Cellular Electrolyte and Volume Changes Induced by Acidosis in the Rabbit Proximal Straight Tubule

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ABSTRACT

Cellular acidosis induced either by high Pco2 or by low HCO3 concentrations has been shown to cause cell swelling in isolated, lumen-collapsed, S2 segments of the rabbit proximal tubule (Sullivan et al., Am J Physiol 1990;258:F830–F839). The swelling is not followed by a volume regulatory response. The ionic basis of the swelling has been investigated by measurement of the cellular K+, Na+, and Cl− content (electron probe) and HCO3 concentration (pH-sensitive fluorescent dye). Cell content of K+, Na+, and Cl− was expressed as a ratio to P content. Exposure to 15% CO2 increased K/P from 0.98 to 1.16, Cl/P from 0.14 to 0.20, and Na/P from 0.09 to 0.11. Cell (HCO3) increased from 22 to 32 mM. Reduction in bath (HCO3) from 25 to 5 mM reduced cell (HCO3) from 24 to 8 mM and increased K/P from 0.75 to 0.90. Na/P fell from 0.13 to 0.09, and Cl/P fell from 0.15 to 0.12. Thus, swelling resulting from acidosis induced by high CO2 was accompanied by an accumulation of K+, Cl−, and HCO3; that resulting from acidosis induced by a fall in (HCO3) was combined with an accumulation of K+ and an unidentified anion. To determine if the swelling induced by a fall in pH might be coupled with depolarization of the basolateral membrane, the effect of 1 mM barium was tested. Barium caused cell volume to increase 10.2%. Cell pH rose from 7.38 to 7.56, K/P increased from 0.63 to 0.73, Na/P did not change, and Cl/P rose from 0.17 to 0.20. Cell (HCO3) increased 10.4 mM. When the pH of the barium-treated tissue was reduced to 7.02 by raising Pco2, additional cell swelling and accumulation of K+ occurred. The effect on cell volume of a reduction of bath (HCO3) from 25 to 5 mM at constant bath pH was determined. Cell pH was not altered. Cell volume decreased 3% initially and then returned to the control level. When the bath (HCO3) was restored to 25 mM, cell volume increased 3.9% and then returned to the baseline. Thus, volume regulation was not impaired. It was concluded that a fall in cell pH induces swelling, and this is coupled with an accumulation of K+. This is probably the result of a pH effect on barium-sensitive and barium-insensitive K+ conductance pathways. The nature of the anions that balance the gain in K+ depends on the means used to induce acidosis.

Key Words: Cell volume regulation, cell pH, HCO3 transport, K+ channels, barium

A number of transport mechanisms in tubular cells can alter the total cellular solute content. Even the neutral ion exchangers, such as the Na+/H+ and the Cl−–HCO3 exchangers working in the presence of CO2 and cellular buffers, can potentially change the osmotic content of the cell and thus induce a net change in volume. Some of these transport systems are activated by a change in cell volume, and by altering cellular solute content, they contribute to volume regulation. However, many of these mechanisms are also affected by other factors such as cell pH and thus have the potential to drive cell volume away from the basal level. This suggests that there may be a complex interplay between the regulation of cell volume and the regulation of cell pH.

We recently reported that mild cellular acidosis, induced either by raising the CO2 tension or by reducing the HCO3 concentration, caused a swelling of the cells in lumen-collapsed S2 segments of the rabbit proximal tubule. This swelling was not corrected by a volume regulatory decrease. We also found that...
the rate of volume regulation after cell swelling induced by hypotonic media was slowed by acidosis (1). In order to gain an understanding of the ionic basis of the acidosis-induced swelling, we have measured the changes that occurred in the cellular content of Na⁺, K⁺, and Cl⁻ and in the cellular concentration of HCO₃⁻ in isolated, lumen-collapsed, S₂ tubular segments. The results indicate that the swelling induced by raising the CO₂ tension was due to the accumulation of K⁺, HCO₃⁻, and Cl⁻. The swelling induced by reducing the bicarbonate concentration was due to the accumulation of K⁺ and an unidentified anion.

METHODS

Preparation

The isolated, lumen-collapsed, tubular segment preparation was used for these studies. In this preparation, solute and water movement across the apical membrane is largely absent, enabling us to focus on the basolateral membrane processes involved in the cellular response to changes in cell pH. In addition, measurements of cell ion content are much simpler and more accurate in the absence of a fluid-filled lumen. It is also easier to make multiple measurements of small changes in volume in this preparation than in the perfused tubule.

The methods used for isolation of the tubular segments and superfusion of the tubule have been described previously (1). Briefly, 1- to 2-mm lengths of S₂ segments were dissected from the superficial cortical zone of kidneys removed from female New Zealand rabbits that were killed by i.v. administration of pentobarbital sodium. The tubules were placed in a thermostated chamber sitting on the stage of an inverted microscope. Each end of the lumen-collapsed tubule was attached to a micropipette that cramped the ends. The chamber (0.5-mL volume) was perfused at 3.2 mL/min with an isotonic medium (see below for composition). The chamber was also superfused by a gas containing CO₂ and O₂, and the pH of the chamber fluid was monitored by a small pH electrode. When the bath solution was changed, the fluid in the chamber was drained except for the drop clinging to the pipette tips and the tubule, and the new solution was immediately introduced. The half-time of exchange was ≪3 s.

Analyses

Cell volume was measured by a video technique described previously (1). Cell pH was measured by epifluorescence by using the pH-sensitive fluorescein derivative, 2,7'-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) and a photometer attached to the microscope (1). The cells were loaded with the fluorochrome by being exposed to a 2 µM solution of the acetoxyethyl derivative for 5 min. The fluorochrome was excited by 1.5-s pulses of light at 450 and 500 nm, and the emission at 535 nm was recorded. A ratio of the intensity of emitted light after excitation at the two wavelengths was calculated (500/450). The ratio readings were calibrated to yield cell pH by using the nigericin, high-potassium procedure as described previously (1). Cellular HCO₃⁻ concentration was calculated from the bathing solution PCO₂ and the cell pH.

Tubular cell content of Na⁺, K⁺, Cl⁻, and P were measured in three series of experiments by methods previously described (2,3). Approximately 12 tubules were dissected from one kidney and were placed in a control isotonic medium (5% CO₂-25 mM HCO₃⁻) maintained at 37°C for a 20-min period. In Series I, after the control incubation period, half of the tubules were placed in an isotonic medium equilibrated with 15% CO₂ and were then removed and prepared for analysis 3.5 to 4.5 min later—a time we have previously shown to be the point at which maximum cell volume was reached (1). The remaining tubules were removed from the control solution and were prepared for analysis. In Series II, the experimental solution containing 5 mM HCO₃⁻ and the time of incubation was 7.5 to 11 min. In Series III, the experimental solution containing 5 mM HCO₃⁻ and the time of incubation was 4 to 8 min before being removed for analysis. Thus, one third of the tubules was exposed to the control solution only; one third was exposed to the control solution and then to 1 mM barium; and one third was exposed to the control solution, then to 1 mM barium, and subsequently to 1 mM barium plus 25% CO₂.

Tubules were prepared for electron probe analysis by being placed individually on a silicon chip. The chips had previously been coated with a 2% solution of poly-l-lysine and either rabbit serum or 6% BSA and dried. The chip with tubule in place was swirled in a 295 mosM ammonium acetate solution for 9 s and was then dried on a chilled brass mount under a stream of N₂ gas. Excess liquid was removed by suction during drying (2). Tubules were analyzed by wavelength dispersive x-ray spectrometry with a Cameca MS 46 electron probe (3). The characteristic x-ray lines were analyzed for K⁺, Cl⁻, and P by using a pentaerythritol crystal and for Na⁺ by using a potassium acid phthalate crystal. The beam diameter was 60 µm, large enough to span the diameter of an individual dried tubule. The accelerating voltage was 11 kV, and the beam current was 200 nA. The electron beam was positioned on the tubule by visual observation. Approximately 10 readings of x-ray
emission counts were made on each tubule with care taken to avoid the ends where damage may have occurred during dissection. Background counts were subtracted for each element for each tubule. To standardize the procedure, the characteristic x-ray intensity counts of each element were quantified by comparison with x-ray counts of known quantities of salt crystals prepared from liquid droplets of known composition. Cellular K\textsuperscript{+}, Na\textsuperscript{+}, and Cl\textsuperscript{-} content was expressed per unit of cellular P to normalize for the cell mass analyzed by the electron beam (3). The 6 to 12 readings for each tubule were averaged to yield a single value for each tubular segment. Because the numbers of control and experimental tubules taken from one rabbit were about equal, the control and experimental values from all experiments were averaged and compared statistically on a group basis.

Solutions

The basic medium used consisted of (millimolar): Na\textsuperscript{+}, 152; Cl\textsuperscript{-}, 119; HCO\textsubscript{3}\textsuperscript{-}, 25; K\textsuperscript{+}, 5; HPO\textsubscript{4}\textsuperscript{2-}, 2.5; Ca\textsuperscript{2+}, 2; Mg\textsuperscript{2+}, 1.2; SO\textsubscript{4}\textsuperscript{2-}, 1.2; glucose, 5; acetate, 5; alanine, 6; citrate, 1; lactate, 4; and butyrate, 0.5; osmolality was 295 mosmol/kg of H\textsubscript{2}O. The dissecting solution also contained 6% albumin, and the NaCl concentration was reduced to maintain osmolality. Both solutions were equilibrated with a gas containing 5% CO\textsubscript{2}-95% O\textsubscript{2}. The low-bicarbonate experimental solution contained 5 mM HCO\textsubscript{3} and an additional 20 mM Cl\textsuperscript{-}. It was equilibrated with a gas containing either 15% CO\textsubscript{2}-85% O\textsubscript{2} or 25% CO\textsubscript{2}-75% O\textsubscript{2}. The low-bicarbonate experimental solution contained 5 mM HCO\textsubscript{3} and an additional 10 mM Cl\textsuperscript{-}. It was equilibrated with a gas containing 5% CO\textsubscript{2}-95% O\textsubscript{2}. The experimental tubules were exposed to the bathing solution for the period of time required to reach peak volume (determined in the previous study) and were then removed for analysis. The results are listed in Table 1 (Series A) and are summarized in Figure 2. The phosphorus content of the tubules in the two groups did not differ. The major change was the increase in K/P of 0.18. Na/P fell by 0.04, and Cl/P decreased by 0.03. Thus, the cell swelling induced by exposure to high CO\textsubscript{2} was accompanied by an increase in the cell content of K\textsuperscript{+}, Cl\textsuperscript{-} (Figure 2), and HCO\textsubscript{3} (Figure 1).

The effect of an isosmotic reduction in bath HCO\textsubscript{3} concentration from 25 to 5 mM was determined in three experiments in which measurements were made on a total of 14 control tubular segments and 13 experimental segments. The results are listed in Table 2 (Series B) and are summarized in Figure 3. The phosphorus content of the tubules in the two groups did not differ. Again, the major change was an increase in K/P (0.15). Na/P fell by 0.04, and Cl/P was reduced by 0.03. Thus, the cell swelling induced by this type of acidosis was also coupled with an accumulation of K\textsuperscript{+}. However, a loss of HCO\textsubscript{3}
TABLE 1. Effect of acidosis on tubule ion content

<table>
<thead>
<tr>
<th>Medium Composition</th>
<th>P</th>
<th>K/P</th>
<th>Na/P</th>
<th>Cl/P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Series A: Effect of High CO₂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% CO₂, 25 mM HCO₃</td>
<td>83.3 ± 2.8</td>
<td>0.98 ± 0.03</td>
<td>0.09 ± 0.01</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>15% CO₂, 25 mM HCO₃</td>
<td>83.9 ± 3.6</td>
<td>1.16 ± 0.03</td>
<td>0.11 ± 0.01</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td><strong>Series B: Effect of Low HCO₃</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% CO₂, 25 mM HCO₃</td>
<td>85.2 ± 2.3</td>
<td>0.75 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>5% CO₂, 5 mM HCO₃</td>
<td>87.1 ± 1.7</td>
<td>0.90 ± 0.03</td>
<td>0.09 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Values are means ± SE. Series A: N = 12 tubules from three rabbits for the control data and 12 tubules from the same rabbits for the experimental data. Series B: N = 14 tubules from three rabbits for the control data and 13 tubules from the same rabbits for the experimental data. NS, not significant.

Figure 2. The effect of high P<sub>CO₂</sub> on cell ion content. The cell content of K⁺, Na⁺, and Cl⁻ are expressed as ratios to the cell phosphorus content, which was not affected by the increase in P<sub>CO₂</sub> (see Table 1). Values are means ± SE. *P < 0.05.

Figure 3. The effect of low bath HCO₃ concentration on cell ion content. The cell contents of K⁺, Na⁺, and Cl⁻ are expressed as ratios to the cell phosphorus content, which was not affected by the reduction in bath HCO₃ concentration (see Table 1). Values are means ± SE. *P < 0.05.

(Figure 1) and smaller reductions in Na⁺ and Cl⁻ content also occurred.

The difference in the control values for K/P in the two series of experiments listed in Table 1 may be related to the fact that the two series of experiments were performed about 5 months apart. Thus, the rabbits used for the two series were not from the same population. However, control and experimental tubules were obtained from each kidney used in these series. Thus, the significant differences within each series can be ascribed to the changes in the medium P<sub>CO₂</sub> or HCO₃ concentration.

Effect of Barium

We hypothesized that the effect of a reduction of cell pH on cell K⁺ content and on cell volume is due to a reduction in K⁺ conductance. To test the plausibility of this hypothesis, we measured the effect of Ba<sup>2+</sup> on cell volume, on cell pH and HCO₃ concentrations, and on cell ion content. We also attempted to determine if prior treatment of the tissue with barium would affect the response to a fall in cell pH.

In an initial series of experiments, after a control period, the bath solution was changed to one containing 1 mM barium and 5% CO₂. Ten minutes later, the solution was changed again to one containing 1 mM barium and 15% CO₂. Application of barium increased cell volume 12.4 ± 1.3% in 2 min, but subsequent exposure to 15% CO₂ in the presence of barium had no additional effect on cell volume (N = 5). Measurement of cell pH in two experiments indicated that barium caused a rise in cell pH from 7.48 to 7.60; however, the subsequent application of 15% CO₂ reduced cell pH to only 7.28, whereas we had previously found that this level of CO₂ had reduced...
TABLE 2. The effect of barium on cell pH and on the response to CO₂

<table>
<thead>
<tr>
<th>Medium Composition</th>
<th>Medium pH</th>
<th>Cell pH</th>
<th>Cell (HCO₃⁻) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% CO₂</td>
<td>7.44 ± 0.01</td>
<td>7.38 ± 0.04</td>
<td>21.0 ± 2.0</td>
</tr>
<tr>
<td>1 mM Ba²⁺, 5% CO₂</td>
<td>7.44 ± 0.01</td>
<td>7.56 ± 0.04b</td>
<td>31.4 ± 2.6b</td>
</tr>
<tr>
<td>1 mM Ba²⁺, 25% CO₂</td>
<td>6.77 ± 0.04c</td>
<td>7.02 ± 0.04c</td>
<td>41.3 ± 4.0c</td>
</tr>
<tr>
<td>1 mM Ba²⁺, 5% CO₂</td>
<td>7.44 ± 0.01</td>
<td>7.56 ± 0.04</td>
<td>30.8 ± 2.5</td>
</tr>
</tbody>
</table>

a Values are means ± SE.
b Significantly different from the control values.
c Significantly different from the values for 1 mM Ba²⁺, 5% CO₂.

cell pH to 7.03 (1). Therefore, we repeated these experiments with 25% CO₂ in order to reduce cell pH to the same level that was reached in the absence of barium. The results of the series in which cell volume was determined are presented in Figure 4. The application of barium increased cell volume 10.2 ± 1.3% in 2 min. This was followed by a modest regulatory volume decrease to 7.5 ± 0.9% above baseline at 9.5 min. Subsequent application of 25% CO₂ then increased cell volume to 18.3 ± 2.5% above baseline at 20 min. In another series of experiments, the changes that occurred in cell pH and cell HCO₃ concentration were determined. The cell pH data are presented in Figure 5. Table 2 includes the solution pH values and the cell pH and HCO₃ concentrations that prevailed during exposure of the tubule to each of the solutions used. The addition of barium to the control medium caused cell pH to rise from 7.38 to a plateau level of 7.56 and the cell HCO₃ concentration to increase from 21 to 31 mM (Figure 5; Table 2). Exposure to barium plus 25% CO₂ reduced cell pH to 7.02 and caused a further increase in cell HCO₃ concentration to 41 mM. Thus, treatment with barium alone increased cell volume and pH and did not prevent the volume gain caused by a reduction in cell pH to 7.0.

The effect of barium alone and in combination with 25% CO₂ on cellular ion content was also determined in four experiments (Figure 6 and Table 3). The protocol for these experiments mimicked that illustrated in Figures 4 and 5. Control tubules were removed from the bathing medium and fixed for analysis just before the addition of barium. Another group of tubules were removed and fixed 10 min after exposure to barium and 5% CO₂. The third group was subsequently exposed to barium plus 25% CO₂ for 4 to 8 min. Thus, with reference to the abscissa in Figures 4 and 5, tubular ion content was measured at 0, 10, and 14 to 18 min. Barium in the presence of 5% CO₂ increased K/P by 0.10, did not affect Na/P, and increased Cl/P by 0.03. Thus, the barium-induced increase in cell volume appeared to be primarily due to cellular accumulation of K⁺ and HCO₃⁻ (Table 2). The exposure of the tissue to 25% CO₂ in the presence of barium resulted in an additional increase in K/P of 0.10; Na/P and Cl/P did not change significantly. Thus, the additional cell swelling induced by CO₂ after exposure of the tissue to barium evidently was...
Effect of HCO₃

Transient volume changes measured in the low HCO₃ experiments had suggested that changes in cell HCO₃ concentration per se might cause changes in cell volume. Figure 7A illustrates the changes in cell volume that resulted from a decrease in bath HCO₃ concentration and a fall in pH (low HCO₃-constant CO₂). These data are taken from a previous report (1). Note that the reduction in bath HCO₃ concentration caused a transient fall in cell volume before the effect

Figure 6. The effect of barium and of barium combined with 25% CO₂ on cell ion content. The cell content of K⁺, Na⁺, and Cl⁻ are expressed as ratios to the cell phosphorus content, which was not affected by barium or by the combination of barium and 25% CO₂ (see Table 3). Values are means ± SE. *Significantly different from the control value (P < 0.05). **Significantly different from the effect of 1 mM barium-5% CO₂ (P < 0.05).

Figure 7. The effect of reducing the bath HCO₃ concentration from 25 to 5 mM on cell volume. (A) Low-HCO₃-constant-CO₂ experiments. Data were presented previously (1). (B) Low-HCO₃-constant-pH experiments. The horizontal lines indicate a V/V₀ value of 1.00. Means ± SE are shown. V₀ for data shown in panel B = 1.062 ± .069 nl/mm tubule length. V₁ and V₀ are as described in the legend to Figure 4.

<table>
<thead>
<tr>
<th>TABLE 3. Effect of barium and 25% CO₂ on tubule ion content*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium Composition</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>5% CO₂</td>
</tr>
<tr>
<td>5% CO₂, 1 mM Ba²⁺</td>
</tr>
<tr>
<td>25% CO₂, 1 mM Ba²⁺</td>
</tr>
<tr>
<td>Group Comparison: P Values</td>
</tr>
<tr>
<td>5% CO₂ versus 5%</td>
</tr>
<tr>
<td>CO₂-Ba²⁺</td>
</tr>
<tr>
<td>5% CO₂ versus 25%</td>
</tr>
<tr>
<td>CO₂-Ba²⁺</td>
</tr>
<tr>
<td>5% CO₂-Ba²⁺ versus</td>
</tr>
<tr>
<td>25% CO₂-Ba²⁺</td>
</tr>
</tbody>
</table>

* Values are means ± SE. Tubules were obtained from 4 rabbits. Each rabbit contributed tubules to the three groups. NS, not significant.

b N = 13 tubules.
c N = 11 tubules.
d N = 9 tubules.
of a fall in cell pH resulted in cell swelling. The return of the bath concentration of HCO$_3$ to 25 mM resulted in a transient increase in cell volume before it returned to the control level. To determine if these transient changes in cell volume were due to changes in cell HCO$_3$ concentration independent of cell pH changes, we performed experiments in which the bath concentration of HCO$_3$ was reduced to 5 mM but the bath pH was maintained constant by a simultaneous reduction in Pco$_2$ (low HCO$_3$-constant pH). The volume changes are presented in Figure 7B. The cell pH and HCO$_3$ concentration changes are listed in Table 4.

The pH of the control and experimental media differed by less than 0.02 pH units (Table 4). After the change to the experimental medium, cell pH did not change significantly; however, the cell HCO$_3$ concentration fell 20 mM. Cell volume dropped by 3.1 ± 1.1% (P < 0.05) in 1 min and then returned to the control level by 4 min (Figure 7B). When the control medium was returned to the chamber, cell volume increased 3.9 ± 0.9% (P < 0.05) above baseline in 1 min before returning to the control level at 13 min. Thus, the changes in cell HCO$_3$ concentration were accompanied by changes in cell volume. In the absence of a pH change, volume regulatory mechanisms evidently returned cell volume to the control level. If cell pH changed at the same time, the volume regulatory adjustments were masked or overridden (Figure 7A).

<table>
<thead>
<tr>
<th>Medium Composition</th>
<th>Medium pH</th>
<th>Cell pH</th>
<th>Cell (HCO$_3$) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mM HCO$_3$ - 5% CO$_2$</td>
<td>7.47 ± 0.04</td>
<td>7.52 ± 0.02</td>
<td>26.6 ± 1.2</td>
</tr>
<tr>
<td>5 mM HCO$_3$ - 1% CO$_2$</td>
<td>7.45 ± 0.04$^b$</td>
<td>7.56 ± 0.03</td>
<td>6.4 ± 0.4$^b$</td>
</tr>
<tr>
<td>25 mM HCO$_3$ - 5% CO$_2$</td>
<td>7.47 ± 0.01</td>
<td>7.50 ± 0.02</td>
<td>25.5 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± 5E.

$^b$ P < 0.001 for paired differences with control values. All other experimental values do not differ significantly from control values.

**DISCUSSION**

Acidosis induced either by high CO$_2$ or by low HCO$_3$ caused cell volume to increase to a new steady state. We emphasize that these volume changes occurred in the absence of an osmotic change in the bathing solutions and were not followed by volume regulatory adjustments (1). When the cellular acidosis and swelling were induced by an increase in Pco$_2$, K$, HCO$_3$, and to a lesser extent, Cl$^{-}$ and Na$^+$ accumulated within the cell (Figures 1 and 2). When the cellular acidosis and swelling were induced by a reduction in bath HCO$_3$ concentration, K$^+$ again accumulated within the cell. However, the cell content of Cl$^{-}$, Na$^+$ (Figure 3), and HCO$_3$ fell (Figure 1).

To what extent do the measured cell solute changes account for the volume of water gained? Table 5 lists the results of calculations based on certain assumptions. An initial cell volume of 1 nL/mm tubular length is assumed, and 60% of this is considered to...
Figure 8. The changes in cell solute and ion content induced by acidosis. Total solute gain was calculated from the measured volume increase; changes in K⁺, Cl⁻, Na⁺, and HCO₃⁻ content were calculated as indicated in the text and in Table 5.

...cellular acidosis has been reported to inhibit, not enhance, Na-K-ATPase activity in the rabbit urinary bladder (15) and in primary cultures and Cl⁻ and the calculations indicate a net loss in the total content of the measured ions. If the gain in K⁺ were balanced by a similar gain in an unidentified anion, the total would approximate the total solute gain. In summary, an increase in cell K⁺ content accounted for a major fraction of the solute gained in both types of acidosis. In the high-Pco₂ experiments, the gain in K⁺ and in the other measured ions could account for the magnitude of the swelling. In the low-HCO₃⁻ experiments, the gain in K⁺ was accompanied by losses in content of the other measured ions; a gain in an unidentified anion may also have occurred.

Cell K⁺ Content

The increase in K⁺ content in tubular cells after the induction of acidosis may be due to an effect of pH on K⁺ conductance of the basolateral membrane. An inhibition of K⁺ efflux coupled with a continued influx via the Na-K-ATPase pump could account for the gain in K⁺ content. A number of studies have documented an inhibitory effect of protons on K⁺ channels in a variety of cell types including renal tubular cells and proximal tubular cells in particular (4–10). Biagi and Sohtell reported that an increase in Pco₂ or a reduction in bath HCO₃⁻ concentration at constant Pco₂ depolarized the basolateral membrane of the rabbit proximal tubule; the depolarization was the consequence of a reduction in the potassium conductance (4). Sasaki et al. confirmed that a reduction in bath HCO₃⁻ concentration at constant Pco₂ depolarized the basolateral membrane of the rabbit proximal straight tubule (11). A very close relationship between cell pH and barium-sensitive K⁺ conductance has been documented in fused cells of the frog distal tubule (6) and in fused, cultured, canine tubular cells (MDCK) (7). In the amphibian cells, the greatest effect of pH occurred in the range 7.5 to 7.1 with half-maximal inhibition occurring at 7.42 (6). In the mammalian cells, the range was 7.4 to 6.9 with half-maximal inhibition at a pH of 7.18 (7).

The volume regulatory response of S₂ cells to an increase in volume involves a loss of K⁺ (12,13) that is at least partially due to an increase in K⁺ conductance (14). Thus, the absence of a volume regulatory response in our experiments in which K⁺ conductance is probably diminished is not surprising. We have also demonstrated that a fall in cell pH slows the volume regulatory response to a hypoosmotic challenge (1). Cell Na content decreased significantly in the low-HCO₃⁻-constant CO₂ experiments (Figure 3), and it may be argued that cell K increased because of a stimulation of the Na-K pump rather than a reduction in membrane K conductance. However, the Na content rose slightly in the high-CO₂ series (Figure 2). In addition, cellular acidosis has been reported to inhibit, not enhance, Na-K-ATPase activity in the rabbit urinary bladder (15) and in primary cultures...
of rat proximal tubular cells (16). It seems unlikely, therefore, that an increase in pump activity is responsible for an increase in cell K⁺ content.

**Changes in Anion Content**

The fall in cell pH should titrate cellular anionic buffers, requiring the net import of anion with K⁺. The anion involved evidently varies depending upon the type of acidosis that is induced. In CO₂-induced acidosis, HCO₃⁻ and, to a lesser extent, Cl⁻ accumulated within the cell. When acidosis was induced by a reduction in bath bicarbonate concentration, cell HCO₃⁻ concentration fell and cell Cl⁻ content was slightly reduced. Thus, an unidentified anion may have accumulated with K⁺. The pH gradient across the cell membrane was reversed during the low HCO₃⁻ acidosis ([pHᵢ] = 6.81; pHₑ = 7.03; e, extracellular; i, intracellular [1]). This may have caused the accumulation of a weak organic acid. However, a similar pH gradient was created in the high-CO₂ experiments in which the K⁺ accumulation was accompanied by HCO₃⁻ and Cl⁻. There was little indication of a sizable accumulation of an unidentified anion in those experiments.

In the high-CO₂ experiments, the increase in cell HCO₃⁻ concentration may have been caused by two factors. First, the increase in Pco₂ in combination with the cellular buffers produced an immediate rise in cell HCO₃⁻ concentration. Second, the probable depolarization of the basolateral membrane, resulting from the fall in K⁺ conductance, would have reduced the driving force for the electrogenic Na⁺-HCO₃ cotransporter. Slowing the rate of this symport would be expected to cause a reduction in the rate of efflux of HCO₃⁻ from the cell (17,18). In the low-HCO₃⁻ experiments, the reduction in the bath HCO₃⁻ would have increased the gradient driving the mechanism and caused the efflux of HCO₃⁻ from the cell.

The cellular Cl⁻ content increased 43% in the high-CO₂ experiments (Figure 2) and fell 20% in the low-HCO₃⁻-constant-CO₂ experiments (Figure 3). Four different modes of Cl⁻ transport have been reported to exist in the basolateral membrane of this tubular segment: Na⁺-dependent Cl⁻-HCO₃⁻ exchange; Na⁺-independent Cl⁻-HCO₃⁻ exchange (18); K⁺-Cl⁻ cotransport (19–21), and a minor conductance pathway (22). It is difficult to determine which of these mechanisms are responsible for these changes in cell Cl⁻ content without additional experiments. The fall in cell Cl⁻ content in the low-HCO₃⁻ CO₂ series contrasts with the rise in cell Cl⁻ activity in similar experiments reported by Sasaki and Yoshiyama (23). However, their experiments were performed on perfused tubules. The source of the Cl⁻ may have been apical Cl⁻ transport mechanisms, which were not functionally optimally in our experiments on nonperfused tubules.

**Cellular Na Content**

Only small changes in cell Na⁺ content occurred in both types of acidosis. It rose slightly in the high-CO₂ experiments and fell slightly in the low-HCO₃⁻-constant-CO₂ experiments. These changes may have occurred as the result of changes in the rate of the Na-HCO₃ cotransporter (17,18). Boron and Boulpaep reported that cell Na⁺ activity fell in amphibian proximal tubules after a reduction in bath HCO₃⁻ concentration at constant Pco₂ (24). Sasaki et al. confirmed that a similar reduction in cell Na⁺ activity was produced by the same maneuver in the rabbit proximal straight tubule (1). A similar fall in cell Na⁺ content occurred in our low-HCO₃⁻-constant-Pco₂ experiments. In the high-CO₂ experiments, the rise in cell Na⁺ content may have been due to inhibition of the Na-HCO₃ cotransporter caused by the pH-induced depolarization of the membrane.

The Effect of Barium

To confirm our hypothesis that the increase in volume after the reduction in cell pH was paired with a fall in the K⁺ conductance of the basolateral membrane, we tested the effect of barium, which reduces K⁺ conductance by blocking certain K⁺ channels. Barium also caused cell swelling (Figure 4) and an increase in cell K⁺ content (Figure 6). Table 5 contains the calculations of the total osmotic solute gained by the cells and the increase in the measured solutes. The results are summarized in Figure 9. The net influx of K⁺ and HCO₃⁻ accounted for the major fraction of the calculated solute gains. There was also a slight gain in Cl⁻ and a small, insignificant gain in Na⁺ content.

Kone et al. have measured the effect of barium on K⁺ fluxes in rabbit proximal tubular segments (21). Barium caused a transient net influx of K⁺ into the segments by blocking K⁺ permeability pathways.
However, other K⁺ efflux mechanisms were subsequently activated. This K⁺ efflux was partly inhibited by quinine and by furosemide. These latter pathways are evidently responsible for the modest regulatory decrease in cell volume that we noted (Figure 4). The data suggest, as did Kone et al. (21), that the increase in cell volume is the factor that activates these mechanisms.

The increase in cell HCO₃⁻ concentration in these experiments is evidently due to depolarization of the basolateral membrane. Depolarization, induced either by raising the bath K⁺ concentration or by the application of barium, has been shown to cause cell alkalization in the rat, frog, and salamander kidney (25-27). This is partly due to the reduction in the gradient driving the Na-HCO₃ cotransporter (27).

Pretreatment of the tissue with barium inhibited the effect of 15% CO₂ on cell volume, but this may have been due to the fact that the combination of the two treatments resulted in a higher cell pH than occurred with 15% CO₂ alone. The use of 25% CO₂ after pretreatment with barium reduced cell pH to the same level that was obtained with 15% CO₂ alone. In that situation, cell volume and cell K⁺ content increased to at least the same extent as noted in the absence of barium. Two factors may be responsible for the additive effects. First, we were unable to use the maximum effective concentration of barium because of its limited solubility in bicarbonate-containing solutions. Second, as Kone et al. reported, the barium inhibition was followed by activation of other K⁺ permeability pathways (21). These pathways, particularly that blocked by quinine, may also be sensitive to pH. The results of the experiments reported here, combined with the known effects of pH and barium on K⁺ conductance, strongly suggest that a reduction in K⁺ conductance of the basolateral membrane accompanies the cell swelling induced by acidosis.

The Response to Low HCO₃⁻–Constant pH

The Na-HCO₃ cotransporter, by transporting net solute in or out of the cell, does have the potential to alter cell volume. It was of interest to determine the effect of that transport on cell volume when cell pH was maintained constant. In these experiments, there was a transient fall of 3.1% in cell volume when the HCO₃⁻ concentration was reduced (Figure 7B). Cell volume returned to the control level and then transiently increased when the bath HCO₃⁻ concentration was restored. These changes in volume reflect the transient membrane potential changes that occurred in similar experiments on the perfused tubule by Biagi and Sohtell (4) and on the nonperfused tubule by Welling and O’Neill (22). In those experiments, the reduction in bath HCO₃⁻ concentration transiently depolarized the membrane by 15 mV. The restoration of the bath HCO₃⁻ concentration caused a transient hyperpolarization. It is reasonable to suggest that both the volume and voltage changes were due to transient changes in the flux of Na⁺ and HCO₃⁻ through an electrogenic cotransporter.

The fact that cell volume returned to baseline in the low-HCO₃⁻–constant-pH experiments despite the net loss of HCO₃⁻ (Figure 7B) indicates that a volume regulatory mechanism must have been activated. It was previously thought that the rabbit proximal straight tubule would not respond to an osmotically induced loss in volume with a regulatory volume increase. However, Rome et al. have recently shown that the tubule will respond when butyrate is present in the bathing fluid (2). Butyrate was included in our solutions. The regulatory volume increase is due to the influx of K⁺, Na⁺, and Cl⁻ (2).

In the low-HCO₃⁻ experiments in which cell pH fell (low-HCO₃⁻–constant-CO₂ series), transient changes in volume were also present (Figure 7A). However, the initial transient fall was followed by a swelling well above baseline. Thus, a reduction in cell pH interfered with the volume regulatory mechanisms and caused additional volume changes.

It must be pointed out that the cellular electrolyte and volume changes reported here occurred in a lumen-collapsed tubule segment and illustrate the effect of acidosis on transport by mechanisms that reside in the basolateral membrane. The extent to which such changes may occur in the lumen-perfused preparation or in vivo remain to be determined.

Summary

The results of these experiments indicate that a fall in cell pH induced cell swelling in the lumen-collapsed S₂ tubule segment. The gain in cell water was accompanied by an increase in cell K⁺ content, which may be the result of a reduction in the K⁺ conductance of the basolateral membrane. The nature of the anions that balanced the gain in K⁺ depended on the means used to induce acidosis. When the acidosis was induced by high CO₂, cellular levels of HCO₃⁻ and Cl⁻ also increased. When the acidosis was caused by a reduction in bath HCO₃⁻ concentration, the cell HCO₃⁻ concentration fell and the Cl⁻ content was also reduced slightly; an unknown anion may have accumulated with K⁺ in the cell.

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REFERENCES

Cellular Electrolytes and Volume in Acidosis


