3^- co-transport were determined from measurements of tracer uptake by membrane vesicles purified simultaneously from the luminal and basolateral side of the same cells. Similar to previous studies using rabbit membrane vesicles [14], and in vivo, microperfused rat proximal tubules [15], an increased level of luminal membrane Na^+-H^+ exchange activity and basolateral membrane Na^+/HCO_3^- cotransport activity was detected in membranes from chronic acid loaded rats. In addition, an increased level of $Cl^-HCO_3^-$ exchange activity was also measured in basolateral membranes from acidotic kidneys. These findings suggest an increased activity of luminal and basolateral membrane acid/base transporters may contribute to the increased capacity of proximal tubules to reabsorb bicarbonate in response to chronic acid loading.

Material and Methods

Chronic acid loading

Male Sprague-Dawley rats (225–250 g) were fed standard rat chow and allowed to drink 1.5% (w/v) NH_4Cl or distilled water for 7 days. Inactin-anesthetized rats (100 mg/kg, i.p.) were placed on a heating pad and arterial blood (1 cm^3) was immediately sampled from the abdominal aorta using a heparin-coated syringe. Blood samples were immediately transferred to a radiometer ABL2 blood gas analyzer to evaluate the acid/base status of each rat by determination of arterial blood pH, p_{CO_2} and HCO_3 concentration.

Membrane preparations

Basolateral membrane vesicles were prepared from acidotic and control kidneys by differential and Percoll density gradient centrifugation as previously described [26]. Basolateral membrane purification was assessed by the enrichment of Na^+/K^+-ATPase specific activity compared to homogenate [31]. Brush-border membrane contamination of the basolateral membrane preparation was assessed by the enrichment of maltase specific activity compared to homogenate [32]. Membrane protein was determined by a sodium dodecyl sulfate (SDS) Lowry assay with bovine serum albumin as the standard [33]. The orientation of basolateral membrane vesicles in the right-side-out and inside-out configuration was estimated from the latency of Na^+/K^+-ATPase activity using SDS as a membrane permeabilizing reagent [34]. Freshly prepared basolateral membrane vesicles were resuspended in 100 mM TMA gluconate, 80 mM Hepes/TMA (pH 7.5) and held overnight at 4°C for flux assays performed the following day.

Upon removing the basolateral membrane fraction from the Percoll density gradient brush-border membrane vesicles were further isolated from the remaining membranes by divalent cation aggregation. Briefly, membranes were centrifuged $200\,000 \times g$ for 60 min to remove the Percoll and resuspended in 250 mM sucrose, 10 mM Hepes/TMA (pH 7.5) using three strokes of a Potter-Elvehjem homogenizer. The $MgCl_2$ concentration of the membrane suspension was brought to 12 mM and the suspension was incubated 15 min on ice. The Mg^{2+}-induced membrane aggregates were pelleted by centrifuging the incubate $2400 \times g$ for 15 min and the resulting supernatant was further centrifuged $30\,000 \times g$ for 30 min. The high-speed pellet was resuspended in sucrose buffer and the $MgCl_2$ incubation was repeated. The incubate was again centrifuged $2400 \times g$ for 15 min and the resulting supernatant centrifuged $31\,000 \times g$ for 30 min to pellet the purified brush-border membrane vesicles. Brush-border membrane purification was assessed by the enrichment of maltase specific activity compared to the homogenate. Contamination of the brush-border membrane preparation by basolateral membrane was assessed by the enrichment of Na^+/K^+-ATPase specific activity compared to homogenate. Brush-border membranes vesicles were resuspended in 100 mM TMA gluconate, 80 mM Hepes/TMA (pH 7.5) and centrifuged $35\,000 \times g$ for 30 min. The brush-border membrane pellet was again resuspended and recentrifuged and the vesicles were used immediately in tracer flux assays.

Isotopic flux measurements

Membrane vesicles were brought to 10–30 mg protein/ml in TMA gluconate buffer and isosmotic solutions of appropriate ionic composition were added to obtain the desired intravesicular solution described for each experiment in the figure and table legends. The membrane suspensions were incubated 1.5 h at room temperature to facilitate transmembrane equilibration of the added media. Membranes assayed for ion-coupled HCO_3 transport activity were gassed continuously during the pre-equilibration period with humidified 90%

N_2/10% CO_2. The extravesicular media were prepared similarly and the final composition for each experiment is given in the figure and table legends. Intravesicular ^{22}Na or ^{36}Cl content was assayed in quadruplicate in the absence or continued presence of 90% N_2/10% CO_2 by a rapid filtration technique previously described [26,30]. Initial rates of Na^+ uptake were determined from 2 s time points using the acoustic signal of a metronome [35]. The uptake was quenched by the rapid addition of isosmotic potassium gluconate, 10 mM Hepes/TMA (pH 7.5) kept at 4°C. The diluted membrane suspension was passed through a 0.65 μm Millipore filter (DAWP) and washed with an additional 9 ml of quench buffer. The filters were dissolved in 3 ml of Ready-Solv HP (Beckman) and counted by scintillation spectroscopy. The process of quenching filtration and washing never exceeded 15s. The timed uptake values obtained were corrected for the nonspecific retention of isotope by the filters.

Fluorescence determinations of Δ pH

Changes in intravesicular pH in response to an imposed pH gradient (pH_o 8/pH_i 6) in the presence and absence of CO_2/HCO_3^- were monitored by Acridine orange fluorescence using a SPEX DM 3000 fluorometer [36]. Fluorescence was measured at excitation and emission wavelengths of 492 and 529 nm, respectively, with a bandpass of 3.6 nm. Aliquots of freshly prepared acidemic and control membrane vesicles were washed into 105 mM TMA gluconate, 91.5 mM potassium gluconate, 10.5 mM Mes/TMA (pH 6) and diluted with solutions of appropriate ionic composition to obtain the desired intravesicular solution described in the figure legend. The membrane suspensions were incubated 120 min at room temperature under continuous gassing with either humidified 100% N_2 or 95% N_2/5% CO_2. The extravesicular or cuvette solutions were prepared similarly and their ionic composition are given in the figure legend. Experiments were initiated by the rapid addition of membranes (15–30 μl) to 2.5 ml of stirred cuvette solutions thermostatically maintained at 25°C. The volumes of acidemic and control membrane were adjusted to deliver equal amounts of protein (130–160 μg). Upon addition of vesicles to the cuvette buffer, an immediate 'pH jump' or quench in fluorescence was noted in response to the imposed pH gradient. No significant difference in the magnitude of the pH jump was observed in the absence or presence of CO_2/HCO_3^- and the time-dependent changes in fluorescence occurring immediately after the pH jump were determined.

Materials

Percoll, Acridine orange, valinomycin and DIDS were purchased from Sigma (St. Louis, MO). $^{22}Na^+$, $^{36}Cl^-$ and [^{14}C]succinate$^-$ were obtained from New England Nuclear (Boston, MA). Inactin was purchased from A. Lockwood Ass. (E. Lansing, MI). Valinomycin was dissolved in 95% ethanol (50 mg/ml) and was added to the membrane suspensions in a 1:200 dilution. All solutions were prepared with distilled-deionized water and passed through a 0.22 μm Millipore filter.

Results

Effect of chronic acid loading on membrane purification

The adequacy of the protocol used to induce acidemia is indicated by the significant reduction of arterial blood pH, p_{CO_2} and [HCO_3] in NH_4Cl-fed rats compared to control rats. The mean ± S.E. values of arterial blood pH, p_{CO_2} and [HCO_3] for acidotic ($n = 23$) vs. control ($n = 24$) rats were respectively 7.23 ± 0.03 vs. 7.37 ± 0.01, 41 ± 0.8 mmHg vs. 46.8 ± 0.7 mmHg and 17.5 ± 1 mM vs. 26.7 ± 0.4 mM.

To evaluate the possible effect of chronic acidemia on basolateral and brush-border membrane purification, membrane marker enzyme activities were compared as shown in Table I. The basolateral and brush-border membrane vesicle preparations from acidemic and control kidneys were essentially identical with regard to the basolateral and brush-border membrane marker enzyme activities Na^+/K^+-ATPase and maltase, respectively. Closer inspection of the data further indicate basolateral and brush-border membrane vesicles were enriched to virtually the same level, 11–12-fold and 14–16-fold, respectively, when prepared from acidemic and control kidneys. In addition to membrane purification, the possible effect of chronic acidemia on basolateral membrane orientation was also assessed by the latency of Na^+/K^+-ATPase activity. According to this analysis the percent distribution of membrane

TABLE I

Na^+/K^+-ATPase and maltase activity of basolateral and brush-border membrane vesicles from acidotic and control kidneys

The mean ± S.E. of six preparations are shown.

	Na^+/K^+-ATPase activity (μmol P_i/mg protein per min)			Maltase activity (*A* unit/mg protein per min)		
	homogenate	BLMV	BBMV	homogenate	BLMV	BBMV
Control	0.46 ± 0.06	5.6 ± 0.9	0.64 ± 0.1	1 ± 0.1	2 ± 0.4	13.6 ± 1
Acidotic	0.48 ± 0.06	5.4 ± 1	65 ± 0.1	0.9 ± 0.06	2 ± 0.5	14.5 ± 0.7

TABLE II

Effect of chronic acidemia on basolateral membrane vesicle orientation

Acidotic and control basolateral membrane in the right-side-out (RSOV), inside-out (ISOV) and leaky/sheet configuration is shown as % of total membrane. The mean ± S.E. of ten membrane preparations is shown.

	RSOV	ISOV	Leaky/sheets
Control	42 ± 2.2	2.1 ± 0.72	56.4 ± 2.6
Acidotic	45.5 ± 4.7	3.7 ± 1.1	53.3 ± 4.4

vesicles among three configurations: right-side-out (RSOV), inside-out (ISOV) and leaky/sheets may be delineated based upon differences in total and ouabain-sensitive enzyme activity in the presence and absence of a membrane permeabilizing agent. As shown in Table II, the percent distribution of acidemic and control basolateral membranes among these three configurations is the same, which indicates chronic acidemia had no effect on basolateral membrane orientation.

Effect of chronic acid loading on brush-border membrane Na^+–H^+ exchange activity

To investigate the possibility that rat proximal tubule cells coordinately upregulate luminal membrane Na^+–H^+ exchange and basolateral membrane HCO_3^- transport in response to chronic acidemia basolateral and brush-border membrane vesicles were prepared simultaneously from acidemic and control kidneys. The effect of chronic acidemia on brush-border membrane Na^+–H^+ exchange was examined by assaying pH gradient-driven Na^+ uptake in the presence and absence of amiloride, a specific inhibitor of Na^+–H^+ exchange [37]. As shown in Table III, in the absence of amiloride, pH gradient-driven Na^+ uptake was approx. 50% greater in brush-border membranes isolated from acidotic compared to control kidneys. In the presence of amiloride,

TABLE III

Effect of chronic acidemia on brush-border membrane Na – H exchange activity

Brush-border membrane vesicles were pre-equilibrated with 72.5 mM TMA gluconate, 71.5 mM potassium gluconate, 52 mM Mes, 42 mM Hepes, 21 mM TMA(OH) (pH 6). The 5 s uptake of ^{22}Na (0.1 mM) occurred from an extravesicular solution containing 56.6 mM TMA gluconate, 71.5 mM potassium gluconate, 10.4 mM Mes, 40 mM mannitol, 42 mM Hepes, 34 mM TMA(OH) (pH 7.5), with or without 1 mM amiloride. Membranes were preincubated with valinomycin (225 μM) for a minimun of 30 min. The data shown are the means ± S.E. of Na^+ uptake (pmol/mg protein) for seven separate experiments each performed with a different membrane preparation. Δ is the amiloride-sensitive Na^+ uptake.

	−Amiloride	+Amiloride	Δ
Control	98 ± 6.7	11.3 ± 2	86.8 ± 7
Acidotic	143.1 ± 1	12.3 ± 1.4	130.1 ± 8.5

TABLE IV

Effect of chronic acidemia on basolateral membrane Na^+/HCO_3^- cotransport activity

Basolateral membrane vesicles were pre-equilibrated with 72.5 mM TMA gluconate, 71.5 mM potassium gluconate, 52 mM Mes, 42 mM Hepes, 21 mM TMA(OH) (pH 6) under 10% CO_2/90% N_2. The 2 s uptake of $^{22}Na^+$ (2 mM) occurred from an extravesicular solution containing 74.6 mM TMA gluconate, 57.3 mM $KHCO_3$, 14.3 mM potassium gluconate, 10.4 mM Mes, 22.8 mM mannitol, 42 mM Hepes, 31.4 mM TMA(OH) (pH 7.5) under 10% CO_2/90% N_2 with or without 1 mM DIDS. Membranes were pre-incubated with valinomycin (225 μM) for a minimum of 30 min. The data shown are the means ± S.E. of Na^+ uptake (nmol/mg protein) for seven separate experiments, each performed with a different preparation. Δ is the DIDS-sensitive Na^+ uptake.

	−DIDS	+DIDS	Δ
Control	1.84 ± 0.749	0.955 ± 0.084	0.888 ± 0.100
Acidotic	2.53 ± 0.146	1.24 ± 0.085	1.29 ± 0.079

Na^+ uptake was much less and essentially the same in both acidotic and control membranes. Thus the magnitude of amiloride-sensitive Na^+ uptake, used here as an index of Na^+/H^+ exchange activity, was approx. 50% greater in brush-border membranes isolated from acidotic compared to control kidneys. The increased amiloride-sensitive Na^+ uptake could not be attributed to a difference in vesicle size, as the Na^+ content of acidotic and control vesicles at equilibrium (2 h) was indistinguishable (184 ± 9 pmol Na^+/mg protein vs. 175 ± 8 pmol Na^+/mg protein). Consistent with previous observations [14,15,38], these results demonstrate the capacity of the rat proximal tubule cell to increase luminal membrane Na^+–H^+ exchange activity as an adaptive response to chronic acidemia.

Effect of chronic acid loading on basolateral membrane HCO_3^- transport

The effect of chronic acidemia on basolateral membrane Na^+/HCO_3^- co-transport was assessed by assaying HCO_3^- gradient-driven Na^+ uptake in the presence and absence of DIDS, an inhibitor of Na^+/HCO_3^- co-transport [30]. As shown in Table IV, in the absence of DIDS, HCO_3^- gradient-driven Na^+ uptake was approx. 40% greater in basolateral membrane vesicles isolated from acidemic kidneys compared to control. Although Na^+ uptake in the presence of DIDS was reduced to a lower level in control compared to acidemic membranes, the magnitude of DIDS-sensitive Na^+ uptake, used here as an index of basolateral membrane Na^+/HCO_3^- cotransport, remained approx. 45% greater in vesicles isolated from acidemic kidneys. Measurements of acidemic and control membrane vesicle Na^+ content at equilibrium were similar (187.2 ± 12 pmol Na^+/mg vs. 206.7 ± 10 pmol (Na^+/mg), suggesting that differences in membrane vesicle size could not account for the increased Na^+ uptake by acidemic

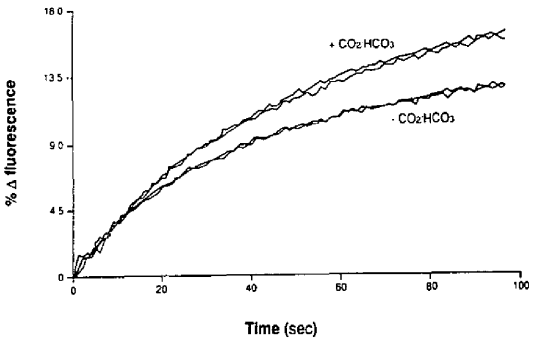

Fig. 1. H^+ and HCO_3^- gradient-induced intravesicular alkalinization. Basolateral membrane vesicles were pre-equilibrated under 100% N_2 with 100 mM TMA gluconate, 88.7 mM potassium gluconate, 6 μM Acridine orange, 10 mM Mes/TMA (pH 6) and under 95% N_2/5% CO_2 with 100 mM TMA gluconate, 87.8 mM potassium gluconate, 0.9 mM $KHCO_3$, 6 μM Acridine orange, 10 mM Mes/TMA (pH 6). Cuvette solutions were gassed in parallel with membrane vesicles and under 100% N_2 were 100 mM TMA gluconate, 88.7 mM potassium gluconate, 6 μM acridine orange, 10 mM Hepes/TMA (pH 8) and under 95% N_2/5% CO_2 were 100 mM TMA gluconate, 88.7 mM $KHCO_3$, 6 μM Acridine orange, 10 mM Hepes/TMA (pH 8). Membranes were pre-incubated with valinomycin (225 μM) for a minimum of 30 min. The rate of intravesicular alkalinization in the presence and absence of CO_2/HCO_3^- is illustrated as the % change in fluorescence following the pH jump. A representative of four experiments each performed with a different membrane preparation is shown.

membranes both in the absence and presence of inhibitor. Furthermore, experiments using Acridine orange fluorescence to monitor changes in intravesicular pH indicated the rate of intravesicular alkalinization resulting from the collapse of an imposed, inside-acid pH gradient were similar in acidemic and control vesicles when measured in the presence and absence of CO_2/HCO_2 as shown in Fig. 1. This would suggest that the increased HCO_3^- gradient-driven Na^+ uptake observed in acidemic vesicles is not the result of differences in the rate of HCO_3^- or H^+ gradient dissipation. The results of these studies performed using brush-border and basolateral membrane vesicles isolated simultaneously from the same rat proximal tubule cells suggest a coordinate upregulation of luminal membrane Na^+–H^+ exchange and basolateral membrane Na^+/HCO_3^- cotransport in response to chronic acidemia. A similar adaptive response to chronic metabolic acidosis has recently been described from studies of the in vivo microperfused rat proximal tubule [15].

Additional experiments were undertaken in an attempt to confirm the suggested acidosis-induced increase in basolateral membrane Na^+-HCO_3^- cotransport as well as explore possible alterations in the molecular properties of Na^+-HCO_3^- cotransport which could account for increased transport activity. Initial rates of HCO_3^- gradient-driven Na^+ uptake by acidemic and control basolateral membrane vesicles were estimated from 2 s uptake values at Na^+ ranging from 0.1 mM to 10 mM. Preliminary determinations of HCO_3^- gradient-driven Na^+ indicated that uptake was linear with time for 2 s at Na^+ concentrations up to 10 mM. The data shown in Fig. 2 may be represented as a linear Hanes-Woolf plot for both acidemic and control membranes, which is consistent with the interaction of Na^+ at a single saturable site. The estimated K_m values for Na^+ uptake by acidemic (3.76 mM) and control membranes (3.74 mM) were essentially identical, which indicates that an increased affinity for Na^+ is not likely to account for the increased transport activity observed. However, the apparent V_{max} value for Na^+/HCO_3^- cotransport by acidemic membranes was increased approx. 45% compared to the value measured for control membranes. The increased apparent maximal rate of Na^+ uptake (V_{max}) suggests the increased Na^+/HCO_3^- cotransport activity observed in acidemic membranes occurs as a result of an increased number of Na^+/HCO_3^- cotransport molecules present in the basolateral membrane or an increased turnover rate for an individual transport event.

Previous membrane vesicle [29] and perfused tubule [27] studies have identified a basolateral membrane Cl^-–HCO_3^- exchange mechanism which, in addition to

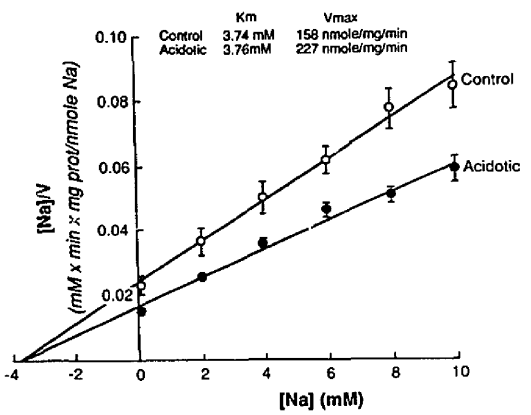

Fig. 2. Kinetic analysis of Na^+/HCO_3^- cotransport. Basolateral membrane vesicles were preequilibrated with 72.5 mM TMA gluconate, 71.5 mM potassium gluconate, 52 mM Mes, 42 mM Hepes, 21 mM TMA(OH) (pH 6) under 10% CO_2/90 N_2. 2 s uptake of ^{22}Na occurred from an extravesicular solution containing 74.6 mM TMA gluconate, 57.3 mM $KHCO_3$, 14.3 mM potassium gluconate, 10.4 mM Mes, 22.8 mM mannitol, 42 mM Hepes, 31.4 mM TMA(OH) (pH 7.5) under 10% CO_2/90% N_2. Na^+ concentration was varied by replacing TMA gluconate with sodium gluconate. Membranes were preincubated with valinomycin (225 μM) for a minimum of 30 min. The data shown represent the mean ± S.E. for six separate experiments performed with six different membrane preparations. The regression lines were calculated by the method of least squares.

Na^+/HCO_3^- cotransport, may serve as a pathway for HCO_3^- efflux from rat proximal tubule cells. To investigate a possible effect of chronic acidemia on basolateral membrane $Cl^- - HCO_3^-$ exchange activity, HCO_3 gradient-driven Cl^- uptake was assayed in the presence and absence of DIDS, an inhibitor of $Cl^- - HCO_3^-$ exchange [29]. As shown in Table V, in the absence of DIDS, HCO_3^- gradient-driven Cl^- uptake was approx. 60% greater in acidemic membrane vesicles compared to control membrane vesicles. In the presence of DIDS, Cl^- uptake was significantly reduced to essentially the same level in acidemic and control membranes. Thus, the magnitude of DIDS-sensitive Cl^- uptake, used to quantitate $Cl^- - HCO_3^-$ exchange activity, was increased more than 2-fold in acidemic compared to control membranes. The Cl^- content of acidemic and control membrane vesicles at equilibrium (2 h) were not distinguishable (10 ± 1 nmol Cl^-/mg protein vs. 10.6 ± 0.6 nmol Cl^-/mg) which indicates the increased Cl^- uptake by acidotic membranes did not result from a relative increase in vesicle size. This finding would suggest the increased capacity for basolateral membrane HCO_3^- transport in response to chronic acidemia may occur as the result of an increased $Cl^- - HCO_3^-$ exchange activity as well as an increase in Na^+/HCO_3^- cotransport.

Finally, the effect of chronic acidemia on basolateral membrane Na^+/succinate$^-$ cotransport was assessed to test whether the acidosis-induced increase in basolateral membrane HCO_3^- transport activity is specific or merely reflects a more general adaptive response of the proximal tubule cell. The timed uptake of succinate by acidemic and control membrane vesicles was assayed in the presence and absence of an inwardly directed Na^+ concentration gradient. As shown at the bottom of Fig. 3, succinate is equally impermeable to acidemic and control membranes when uptake was measured for 60 s in the absence of Na^+. Consistent with the known presence of a basolateral membrane Na^+/dicarboxylate$^-$ co-transport mechanism [39,40], an inwardly directed Na^+ gradient markedly stimulated the rate of succinate uptake by acidemic and control membrane to a similar level for each time point examined. The similar level of Na^+/succinate$^-$ cotransport activity measured in acidemic and control membranes suggests the proximal tubule cell does not adapt to chronic acidemia by expressing a general increase in basolateral membrane transport activity.

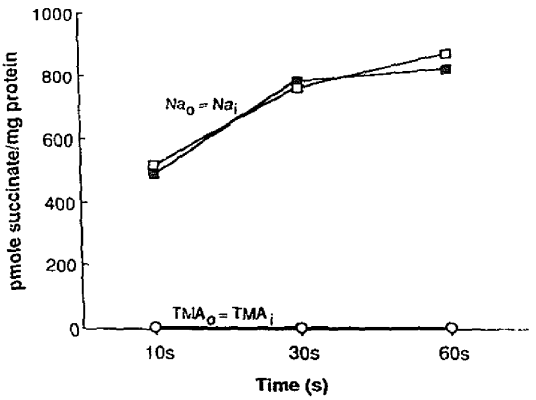

Fig. 3. Effect of chronic acid loading on basolateral membrane Na^+/succinate$^-$ cotransport. Basolateral membrane vesicles were pre-equilibrated with 125 mM TMA-Cl, 50 mM KCl, 20 mM Hepes, 10 mM TMA(OH) (pH 7.5). Succinate (14 μM) uptake occurred from an extravascular solution containing 100 mM NaCl, 25 mM TMA-Cl or 125 mM TMA-Cl, 50 mM KCl, 20 mM Hepes, 10 mM TMA(OH) (pH 7.5). Membranes were preincubated with valinomycin (225 μM) for a minimum of 30 min. A representative experiment of four independent observations is illustrated.

TABLE V

Effect of chronic acidemia on basolateral membrane $Cl^- - HCO_3^-$ exchange activity

Basolateral membrane vesicles were pre-equilibrated with 52.5 mM TMA gluconate, 57.3 mM $KHCO_3$, 52 mM mannitol, 45.2 mM Hepes, 22.6 mM TMA(OH) (pH 7.5) under 10% CO_2/90% N_2. The 10 s uptake of $^{36}Cl^-$ (5 mM) occurred from an extravesicular solution containing 51.4 mM TMA gluconate, 57.2 mM K, 59 mM gluconate, 47.3 mM Mes, 29 mM mannitol, 9 mM Hepes, 23.3 mM TMA(OH) (pH 6) under 10% CO_2/90% N_2 with or without 1 mM DIDS. Membranes were pre-incubated with valinomycin (225 μM) for a minimum of 30 min. The data shown are the means ± S.E. of Cl^- uptake (nmol/mg protein) for seven separate experiments, each performed with a different membrane preparation. Δ is the DIDS-sensitive Cl^- uptake.

	−DIDS	+DIDS	Δ
Control	3.59 ± 0.197	1.99 ± 0.100	1.59 ± 0.138
Acidotic	5.72 ± 0.560	1.815 ± 0.052	3.90 ± 0.520

Discussion

The renal proximal tubule adapts to chronic acid loading by increasing its capacity for transcellular bicarbonate reabsorption [1–5]. When examined at the cell membrane level this adaptive response appears to result in part from an increased $Na^+ - H^+$ exchange activity at the luminal side of the cell [10–14]. In addition to resulting from an increased proton secretory capacity at the luminal membrane, the increased capacity for transcellular bicarbonate reabsorption may also reflect an increased capacity for bicarbonate transport at the basolateral membrane. Accordingly, we investigated the possible coordinate upregulation of luminal membrane $Na^+ - H^+$ exchange and basolateral membrane $Cl^- - HCO_3^-$ exchange and Na^+/HCO_3^- cotransport by assaying transport activity in renal cortical membrane vesicles prepared from chronic acid-loaded

rats. The results obtained suggest that proximal tubule cells respond to chronic acid loading by increasing both luminal and basolateral membrane acid/base transport activities mediating transcellular bicarbonate reabsorption.

To assess a possible acidosis-induced increase in luminal and basolateral membrane acid/base transport both brush-border and basolateral membrane vesicles were prepared simultaneously from rat kidney cortex. The adequacy of the protocol used to induce chronic acidemia is indicated by the marked reduction of arterial blood pH, p_{CO_2} and HCO_3^- concentration when comparing acid loaded to control rats. The effect of acute changes in respiration on blood gas measurements was minimized by using the anesthetic Inactin and drawing blood samples as quickly as possible. Despite this clear perturbation of acid/base balance, the brush-border and basolateral membranes isolated from chronic acid loaded and control rat kidneys were essentially indistinguishable with regard to membrane marker enzyme activities or the enrichment of these activities. This observation suggests that differences in the degree of membrane purification could not account for acidemic vs. control differences in brush-border or basolateral membrane acid/base transport activities. A possible effect of chronic acid loading on basolateral membrane orientation was assessed by determining the percent distribution of membrane among three configurations: right-side-out vesicles, inside-out vesicles, leaky vesicles and/or open sheets. The percent distribution of basolateral membrane among these three configurations was essentially identical for membranes isolated from acidemic and control kidneys. This observation suggests acidemic vs. control differences in basolateral membrane acid/base transport activity would not result from possible asymmetric affinities at the intra- and extravesicular side of the transport proteins.

The effect of chronic acid loading on the luminal membrane Na^+–H^+ exchange activity was assessed by measuring the magnitude of amiloride-sensitive, pH gradient-driven Na^+ uptake in brush-border membrane vesicles isolated from acidemic and control kidneys. Consistent with previous observations made in rat [10,13], rabbit [12] and dog [11] brush-border membrane Na^+–H^+ exchange activity was increased approx. 50% in acidemic compared to control membranes. Where it has been examined previously in rat [10,38], the increased Na^+–H^+ exchange activity was not due to less rapid pH gradient dissipation, changes in stoichiometry or electrogenicity, altered affinity for Na^+ or H^+ or changes in the intracellular H^+ modifier site. Kinetic analysis of initial rates of Na^+ uptake does suggest, however, that the increase in brush-border membrane exchange activity results from either an increased turnover rate or an increased number of exchanger molecules [10,12–14]. Similar to chronic acid loading the proximal tubule increases brush-border membrane Na^+–H^+ exchange activity in response to parathyroidectomy [11], K^+ depletion [16], glucocorticoid administration [17] and increased glomerular filtration rate [18–20]. Studies of [^{3}H]ethylpropylamiloride binding to brush-border membranes isolated from remnant kidneys suggest the increased Na^+–H^+ exchange activity in response to increased filtration rate results from an increased turnover rate rather than an increased number of transport molecules [20].

The observed increase in brush-border membrane Na^+–H^+ exchange activity serves to demonstrate and verify the adaptive capacity of rat proximal tubule cells in response to the acid-loading protocol used in the present investigation. In addition to targeting an adaptive increase in luminal membrane proton secretion, the proximal tubule cell may also respond to chronic acid loading by increasing its capacity for basolateral membrane bicarbonate efflux. As a result of such a coordinate increase in luminal and basolateral membrane acid/base transport activities the cell would be well-poised to maintain cell pH while accomodating increased levels of transcellular bicarbonate reabsorption. A possible effect of chronic loading on basolateral membrane bicarbonate transport was assessed by measuring the activity of both Na^+/HCO_3^- cotransport and Cl^-–HCO_3^- exchange in membrane vesicles prepared from acidemic and control kidneys. First, the magnitude of basolateral membrane Na^+/HCO_3^- cotransport activity was quantified by measuring the level of DIDS-sensitive, HCO_3^- gradient-driven Na^+ uptake. Similar to the increase in Na^+–H^+ exchange activity observed in brush-border membranes, the activity of basolateral membrane Na^+/HCO_3^- cotransport was increased approx. 45% in acidemic versus control membrane vesicles. The observed increase in basolateral membrane Na^+/HCO_3^- cotransport activity was unlikely to result from a less rapid HCO_3^- gradient dissipation, as Acridine orange fluorescence studies of intravesicular pH change suggest that H^+ and HCO_3^- permeabilities were similar in acidemic and control membrane vesicles. To explore possible alterations in the molecular properties of Na^+/HCO_3^- cotransport which may account for the increased transport activity in acidemic membranes kinetic studies of Na^+/HCO_3^- cotransport were undertaken. Kinetic analysis suggests the increased Na^+/HCO_3^- cotransport activity observed in acidemic membranes does not result from an increased affinity for Na^+ but may result from either an increased number of transport molecules or a modification increasing the rate-limiting step in turnover. Future identification of radiolabelled probes for Na^+/HCO_3^- cotransport molecules may facilitate a distinction between these two possibilities. Although not examined in the present investigation the increased maximal rate of Na^+/HCO_3^- cotransport observed in acidemic mem-

branes may have also resulted from an effect to increase the cotransporters affinity for HCO_3^-. We do not favor this possibility as the HCO_3 concentration (60 mM) used to measure initial rates of Na^+ uptake was well in excess of intra- and extracellular levels spanning the basolateral membrane and therefore likely to saturate the cotransport mechanism. The results of these rat membrane vesicle studies agree with and extend a previous in vivo observation of increased basolateral membrane Na^+/HCO_3^- cotransport activity [15] by demonstrating an effect of chronic acid loading to increase the apparent maximal rate (V_{max}) of Na^+/HCO_3^- cotransport. Membrane vesicle studies also suggest rabbit proximal tubule cells adapt to chronic acid loading by increasing the maximal rate of basolateral membrane Na^+/HCO_3^- cotransport [14].

In addition to Na^+/HCO_3^- cotransport, the presence of a basolateral membrane Cl^-–HCO_3^- exchange mechanism suggests that a second pathway may mediate bicarbonate efflux from proximal tubule cells [27–30]. To determine whether proximal tubule cells respond to chronic acid loading by increasing the activity of Cl^-–HCO_3^- exchange as well as Na^+/HCO_3^- cotransport the level of DIDS-sensitive, HCO_3^- gradient-driven Cl^- uptake was compared in basolateral membrane isolated from acidemic and control kidneys. The observed 2-fold increase in Cl^-–HCO_3^- exchange activity suggests that both basolateral membrane bicarbonate efflux pathways are upregulated to accomodate an increased capacity for cellular bicarbonate elimination. Finally, the level of Na^+/succinate$^-$ cotransport was measured in acidemic and control membranes in a limited attempt to assess the specificity of the adaptive increase in basolateral membrane transport activity. The observed lack of an effect on Na^+/succinate$^-$ cotransport activity suggests proximal tubule cells may selectively increase basolateral membrane transport activity as an adaptive response to chronic acid loading. A description of increased Na^+/glutamine cotransport in response to chronic acid loading further attests to the specificity of the adaptive increase in basolateral membrane transport activity [41].

In conclusion, the demonstrated increase in basolateral membrane Na^+/HCO_3^- cotransport and Cl^-–HCO_3^- exchange activity suggests proximal cells coordinately upregulate basolateral as well as luminal acid/base transport pathways to accomodate an increased capacity for transcellular bicarbonate reabsorption in response to chronic acid loading. Future studies will determine whether the associated increase in luminal and basolateral membrane acid/base transport activity is uniquely expressed in response to chronic acid loading or is also present where, so far, only an increased brush-border membrane Na^+–H^+ exchange activity has been described.

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