Effect of In Vitro Metabolic Acidosis on Luminal Na^+/H^+ Exchange and Basolateral $Na^+:HCO_3^-$ Cotransport in Rabbit Kidney Proximal Tubules

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Abstract

The aim of this study was to evaluate the role of the kidney in mediating the signals involved in adaptive changes in luminal Na^+/H^+ exchange and basolateral $Na^+:HCO_3^-$ cotransport systems in metabolic acidosis. Proximal tubular suspensions were prepared from rabbit kidney cortex and incubated in acidic (A) or control (C) media (pH 6.9 vs 7.4, 5% CO_2) for 2 h. Brush border membrane (BBM) and basolateral membrane (BLM) vesicles were isolated from the tubular suspensions and studied for the activity of Na^+/H^+ exchange and $Na^+:HCO_3^-$ cotransport. Influx of 1 mM ^{22}Na at 10 s (pH_6 7.5, pH_i 6.0) into BBM vesicles was 68% higher in group A compared to group C. The increment in Na^+ influx in the group A was amiloride sensitive, suggesting that Na^+/H^+ exchange was responsible for the observed differences. Kinetic analysis of Na^+ influx showed a K_m of 8.1 mM in C vs 9.2 in A and V_{max} of 31 nmol/mg protein per min in group C vs 57 in A. Influx of 1 mM ^{22}Na at 10 s (pH_0 7.5, pH_i 6.0, 20% CO_2, 80% N_2) into BLM vesicles was 83% higher in the group A compared to C. The HCO_3-dependent increment in ^{22}Na uptake in group A was 4,4'-diisothiocyano-2,2'-stilbene disulfonic acid sensitive, suggesting that $Na^+:HCO_3^-$ cotransport accounted for the observed differences. Kinetic analysis of Na^+ influx showed a K_m of 11.4 mM in C vs 13.6 in A and V_{max} of 35 nmol/mg protein per min in C vs 64 in A. The presence of cyclohexamide during incubation in A medium had no effect on the increments in ^{22}Na uptake in group A. We conclude that the adaptive increase in luminal Na^+/H^+ exchange and basolateral $Na^+:HCO_3^-$ cotransport systems in metabolic acidosis is acute and mediated via direct signal(s) at the level of renal tubule. (*J. Clin. Invest.* 1992. 90:211–218.) Key words: in vitro acidosis • proximal tubule • Na^+/H^+ exchange • $Na^+:HCO_3^-$ cotransport

Introduction

The majority of HCO_3^- filtered at the glomerulus is reabsorbed in the kidney proximal tubule via the luminal Na^+/H^+ exchanger and basolateral $Na^+:HCO_3^-$ cotransporter acting in series (1–5). The Na^+/H^+ exchanger is an electroneutral, amiloride-sensitive process that regulates blood pH by secreting H^+ in exchange for Na^+ subsequently leading to HCO_3^- reabsorption in the proximal tubule (1). This transport process is involved in pH regulation in almost every epithelial and most nonepithelial tissues including cardiac, vascular smooth, and skeletal muscle (6). The $Na^+:HCO_3^-$ cotransport system, which is responsible for the transport of HCO_3^- from the kidney proximal tubule cell to blood, represents an electrogenic process (2–5) with an apparent stoichiometry of three equivalents of base per Na^+ ion (7, 8). This transport process is involved in HCO_3^- transport in multiple types of cells including corneal endothelial cells (9), gastric parietal cells (10), cells of cortical thick ascending limb of Henle (11), hepatocytes (12), and glial cells (13), suggesting it is also of general physiologic significance. Recent studies have suggested that the actual ionic mechanism involves the cotransport of Na^+, $CO_3^=$, and HCO_3^- in a 1:1:1 ratio on distinct sites (14).

Since the bulk of HCO_3^- reabsorption in the proximal tubule occurs via the luminal Na^+/H^+ exchanger and basolateral $Na^+:CO_3^=:HCO_3^-$ cotransporter, it might be predicted that alterations in proximal tubular acidification in pathologic states should ultimately result from changes in the activity of these transporters. One frequent clinical condition affecting the pH homeostasis of blood is metabolic acidosis. Primary metabolic acidosis, a condition manifested by decreased serum [HCO_3^-] and pH, has been shown to be associated with an increased ability of the renal tubules to reabsorb HCO_3^- (15, 16). The few studies which have evaluated the luminal Na^+/H^+ exchanger and or basolateral $Na^+:HCO_3^-$ cotransporter in metabolic acidosis suggest that the activity of these two transport processes are increased (17–19). Whether the alteration in the activity of the luminal Na^+/H^+ exchanger and basolateral $Na^+:HCO_3^-$ cotransporter in metabolic acidosis is due to systemic effects or is mediated locally at the level of the kidney is not known. Furthermore, the time course of these adaptive changes, i.e., hours vs days, has not been determined.

The purpose of the present studies was to examine whether the effect of acidic pH on the luminal Na^+/H^+ exchanger and basolateral $Na^+:HCO_3^-$ cotransporter is direct and mediated via signals at the level of the renal tubule. In addition, we wanted to determine the rapidity with which these adaptive changes occur. To address these questions, proximal tubular suspensions were prepared and incubated in acidic and normal media. After timed incubation, brush border and basolateral membrane vesicles were isolated to study the activity of these transporters. The result demonstrate that exposure of proximal tubules to an acidic pH results in adaptive increases in the luminal Na^+/H^+ exchanger and basolateral $Na^+:CO_3^=:HCO_3^-$ cotransporter activities. The effect is apparently due to a change in the V_{max} of the transporters. The results also demonstrate that the effect of acidic pH on these two transporters is mediated directly at the level of the renal tubule and is independent of the systemic factor(s).

Methods

Tubular suspension. Male New Zealand White rabbits were killed by intravenous sodium pentobarbital. Tubular suspensions were prepared

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Received for publication 28 August 1991 and in revised form 30 December 1991.

The Journal of Clinical Investigation, Inc.
Volume 90, July 1992, 211–218

as described by Khan et al. (20). Briefly, renal cortices of two rabbits were sliced using a microtome and placed in a cold bicarbonate Ringer's solution gassed with 95% O_2, 5% CO_2. The cortical suspension was digested for 60 min at 37°C using collagenase and hyaluronidase. After digestion, the suspension was filtered and the tubules were isolated by centrifugation as described (20). The proximal tubular suspension was then incubated in either regular Ringer's (pH 7.4, [HCO_3^-] 24 mM, 5% CO_2) or an acidic solution (pH 6.9, [HCO_3^-] 8 mM, 5% CO_2) for 2 h (1 h at 4°C followed by 1 h at 37°C). After incubation, the tubules were homogenized, centrifuged, and membrane vesicles were isolated. The homogenizing solution for brush border membrane (BBM)[1] vesicles preparation consisted of 10 mM mannitol, and 2 mM Tris titrated to pH 7.1 with HCl (21). The homogenizing solution for basolateral membrane (BLM) vesicles preparation consisted of 250 mM sucrose, 2 mM EDTA, and 10 mM Hepes titrated to pH 7.6 with tetramethyl ammonium (TMA) hydroxide.

Membrane vesicles preparation. BBM vesicles were isolated from tubular suspensions by a Ca^{++} aggregation method (21) as employed previously (22). BLM vesicles were isolated from tubular suspensions by differential and Percoll gradient centrifugation previously described (23, 24). Both BBM and BLM vesicles were frozen and stored at −70°C and used within two weeks of preparation. The purification of the brush border membrane vesicles relative to the initial cortical homogenate was 10.9±2.1-fold for the control and 11.8±1.8-fold for the acidotic group based on the enrichment in specific activity of alkaline phosphatase ($P > 0.05$). Purification of BLM vesicles relative to the initial cortical homogenate was 11.2±2.2 ($n = 4$)-fold for the control and 10.6±1.4 ($n = 4$)-fold for the acidotic group based on the enrichment in specific activity of Na^+, K^+-ATPase ($P > 0.05$). Additionally, HCO_3-dependent ^{22}Na uptake in BBM vesicles from acidic and control tubules could not be detected, suggesting little contamination of BBM with BLM vesicles in control and acidic groups. BBM vesicle size, calculated from external Na^+ concentration and equilibrium values for $^{22}Na^+$ uptake was 1.07±0.18 μl/mg protein in the control group (C) and 1.26±0.21 ($n = 3$) in the acidotic group (A) ($P > 0.05$). BLM vesicle size was 1.28±0.23 μl/mg protein in group C vs 1.15±0.20 ($n = 3$) in group A ($P > 0.05$).

^{22}Na transport measurements. In general, the timed uptake of ^{22}Na by membrane vesicles suspension were assayed at room temperature in quadruplicate by a rapid filtration technique as previously described (23, 24). The ice-cold medium used to dilute and wash the vesicles at the termination of the uptake period consisted of 170 mM K^+ gluconate and 10 mM Hepes titrated to pH 7.5 with TMA hydroxide. Thereafter, each filter (0.45 μM, DAWP; Millipore Corp., Bedford, MA) was placed in 3 ml of scintillation fluid and radioactivity assayed by liquid scintillation spectroscopy. The membrane vesicles and all experimental media were continuously gassed with 100% N_2 or 20% CO_2/80% N_2. The final composition of the experimental media and other details of the protocols are given in the figure legends. All experiments were performed using vesicles treated with valinomycin (0.5 mg/ml) and preequilibrated in media of appropriate composition to ensure that $[K^+]_i = [K^+]_o$ during uptake measurements. For kinetic studies, uptake was measured at 4 s. Uptake of ^{22}Na into BBM and BLM vesicles are linear at these times (24). The intravesicular buffering capacity was measured as described previously (25). Briefly, the BBM and BLM vesicles from control and acidotic tubules were preequilibrated at pH 6 as described in the legends to Figs. 1 and 5. The membrane vesicles were lysed and the buffering capacity of the solutions was measured by titrating the pH of the solutions to 7.0 using potassium hydroxide. The buffering capacity of the BBM vesicles from control and acidotic tubule were 3.84±0.44 μmol/mg protein per pH unit and 3.59±0.62, respectively ($P > 0.05$, $n = 3$). The buffering capacity of the BLM vesicles from control and acidotic tubule were 4.20±0.61 μmol/mg protein per pH unit and 4.31±0.71, respectively ($P > 0.05$, $n = 3$).

Data analysis. The data are expressed as means±SEM. Statistical analysis was determined using Student's t test with $P < 0.05$ being considered statistically significant.

Materials. We purchased $^{22}Na^+$ from New England Nuclear (Boston, MA). Valinomycin, cyclohexamide, amiloride, 4,4′-diisothiocyano-2,2′-stilbene disulfonic acid (DIDS), Ficoll, and Percoll were obtained from Sigma Chemical Co. (St. Louis, MO). Valinomycin was added to the membrane suspension in a 1:100 dilution from a stock solution in 95% ethanol.

Results

To examine for possible effects of in vitro metabolic acidosis on the luminal Na^+/H^+ exchanger, the influx of Na^+ into BBM vesicles isolated from acidotic and control tubular suspensions was measured in the presence of an inwardly directed pH gradient. The time course of pH-dependent $^{22}Na^+$ uptake in these vesicles is shown in Fig. 1. An inward pH gradient resulted in a significant increase in ^{22}Na influx in vesicles from the acidotic group as compared to control. The influx of Na^+ into the BBM vesicles was 1.6-fold above the equilibrium value in the acidotic group, whereas it barely displayed an overshoot pattern in control group. There was no significant difference in the equilibrium values between the two groups. These results suggest that in vitro metabolic acidosis activates the Na^+/H^+ exchange system. To determine if the increment in Na^+ influx was indeed due to increased activity of Na^+/H^+ exchange system, Na^+ influx was measured in the presence and absence of 1 mM amiloride (Fig. 2). $^{22}Na^+$ influx was 68% higher in vesicles from acidotic tubules compared to control (312±51 pmol/mg

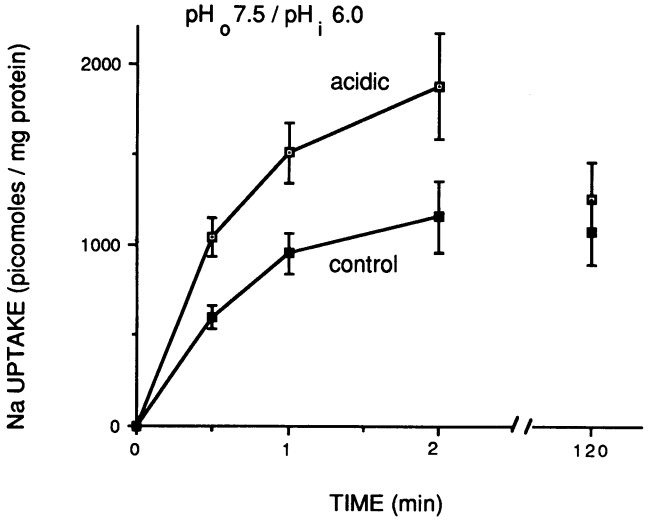

Figure 1. Effect of pH gradient on Na^+ influx. Brush border membrane vesicles from acidotic and control tubular suspensions were preequilibrated for 120 min at 20°C in a medium consisting of 52 mM TMA gluconate, 165 mM potassium gluconate, 52 mM Mes, 42 mM Hepes, 21 mM TMA hydroxide, pH 6.0. Uptake of 1 mM $^{22}Na^+$ into brush border membrane vesicles was assayed in the presence of a medium consisting of 52 mM TMA gluconate, 165 potassium gluconate, 31 mM mannitol, 10 mM 2-[*N*-Morpholino]-ethanesulfonic acid, 42 mM Hepes, 31 mM TMA hydroxide, pH 7.5. Values shown for uptake represent mean±SE for experiments performed in quadruplicate on four different membrane preparations.

1. *Abbreviations used in this paper:* A, acidotic; BBM, brush border membrane; BLM, basolateral membrane; C, control; DIDS, 4,4′-diisothiocyano-2,2′-stilbene disulfonic acid; PKC, protein kinase C; TMA, tetramethyl ammonium.

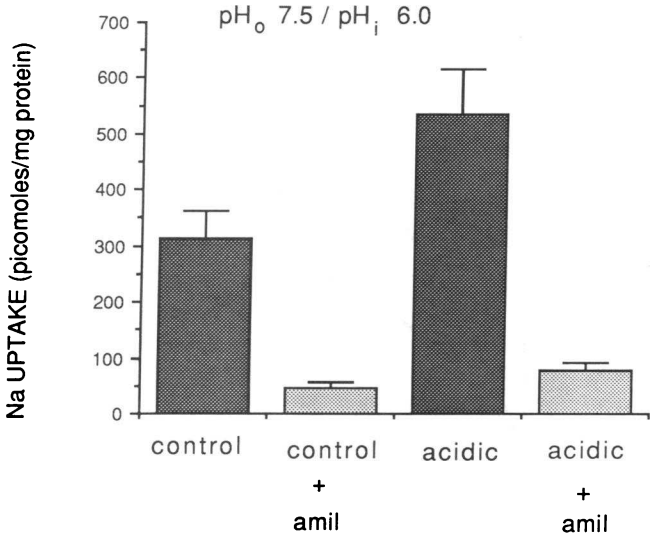

Figure 2. Effect of amiloride on pH-dependent Na^+ influx. The 10-s uptake of $^{22}Na^+$ into brush border membrane vesicles preequilibrated in pH 6.0 medium was assayed in the presence of pH 7.5 medium as described in Fig. 1. Amiloride (1 mM) was added as the hydrochloride salt to the external solution. Valinomycin was added at the start of preequilibration period. Values shown for uptake represent mean±SE for experiments performed in quadruplicate on four different membrane preparations.

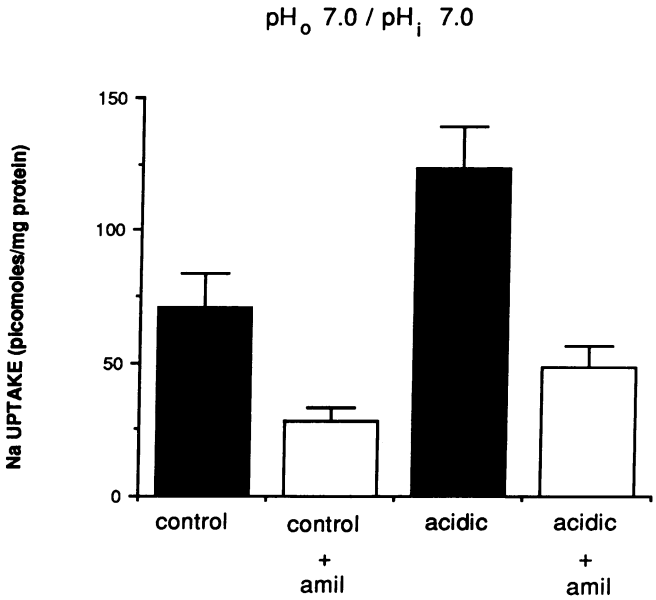

Figure 3. Effect of in vitro acidosis on Na^+/H^+ exchange measured in absence of pH gradient. Brush border membrane vesicles from acidotic and control tubular suspensions were preequilibrated for 120 min at 20°C in a medium consisting of 52 mM TMA gluconate, 165 mM potassium gluconate, 39 mM mannitol, 13 mM Mes, 42 mM Hepes, 21 mM TMA hydroxide, pH 7.0. The 10-s uptake of 1 mM $^{22}Na^+$ into brush border membrane vesicles was assayed in the presence of a similar medium at pH 7.0. Values shown for uptake represent mean±SE for experiments performed in quadruplicate on four different membrane preparations.

protein in group C vs 533±81 in A, $P < 0.02$). The increment in $^{22}Na^+$ influx in the acidotic group was abolished in the presence of 1 mM amiloride, suggesting that the observed difference between the two groups resulted predominantly from increased activity of the Na^+/H^+ exchanger in the acidotic group. The magnitude of influx and overshoot in BBM vesicles isolated from proximal tubular suspensions is less than that in BBM vesicles isolated directly from kidney homogenates (25). It has been reported previously that incubation of tubular suspensions results in a time-dependent decrease in the activity of the Na^+/H^+ exchanger (20). In the above experiments, the initial rate of ^{22}Na influx was measured in the presence of an inward pH gradient (pH_o = 7.5, pH_i = 6.0). It is possible, therefore, that the increased activity in the luminal Na^+/H^+ exchanger in BBM vesicles isolated from acidotic vesicles was secondary to a decreased proton permeability of the vesicles. Such a change in proton permeability could retard the rate of H^+ gradient dissipation across the vesicles and indirectly increase the activity of the Na^+/H^+ exchanger. To examine this possibility, vesicles were incubated at pH 7.5 and the 10-s influx of 1 mM ^{22}Na into the vesicles was assayed in the absence of a pH gradient ($pH_o = pH_i = 7.0$). As demonstrated in Fig. 3, the vesicles from acidotic group had a > 75% increase in ^{22}Na influx compared to the control group (71±13 pmol/mg protein in C vs 123±16 in A, $P < 0.02$) suggesting that the increased activity of the luminal Na^+/H^+ exchanger in BBM vesicles isolated from acidotic proximal tubules was independent of the pH gradient imposed at the beginning of the experiments shown in Fig. 2. To examine the mechanism of increased activity of the Na^+/H^+ exchanger in vesicles isolated from acidotic proximal tubule suspensions, we evaluated the kinetics of this exchanger by measuring initial rates (4 s) of $^{22}Na^+$ influx as a function of the $[Na^+]$. Hanes-Woolf plots of the results (Fig. 4) demonstrate an increase in the V_{max} for Na^+ with no significant difference in K_m in the acidotic group compared to control. This is consistent with either an increase in the number and/or turnover rate of the Na^+/H^+ transporters.

We next evaluated the activity of the $Na^+:CO_3^=:HCO_3^-$ cotransporter in BLM vesicles isolated from control and acidotic tubular suspensions. In the experiments illustrated in Fig. 5, the time course of Na^+ influx into these vesicles was assayed in the presence of an inward pH and HCO_3^- gradient (pH_o/pH_i = 7.5/6.0, 20% CO_2). The influx of Na^+ into the BLM vesicles was twofold above the equilibrium value in acidotic group, whereas it showed a minimal overshoot pattern in the control group. There was no significant difference in the equilibrium values in the two groups. To further examine this process, HCO_3^--dependent Na^+ influx into BLM vesicles was assayed in the presence and absence of 0.5 mM DIDS, an inhibitor of the $Na^+:CO_3^=:HCO_3^-$ cotransporter (Fig. 6). Na^+ influx in vesicles isolated from acidotic tubule suspensions was 83% greater than that in control vesicles (347±49 nmol/mg protein in C vs 635±74 in A, $P < 0.01$). Furthermore, the increment in $^{22}Na^+$ influx in the acidotic group was abolished when DIDS was added to the external solution, suggesting that majority of the observed difference between the acidotic and control groups was mediated predominantly via $Na^+:CO_3^=:HCO_3^-$ cotransport. In these experiments, ^{22}Na influx was measured in the presence of an inward pH and HCO_3^- gradient (pH_o = 7.5, pH_i = 6.0, 20% CO_2). It is possible, therefore, that the increased activity of the $Na^+:HCO_3^-$ cotransporter in BLM vesicles isolated from acidotic tubules was secondary to a decreased proton permeability of the vesicles. Such a change in permeability could retard the rate of H^+ and HCO_3^- gradient dissipation

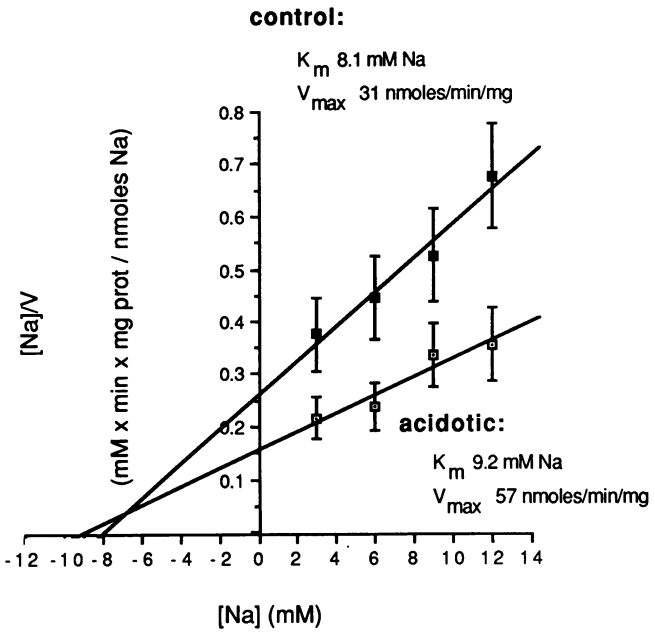

Figure 4. Kinetics of pH-dependent Na^+ influx into BBM vesicles. Brush border membrane vesicles from acidotic and control tubule suspensions were preequilibrated for 120 min at 20°C in a medium consisting of 67 mM TMA gluconate, 149 mM potassium gluconate, 52 mM Mes, 42 mM Hepes, 21 mM TMA hydroxide, pH 6.0. The 4-s uptake of $^{22}Na^+$ into vesicles was assayed in the presence of a medium consisting of 67 mM TMA gluconate, 149 potassium gluconate, 31 mM mannitol, 10 mM Mes, 42 mM Hepes, 31 mM TMA hydroxide, pH 7.5. The Na^+ concentration was varied by replacing TMA gluconate with sodium gluconate in the uptake medium. Valinomycin was added at the start of preequilibration period. Values shown for uptake represent mean±SE for experiments performed in quadruplicate on four different membrane preparations.

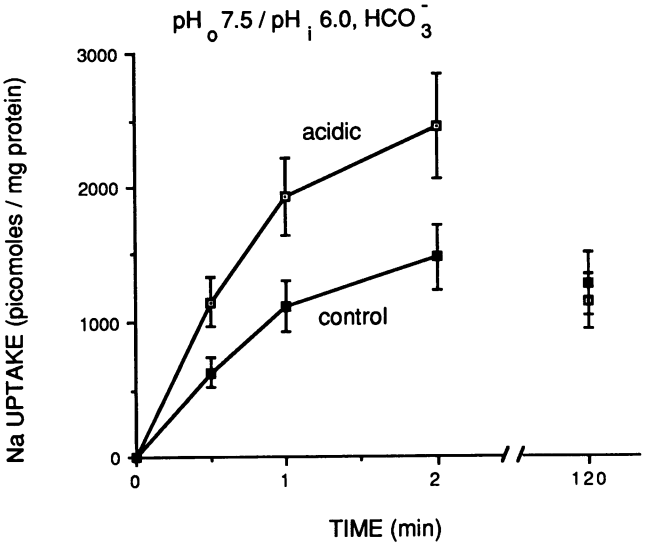

Figure 5. Effect of HCO_3^- gradient on Na^+ influx into BLM vesicles. Basolateral membrane vesicles from acidotic and control tubular suspensions were preequilibrated for 120 min at 20°C in a medium consisting of 52 mM TMA gluconate, 165 mM potassium gluconate, 52 mM Mes, 42 TMA hydroxide, pH 6.0, gassed with 20% CO_2. Uptake of 1 mM $^{22}Na^+$ into vesicles was assayed in the presence of a medium consisting of 52 mM TMA gluconate, 51 potassium gluconate, 114 mM potassium bicarbonate, 31 mM mannitol, 10 mM Mes, 31 mM TMA hydroxide, pH 7.5, gassed with 10% CO_2. Values shown for uptake represent mean±SE for experiments performed in quadruplicate on four different membrane preparations.

across the vesicles and indirectly increase the activity of the Na^+:HCO_3^- cotransporter. To examine this possibility, the BLM vesicles were incubated at pH 7.5. An outward Na^+ gradient was imposed (Na_i/Na_o = 50/2 mM) and HCO_3-dependent Na^+-Na^+ exchange (26) was measured in the presence of a media at pH 7.5 (pH_o = pH_i = 7.5, 10% CO_2). As demonstrated in Fig. 7, vesicles from acidotic group had a > 78% increase in ^{22}Na influx compared to the control group (180±32 nmol/mg protein in C vs 320±43 in A, $P < 0.02$) suggesting that the increased activity of the Na^+:HCO_3^- cotransporter in BLM vesicles isolated from acidotic proximal tubule suspensions was due to direct effect of acidosis on the Na^+:HCO_3^- cotransporter. To examine the mechanism of increased activity of the Na^+:$CO_3^=$:HCO_3^- cotransporter in BLM vesicles from acidotic tubule suspensions, we evaluated the kinetics of this transport process. The initial rate (4 s) of $^{22}Na^+$ influx in basolateral membrane vesicles isolated from control and acidotic tubule suspension was measured as a function of the [Na^+]. Hanes-Woolf plots of the results (Fig. 8) demonstrate that the V_{max} for Na^+ was increased in the acidotic group with no significant change in K_m. These results are consistent with either an increase in numbers or turn over rates of the transporter.

To determine the potential role of new protein synthesis in the adaptive increases observed in the luminal Na^+/H^+ exchanger and basolateral Na^+:HCO_3^- cotransporter, proximal tubular suspensions were incubated in the presence and absence of 0.05 mM cyclohexamide in the acidic media. BBM and BLM vesicles were isolated and the activity of the luminal Na^+/H^+ exchanger and basolateral Na^+:HCO_3^- cotransporter

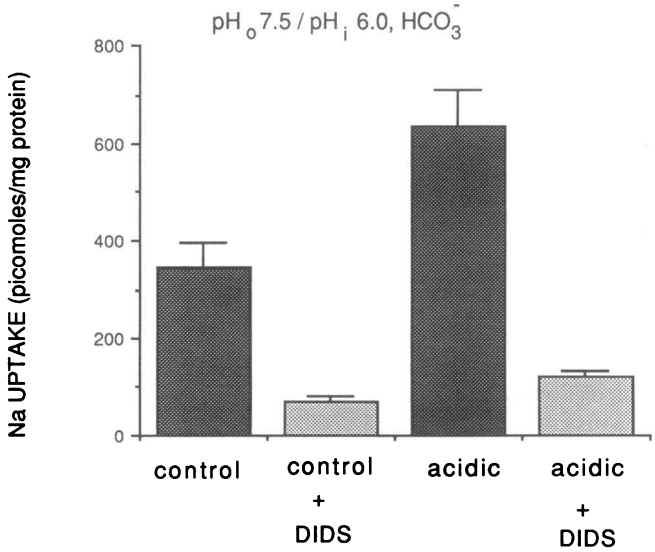

Figure 6. Effect of DIDS on HCO_3^--dependent Na^+ influx in BLM vesicles. The 10-s uptake of 1 mM $^{22}Na^+$ into the basolateral membrane vesicles preequilibrated in pH 6.0 medium was assayed in the presence of 57 mM HCO_3^-, 20% CO_2 at pH 7.5 as described in Fig. 5. DIDS (0.5 mM) was added as the disodium salt to the external solution. Valinomycin was added at the start of the preequilibration period. Values shown for uptake represent mean±SE for experiments performed in quadruplicate on four different membrane preparations.

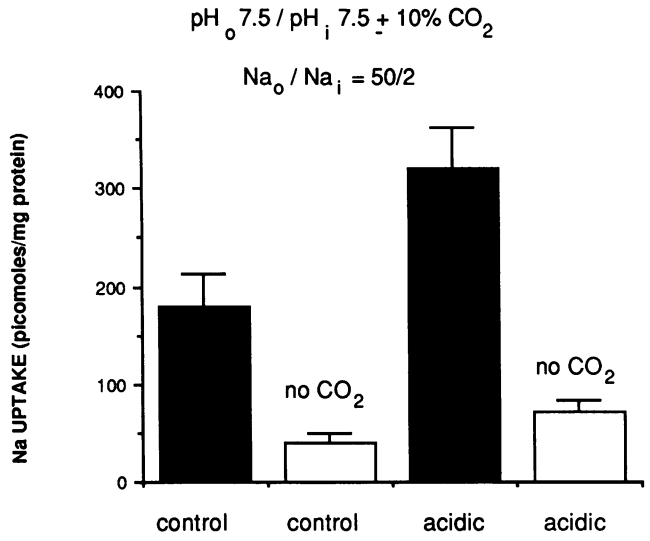

Figure 7. Effect of in vitro acidosis on $Na^+:CO_3^=:HCO_3^-$ cotransport measured in absence of pH gradient. Basolateral membrane vesicles isolated from acidotic and control tubular suspensions were preequilibrated for 120 min at 20°C in a medium consisting of 52 mM TMA gluconate, 50 mM Na gluconate, 42 mM Hepes, 21 mM TMA hydroxide, pH 7.5 that either contained an additional 57 mM potassium gluconate and was gassed with 100% N_2 or contained 57 mM K-HCO_3^- and gassed with 10% CO_2, 90% N_2. The 10-s uptake of 2 mM $^{22}Na^+$ into basolateral membrane vesicles was assayed in the presence of a medium consisting of 52 mM TMA gluconate, 48 mM TMA gluconate, 2 mM Na gluconate, 42 mM Hepes, 21 mM TMA hydroxide, pH 7.5 that either contained an additional 57 mM potassium gluconate and was gassed with 100% N_2 or contained 57 mM K-HCO_3^- and gassed with 10% CO_2, 90% N_2. Values shown for uptake represent mean±SE for experiments performed in quadruplicate on four different membrane preparations.

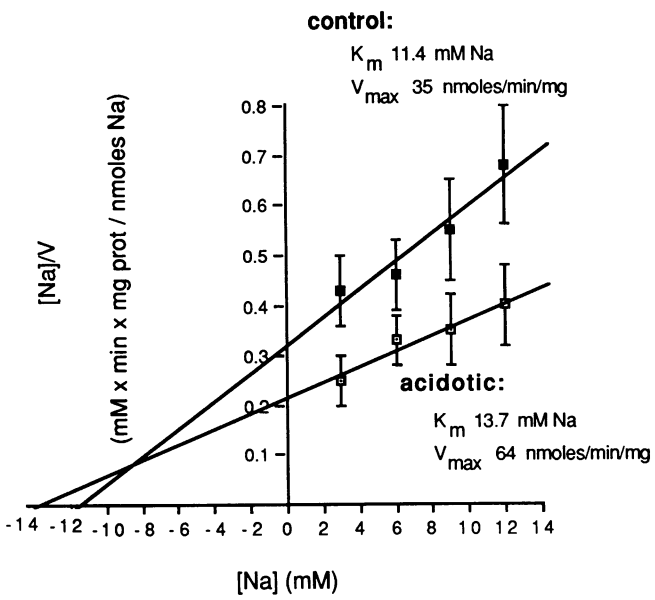

Figure 8. Kinetics of HCO_3^--dependent Na^+ influx into BLM vesicles. Basolateral membrane vesicles from acidotic and control tubular suspensions were preequilibrated for 120 min at 20°C in a medium consisting of 67 mM TMA gluconate, 149 mM potassium gluconate, 52 mM Mes, 42 Hepes, 21 mM TMA hydroxide, pH 6.0 and gassed with 20% CO_2. The 4-s uptake of $^{22}Na^+$ into vesicles was assayed in the presence of a medium consisting of 67 mM TMA gluconate, 35 potassium gluconate, 114 mM potassium bicarbonate, 31 mM mannitol, 10 mM Mes, 42 mM Hepes, 31 mM TMA hydroxide pH 7.5. The Na^+ concentration was varied by replacing TMA gluconate with sodium gluconate in the uptake medium. Valinomycin was added at the start of preequilibration period. Values shown for uptake represent mean±SE for experiments performed in quadruplicate on four different membrane preparations.

was assayed as in Figs. 1 and 4, respectively. The results, shown in Table I, demonstrate that cyclohexamide did not prevent increases in the activities of these two transport processes. Incubation of the tubular suspensions in the control media with cyclohexamide for 2 h did not have any significant effect on the activities of the luminal Na^+/H^+ exchanger and basolateral $Na^+:HCO_3^-$ cotransporter (the pH-dependent $^{22}Na^+$ influx into the BBM vesicles was 335.6±27.6 pmol/mg protein per 10 s for the control vs 309.2±28.5 for the cyclohexamide-treated group ($P > 0.05$, $n = 4$). The HCO_3^- dependent $^{22}Na^+$ influx into the BLM vesicles was 357.3±34.4 pmol/mg protein per 10 s for the control vs 325.2±34.1 for the cyclohexamide-treated group ($P > 0.05$, $n = 4$).

We have performed further studies evaluating the effect of shorter incubation periods of the tubular suspensions at less extreme acidic pH on the activities of the acidification transport processes. In a series of experiments, the proximal tubular suspensions were incubated at pH 7.1 for 50 min at 37°C. The BBM and BLM vesicles were isolated and studied for Na^+/H^+ exchange and $Na^+:HCO_3^-$ cotransport activity. The pH-dependent $^{22}Na^+$ influx into the BBM vesicles was 412.2±30.6 pmol/mg protein per 10 s for the control vs 540.7±42.3 for the acidotic group ($P < 0.05$, $n = 4$). The $^{22}Na^+$ influx in the presence of 1 mM amiloride was 66.7±9.1 pmol/mg protein per 10 s for the control vs 83.3±10.4 for the acidotic group ($P > 0.05$, $n = 4$). The HCO_3-dependent $^{22}Na^+$ influx into the BLM vesicles was 482.2±47.6 pmol/mg protein per 10 s for the control vs 641.3±49.6 for the acidotic group ($P < 0.05$, $n = 4$). The $^{22}Na^+$ influx in the presence of 0.5 mM DIDS was 74.5±9.1 pmol/mg protein per 10 s for the control vs 106.5±17.1 for the acidotic group ($P > 0.05$, $n = 4$).

Discussion

Much evidence suggests that systemic pH plays a key role in the regulation of HCO_3^- reabsorption in the proximal tubule (27, 28). Metabolic acidosis, a condition manifested by a decrease in serum pH and $[HCO_3^-]$, is associated with an increase in the rate of HCO_3^- reabsorption in the proximal tubule (15, 16). This has been shown to be due to increased activity of the luminal Na^+/H^+ exchanger and basolateral $Na^+:HCO_3^-$ cotransporter (17–19). Studies in membrane vesicles isolated from acidotic rabbits have demonstrated that there is a parallel adaptive increase in these two transport processes (18). Kinetic studies suggest that the adaptive changes are due to an increased V_{max} of these two transport processes (18). In these experiments, the animals were given diet supplemented with NH_4Cl in their drinking water for several days. These studies, therefore, evaluated the effect of chronic metabolic acidosis in the intact animal. Whether the processes responsible for mediating the adaptive changes in the Na^+/H^+ exchanger and $Na^+:HCO_3^-$ cotransporter in metabolic acidosis involve a direct signal at the level of the kidney or result from systemic factors was not answered by these experiments. Furthermore,

Table I. Effect of Cyclohexamide on Na^+ Influx in BBM and BLM Vesicles Isolated from Acidotic Tubular Suspensions

	Acidic	Acidic (+ cyclohexamide)
$^{22}Na^+/H^+$ exchange (BBM)	498±66	459±57
$^{22}Na^+:HCO_3^-$ cotransport (BLM)	610±84	574±76
	Activity: pmol/mg protein	

The 10-s uptake of 1 mM $^{22}Na^+$ into the BBM and BLM vesicles preequilibrated in pH 6.0 medium was assayed in the presence of inward pH gradient (BBM) and inward pH and HCO_3^- gradient (BLM). The composition of the media was similar to Fig. 2 (BBM) and Fig. 6 (BLM). 1 mM amiloride or DIDS was added to the external solution. Valinomycin was added at the start of the preequilibration period. Values shown for uptake represent mean±SE for experiments performed in quadruplicate on four different membrane preparations.

the rapidity of the adaptive changes under these conditions was not determined.

The experiments demonstrated in Figs. 1–6 indicate that the signal(s) responsible for the adaptive increases in the activity of Na^+/H^+ exchanger and basolateral $Na^+:HCO_3^-$ cotransporter in acute metabolic acidosis resides in the kidney. The nature of the mediating signal(s) responsible for these adaptive changes remain speculative.

Results of the experiments demonstrated in Figs. 1–3 indicate that in vitro metabolic acidosis increases the rate of pH-dependent Na^+ influx in renal cortical BBM vesicles. The increase in Na^+ influx was amiloride sensitive, suggesting that Na^+/H^+ exchange was responsible for the increase. Moreover, our results suggest that increased activity of the Na^+/H^+ exchanger is due to an increase in the V_{max} with no significant change in the K_m of the exchange process.

We also studied the effect of in vitro metabolic acidosis on pathways involved in the exit of HCO_3^- across the basolateral membrane of proximal tubule cells. The results show that HCO_3^- dependent ^{22}Na influx into BLM vesicles isolated from acidotic tubules was increased compared to control group. The increase in HCO_3^--dependent Na^+ influx was DIDS sensitive suggesting that increased activity of the $Na^+:CO_3^=:HCO_3^-$ cotransporter was responsible for the results. Kinetic studies evaluating the nature of these adaptive changes showed that there was an increase in V_{max} with no change in K_m.

The cellular mechanism(s) by which in vitro metabolic acidosis increases Na^+/H^+ exchange and basolateral $Na^+:HCO_3^-$ cotransport remains unknown. In rabbits with chronic metabolic acidosis, the increase in Na^+/H^+ exchange and $Na^+:HCO_3^-$ cotransport was found to be due to an increase in V_{max} for the transporters (17, 18). This is similar to the results of the present studies. An increase in V_{max} under these conditions is consistent with either an increase in the number or turnover rate of these transport proteins. Given the brief time of exposure of the tubular suspensions to an acidic pH, synthesis of new transport protein seems unlikely. Failure of cyclohexamide to prevent the adaptive changes in the Na^+/H^+ exchanger and $Na^+:HCO_3^-$ cotransporter also lends support to this conclusion. One possible mechanism, however, might be increased incorporation of existing intracellular transport proteins into the membranes upon exposure of the tubules to an acidic pH. This could possibly occur via recruitment of endosomal vesicles to the luminal and basolateral membranes. Recently, Sabolic et al. demonstrated the presence of an amiloride-sensitive Na^+/H^+ exchanger in endosomal vesicles isolated from rabbit kidney proximal tubule cells (29). In these cells, lowering cell pH by increasing pCO_2-stimulated incorporation of acid-extruding endosomes into the cell membrane (30). Incubation of proximal tubular suspensions from rabbit or dog kidneys in acidic medium results in cellular acidosis (31, 32). It is possible, therefore, that acidic medium lowers the proximal tubule cell pH which in turn stimulates the exocytosis of endosomal vesicles to the luminal membrane. This would lead to extrusion of acid via luminal Na^+/H^+ exchange. The role of the basolateral $Na^+:HCO_3^-$ cotransporter under these circumstances is more complicated. Based on the driving forces for H^+ and HCO_3^- transport across the luminal and basolateral membrane, reduction of the pH in the incubating medium should decrease the activity of the Na^+/H^+ by decreasing the favorable gradient for H^+ extrusion. The increased activity in luminal Na^+/H^+ exchange shown in Figs. 1–4, and as demonstrated in intact animals with metabolic acidosis (17–19), can not be explained on the basis of the effect of decreased luminal pH (1). A major portion of the intracellular acidosis that is observed in proximal tubule cell suspensions incubated in acidic medium (31, 32) results from increased base extrusion via the basolateral $Na^+:HCO_3^-$ cotransporter (2–5). Reduction of peritubular $[HCO_3^-]$ increases the driving force for the exit of HCO_3^- via this cotransporter across the basolateral membrane (2–5). This leads to acidification of the cells providing more substrate for the Na^+/H^+ exchanger in the luminal membrane whose activity subsequently increases. Whether there is also an increased permeability of the proximal tubule cells to protons in acidic medium remains speculative. Such a phenomenon could also increase the cellular proton concentration and increase the activity of the luminal Na^+/H^+ exchanger independent of the activity of the basolateral $Na^+:HCO_3^-$ cotransporter.

The cellular mechanism for increased activity of the $Na^+:HCO_3^-$ cotransporter in basolateral membrane vesicles harvested from acidic tubules also remains undetermined. An increase in V_{max} is consistent with either an increase in turnover rate or incorporation of new transport proteins. To our knowledge, endosomes containing a $Na^+:HCO_3^-$ cotransporter have not been isolated from the proximal tubule.

Whether incubation of proximal tubular suspensions in acidic medium affects the fluidity of the membrane vesicles is not clear. It has been demonstrated that changes in the fluidity of BBM vesicles can affect the activity of certain transport proteins (33, 34). Studies by Ives et al. have shown that alterations in membrane fluidity have no effect on the rate of Na^+/H^+ exchange in BBM vesicles isolated from rabbit kidney cortex (35).

Another possible explanation for the increased activity of the luminal Na^+/H^+ exchanger and the basolateral $Na^+:HCO_3^-$ cotransporter in in vitro metabolic acidosis might be via the activation of protein kinases. Protein kinase C (PKC) activation increases the activity of Na^+/H^+ exchange by phosphorylating the transporter protein (36). Preliminary results on BLM vesicles have demonstrated that protein kinase C also

activates the Na^+:HCO_3^- cotransporter (37). It is possible, therefore, that acidic pH, either via extracellular or intracellular signals activates protein kinase C which in turn phosphorylates the Na^+/H^+ exchanger and Na^+:HCO_3^- cotransporter proteins subsequently stimulating the activity of these two transport proteins.

Studies evaluating the effect of pH on Na^+/H^+ exchange system have shown that preincubation of cultured proximal tubule cells in acidic medium for two days increases the activity of the Na^+/H^+ exchanger (38). This suggests that the increased activity of the Na^+/H^+ exchanger that is observed in metabolic acidosis is mediated via signal(s) at the level of proximal tubule cells. Preliminary studies examining the mechanisms of adaptive increases in Na^+/H^+ exchange systems in respiratory acidosis have shown that inhibition of PKC in cultured proximal tubule cells prevented the upregulation of the Na^+/H^+ exchanger in acidic medium (39). This suggests that PKC is involved in mediating the Na^+/H^+ exchanger response to acidic medium. The increased activity of the Na^+/H^+ exchanger that was observed in the above studies (38, 39) are in agreement with the results of our experiments. The present studies further demonstrate that in vitro acidosis activates the basolateral Na^+:HCO_3^- cotransporter. In addition, these experiments suggest that the adaptive responses to in vitro acidosis are acute and occur in a matter of hours. Indeed, incubation of the proximal tubular suspensions in less extreme acidic pH (7.1) and shorter exposure (50 min) also resulted in adaptive increases in the luminal Na^+/H^+ exchanger and the basolateral Na^+/HCO_3^- cotransporter (see Results). These data strongly suggest that the effect of in vitro metabolic acidosis is acute. They further suggest that the degree of adaptive increase in these two transport processes are dependent on the severity of acidosis and duration of exposure to the acidic medium (see Results). Based on the differential effect of cyclohexamide in acute (Table I) vs chronic (38) acid incubation, one can conclude that the cellular mechanisms responsible for the upregulation of Na^+/H^+ exchange system are different under these two conditions. Indeed, a recent study shows that chronic acid exposure is associated with increased expression of the Na^+/H^+ exchanger cDNA suggesting that chronic acidosis induces transcriptional changes in the Na^+/H^+ exchanger gene (40).

In summary, in vitro metabolic acidosis causes parallel adaptive increases in the luminal Na^+/H^+ exchanger and basolateral Na^+:HCO_3^- cotransporter in the rabbit proximal tubule. The signal(s) responsible for evoking these adaptive changes reside at the level of the renal tubule and is (are) independent of systemic factor(s). These adaptive changes are likely responsible for increased ability of the proximal tubule to reabsorb filtered HCO_3^- in systemic acidosis.

Acknowledgments

The excellent secretarial assistance of Marcia A. Harmon is acknowledged.

These studies were supported by a grant-in-aid from the American Heart Association-Indiana Affiliate, Inc., and by a grant from the Department of Veterans Affairs (M. Soleimani).

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