A pH Modifier Site Regulates Activity of the Na\textsuperscript{+}:HCO\textsubscript{3} Cotransporter in Basolateral Membranes of Kidney Proximal Tubules

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Abstract

HCO\textsubscript{3} exit across the basolateral membrane of the kidney proximal tubule cell is mediated via an electrogenic Na\textsuperscript{+}:HCO\textsubscript{3} cotransporter. In these experiments, we have studied the effect of internal pH on the activity of the Na\textsuperscript{+}:HCO\textsubscript{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\textsuperscript{+}, an increasing concentration of HCO\textsubscript{3} will be associated with an increasing driving force for Na\textsuperscript{+}:HCO\textsubscript{3} cotransport across the vesicles. Our experimental approach was to pre-equilibrate the membrane vesicles with 1 mM \textsuperscript{22}Na\textsuperscript{+} at pH 6.8-8.0 and known concentrations of HCO\textsubscript{3}. The vesicles were diluted 1:100 into Na\textsuperscript{+}-free solution at pH 7.4 and the net flux of \textsuperscript{22}Na\textsuperscript{+} was assayed over 5 s. The results demonstrate that the net flux of Na\textsuperscript{+} was significantly higher at pH 7.2 than at pH 8.0 despite much higher [HCO\textsubscript{3}] at pH 8.0. This suggests that an internal pH-sensitive site regulates the activity of the Na\textsuperscript{+}:HCO\textsubscript{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\textsuperscript{+}/H\textsuperscript{+} exchanger and the basolateral Na\textsuperscript{+}:HCO\textsubscript{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\textsubscript{3} is reabsorbed in the proximal tubule of the kidney. This results predominantly from the combined actions of the brush border membrane Na\textsuperscript{+}/H\textsuperscript{+} exchanger and the basolateral membrane (BLM)\textsuperscript{1} Na\textsuperscript{+}:HCO\textsubscript{3} cotransport system acting in series (1–5). The Na\textsuperscript{+}:HCO\textsubscript{3} cotransport system represents an electrogenic process (1, 3–5) with an apparent stoichiometry of three equivalents of base per Na\textsuperscript{+} ion (6, 7). Recent studies have suggested that the actual ionic mechanism involves the cotransport of Na\textsuperscript{+}, CO\textsubscript{3} and HCO\textsubscript{3} in a 1:1:1 ratio (8). (Thus, the terms Na\textsuperscript{+}:CO\textsubscript{3}:HCO\textsubscript{3} cotransporter and Na\textsuperscript{+}:HCO\textsubscript{3} transporter will be used interchangeably in this paper.) This latter process is involved in HCO\textsubscript{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\textsuperscript{+}:HCO\textsubscript{3} cotransporter has not been determined. Studies evaluating the pH sensitivity of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger in the luminal membranes of renal proximal tubules have demonstrated that the activity of this exchanger increases with decreasing pH, (14). This is predominantly due to the presence of a H\textsuperscript{+} stimulatory site on the Na\textsuperscript{+}/H\textsuperscript{+} exchanger protein. If the basolateral Na\textsuperscript{+}:HCO\textsubscript{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\textsubscript{3} reabsorption by proximal tubules. Accordingly, we have attempted to study the pH sensitivity of the Na\textsuperscript{+}:HCO\textsubscript{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 than alkaline pH, despite a more favorable driving force for Na\textsuperscript{+}:HCO\textsubscript{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\textsuperscript{+}K\textsuperscript{-}-ATPase.

We evaluated the sidedness of the final vesicle preparation by assessing the sensitivity of the Na\textsuperscript{+},K\textsuperscript{-}-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 \textsuperscript{22}Na\textsuperscript{+} (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 \textsuperscript{22}Na\textsuperscript{+} were assayed over a 5-s interval. Na\textsuperscript{+} uptake in the presence of an imposed HCO\textsubscript{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.

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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$_2$::HCO$_3^-$ cotransport system will be at equilibrium and will mediate no net flux when

$$\frac{[Na^+_i]}{[Na^+]_o} = \frac{[HCO_3^-]_o}{[HCO_3^-]_i} \cdot \frac{[CO_2]_i}{[CO_2]_o} \cdot \frac{[CO_3^2-]_i}{[CO_3^2-]_o} \cdot (7)$$

(Subscripts i and o refer to intravesicular and extravesicular concentrations of a given substance or pH. When $[Na^+_i]/[Na^+]_o$ exceeds $[HCO_3^-]_o/[HCO_3^-]_i \cdot [CO_2]_o/[CO_2]_i \cdot [CO_3^2-]_i/[CO_3^2-]_o$, there will be net efflux of $^{22}$Na$^+$ along with internal HCO$_3^-$. Finally, when $[Na^+_i]/[Na^+]_o$, is less than $[HCO_3^-]_o/[HCO_3^-]_i \cdot [CO_2]_o/[CO_2]_i \cdot [CO_3^2-]_i/[CO_3^2-]_o$, 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-glucuronate, Hepes, MES, or TMA-hydroxide at pH 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$^{\infty} + \Delta \mu$HCO$_3^{\infty}$), is less favorable at pH 7.0 and 7.2 than at pH 7.4 or higher. At pH 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$_2^-$ concentrations, we investigated the possibility that inhibition of the Na$^+$:CO$_2$::HCO$_3^-$ cotransporter at more alkaline pH might be due to an inhibitory effect of CO$_2^-$ 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$_2$ 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$_a$ and pK$_a$ of carbonic acid to be 6.1 and 10.1, respectively, the calculated [CO$_2^-$], 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$_2^-$], 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$_3^2$-]. (These experiments do not rule out the possibility that the inhibition of Na$^+$:HCO$_3^-$ cotransporter at pH 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$^+$: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$_3^-$] 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-glucuronate, 42 mM Hepes, 21 mM TMA-OH which also contained 11.4 mM K-HCO$_3$, 170 mM K-glucuronate, 6 mM mannitol titrated to pH 6.8 with 17 mM MES; or 18 mM K-HCO$_3$, 163 mM K-glucuronate, and 10 mM mannitol titrated to pH 7.0 with 13 mM MES; or 28 mM K-HCO$_3$, 153 mM K-glucuronate, and 15 mM mannitol titrated to pH 7.2 with 8 mM MES; or 45 mM K-HCO$_3$, 136 mM K-glucuronate, 21 mM mannitol, and was titrated to pH 7.4 with 2 mM MES; or 71 mM K-HCO$_3$, 110 mM K-glucuronate, 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-glucuronate, 45 mM K-HCO$_3$, 136 K-glucuronate, 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-glucuronate 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 [HCO₃⁻], increasing pH inhibited the activity of the Na⁺:HCO₃⁻ cotransporter suggesting that the inhibition observed at higher pH was not due to an increase in [HCO₃⁻]. In the above experiments, the changes in the partial pressure of CO₂ at constant pH, was associated with changes in the external HCO₃⁻ concentration. This, however, resulted in an increased outward driving force for movement of Na⁺:HCO₃⁻ at pH 8.0, 1.5% CO₂ as external [HCO₃⁻] was lowered from 45 to 7 mM. Furthermore, when we assayed the efflux of ²²Na⁺ at external pH 6.0, a pH at which there is minimal [HCO₃⁻] at any PCO₂, the efflux of ²²Na⁺ decreased significantly at pH 8.0 and was maximal around pH 7.0–7.2 (data not shown).

We next evaluated the effect of increasing concentrations of CO₃⁻ and HCO₃⁻ at constant pH. Membrane vesicles were incubated at pH 7.2 and gassed with either 10 or 60% CO₂. The net change in intravesicular content of ²²Na⁺ 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 HCO₃⁻ and CO₃⁻ did not inhibit the activity of the cotransporter.

To further confirm that the ²²Na⁺ efflux observed in the above experiments was mediated via the Na⁺:CO₂⁻:HCO₃⁻ cotransporter, the effect of the disulfonic stilbene DIDS on the rate of ²²Na⁺ 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 ²²Na⁺ flux was abolished confirming that the observed net fluxes were mediated via the Na⁺:HCO₃⁻ cotransporter.

![Figure 2](image2.png)

Figure 2. Effect of internal pH on ²²Na⁺ efflux at constant [CO₃⁻]. Basolateral membrane vesicles were preequilibrated for 120 min in a medium consisting of 52 mM TMA-glucanate, 42 mM Hapes, 21 mM TMA-OH that in addition contained 28 mM K-HCO₃, 242 mM K-glucanate, and 15 mM mannitol titrated to pH 7.2 with 8 mM MES and gassed with 10% CO₂ or which contained 28 mM K-HCO₃ and 242 K-glucanate titrated to pH 8.0 with 23 mM TMA-OH and gassed with 1.5% CO₂. Intravesicular content of ²²Na⁺ was measured before and 5 s after 1:100 dilution and reincubation of the vesicles in a medium of final composition 52 mM TMA-glucanate, 42 mM Hapes, 21 mM mannitol, and 21 mM TMA-OH titrated to pH 7.4 with 2 mM MES which in addition contained 45 mM K-HCO₃ and 225 mM K-glucanate and gassed with 10% CO₂ or 7 mM K-HCO₃ and 263 mM K-glucanate and gassed with 1.5% CO₂. 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⁺:CO₂⁻:HCO₃⁻ cotransporter is a reversible process or could be due to irreversible inactivation of the transport protein, we measured the net flux of ²²Na⁺ at pH 8.0/pH₆ 7.4, washed the vesicles, reincubated them at pH 7.2, and assayed the net change in intravesicular content of ²²Na⁺ at pH 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 ²²Na⁺ 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⁺] = [K₆] 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 ²²Na⁺ 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⁺:HCO₃⁻ cotransporter. It is possible that this membrane potential might have a regulatory role on the Na⁺:HCO₃⁻ cotransporter, i.e., by altering the affinity of the modifier site to H⁺. To address this possibility, an outward [K⁺] gradient was imposed (K₆/K₆ = 180/18 mEq) across the vesicles and the rate of Na⁺:HCO₃⁻ cotransport was measured under conditions similar to those in Fig. 1. The results, shown in Table I, demonstrate that the inhibitory effect
observed at alkaline pH is independent of membrane potential. The HCO₃⁻-dependent ²²Na⁺ flux, measured by subtracting the ²²Na⁺ flux in the absence of CO₂ from total flux, was significantly higher at pH 7.0–7.4 than pH 8.0. In the presence of an outward [K⁺] gradient, the magnitude of HCO₃⁻-dependent ²²Na⁺ efflux was higher for any given pH, when compared with the absence of a [K⁺] gradient. This reflects the electrogenicity of the Na⁺:HCO₃⁻ cotransporter as an outward [K⁺] gradient generates an inside negative membrane potential and this increases the rate of Na⁺:HCO₃⁻ exit across the vesicles.

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

<table>
<thead>
<tr>
<th>pH_i</th>
<th>6.8</th>
<th>7.2</th>
<th>7.6</th>
<th>8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCO₃⁻-dependent Na flux (% of initial content)</td>
<td>-17.6±3.5</td>
<td>-34.2±6.2</td>
<td>-25.4±5.2</td>
<td>-13.5±4.2</td>
</tr>
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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-HCO₃ was replaced with equimolar concentration of choline-HCO₃ and a portion of K-glucolate was replaced with equimolar TMA-glucolate to keep the concentration of K-glucolate at 18 mM. The net flux of ²²Na was measured over 5-s period. The experiment was performed in the presence and absence of CO₂. The HCO₃⁻-dependent ²²Na net flux was measured by subtracting the HCO₃⁻-independent ²²Na flux from total flux. Values shown for HCO₃⁻-dependent ²²Na 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\textsuperscript{+}:HCO\textsubscript{3}\textsuperscript{−} 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\textsubscript{3}\textsuperscript{−} extruding processes, particularly the Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} 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\textsuperscript{+}:CO\textsubscript{3}\textsuperscript{−}:HCO\textsubscript{3}\textsuperscript{−}cotransporter in the renal proximal tubule act in series to reclaim filtered HCO\textsubscript{3}\textsuperscript{−}. Activity of the luminal Na\textsuperscript{+}/H\textsuperscript{+} exchanger and basolateral Na\textsuperscript{+}:CO\textsubscript{3}\textsuperscript{−}:HCO\textsubscript{3}\textsuperscript{−} cotransporter is an inhibitory profile at alkaline pH and more active at acidic (physiologic) pH. The luminal Na\textsuperscript{+}/H\textsuperscript{+} and basolateral Na\textsuperscript{+}:CO\textsubscript{3}\textsuperscript{−}:HCO\textsubscript{3}\textsuperscript{−} cotransporter in the renal proximal tubule act in series to reclaim filtered HCO\textsubscript{3}\textsuperscript{−}. Activity of the luminal Na\textsuperscript{+}/H\textsuperscript{+} exchanger leads to alkalization of the cell providing more substrate for the basolateral Na\textsuperscript{+}:CO\textsubscript{3}\textsuperscript{−}:HCO\textsubscript{3}\textsuperscript{−} cotransporter. The inhibitory effect of an alkaline pH on HCO\textsubscript{3}\textsuperscript{−} 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\textsubscript{3}:HCO\textsubscript{3} cotransporter will be maximally functional at pH 7.0–7.2. This suggests that the activity of the luminal Na\textsuperscript{+}/H\textsuperscript{+} exchanger is coupled to the activity of the basolateral Na\textsuperscript{+}:CO\textsubscript{3}:HCO\textsubscript{3} cotransporter via cell pH rather than by the substrate concentration. According to this hypothesis, an increase in the activity of the luminal Na\textsuperscript{+}/H\textsuperscript{+} exchanger at physiologic or mildly acidic (7.0) cell pH is associated with an increase in the activity of the basolateral Na\textsuperscript{+}:CO\textsubscript{3}:HCO\textsubscript{3} cotransporter. This maintains the cell pH in a narrow range and provides maximal capacity for reabsorption of HCO\textsubscript{3}\textsuperscript{−} from the luminal fluid.

Studies in brush border membrane vesicles have shown that the activity of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger increases with decreasing pH, (14). Those studies also demonstrated the presence of a H\textsuperscript{+} stimulatory modifier site on the Na\textsuperscript{+}/H\textsuperscript{+} exchanger protein. One can speculate that pH-sensitive sites located on the Na\textsuperscript{+}/H\textsuperscript{+} and Na\textsuperscript{+}:HCO\textsubscript{3}\textsuperscript{−} 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\textsubscript{3}\textsuperscript{−} reabsorption. The molecular mechanism of the stimulatory effect of acidic pH and inhibitory effect of alkaline pH on the activity of the Na\textsuperscript{+}:HCO\textsubscript{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\textsuperscript{+}/H\textsuperscript{+} 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\textsuperscript{+}/H\textsuperscript{+} exchanger and basolateral Na\textsuperscript{+}:HCO\textsubscript{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\textsuperscript{+}/H\textsuperscript{+} exchanger at physiologic or mildly acidic pH is matched by maximal activity of the basolateral Na\textsuperscript{+}:HCO\textsubscript{3} transporter. This will mitigate against any significant changes in cell pH and provide maximal capacity for reabsorption of filtered HCO\textsubscript{3}\textsuperscript{−} from the tubule lumen.

The physiologic significance of pH sensitivity of the Na\textsuperscript{+}:CO\textsubscript{3}:HCO\textsubscript{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\textsuperscript{+}/H\textsuperscript{+} exchanger and basolateral Na\textsuperscript{+}:CO\textsubscript{3}:HCO\textsubscript{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\textsuperscript{+}:CO\textsubscript{3}:HCO\textsubscript{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\textsuperscript{+}:CO\textsubscript{3}:HCO\textsubscript{3} cotransporter plays a more important role in regulating proximal tubule cell pH than the luminal Na\textsuperscript{+}/H\textsuperscript{+} exchanger.

In conclusion, the results of our studies suggest that the Na\textsuperscript{+}:HCO\textsubscript{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\textsuperscript{+}:HCO\textsubscript{3} cotransporter in acid-base disorders. Further studies will be necessary to evaluate these possibilities.

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References


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