J. Membrane Biol. 144, 209–216 (1995)

The Journal of
Membrane
Biology

Differential Regulation of Na^+/H^+ Exchange and H^+-ATPase by pH and HCO_3^- in Kidney Proximal Tubules

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Received: 26 August 1994/Revised: 7 November 1994

Abstract. This study examines the effects of acute in vitro acid-base disorders on Na^+/H^+ and H^+-ATPase transporters in rabbit kidney proximal tubules (PT). PT suspensions were incubated in solutions with varying acid base conditions for 45 min and utilized for brush border membrane (BBM) vesicles preparation. BBM vesicles were studied for Na^+/H^+ exchange activity (assayed by $^{22}Na^+$ influx) or abundance (using NHE-3 specific antibody) and H^+-ATPase transporter abundance (using antibody against the 31 kDa subunit). The Na^+/H^+ exchanger activity increased by 55% in metabolic acidosis (pH 6.5, HCO_3^- 3 mM) and decreased by 41% in metabolic alkalosis (pH 8.0, HCO_3^- 90 mM). The abundance of NHE-3 remained constant in acidic, control, and alkalotic groups. H^+-ATPase abundance, however, decreased in metabolic acidosis and increased in metabolic alkalosis by 57% and 42%, respectively. In PT suspensions incubated in isohydric conditions (pH 7.4), Na^+/H^+ exchanger activity increased by 29% in high HCO_3^- group (HCO_3^- 96 mM) and decreased by 16% in the low HCO_3^- groups (HCO_3^- 7mM. The NHE-3 abundance remained constant in high, normal, and low [HCO_3^-] tubules. The abundance of H^+-ATPase, however, increased by 82% in high [HCO_3^-] and decreased by 77% in the low [HCO_3^-] tubules. In PT suspensions incubated in varying pCO_2 and constant [HCO_3^-], Na^+/H^+ exchanger activity increased by 35% in high pCO_2 (20% pCO_2, respiratory acidosis) and decreased by 32% in low pCO_2 (1.5% pCO_2, respiratory alkalosis) tubules. The NHE-3 abundance remained unchanged in high, normal, and low pCO_2 tubules. However, the H^+-ATPase abundance increased by 74% in high pCO_2 and decreased by 69% in low pCO_2 tubules.

The results of these studies suggest that the luminal Na^+/H^+ exchanger is predominantly regulated by pH whereas H^+-ATPase is mainly regulated by [HCO_3^-] and/or pCO_2. They further suggest that the adaptive changes in H^+-ATPase transporter are likely mediated via endocytic/exocytic pathway whereas the adaptive changes in Na^+/H^+ exchanger are via the nonendocytic/exocytic pathway.

Key words: Na^+/H^+ exchange — H^+-ATPase — Proximal tubules — Kidney — Acid-base — pH

Introduction

The major mechanism for the reabsorption of HCO_3^- in the kidney proximal tubule is by a process of active acid secretion mediated via Na^+/H^+ and H^+-ATPase transporters (Aronson, 1983; Alpern, 1990; Krapf, 1993). The Na^+/H^+ exchanger mediates an electroneutral, amiloride-sensitive process that regulates blood pH by secreting H^+ in exchange for Na^+ subsequently leading to HCO_3^- and Na^+ reabsorption (Aronson, 1983; Alpern, 1990; Krapf, 1993). Recent studies have demonstrated that NHE-3 is the Na^+/H^+ exchanger isoform that is present in luminal membranes of proximal tubules (Biemesderfer, 1993; Bookstein, 1994; Soleimani, 1994*a;* Soleimani, 1994*c*). The H^+-ATPase transporter mediates an electrogenic, amiloride-insensitive, NEM-sensitive process that

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secrets H^+ into the lumen utilizing the energy generated by ATP hydrolysis (Alpern, 1990; Krapf, 1993). The renal H^+-ATPase has been purified and found to be a large multisubunit protein (Brown, 1987; Brown, 1988). H^+-ATPase is responsible for 30–40% of HCO_3^- reabsorption in proximal tubule while Na^+/H^+ exchange mediates the rest (Kurtz, 1987; Preisig, 1987).

Since the reabsorption of HCO_3^- in the proximal tubule occurs via the luminal Na^+/H^+ exchanger and H^+-ATPase transporter, it would be anticipated that alterations in proximal tubular acidification in pathologic states should result from changes in the activity of the Na^+/H^+ exchanger and the H^+-ATPase. Studies evaluating *chronic* metabolic acid-base disorders suggest that luminal Na^+/H^+ exchange is increased in metabolic acidosis and decreased in metabolic alkalosis (Akiba, 1987; Preisig, 1988; Hamm, 1992). Similarily, in *acute* acid-base disorders, metabolic acidosis increased whereas metabolic alkalosis decreased the Na^+/H^+ exchanger (Soleimani, 1992, 1994*b*). Chronic respiratory acidosis was also found to be associated with increased luminal Na^+/H^+ exchange activity (Talor, 1987; Krapf, 1989). The possible adaptive alterations in H^+-ATPase transporter in response to acid-base disorders is less well understood. In rats with *chronic* metabolic acidosis or alkalosis, the abundance of proximal tubule H^+-ATPase was not significantly different from the control groups as assessed by immunocytochemical methods (Bastani, 1991). Luminal bicarbonate concentration appears to be an important determinant of H^+-ATPase activity in proximal tubules. Increasing luminal $[HCO_3^-]$ at constant pH (pH 7.4, HCO_3^- 25–50 mM) was associated with increased HCO_3^- reabsorption in rat perfused proximal tubules (Bank, 1989). These effects were observed even when Na^+/H^+ exchange was inhibited with amiloride (Bank, 1989). These results suggest that the proximal tubule H^+-ATPase activity is upregulated thereby increasing HCO_3^- reabsorption in the presence of increased luminal $[HCO_3^-]$ (Bank, 1989). Increased luminal flow rate was shown to be associated with increased H^+-ATPase activity in one (Maddox, 1993) but not other investigations (Preisig 1987). However, the cellular mechanisms that are involved in regulation of proximal tubule Na^+/H^+ exchange and H^+-ATPase in these acute acid-base disorders are poorly understood. Therefore, the present studies examined the effect of acute acid-base disorders on luminal H^+-ATPase transporter and Na^+/H^+ exchange using membrane vesicles isolated from tubular suspensions incubated in various acid-base conditions. The results demonstrate differential regulation of Na^+/H^+ exchange and H^+-ATPase by pH and HCO_3^-/CO_2. The results further suggest that the adaptive regulation of H^+-ATPase is via alterations in transporter abundance at the luminal membrane. The Na^+/H^+ exchange adaptation is most likely via a post translational modification such as protein phosphorylation.

Materials and Methods

Tubular Suspension and Membrane Vesicles Preparation

Male New Zealand white rabbits were killed by intravenous sodium pentobarbital. Tubular suspensions were prepared from kidney cortex using Percoll gradient centrifugation as described previously (Soleimani, 1992). The proximal tubular suspensions were then incubated in modified Krebs-HCO_3 Ringer solution of varying pH, HCO_3^-, or pCO_2 for 45 min at 37°C. The pH was adjusted by replacing NaCl with known concentrations of $NaHCO_3^-$. After incubation, the tubules were homogenized in a buffer containing 10 mM mannitol, and 2 mM Tris titrated to pH 7.1 with HCl (Soleimani, 1992). Brush border membrane (BBM) vesicles were isolated from this homogenate by a Ca^{++} aggregation method (Evers, 1978) as employed before (Soleimani, 1990, 1992). BBM vesicles were stored at –70°C and used within two weeks of preparation. The purification of BBM vesicles relative to the initial cortical homogenate was 8–11-fold as determined by alkaline phosphatase enrichment and was similar in the different experimental groups. BBM vesicle size, calculated from external Na^+ concentration and equilibrium values for $^{22}Na^+$ uptake was also not significantly different between groups.

Sodium Dodecyl Sulfate-polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblot Analysis

Brush border membranes were solubilized and subjected to a SDS-PAGE according to the Laemmli protocol (Laemmli, 1970). Proteins were electrophoretically transferred to nitrocellulose at 200 mA for 15 hr. Strips of nitrocellulose were blocked in 0.1% Tween 20/phosphate buffered saline (PBST) and then incubated with either monoclonal antibody against the 31-kDa subunit of H^+-ATPase or NHE-3 specific antiserum at 1:400 dilution, respectively for 2 hr. The NHE-3 specific antiserum was generated using a fusion protein method as described recently (Bookstein, 1994: Soleimani, 1994*a;* Soleimani, *in press*). The H^+-ATPase specific antibody, a generous gift from Dr. Stephen Gluck at Washington University, was generated against a 10 amino acid synthetic peptide derived from the predicted sequence of the carboxy terminal of the 31 kD subunit of the transporter (Bastani, 1991). Immunodetection of NHE-3 was accomplished by addition of alkaline phosphatase-conjugated goat anti-rabbit IgG antibody and developed with nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) dissolved in *N,N,*-dimethylformamide (DMF). Immunodetection of H^+-ATPase was by enhanced chemiluminescence (ECL) (Amersham) using horse raddish peroxidase-conjugated goat anti-rabbit IgG antibody. Western blots were scanned using Image-1/MetaMorph Program from Universal Imaging.

$^{22}Na^+$ Transport Measurements

The timed uptake of $^{22}Na^+$ by membrane vesicle suspensions was assayed at room temperature in quadruplicate by a rapid filtration technique as previously described (Grassl, 1986; Soleimani, 1989*a,b*). Vesicles and all experimental media were continuously gassed with 100% 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 pre-equilibrated in media of appropriate composition to ensure that $[K^+]_i = [K^+]_o$ during uptake measurement. The intravesicular buffering

capacity, measured as previously described (Ives, 1985) and used earlier (Soleimani, 1992), showed no differences among the three experimental groups.

Data Analysis

The data are expressed as means ± SEM. Statistical analysis was determined using ANOVA test with $P < 0.05$ considered statistically significant.

Materials

$^{22}Na^+$ was purchased from New England Nuclear (Boston, MA). Valinomycin, amiloride, nitrocellulose filters and nitro blue tetrazolium (NBT) were purchased from Sigma Chemicals (St. Louis, MO). Valinomycin was added to the membrane suspension in a 1:100 dilution from a stock solution in 95% ethanol. Alkaline phosphatase-conjugated anti-rabbit IgG and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) were purchased from Boehringer Mannheim (Indianapolis, IN). BCECF and nigericin were purchased from Molecular Probe (Eugene, OR). ECL lit was purchased from Amersham. SDS, acrylamide, and *NN'*-methylenebisacrylamide were purchased from Bi-Rad (Hercules, CA).

Results

We first examined the effect of in vitro metabolic acidosis and alkalosis on luminal Na^+/H^+ exchange. Proximal tubular suspensions were incubated in regular Ringer (pH 7.4, HCO_3^- 24 mM, acidic (pH 6.5, HCO_3^- 3 mM), or alkaline media (pH 8.0, HCO_3^- 90 mM) and gassed with 5% CO_2 for 45 min at 37°C. The influx of $^{22}Na^+$ into BBM vesicles isolated from these acidotic, control, and alkalotic tubular suspensions was measured in the presence of an inwardly directed pH gradient (pH_o/pH_i = 7.5/6.0), ± 1 mM amiloride, Na^+/H^+ exchange activity was calculated as the amiloride-sensitive $^{22}Na^+$ influx by subtracting the amiloride-insensitive component from the total count. As shown in Fig. 1, Na^+/H^+ exchange activity increased by 55% in metabolic acidosis and decreased by 41% in metabolic alkalosis ($P < 0.02$ and < 0.04, respectively).

To determine if alterations in Na^+/H^+ exchange activity in Fig. 1 result from changes in the number of the exchanger, BBM proteins isolated from the tubular suspension used in Fig. 1 were subjected to SDS-PAGE, electrophoretically transferred to nitrocellulose membrane, and blotted against NHE-3 antiserum. NHE-3 is a 95–100 kDa protein (Bookstein, 1994; Soleimani, 1994*a;* Soleimani, *in press*) and is localized to the luminal membranes of kidney proximal tubules and intestine (Biemesderfer, 1993; Bookstein, 1994; Soleimani, 1994*a, c*). A representative immunoblot (four separate NHE-3 immunoblots were performed for each acid base disorder) is shown in Fig. 2. As indicated, the abundance of NHE-3 remained unchanged in vesicles isolated from acidotic, control, and alkalotic tubules. Two smaller proteins at 90 and 70 kD area were also occasionally detected with NHE-3 immune serum. The immunodetection of the these two proteins was not consistent, suggesting the presence of a proteolytic breakdown product of the 95–100 kDa protein. To examine if these metabolic acid base disorders affected the H^+-ATPase transporter, BBM proteins isolated from proximal tubule suspensions exposed to acid, control, or alkaline solution

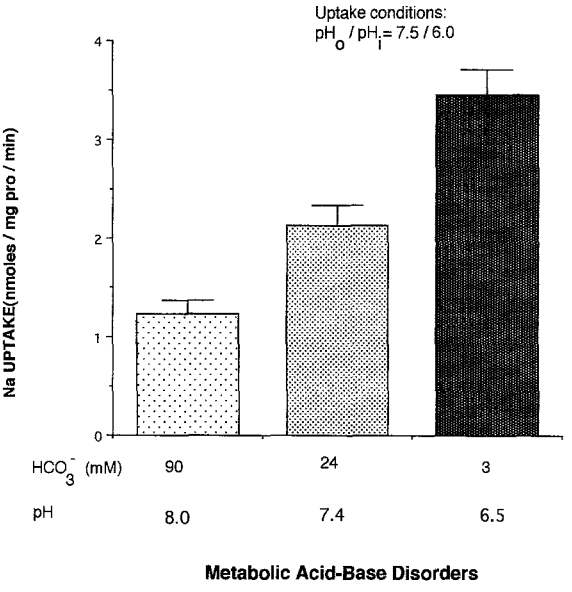

Fig. 1. Effect of in vitro metabolic acidosis and alkalosis on amiloride-sensitive $^{22}Na^+$ influx in brush border membranes. BBM vesicles from acidotic, control, or alkalotic tubular suspensions were pre-equilibrated for 120 min at 20°C in a medium consisting of (mM) 92 TMA gluconate, 60 potassium gluconate, 52 MES, 42 HEPES, 21 TMA-hydroxide, pH 6.0. 10 sec uptake of 1 mM $^{22}Na^+$ into BBM vesicles was assayed in the presence of a medium consisting of (mM) 92 TMA gluconate, 60 potassium gluconate, 31 mannitol, 10 MES, 42 HEPES, 31 TMA hydroxide, pH 7.5, ±1 mM amiloride. Values shown for uptake represent mean ± SE for experiments performed in quadruplicate on three different membrane preparations.

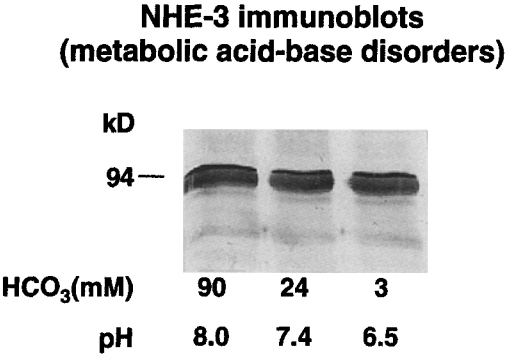

Fig. 2. Effect of in vitro metabolic acidosis and alkalosis on NHE-3 abundance in BBM. Representative immunoblots of BBM protein (100μg) from acidotic, control, or alkalotic tubular suspensions against NHE-3 antiserum (1/400).

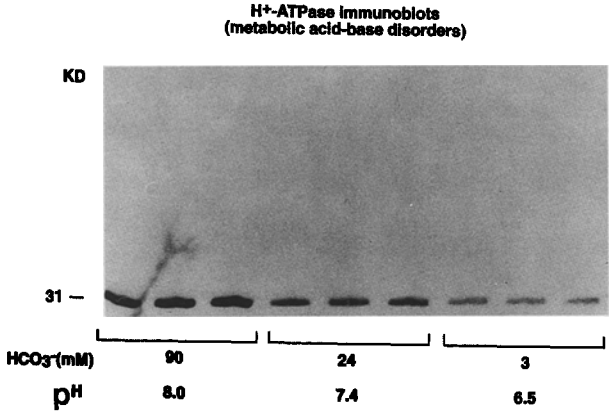

Fig. 3. Effect of in vitro metabolic acidosis and alkalosis on H^+-ATPase abundance in BBM. Representative immunoblots of BBM protein (100μg) from acidotic, control, or alkalotic tubular suspensions against H^+-ATPase 31 kDa subunit specific antibody.

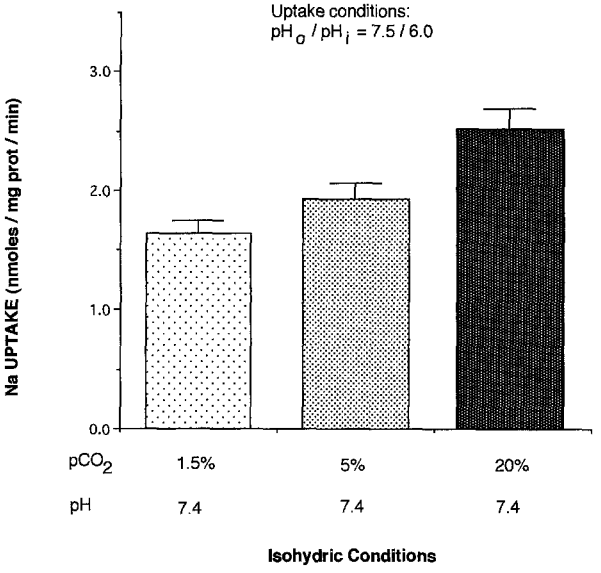

Fig. 4. Effect of in vitro isohydric conditions on amiloride-sensitive $^{22}Na^+$ influx in brush border membranes. BBM vesicles were isolated from tubular suspensions incubated in isohydric conditions (constant pH and low, normal, or high bicarbonate/pCO_2) and pre-equilibrated in a medium similar to Fig. 1. Ten-second uptake of 1 mM $^{22}Na^+$ into BBM vesicles was assayed as described in Fig. 1. Values shown for uptake represent mean ± SE for experiments performed in quadruplicate on three different membrane preparations.

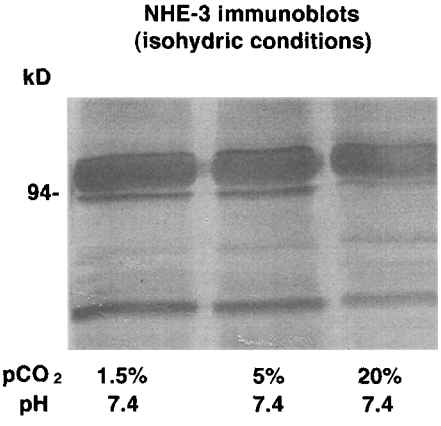

Fig. 5. Effect of in vitro isohydric conditions on NHE-3 abundance in BBM. Representative immunoblots of BBM protein (100μg) from tubules incubated in isohydric conditions (low bicarbonate, control, or high bicarbonate) against NHE-3 specific antiserum.

were subjected to immunoblot analysis utilizing a monoclonal antibody against the 31-kDa H^+-ATPase subunit. A representative immunoblot (three separate H^+-ATPase immunoblots were performed for each acid base disorder) is shown in Fig. 3. The abundance of H^+-ATPase decreased >57% in metabolic acidosis and increased >42% in metabolic alkalosis (*43% + 8.1 in Acidosis vs. 100% in C, $P < 0.05$ and 142% + 10.8 in Alkalosis vs. 100% in C, $P < 0.05$*). The results of the above studies (Figs. 1–3) suggest that H^+-ATPase transporter and Na^+/H^+ exchange are differentially regulated in metabolic acidosis or alkalosis. Moreover, the adaptive regulation of H^+-ATPase, unlike that of the Na^+/H^+ exchanger, appears to be mediated via alterations in the abundance of the transporter at the BBM.

In the above experiments, both pH and $[HCO_3^-]$ of the tubular suspensions were varied and therefore their separate roles could not be determined. To examine the role of HCO_3^- independent of pH, tubular suspensions were incubated at constant pH (isohydric conditions, pH 7.4) and varying $[HCO_3^-]$ and pCO_2. The influx of $^{22}Na^+$ into BBM vesicles isolated from high HCO_3^- (96 mM, 20% CO_2), control (24 mM, 5% CO_2), or low HCO_3^- (7 mM, 1.5% CO_2) groups was measured as described for Fig. 1. As shown in Fig. 4, the amiloride-sensitive $^{22}Na^+$ influx increased by 29% in high HCO_3^- ($P < 0.05$) and decreased by only 16% ($P > 0.05$) in low HCO_3^- groups. We examined whether or not these alterations in Na^+/H^+ exchanger activity are due to changes in the antiporter abundance. As shown in Fig. 5, immunoblot analysis with NHE-3 antiserum showed that the abundance of NHE-3 was unchanged in vesicles isolated from tubules exposed to low, normal, or high $[HCO_3^-]$. In contrast, alteration in $[HCO_3^-]$ affected the abundance of H^+-ATPase. Brush border membranes isolated from the high HCO_3^- group (HCO_3^- 96 mM, pCO_2 20%) showed 82% increase whereas membranes from the low HCO_3^- group (HCO_3^- 7 mM, pCO_2 1.5%) showed a 77% decrease in H^+-ATPase abundance as compared to control (*182% + 20.8 in Hi Bicarb vs. 100% in C, $P < 0.05$ and 23% + 6.9 in Low Bicarb vs. 100% in C, $P < 0.05$*). The results of the experiments in Figs. 4–6 suggest that increased $[HCO_3^-]/pCO_2$ increases Na^+/H^+ exchange activity and H^+-ATPase abundance in the luminal membranes of the proximal tubules. The increase in Na^+/H^+ exchange activity, however, does not appear to be due to an alteration in the abundance of the transporter.

We next examined the effects of pCO_2 independent

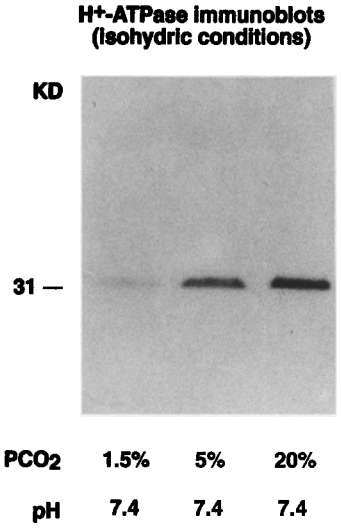

Fig. 6. Effect of in vitro isohydric conditions on H^+-ATPase abundance in BBM. Representative immunoblots of BBM protein (100μg) from tubules incubated in isohydric conditions (low bicarbonate, control, or high bicarbonate) against H^+-ATPase 31 kDa subunit specific antibody.

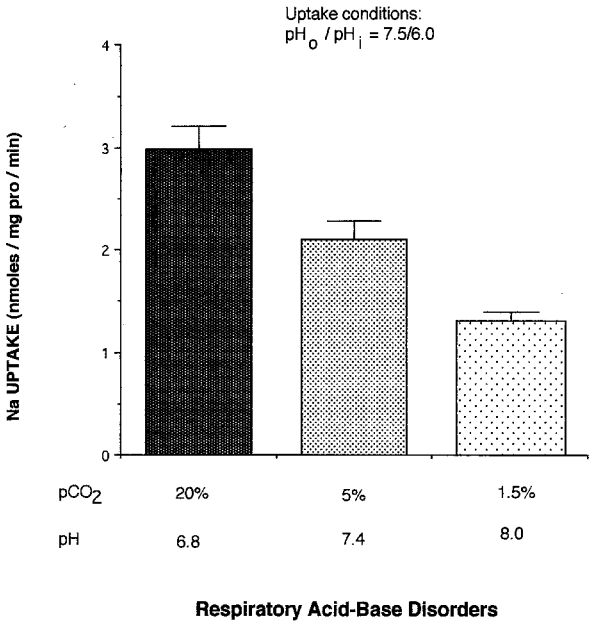

Fig. 7. Effect of in vitro respiratory acidosis and alkalosis on amiloride-sensitive $^{22}Na^+$ influx in BBM. BBM vesicles were isolated from tubular suspensions incubated in respiratory acidosis, control, or respiratory alkalosis conditions and pre-equilibrated in a medium similar to Fig. 1. Ten-second uptake of 1 mM $^{22}Na^+$ into BBM vesicles was assayed as described in Fig. 1. Values shown for uptake represent mean ± SE for experiments performed in quadruplicate on three different membrane preparations.

of HCO_3^-, by incubating tubular suspensions at constant [HCO_3^-] (24 mM) and varying pCO_2 of 1.5% (pH 7.95), 5% (pH 7.4), or 20% (pH 6.8). The influx of $^{22}Na^+$ into BBM vesicles isolated from high pCO_2 (20% pCO_2, pH 6.80), control (5% pCO_2, pH 7.4), or low pCO_2 (1.5% pCO_2, pH 7.95) groups was measured as described above. As shown in Fig. 7, the amiloride-sensitive $^{22}Na^+$ influx increased by 35% in high pCO_2 and decreased by 32% in low pCO_2 groups ($P < 0.05$ and $P < 0.04$, respectively).

To determine whether alterations in the Na^+/H^+ exchanger activity in Fig. 7 result from changes in the antiporter abundance, BBM proteins that were isolated from the tubular suspensions exposed to various pCO_2s were subjected to immunoblotting using NHE-3 antiserum. As indicated in Fig. 8, altering pCO_2 did not affect the abundance of NHE-3.

The effect of pCO_2 concentration on the abundance of H^+-ATPase was next examined. The results, shown in Fig. 9, indicate that brush border membranes from high pCO_2 group (pCO_2 20%, pH 6.8) showed increase whereas membranes from the low pCO_2 tubules (pCO_2 1.5%, pH 7.95) showed decrease in H^+-ATPase abundance (*174% + 13.8 in Hi pCO_2 vs. 100% in C, $P < 0.05$ and 31% + 5.18 in Low pCO_2 vs. 100% in C, $P < 0.05$*).

Discussion

In the present study, the acute adaptive regulation of Na^+/H^+ exchange and H^+-ATPase in proximal tubules was examined. Proximal tubule suspensions were incubated under various acid-base conditions for 45 min and utilized for BBM vesicles isolation. For Na^+/H^+ exchange, the activity of the antiporter was assayed by influx of pH-dependent, amiloride-sensitive $^{22}Na^+$ in BBM vesicles. The abundance of Na^+/H^+ exchange protein was determined by immunoblot analysis using NHE-3 specific antiserum. The abundance of H^+-ATPase in BBM was analyzed by immunoblot analysis using a specific antibody against the 31 kDa subunit. Na^+/H^+ exchange activity was increased both in metabolic and respiratory acidosis and decreased both in metabolic and respiratory alkalosis (Figs. 1 and 7). When tubules were incubated in solutions with constant pH and varying [HCO_3^-] and pCO_2, the antiporter activity increased in high [HCO_3^-] and pCO_2 and decreased (though not statistically significant) in low [HCO_3^-] and pCO_2 groups (Fig. 4). Taken together, these experiments suggest that the Na^+/H^+ exchanger is predominantly regulated by the pH (Figs. 1 and 7) and/or pCO_2 (Figs. 4 and 7) but not bey the [HCO_3^-] (Fig. 1). The abundance of NHE-3 remained constant in various acid base disorders suggesting that the adaptive regulation in the Na^+/H^+ exchanger in acute acid base disorders is mediated via process(es) that is (are) independent of transporter abundance at the luminal membrane.

In contrast, the abundance of the proximal tubule H^+-ATPase in the luminal membrane in acid base disorders was found to be altered. The H^+-ATPase abundance increased in metabolic alkalosis and decreased in meta-

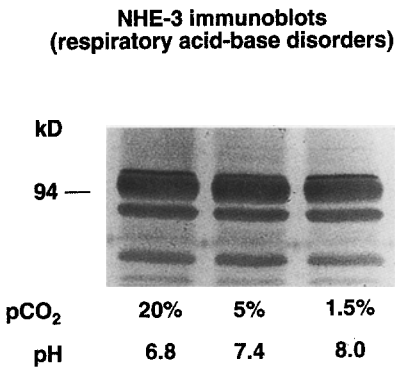

Fig. 8. Effect of in vitro respiratory acidosis and alkalosis on NHE-3 abundance in BBM. Representative immunoblots of BBM protein from different tubular suspensions (respiratory acidosis, control, or respiratory alkalosis) against NHE-3 specific antiserum.

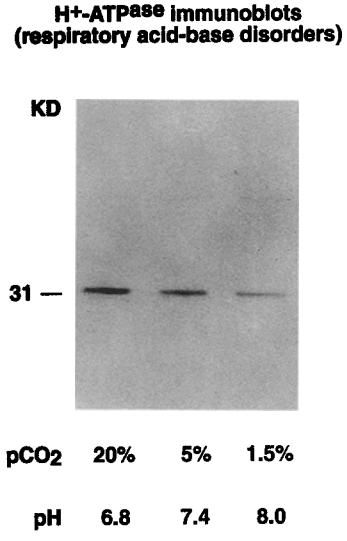

Fig. 9. Effect of in vitro respiratory acidosis and alkalosis on H^+-ATPase abundance in BBM. Representative immunoblots of BBM protein from different tubular suspensions (respiratory acidosis, control, or respiratory alkalosis) against H^+-ATPase 31 kDa subunit specific antibody.

bolic acidosis (Fig. 3). In BBM proteins isolated from tubules exposed to various pCO_2s abundance of the H^+-ATPase was increased in respiratory acidosis and decreased in respiratory alkalosis (Fig. 9). When tubules were incubated in solutions with constant pH and varying $[HCO_3^-]$ and pCO_2, the H^+-ATPase abundance increased in high $[HCO_3^-]$ and pCO_2 and decreased in low $[HCO_3^-]$ and pCO_2 groups (Fig. 6). These experiments suggest that the H^+-ATPase is predominantly regulated by the $[HCO_3^-]$ (Figs. 3 and 6) and/or pCO_2 (Figs. 6 and 9) but not by the pH (Fig.3). Assessment of H^+-ATPase transporter activity in BBM vesicles isolated from tubular suspensions would be difficult. This is due to right sidedness of the vesicles and lack of accessibility of ATP to its binding site in the inner surface of the vesicles. While the abundance of H^+-ATPase may not reflect the functional activity of this pump under in vivo conditions, the changes that occur in the number of the transporter in the brush border membrane suggest that H^+-ATPase is acutely regulated by changes in the acid-base status of the environment. It should be mentioned that distinguishing between an effect of increased HCO_3^- alone from increased pCO_2 in a H^+ transporting epithelium would be difficult. This is because that H^+ secretion into a solution of high HCO_3^- concentration results in the formation of large amount of pCO_2. The resulting pCO_2 would diffuse into the cells and could stimulate H^+ secretion.

In experiments by Gluck et al. (1982) increasing the media pCO_2 in turtle bladder was associated with increased proton secretion via a membrane fusion-dependent process of H^+-ATPase. These findings have been confirmed by other investigators (Stetson, 1983). Induction of acute respiratory acidosis in rat medulla (Madsen, 1983) and rabbit proximal tubule cells (Schwartz, 1985) has been shown to be associated with enhanced incorporation of acid extruding endosomes into the cell membrane. The results of our experiments illustrated that elevation in pCO_2 increased and reduction in pCO_2 decreased the abundance of H^+-ATPase in brush border membranes. These results confirm the findings by the above investigators that pCO_2 is an important regulator of H^+-ATPase and further suggest that in proximal tubule cell this regulation occurs via the movement of this transporter to and from the cell membrane.

While the results of the above experiments (Figs. 1–9) suggest that adaptive regulation of H^+-ATPase in acute acid-base disorders is most likely via the exo/endocytosis pathway, the molecular mechanism of the Na^+/H^+ exchanger regulation is less well understood. The abundance of the Na^+/H^+ exchanger isoform NHE-3 remained constant in various acid base disorders (Figs. 2, 5, and 8). A recent study examining the effect of acute acid-base disorders demonstrated that the increased activity of the Na^+/H^+ exchanger in acute in vitro metabolic acidosis was due to increased V_{max} and was insensitive to new protein synthesis inhibitors (Soleimani, 1992). The abundance of Na^+/H^+ exchanger isoform NHE-3, also, remained unchanged in acute in vitro metabolic acidosis (Fig. 2). Taken together, these results suggest that increased Na^+/H^+ exchanger activity in acute metabolic acidosis most likely results from activation of currently inactive membrane proteins. The most plausible explanation in this regard would be activation of the Na^+/H^+ exchanger via a phosphorylation-dependent process. A recent study demonstrated that activation of protein kinase C may be partially responsible for the adaptive upregulation of the Na^+/H^+ exchanger in acute in vitro metabolic acidosis (Soleimani, 1994*b*). The alteration in the Na^+/H^+ exchanger activity that is observed in metabolic acidosis and alkalosis (Fig. 1) or other acid

base disorders (Figs. 4 and 7) suggest that activation or inactivation of protein kinases may be responsible for acute regulation of this exchanger in several acid-base disorders.

Whether the effect of pH, $[HCO_3^-]$, or pCO_2 on regulation of Na^+/H^+ and H^+-ATPase transporters is via intracellular or extracellular sensors is not yet known. With regard to their clinical relevance, these results suggest that in respiratory acidosis, upregulation of both transporters would lead to increased HCO_3^- reabsorption from the proximal tubule. In respiratory alkalosis, however, down regulation of both transporters should lead to decreased HCO_3^- reabsorption. Similarly, in isohydric conditions, Na^+/H^+ and H^+-ATPase transporters are both upregulated in high $[HCO_3^-]$ and down regulated in low $[HCO_3^-]$ media. The increased abundance of the luminal membrane H^+-ATPase in metabolic alkalosis might be of clinical significance and suggests that this transporter could be responsible for increased proximal tubule HCO_3^- reabsorption in metabolic alkalosis.

In conclusion, the results of the above experiments suggest that the proximal tubule luminal Na^+/H^+ exchanger is predominantly regulated by pH and/or pCO_2 whereas H^+-ATPase is mainly regulated by $[HCO_3^-]$ and/or pCO_2. They further suggest that the adaptive changes in H^+-ATPase transporter are mediated via alteration in the number of transporters, whereas the adaptive changes in Na^+/H^+ exchanger are likely due to regulatory modifications such as phosphorylation of the exchanger.

These studies were supported by a Merit Review Grant from the Department of Veterans Affairs and a grant-in-aid from the American Heart Association (to M.S.), a Baxter Health Care Grant (to B.B.), and the National Institute of Health Grants DK 38510 (to E.B.C. and M.C.R.) and DK 42086 (to E.B.C.).

The excellent technical assistance of Yollanda J. Hattabaugh, Gwen L. Bizal, and L. Yang is greatly appreciated. Portions of these studies were presented at the annual meeting of the American Society of Nephrology, Boston, MA, November 1993, and published in abstract form (J.Am.Soc.Neph. **4:**840*A*, 1993)

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