A pH Modifier Site Regulates Activity of the Na^+:HCO_3^- Cotransporter in Basolateral Membranes of Kidney Proximal Tubules

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Abstract

HCO_3^- exit across the basolateral membrane of the kidney proximal tubule cell is mediated via an electrogenic Na^+:HCO_3^- cotransporter. In these experiments, we have studied the effect of internal pH on the activity of the Na^+:HCO_3^- cotransport system in basolateral membrane vesicles isolated from rabbit renal cortex. Equilibrium thermodynamics predicts that in the presence of constant intravesicular concentration of Na^+, an increasing concentration of HCO_3^- will be associated with an increasing driving force for Na^+:HCO_3^- cotransport across the vesicles. Our experimental approach was to preequilibrate the membrane vesicles with 1 mM $^{22}Na^+$ at pH_i 6.8–8.0 and known concentrations of HCO_3^-. The vesicles were diluted 1:100 into Na^+-free solution at pH 7.4 and the net flux of $^{22}Na^+$ was assayed over 5 s. The results demonstrate that the net flux of Na^+ was significantly higher at pH_i 7.2 than pH_i 8.0 despite much higher [HCO_3^-] at pH_i 8.0. This suggests that an internal pH-sensitive site regulates the activity of the Na^+:HCO_3^- cotransporter. This modifier site inhibits the cotransporter at alkaline pH despite significant base concentration and is maximally functional around physiologic pH. The combination of modifier sites on the luminal Na^+/H^+ exchanger and the basolateral Na^+:HCO_3^- cotransporter should help maintain intracellular pH in a narrow range with changes in extracellular pH. (*J. Clin. Invest.* 1991. 88:1135–1140.) Key words: renal acidification • regulation • vesicles • intracellular pH

Introduction

More than 85% of the filtered load of HCO_3^- is reabsorbed in the proximal tubule of the kidney. This results predominantly from the combined actions of the brush border membrane Na^+/H^+ exchanger and the basolateral membrane (BLM)[1] Na^+:HCO_3^- cotransport system acting in series (1–5). The Na^+:HCO_3^- cotransport system represents an electrogenic process (1, 3–5) with an apparent stoichiometry of three equivalents of base per Na^+ ion (6, 7). 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 (8). (Thus, the terms Na^+:$CO_3^=$:HCO_3^- cotransporter and Na^+:HCO_3^- transporter will be used interchangeably in this paper.) This latter process is involved in HCO_3^- transport in multiple types of cells including corneal endothelial cells (9), gastric parietal cells (10), cells of the cortical thick ascending limb of Henle (11), hepatocytes (12), and glial cells (13), suggesting that it is of general physiologic significance.

The pH sensitivity of the Na^+:HCO_3^- cotransporter has not been determined. Studies evaluating the pH sensitivity of the Na^+/H^+ exchanger in the luminal membranes of renal proximal tubules have demonstrated that the activity of this exchanger increases with decreasing pH_i (14). This is predominantly due to the presence of a H^+ stimulatory site on the Na^+/H^+ exchanger protein. If the basolateral Na^+:HCO_3^- cotransporter is affected by pH, elucidation of the pH sensitivity profile of this cotransporter would be very helpful in understanding the intracellular physiologic processes leading to HCO_3^- reabsorption by proximal tubules. Accordingly, we have attempted to study the pH sensitivity of the Na^+:HCO_3^- cotransporter in proximal tubular basolateral membrane vesicles using a thermodynamic approach. We find that the cotransporter is sensitive to pH with transport being greater at physiologic pH_i than alkaline pH_i despite a more favorable driving force for Na^+:HCO_3^- cotransport at alkaline pH.

Methods

Basolateral membrane vesicles were prepared from rabbit renal cortex by a modification of differential and percoll gradient centrifugation (15, 16). The vesicles were suspended in a medium consisting of 250 mM sucrose, 10 mM Hepes titrated to pH 7.5 with tetramethylammonium (TMA) hydroxide, and then frozen and stored at −70°C until used. The final vesicle preparation was enriched 10–12-fold in basolateral membranes relative to initial homogenate based on the specific activity of Na^+,K^+-ATPase.

We evaluated the sidedness of the final vesicle preparation by assessing the sensitivity of the Na^+,K^+-ATPase to ouabain before and after disruption of the vesicles by detergent (17). The results of these studies indicated that vesicles had a predominant right-side orientation with < 15% of them having inside-out orientation. These results are similar to those of other investigators (16).

Intravesicular content of $^{22}Na^+$ (0.14–0.23 mg of membrane protein per sample) was assayed in quadruplicate by rapid filtration, as previously described (15). The ice-cold medium used to dilute and wash the vesicles consisted of 170 mM K gluconate, 10 mM Hepes titrated to pH 7.5 with TMA hydroxide. In general, net fluxes of $^{22}Na^+$ were assayed over a 5-s interval. Na^+ uptake in the presence of an imposed HCO_3^- gradient is linear with time for > 5 s in rabbit renal basolateral membrane vesicles (18), indicating that dissipation of imposed ion gradients is minimal within this time interval. Other details of the experimental protocols are described in the figure legends.

Portions of these studies were presented at the Annual Meeting of the American Society of Nephrology, Washington, DC, December 1990, and published in abstract form (1990. *J. Am. Soc. Nephrol.* 1:260A).

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1. *Abbreviation used in this paper:* BLM, basolateral membrane.

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We purchased $^{22}Na^+$ from New England Nuclear (Boston, MA). Valinomycin, DIDS, 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 and Discussion

Our approach was to measure the rate of HCO_3^--dependent $^{22}Na^+$ efflux from BLM vesicles at varying internal pH. Equilibrium thermodynamics predict that in the absence of a transmembrane electrical potential difference, the $Na^+:CO_3^=:HCO_3^-$ cotransport system will be at equilibrium and will mediate no net flux when

$$[Na]_i/[Na]_o = [HCO_3^-]_o\,[CO_3^=]_o/[HCO_3^-]_i\,[CO_3^=]_i \quad (7).$$

(Subscripts i and o refer to intravesicular and extravesicular concentrations of a given substance or pH. When

$[Na]_i/[Na]_o$ exceeds $[HCO_3^-]_o\,[CO_3^=]_o/[HCO_3^-]_i\,[CO_3^=]_i$,

there will be net efflux of $^{22}Na^+$ along with internal HCO_3^-. Finally, when

$[Na]_i/[Na]_o$ is less than $[HCO_3^-]_o\,[CO_3^=]_o/[HCO_3^-]_i\,[CO_3^=]_i$,

there will be net influx of $^{22}Na^+$ along with external HCO_3^-. (Substitution of three equivalents of HCO_3^- in the above equations will yield the same results). In the first experiment, we tested the effect of internal pH on the rate of $^{22}Na^+$ flux occurring via the $Na^+:HCO_3^-$ cotransporter. Vesicles were preequilibrated with 1 mM $^{22}Na^+$ and known concentrations of TMA-gluconate, Hepes, MES, or TMA-hydroxide at pH_i 6.8, 7.0, 7.2, 7.4, 7.6, and 8. The vesicles were gassed with 10% CO_2 and incubated in the presence of known concentrations of K^+-HCO_3^- at different pH_i and valinomycin (0.5 mg/ml) to ensure that $K_i = K_o$. The vesicles were diluted 1:100 into a Na^+-free medium at pH 7.4, 10% CO_2, and the flux of 1 mM $^{22}Na^+$ was assayed over 5 s. As demonstrated in Fig. 1, *bottom,* the net efflux of Na^+ from vesicles with internal pH 7.0 and 7.2 was more than that of vesicles with internal pH 7.4, 7.6, and 8.0. At internal pH 8, there was no significant net $^{22}Na^+$ flux. This internal pH-sensitive Na^+ efflux is only observed in the presence of CO_2/HCO_3^- in the medium as the 5-s net flux of $^{22}Na^+$ in the absence of HCO_3^- was not significantly different than background (Fig. 1, *top*). The thermodynamic driving force for net Na^+ efflux via the Na^+/HCO_3^- cotransporter, ($\Delta\mu Na^{i\text{-}o} - 3\Delta\mu HCO_3^{o\text{-}i}$), is less favorable at pH_i 7.0 and 7.2 than at pH_i 7.4 or higher. At pH_i 8.0, the driving force for $^{22}Na^+$ efflux is 260-fold higher than at pH 7.2. However, at internal pH 8.0, there was no significant net flux of Na^+.

Because the changes observed in the preceding experiment were associated with changes in HCO_3^- and $CO_3^=$ concentrations, we investigated the possibility that inhibition of the $Na^+:CO_3^=:HCO_3^-$ cotransporter at more alkaline pH might be due to an inhibitory effect of $CO_3^=$ or HCO_3^-. Such would be the case if an inhibitory modifier site was activated after saturation of the substrate binding site. An example of this sort of phenomenon is the red cell anion exchanger which is inhibited in the presence of high chloride concentration (19). To investigate this possibility, we assayed the activity of the $Na^+:HCO_3^-$ cotransporter in the presence of constant $CO_3^=$ concentration and varying internal pH. Vesicles were preequilibrated at pH 7.2 and 8.0, and gassed with 60 and 1.5% CO_2. Assuming pK_1 and pK_2 of carbonic acid to be 6.1 and 10.1, respectively, the calculated $[CO_3^=]_i$ will be constant under these circumstances. The net change in intravesicular content of $^{22}Na^+$ was then assayed over 5 s immediately following the 1/100 dilution of the vesicles into Na^+-free medium at pH 7.4. As demonstrated in Fig. 2, in the presence of constant $[CO_3^=]$, increasing pH inhibited the activity of the $Na^+:HCO_3^-$ cotransporter suggesting that the inhibition observed at higher pH was not due to an increase in $[CO_3^=]$. (These experiments do not rule out the possibility that the inhibition of $Na^+:HCO_3^-$ cotransport at pH_i 8.0 could be due to lower intravesicular HCO_3^- concentration. This possibility, though unlikely, cannot be excluded by this experiment.) The goal of the next experiment was to study the role of $[HCO_3^-]$ on the activity of the $Na^+:CO_3^=:HCO_3^-$ cotransporter. Membrane vesicles were preequilibrated at pH 7.2 and 8.0, and gassed with 10 and 1.5% CO_2. The $[HCO_3^-]_i$ was kept at 28 mM in both sets of experiments. The net change in intravesicular content of $^{22}Na^+$ was then assayed over 5 s immediately after the 1/100 dilution of the membrane vesicles into Na^+-free medium at pH 7.4. As demonstrated in Fig. 3, in the presence of

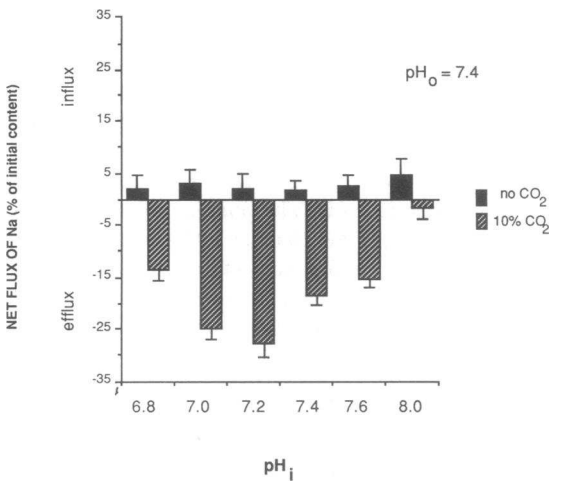

Figure 1. Effect of internal pH on $^{22}Na^+$ efflux. (*Bottom*) Basolateral membrane vesicles were preequilibrated for 120 min in a medium consisting of 52 mM TMA-gluconate, 42 mM Hepes, 21 mM TMA-OH which also contained 11.4 mM K-HCO_3, 170 mM K-gluconate, 6 mM mannitol titrated to pH 6.8 with 17 mM MES; or 18 mM K-HCO_3, 163 mM K-gluconate, and 10 mM mM mannitol titrated to pH 7.0 with 13 mM MES; or 28 mM K-HCO_3, 153 mM K-gluconate, and 15 mM mannitol titrated to pH 7.2 with 8 mM MES; or 45 mM K-HCO_3, 136 mM K-gluconate, 21 mM mannitol, and was titrated to pH 7.4 with 2 mM MES; or 71 mM K-HCO_3, 110 mM K-gluconate, and 21 mM mannitol titrated to pH 7.6 with 2 mM TMA-OH; or contained 181 mM K-HCO_3 titrated to pH 8.0 with 23 mM TMA-OH. Intravesicular content of $^{22}Na^+$ was measured before and 5 s after 1:100 dilution and reincubation of the vesicles in a medium of final composition 52 mM TMA-gluconate, 45 mM K-HCO_3, 136 K-gluconate, 21 mM mannitol, 42 mM Hepes and 21 mM TMA-OH titrated to pH 7.4 with 2 mM MES. The vesicles and the external solutions were gassed with 10% CO_2. (*Top*) K-HCO_3 was replaced with an equimolar amount of K-gluconate in the no-CO_2 experiments. Values shown for net flux represent mean±SE for four separate experiments performed in quadruplicate on four different membrane preparation.

constant [HCO_3^-], increasing pH inhibited the activity of the Na^+:HCO_3^- cotransporter suggesting that the inhibition observed at higher pH was not due to an increase in [HCO_3^-]. In the above two experiments, the changes in the partial pressure of CO_2 at constant pH_o was associated with changes in the external HCO_3^- concentration. This, however, resulted in an increased outward driving force for movement of Na^+:HCO_3^- at pH_i 8.0, 1.5% CO_2 as external [HCO_3^-] was lowered from 45 to 7 mM. Furthermore, when we assayed the efflux of $^{22}Na^+$ at external pH 6.0, a pH at which there is minimal [HCO_3^-] at any P_{CO_2}, the efflux of $^{22}Na^+$ decreased significantly at pH_i 8.0 and was maximal around pH_i 7.0–7.2 (data not shown).

We next evaluated the effect of increasing concentrations of $CO_3^=$ and HCO_3^- at constant pH_i. Membrane vesicles were incubated at pH 7.2 and gassed with either 10 or 60% CO_2. The net change in intravesicular content of $^{22}Na^+$ was then assayed over 5 s immediately after the 1/100 dilution of the membrane vesicles into Na^+-free medium at pH 7.4. As shown in Fig. 4, the higher concentrations of HCO_3^- and $CO_3^=$ did not inhibit the activity of the cotransporter.

To further confirm that the $^{22}Na^+$ efflux observed in the above experiments was mediated via the Na^+:$CO_3^=$:HCO_3^- cotransporter, the effect of the disulfonic stilbene DIDS on the rate of $^{22}Na^+$ flux across the membrane vesicles was evaluated (1, 3–5). The results, shown in Fig. 5, demonstrate that in the presence of 100 μM DIDS the $^{22}Na^+$ flux was abolished confirming that the observed net fluxes were mediated via the Na^+:HCO_3^- cotransporter.

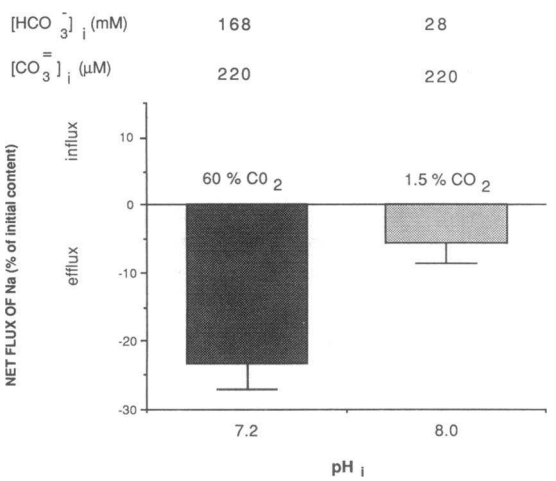

Figure 2. Effect of internal pH on $^{22}Na^+$ efflux at constant [$CO_3^=$]. Basolateral membrane vesicles were preequilibrated for 120 min in a medium consisting of 52 mM TMA-gluconate, 42 mM Hepes, 21 mM TMA-OH that in addition contained 168 mM K-HCO_3, 102 mM K-gluconate, and 15 mM mannitol titrated to pH 7.2 with 8 mM MES, and gassed with 60% CO_2 or containing 28 mM K-HCO_3, and 242 K-gluconate titrated to pH 8.0 with 23 mM TMA-OH and gassed with 1.5% CO_2. Intravesicular content of $^{22}Na^+$ was measured before and 5 s after 1:100 dilution and reincubation of the vesicles in a medium of final composition 52 mM TMA-gluconate, 42 mM Hepes, 21 mM TMA-OH and 21 mM mannitol titrated to pH 7.4 with 2 mM MES that in addition contained 270 mM K-HCO_3^- and was gassed with 60% CO_2 or 7 mM K-HCO_3^-, 263 mM K-gluconate and gassed with 1.5% CO_2. Values shown for net flux represent mean±SE for four separate experiments performed in quadruplicate on four different membrane preparation.

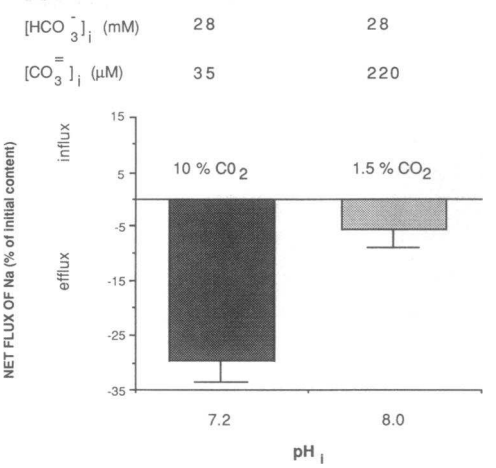

Figure 3. Effect of internal pH on $^{22}Na^+$ efflux at constant [HCO_3^-]. Basolateral membrane vesicles were preequilibrated for 120 min in a medium consisting of 52 mM TMA-gluconate, 42 mM Hepes, 21 mM TMA-OH that in addition contained 28 mM K-HCO_3, 242 mM K-gluconate, and 15 mM mannitol titrated to pH 7.2 with 8 mM MES and gassed with 10% CO_2 or which contained 28 mM K-HCO_3 and 242 K-gluconate titrated to pH 8.0 with 23 mM TMA-OH and gassed with 1.5% CO_2. Intravesicular content of $^{22}Na^+$ was measured before and 5 s after 1:100 dilution and reincubation of the vesicles in a medium of final composition 52 mM TMA-gluconate, 42 mM Hepes, 21 mM mannitol, and 21 mM TMA-OH titrated to pH 7.4 with 2 mM MES which in addition contained 45 mM K-HCO_3^- and 225 mM K-gluconate and gassed with 10% CO_2 or 7 mM K-HCO_3^- and 263 mM K-gluconate and gassed with 1.5% CO_2. Values shown for net flux represent mean±SE for four separate experiments performed in quadruplicate on four different membrane preparation.

To determine whether the inhibitory effect of alkaline pH on activity of the $Na^+CO_3^=$:HCO_3^- cotransporter is a reversible process or could be due to irreversible inactivation of the transport protein, we measured the net flux of $^{22}Na^+$ at pH_i 8.0/pH_o 7.4, washed the vesicles, reincubated them at pH_i 7.2, and assayed the net change in intravesicular content of $^{22}Na^+$ at pH_o 7.4. The results, shown in Fig. 6, indicate that the inhibitory effect of alkaline pH on the activity of the cotransporter is a reversible process because lowering the intravesicular pH from 8.0 to 7.2 reversed the inhibition and increased $^{22}Na^+$ efflux. This also rules out the possibility that the inhibitory effect of alkaline pH is due to loss of vesicle integrity.

The above experiments were performed with [K_o] = [K_i] and in the presence of potassium ionophore valinomycin. This will prevent the generation of any membrane potential and maintains the membrane potential at zero during the $^{22}Na^+$ net flux measurement. Under physiologic conditions, the basolateral membranes in the kidney proximal tubule maintains a membrane potential of −50 to −70 mV. This, in fact, functions as the driving force for the exit of Na^+:HCO_3^- cotransporter. It is possible that this membrane potential might have a regulatory role on the Na^+:HCO_3^- cotransporter, i.e., by altering the affinity of the modifier site to H^+. To address this possibility, an outward [K^+] gradient was imposed (K_i/K_o = 180/18 mEq) across the vesicles and the rate of Na^+:HCO_3^- cotransport was measured under conditions similar to those in Fig. 1. The results, shown in Table I, demonstrate that the inhibitory effect

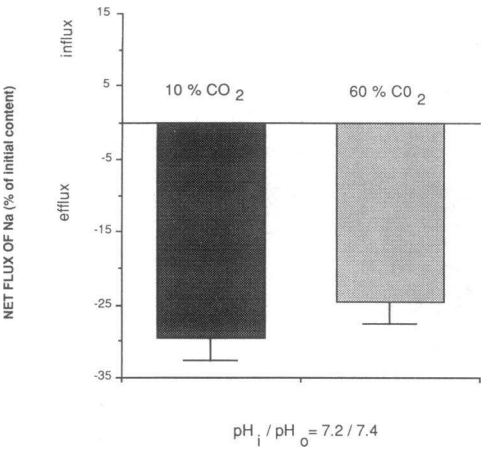

Figure 4. Effect of increasing internal [HCO_3^-] and [CO_3^-] on $^{22}Na^+$ efflux. Basolateral membrane vesicles were preequilibrated for 120 min in a medium consisting of 52 mM TMA-gluconate, 42 mM Hepes, 21 mM TMA-OH, and 15 mM mannitol titrated to pH 7.2 with 8 mM MES which in addition contained 168 mM K-HCO_3 and 102 mM K-gluconate and gassed with 60% CO_2 or 8 mM K-HCO_3 and 242 K-gluconate and gassed with 10% CO_2. Intravesicular content of $^{22}Na^+$ was measured before and 5 s after 1:100 dilution and reincubation of the vesicles in a medium of final composition 52 mM TMA-gluconate, 42 mM Hepes, 21 mM TMA-OH, and 21 mM mannitol titrated to pH 7.4 with 2 mM MES which in addition contained 270 mM K-HCO_3^- and gassed with 60% CO_2 or 45 mM K-HCO_3^- and 235 mM K-gluconate and gassed with 10% CO_2. Values shown for net flux represent mean±SE for four separate experiments performed in quadruplicate on four different membrane preparation.

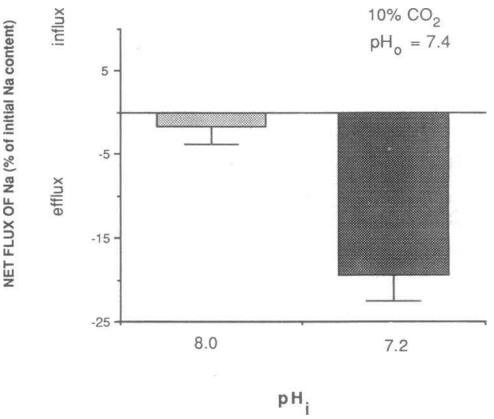

Figure 6. Reversal of the inhibitory effect of alkaline pH. Basolateral membrane vesicles were preequilibrated for 120 min in a medium consisting of 52 mM TMA-gluconate, 180 mM K-HCO_3, 42 mM Hepes, and 34 mM TMA-OH at pH 8.0 and gassed with 10% CO_2. Intravesicular content of $^{22}Na^+$ was measured before and 5 s after 1:100 dilution and reincubation of the vesicles in a medium of final composition 52 mM TMA-gluconate, 45 mM K-HCO_3^-, 135 mM K-gluconate, 42 mM Hepes, 11 mM mannitol, 21 mM TMA-OH, and 2 mM MES at pH 7.4, and gassed with 10% CO_2. The vesicles were washed and reincubated in a medium consisting of 52 mM TMA-gluconate, 28 mM K-HCO_3, 152 mM K-gluconate, 42 mM Hepes, 5 mM mannitol, 8 mM MES, and 21 mM TMA-OH at pH 7.2, and gassed with 10% CO_2. Intravesicular content of $^{22}Na^+$ was measured before and 5 s after 1:100 dilution and reincubation of the vesicles in an external medium similar to the above. Values shown for net flux represent mean±SE for four separate experiments performed in quadruplicate on four different membrane preparation.

observed at alkaline pH is independent of membrane potential. The HCO_3^--dependent $^{22}Na^+$ flux, measured by subtracting the $^{22}Na^+$ flux in the absence of CO_2 from total flux, was significantly higher at pH_i 7.0–7.4 than pH_i 8.0. In the presence of an outward [K^+] gradient, the magnitude of HCO_3-dependent $^{22}Na^+$ efflux was higher for any given pH_i when compared with the absence of a [K^+] gradient. This reflects the electrogenicity of the Na^+:HCO_3^- cotransporter as an outward [K^+] gradient generates an inside negative membrane potential and this increases the rate of Na^+:HCO_3^- exit across the vesicles.

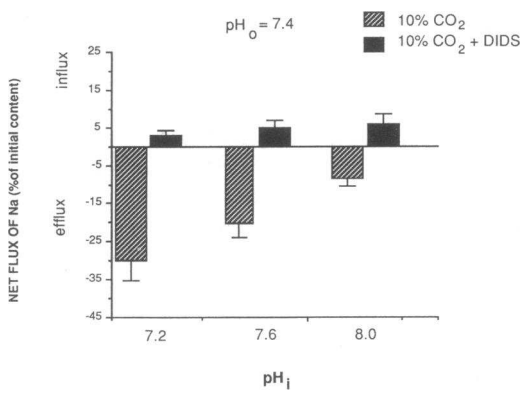

Figure 5. Effect of DIDS on HCO_3-dependent ^{22}Na flux. Basolateral membrane vesicles were pretreated with 100 μM DIDS for 30 min. The vesicles were washed and preequilibrated for 120 min at pH_i 7.2, 7.6, and 8.0 as described in Fig. 1. The intravesicular content of $^{22}Na^+$ was measured before and 5 s after 1:100 dilution and reincubation of the vesicles in a medium similar to Fig. 1. The vesicles and the external solutions were gassed with 10% CO_2. Values shown for net flux represent mean±SE for four separate experiments performed in quadruplicate on four different membrane preparation.

Table I. Effect of Internal pH on $^{22}Na^+$ Efflux in the Presence of Outward [K^+] Gradient

pH_i	6.8	7.2	7.6	8.0
HCO_3^--dependent Na flux (% *of initial content*)	−17.6±3.5	−34.2±6.2	−25.4±5.2	−13.5±4.2

Membrane vesicles were preequilibrated for 120 min in a medium identical to Fig. 1. The external solution was similar to Fig. 1 except that K-HCO_3^- was replaced with equimolar concentration of choline-HCO_3^- and a portion of K-gluconate was replaced with equimolar TMA-gluconate to keep the concentration of K-gluconate at 18 mM. The net flux of ^{22}Na was measured over 5-s period. The experiment was performed in the presence and absence of CO_2. The HCO_3-dependent ^{22}Na net flux was measured by subtracting the HCO_3-independent ^{22}Na flux from total flux. Values shown for HCO_3-dependent ^{22}Na net flux represent mean±SE for four separate experiments performed in quadruplicate on four different membrane preparation.

The results of the above experiments are compatible with the presence of an internal pH modifier site which regulates the activity of the $Na^+:HCO_3^-$ cotransporter. This modifier site inhibits the activity of the cotransporter at alkaline pH and displays a maximal functional activity around physiologic pH. This is in contrast with the pH sensitivity of the other HCO_3^- extruding processes, particularly the Cl^-/HCO_3^- exchanger. Two recent studies evaluating the pH sensitivity of the latter exchanger in lymphocytes and intestinal luminal membrane vesicles demonstrated that an alkaline pH stimulated this exchanger (20, 21). The $Na^+:CO_3^-:HCO_3^-$ cotransporter displays an inhibitory profile at alkaline pH and is more active at acidic (physiologic) pH. The luminal Na^+/H^+ exchanger and basolateral $Na^+:CO_3^-:HCO_3^-$ cotransporter in the renal proximal tubule act in series to reclaim filtered HCO_3^-. Activity of the luminal Na^+/H^+ exchanger leads to alkalinization of the cell providing more substrate for the basolateral $Na^+:CO_3^-:HCO_3^-$ cotransporter. The inhibitory effect of an alkaline pH on HCO_3^- transport across the basolateral membrane does not fit into this scheme. Intracellular pH measurements in rat proximal tubule cells show a baseline pH of 7.1–7.2 under normal conditions (1, 6). The results of our experiments demonstrate that the internal modifier site of the basolateral $Na:CO_3^-:HCO_3^-$ cotransporter will be maximally functional at pH 7.0–7.2. This suggests that the activity of the luminal Na^+/H^+ exchanger is coupled to the activity of the basolateral $Na^+:CO_3^-:HCO_3^-$ cotransporter via cell pH rather than by the substrate concentration. According to this hypothesis, an increase in the activity of the luminal Na^+/H^+ exchanger at physiologic or mildly acidotic (7.0) cell pH is associated with an increase in the activity of the basolateral $Na^+:CO_3^-:HCO_3^-$ cotransporter. This maintains the cell pH in a narrow range and provides maximal capacity for reabsorption of HCO_3^- from the luminal fluid.

Studies in brush border membrane vesicles have shown that the activity of the Na^+/H^+ exchanger increases with decreasing pH_i (14). Those studies also demonstrated the presence of a H^+ stimulatory modifier site on the Na^+/H^+ exchanger protein. One can speculate that pH-sensitive sites located on the Na^+/H^+ and $Na^+:HCO_3^-$ transport systems enhance the ability of these two transport processes to extrude intracellular acid and base loads and thereby regulate both cell pH and HCO_3^- reabsorption. The molecular mechanism of the stimulatory effect of acidic pH and inhibitory effect of alkaline pH on the activity of the $Na^+:HCO_3^-$ transporter remains speculative. One possible explanation is that this cotransporter contains titratable amino groups that are facing inward. Titration of amino groups may lead to conformational changes in the transport protein which affect its activity. Such a candidate is histidine which has a pKa in the range of 6.8–7.2. Studies of the luminal Na^+/H^+ exchanger are suggestive of the presence of a histidine amino group in the exchanger. It is conceivable, therefore, that one or more histidyl groups on the luminal Na^+/H^+ exchanger and basolateral $Na^+:HCO_3^-$ transporter may represent pH-sensitive sites that coordinate and regulate the activity of these two transport proteins. According to this scheme, increased activity of the luminal Na^+/H^+ exchanger at physiologic or mildly acidic pH is matched by maximal activity of the basolateral $Na^+:HCO_3^-$ transporter. This will mitigate against any significant changes in cell pH and provide maximal capacity for reabsorption of filtered HCO_3^- from the tubule lumen.

The physiologic significance of pH sensitivity of the $Na^+:CO_3^-:HCO_3^-$ cotransporter and its role in cell alkalosis has to be examined in intact tubules. Using the pH-sensitive dye BCECF, Krapf et al. evaluated the role of the luminal Na^+/H^+ exchanger and basolateral $Na^+:CO_3^-:HCO_3^-$ cotransporter in cell pH regulation in the intact perfused tubule (22). Increasing luminal pH increased and decreasing luminal pH decreased cell pH. Changes in cell pH were enhanced significantly in the presence of basolateral SITS suggesting that the $Na^+:CO_3^-:HCO_3^-$ cotransporter was an important determinant of cell pH. Simultaneous changes in luminal and peritubular pH invoked larger changes in cell pH that were SITS sensitive compared to changes in luminal pH alone. These results are compatible with the view that the basolateral $Na^+:CO_3^-:HCO_3^-$ cotransporter plays a more important role in regulating proximal tubule cell pH than the luminal Na^+/H^+ exchanger. As to the pH sensitivity of the basolateral $Na^+:CO_3^-:HCO_3^-$ cotransporter, these studies did not measure the rate of pH_i recovery from alkaline loads at varying alkaline pH_i. Comparison between cell pH response at acidic and alkaline pH is complicated by the fact that at acidic cell pH (induced by low luminal pH), the basolateral $Na^+:CO_3^-:HCO_3^-$ cotransporter is reversed and works as an "alkaline loader." This is also the case when both luminal and peritubular pH and HCO_3^- are increased (22).

In conclusion, the results of our studies suggest that the $Na^+:HCO_3^-$ cotransporter is more functional at or around physiologic pH. This is due to the presence of a pH-sensitive regulatory site. The results further suggest that either proximal tubule cells are not well prepared for intracellular alkalosis or that there may be an adaptive shift in pH sensitivity of the $Na^+:HCO_3^-$ cotransporter in acid-base disorders. Further studies will be necessary to evaluate these possibilities.

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References

1. Boron, W. F., and E. L. Boulpaep. 1983. Intracellular pH regulation in the renal proximal tubule of the salamander. Basolateral HCO_3^- transport. *J. Gen. Physiol.* 81:53–94.

2. Aronson, P. S. 1983. Mechanisms of active H^+ secretion in the proximal tubule. *Am. J. Physiol.* 245:F647–F659.

3. Preisig, P. A., and R. J. Alpern. 1989. Basolateral membrane H-OH-HCO_3 transport in the proximal tubule. *Am. J. Physiol.* 256:F751–F756.

4. Alpern R. J. 1990. Cell mechanisms of proximal tubule acidification. *Physiol. Rev.* 70:79–114.

5. Aronson, P. S., M. Soleimani, and S. M. Grassl. 1991. Properties of the renal Na^+-HCO_3^- cotransporter. *Semin. Nephrol.* 11:28–36.

6. Yoshitomi, K., B.-C. Burckhardt, and E. Fromter. 1985. Rheogenic sodium-bicarbonate cotransport in the peritubular cell membrane of rat renal proximal tubule. *Pfluegers Arch. Eur. J. Physiol.* 405:360–366.

7. Soleimani, M., S. M. Grassl, and P. S. Aronson. 1987. Stoichiometry of Na^+-HCO_3^- cotransport in basolateral membrane vesicles isolated from rabbit renal cortex. *J. Clin. Invest.* 79:1276–1280.

8. Soleimani, M., and P. S. Aronson. 1989. Ionic mechanism of Na-HCO_3 cotransport in rabbit renal basolateral membrane vesicles. *J. Biol. Chem.* 264:18302–18308.

9. Jentch, T. J., T. R. Stahlknecht, H. Hollwede, D. G. Fischer, S. K. Keller, and M. Wiederholt. 1985. A bicarbonate-dependent process inhibitable by disulfonic stilbenes and a Na^+/H^+ exchanger mediate $^{22}Na^+$ uptake into cultured bovine corneal endothelium. *J. Biol. Chem.* 260:795–801.

10. Curci, S., L. Debellis, and E. Frömter. 1987. Evidence for rheogenic sodium bicarbonate cotransport in the basolateral membrane of oxyntic cells of frog gastric fundus. *Pfluegers Arch. Eur. J. Physiol.* 408:497–504.

11. Krapf, R. 1988. Basolateral membrane $H/OH/HCO_3$ transport in the rat cortical thick ascending limb. *J. Clin. Invest.* 82:234–241.

12. Fitz, J. C., M. Persico, and B. F. Scharschmidt. 1989. Electrophysiological evidence for Na-coupled bicarbonate transport in cultured rat hepatocyte. *Am. J. Physiol.* 256:G491–G500.

13. Astion, M. L., A. L. Obaid, and R. K. Orkand. 1989. Effects of barium and bicarbonate on glial cells of necturus optic nerve. *J. Gen. Physiol.* 93:731–744.

14. Aronson, P. S., J. Nee, and M. A. Suhm. 1982. Modifier role of internal H^+ in activating the Na/H exchanger in renal microvillus membrane vesicles. *Nature (Lond.).* 299:161–163.

15. Grassl, S. M., and P. S. Aronson. 1986. Na^+/HCO_3^- co-transport in basolateral membrane vesicles isolated from rabbit renal cortex. *J. Biol. Chem.* 261:8778–8783.

16. Akiba, T., R. J. Alpern, J. Eveloff, J. Calamina, and D. G. Warnock. 1986. Electrogenic sodium/bicarbonate cotransport in rabbit renal cortical basolateral membrane vesicles. *J. Clin. Invest.* 78:1472–1478.

17. Soleimani, M., and P. S. Aronson. 1989. Effects of acetazolamide on Na^+-HCO_3 cotransport in basolateral membrane vesicles isolated from rabbit renal cortex. *J. Clin. Invest.* 83:945–951.

18. Soleimani, M., J. A. Bergman, M. A. Hosford, and T. D. McKinney. 1990. Potassium depletion increases luminal Na^+/H^+ exchange and basolateral Na^+:CO_3^-:HCO_3^- cotransport in rat renal cortex. *J. Clin. Invest.* 86:1076–1083.

19. Passow, H. 1986. Molecular aspects of band 3 protein-mediated anion transport across the red blood cell membrane. *Rev. Physiol. Biochem. Pharmacol.* 103:62–223.

20. Mugharbil, A., R. G. Knickelbein, P. S. Aronson, and J. W. Dobbins. 1990. *Am. J. Physiol.* G666–G670.

21. Mason, M. J., J. D. Smith, J. D. Garcia-Sato, and S. Grinstein. 1989. Internal pH-sensitive site couples Cl-HCO_3 exchange to Na/H antiport in lymphocytes. *Am. J. Physiol.* 256:C428–C433.

22. Krapf, R., C. A. Berry, R. J. Alpern, and F. C. Rector, Jr. 1988. Regulation of cell pH by ambient bicarbonate, carbon dioxide tension, and pH in the rabbit proximal convoluted tubule. *J. Clin. Invest.* 81:381–389.