Effect of metabolic acidosis on NaCl transport in the proximal tubule

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Wang, Tong, Allan L. Egbert, J. R., Peter S. Aronson, and Gerhard Giebish. Effect of metabolic acidosis on NaCl transport in the proximal tubule. Am. J. Physiol. 274 (Renal Physiol. 43): F1015–F1019, 1998.—In metabolic acidosis, the capacity of the proximal tubule for bicarbonate absorption is enhanced, whereas NaCl reabsorption is inhibited. Recent evidence indicates that transcellular NaCl absorption in the proximal tubule is mediated by apical membrane Cl−/formate exchange and Cl−/oxalate exchange, in parallel with recycling of these organic anions. We evaluated whether the effect of metabolic acidosis to inhibit NaCl reabsorption in the proximal tubule is due at least in part to inhibition of organic anion-dependent NaCl transport in this nephron segment. Absorption rates of bicarbonate (JHCO3), chloride (JC), and fluid (Jv) were measured in rat proximal tubule segments microperfused in situ. We confirmed that metabolic acidosis stimulates JHCO3 in tubules microperfused with 25 mM HCO3, pH 7.4. For measurements of JC, tubules were microperfused with a low-bicarbonate (5 mM), high-chloride solution, simulating conditions in the late proximal tubule. Under these conditions, baseline JC and Jv measured in the absence of formate and oxalate were not significantly different between control and acidic rats. However, whereas addition of 50 μM formate or 1 μM oxalate to luminal and capillary perfusates markedly stimulated JC and Jv, in control rats, formate and oxalate failed to stimulate JC and Jv in acidic rats. We conclude that metabolic acidosis markedly downregulates organic anion-stimulated NaCl absorption, thereby allowing differential regulation of proximal tubule NaHCO3 and NaCl transport.

pH; anion exchange; formate; oxalate; sodium/proton exchange

IN METABOLIC ACIDOSIS, the capacity of the proximal tubule for bicarbonate absorption is enhanced (2, 8, 11, 17), whereas NaCl reabsorption is inhibited (5, 13, 16, 22), with the latter contributing to increased urinary excretion of NaCl (7, 18). Enhanced bicarbonate transport capacity during metabolic acidosis results at least in part from increased apical membrane Na+/H+ exchange activity (1, 6, 9, 15, 21, 23), which is a consequence of enhanced expression of NHE3 (3, 29).

The reabsorption of NaCl in the proximal tubule involves passive paracellular and active transcellular mechanisms (4). Transcellular NaCl absorption is markedly stimulated by formate and oxalate (12, 19, 20, 25, 27, 28). Evidence indicates that formate-stimulated NaCl absorption results from apical membrane Cl−/formate exchange operating in parallel with H+/coupled formate transport and Na+/H+ exchange (4, 25). In contrast, oxalate-stimulated NaCl transport is not dependent on Na+/H+ exchange but takes place by Cl−/oxalate exchange in parallel with oxalate/sulfate exchange and Na+/sulfate cotransport (25).

Given the dual roles of apical membrane Na+/H+ exchange in mediating both NaHCO3 and a portion of transcellular NaCl reabsorption in the proximal tubule, one would expect that the stimulation of Na+/H+ exchange activity during metabolic acidosis would lead to enhanced rather than reduced NaCl reabsorption, as is actually observed. We therefore evaluated the effect of metabolic acidosis on transcellular chloride absorption in the rat proximal convoluted tubule. We find that chronic metabolic acidosis markedly downregulates organic anion-stimulated NaCl absorption, thereby allowing differential regulation of proximal tubule NaHCO3 and NaCl transport.

METHODS

Induction of metabolic acidosis. Male Sprague-Dawley rats (200–250 g) were obtained from Harlan (Indianapolis, IN). Metabolic acidosis was produced by providing 1.5% NH4Cl with 5% sucrose in the drinking water and feeding a 1:1 (ml/g) mixture of 3% NH4Cl and powdered rat chow (Prolab RMH 3200; Agway, Syracuse, NY) for 5 days (29). Control rats received the same diet without NH4Cl and were provided with 5% sucrose and no NH4Cl in the drinking water. A similar amount of food (25 g) was given daily to both control and acidic animals, an amount that was completely consumed.

Microperfusion. In vivo microperfusion experiments were performed as described previously (27), including anesthesia and surgical preparation. Simultaneous microperfusion of both proximal convoluted tubule and peritubular capillaries was performed (25). Rates of HCO3− (JHCO3) and Cl− (JC) reabsorption were calculated per unit of tubule length, as previously described (24, 25).

Solution composition. In the experiments in which JHCO3 was measured, the composition of the intraluminal perfusion solution was (in mM) 115 NaCl, 25 NaHCO3, 4.0 potassium chloride, 1.0 calcium chloride, 5.0 sodium acetate, 2.0 dibasic sodium phosphate, and 0.5 monobasic sodium phosphate, pH 7.4. In the experiments in which JC was measured, the intraluminal solution was (in mM) 140 NaCl, 5.0 NaHCO3, 4.0 potassium chloride, 2.0 calcium chloride, 1.0 magnesium sulfate, 1.0 dicyclic sodium phosphate, and 1.0 monobasic sodium phosphate, pH 6.7. In both sets of experiments, the composition of the capillary perfusate was (in mM) 115 NaCl, 25 NaHCO3, 4.0 potassium chloride, 2.0 calcium chloride, 5.0 sodium acetate, 2.0 dicyclic sodium phosphate, and 1.0 magnesium sulfate, pH 7.4. Formate (50 μM) and oxalate (1 μM) were added as sodium salts to both intraluminal and peritubular perfusion solutions.

All solutions for both series of experiments were equilibrated at room temperature with a 5% CO2-95% O2 mixture before use. Solution pH was then titrated with NaOH or HCl as required. The osmolality values of the intratubular and...
capillary perfusates were adjusted to 289–292 mosmol/kg H2O.

Measurement of plasma formate. Blood samples (0.5–1.0 ml) were withdrawn from the carotid artery of anesthetized rats after completion of micropuncture experiments. Serum samples were stored at −20°C until the time of formate measurement. Serum formate concentration was measured as described previously (25).

Statistics. Data are given as means ± SE. Student’s t-test was used when a single experimental group was compared with a control group. Several experimental groups were compared with a control group by use of Dunnett’s test. Differences were considered significant if P < 0.05.

RESULTS

Metabolic acidosis was induced by administration of NH4Cl in both food and drinking water. As indicated in Table 1, this resulted in a pronounced metabolic acidosis with a decrease in plasma HCO3− concentration from 27 to 15 meq/l and a decline in pH from 7.35 to 7.07.

In the first series of experiments, we sought to confirm that chronic metabolic acidosis leads to stimulation of HCO3− reabsorption in the proximal tubule. As indicated in Table 2, the induction of metabolic acidosis was associated with a 44% increase in HCO3− reabsorption, from 146 to 211 pmol·mm−1·min−1. These findings confirm previous observations indicating that metabolic acidosis stimulates proximal tube HCO3− reabsorption (2, 8, 11, 17). Indeed, because tubule lumens and capillaries were simultaneously perfused with solutions containing 25 mM HCO3− in both control and acidotic animals, these findings confirm that the intrinsic capacity of the proximal tubule to mediate transcellular HCO3− absorption is enhanced by chronic metabolic acidosis.

Because transcellular NaCl reabsorption in the proximal tubule is stimulated by formate, we next evaluated whether metabolic acidosis of this magnitude affects plasma formate concentration. As illustrated in Fig. 1, plasma formate levels were not affected by metabolic acidosis (114.5 vs. 117.2 μM).

Table 1. Acid-base status of control and acidotic rats

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Blood pH</th>
<th>Pco2, mmHg</th>
<th>[HCO3], mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>7.35 ± 0.01</td>
<td>49.2 ± 1.5</td>
<td>27.0 ± 0.9</td>
</tr>
<tr>
<td>Acidosis</td>
<td>16</td>
<td>7.07 ± 0.02*</td>
<td>49.3 ± 2.4</td>
<td>15.0 ± 1.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE; N, no. of animals. Metabolic acidosis was induced by administration of 1.5% NH4Cl in the drinking water and in the food for 5 days. [HCO3], bicarbonate concentration. *Significant difference from control value (P < 0.05).

We next assessed whether metabolic acidosis affects the intrinsic capacity of the proximal tubule to mediate transcellular NaCl absorption. In both control and acidotic rats, the lumen perfusate contained 5 mM HCO3− at pH 6.7, whereas the capillary perfusate contained 25 mM HCO3− at pH 7.4. An outward transtubular Cl− gradient of 25 mM was imposed, mimicking conditions in the late proximal tubule. Under these conditions, any differences in transport between control and acidotic animals must reflect intrinsic adaptations of the tubule.

As indicated in Table 3 and Fig. 2, the baseline rates of Cl− (JCl) and fluid absorption (Jv) were not different between control and acidotic animals. However, whereas addition of formate to the perfusion solutions markedly stimulated JCl and Jv in tubules in control animals, confirming previous results (25, 27, 28), formate completely failed to stimulate JCl or Jv in acidotic rats.

An additional series of experiments focused on the effect of metabolic acidosis on the stimulation of proximal tubule transport by oxalate. As indicated in Table 4 and Fig. 3, the baseline rates of JCl and Jv were again not different between control and acidotic animals. Yet, whereas addition of oxalate to the perfusion solutions sharply enhanced JCl and Jv in tubules in control animals, confirming previous findings (25, 27, 28), oxalate also completely failed to stimulate JCl or Jv in acidotic rats.

DISCUSSION

The principal mechanisms involved in transcellular NaCl and NaHCO3 absorption in the proximal tubule are illustrated in Fig. 4. According to this scheme, apical membrane Na+/H+ exchange is involved in both

Table 2. Effects of metabolic acidosis on bicarbonate and fluid absorption

<table>
<thead>
<tr>
<th></th>
<th>Vw, nl/min</th>
<th>L, mm</th>
<th>[HCO3], mmol/l</th>
<th>[HCO3], mmol/l</th>
<th>Jv, nl·min−1·mm−1</th>
<th>JHCO3, pmol·min−1·mm−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>20.11 ± 0.04</td>
<td>1.99 ± 0.14</td>
<td>24.8 ± 0.14</td>
<td>12.34 ± 1.44</td>
<td>1.76 ± 0.32</td>
</tr>
<tr>
<td>Acidosis</td>
<td>12</td>
<td>20.13 ± 0.03</td>
<td>1.79 ± 0.10</td>
<td>25.7 ± 0.12</td>
<td>9.19 ± 1.20</td>
<td>2.23 ± 0.21</td>
</tr>
</tbody>
</table>

Values are means ± SE; N, no. of perfused tubules. Vw, perfusion rates; L, tubular length; [HCO3], bicarbonate concentration in original perfusate; [HCO3], bicarbonate concentration in collected fluid; Jv, fluid absorption rate; JHCO3, bicarbonate absorption rate. *Significant difference from control value (P < 0.05).
processes. Shown on the left, Na\(^+/\)H\(^+\) exchange unmatched by Cl\(^-/\)base exchange leads to uptake of NaHCO\(_3\) from the tubule fluid (10). Reabsorbed HCO\(_3\) then exits across the basolateral membrane via the Na\(^+/\)HCO\(_3\) cotransporter (10). Na\(^+\) leaves the cell via the Na\(^+/\)HCO\(_3\) cotransporter and the Na-K-ATPase. Shown on the right, operation of the Na\(^+/\)H\(^+\) exchanger in parallel with Cl\(^-/\)formate exchange and H\(^+\)-coupled formate recycling results in cell uptake of NaCl from the tubule fluid. The principal route of Cl\(^-\) exit across the basolateral membrane is through Cl\(^-\) channels and that of Na\(^+\) is via the Na-K-ATPase. An additional component of NaCl transport across the apical membrane is mediated by Cl\(^-\)/oxalate exchange in parallel with Na\(^+\)-sulfate cotransport and sulfate/oxalate exchange.

The dual role of Na\(^+/\)H\(^+\) exchange in mediating both NaHCO\(_3\) and NaCl reabsorption could in principle impair the ability to regulate these two processes independently in response to metabolic acidosis. In this acid-base disorder, there is upregulation of apical membrane Na\(^+/\)H\(^+\) exchange activity secondary to increased NHE3 protein abundance (3, 29). This adaptation is appropriate for augmenting proximal tubule HCO\(_3\) absorption and NH\(_4\) secretion. Interestingly, despite the increased Na\(^+/\)H\(^+\) exchange activity, we now find dramatic downregulation of formate-induced NaCl transport in metabolic acidosis. In addition, there is also a sharp decline in oxalate-induced NaCl transport in this condition. Thus both of the mechanisms for organic anion-induced NaCl absorption illustrated in Fig. 4 are virtually abolished in metabolic acidosis.

There are several possible mechanisms by which metabolic acidosis could downregulate organic anion-induced NaCl transport. First, it is possible that the activities of the apical Cl\(^-/\)anion exchangers (Cl\(^-/\)for-

Table 3. Effects of metabolic acidosis on formate-induced fluid and chloride absorption

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>(V_o) nL/min</th>
<th>(J_v) nL/min</th>
<th>(J_{cl}) pmol/min/m(^2)</th>
<th>(J_{cl}) pmol/min/m(^2)</th>
<th>(J_{cl}) pmol/min/m(^2)</th>
<th>(J_{cl}) pmol/min/m(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>20.23 ± 0.02</td>
<td>1.97 ± 0.15</td>
<td>149.5 ± 2.1</td>
<td>131.8 ± 4.3</td>
<td>2.02 ± 0.19</td>
<td>435.4 ± 46.7</td>
</tr>
<tr>
<td>Control + formate</td>
<td>10</td>
<td>20.11 ± 0.02</td>
<td>1.63 ± 0.14</td>
<td>146.8 ± 1.7</td>
<td>129.5 ± 1.5</td>
<td>2.85 ± 0.22*</td>
<td>579.2 ± 46.5*</td>
</tr>
<tr>
<td>Acidosis</td>
<td></td>
<td>20.23 ± 0.02</td>
<td>1.93 ± 0.13</td>
<td>146.8 ± 2.1</td>
<td>130.6 ± 3.1</td>
<td>1.95 ± 0.22</td>
<td>436.7 ± 20.8</td>
</tr>
<tr>
<td>Acidosis + formate</td>
<td>14</td>
<td>20.05 ± 0.04</td>
<td>1.69 ± 0.16</td>
<td>147.8 ± 1.0</td>
<td>136.7 ± 2.7</td>
<td>1.63 ± 0.16</td>
<td>370.4 ± 31.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; N, no. of perfused tubules. [Cl\(_o\)], chloride concentration in original perfusate; [Cl\(_l\)], chloride concentration in collected fluid; \(J_{cl}\), chloride absorption. Formate (50 µM) was added to both luminal and capillary perfusates. *Significant difference from control value (P < 0.05).

![Fig. 2. Effect of formate (50 µM) on chloride (\(J_{cl}\) top) and fluid (\(J_v\) bottom) absorption in control and acidotic rats. + formate, with formate; -- formate, without formate.](http://ajprenal.physiology.org/)

![Fig. 3. Effect of oxalate (1 µM) on chloride (top) and fluid (bottom) absorption in control and acidotic rats.](http://ajprenal.physiology.org/)
mate and Cl\textsuperscript{−}/oxalate) are inhibited. Second, the processes involved in recycling formate and oxalate might be compromised in metabolic acidosis so that they no longer can sustain organic anion-induced NaCl absorption. In this regard, it should be noted that expression of the Na\textsuperscript{+}-sulfate cotransporter is markedly reduced in metabolic acidosis (14), a process required for oxalate recycling as illustrated in Fig. 4. Finally, it is possible that the mechanism(s) involved in mediating Cl\textsuperscript{−} exit across the basolateral membrane are inhibited by metabolic acidosis. As shown in Fig. 4, the principal pathway for Cl\textsuperscript{−} exit is via Cl\textsuperscript{−} channels, although other mechanisms, such as K\textsuperscript{+}−Cl\textsuperscript{−} cotransport and Na\textsuperscript{+}-dependent Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange, may also contribute. Clearly, further studies will be necessary to identify which of the apical or basolateral transport pathways directly or indirectly involved in transcellular Cl\textsuperscript{−} absorption are altered by metabolic acidosis.

In addition to the transcellular route of NaCl absorption indicated in Fig. 4, an important component of NaCl transport in the proximal tubule is passive and paracellular (4). The magnitude of this component depends on the generation of an outwardly directed Cl\textsuperscript{−} concentration gradient due to isosmotic NaHCO\textsubscript{3} reabsorption in the early proximal tubule (4). It has been previously shown that, because the concentration of HCO\textsubscript{3}\textsuperscript{−} in the glomerular filtrate is reduced in metabolic acidosis, there is a smaller absolute decrement in luminal HCO\textsubscript{3}\textsuperscript{−} concentration along the proximal tubule, compared with normal conditions. Thus, in meta-

**Table 4. Effects of metabolic acidosis on oxalate-induced fluid and chloride absorption**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>(V_o), nl/min</th>
<th>L, mm</th>
<th>[Cl\textsubscript{o}], mM</th>
<th>[Cl\textsubscript{L}], mM</th>
<th>(J_V), nl·min(^{-1})·mm(^{-1})</th>
<th>(J_{Cl}), pmol·min(^{-1})·mm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>20.10 ± 0.03</td>
<td>2.02 ± 0.18</td>
<td>148.3 ± 1.4</td>
<td>144.6 ± 3.2</td>
<td>2.49 ± 0.24</td>
<td>395.5 ± 33.4</td>
</tr>
<tr>
<td>Control + oxalate</td>
<td>11</td>
<td>20.18 ± 0.01</td>
<td>1.72 ± 0.15</td>
<td>148.5 ± 3.2</td>
<td>143.0 ± 2.8</td>
<td>3.61 ± 0.29*</td>
<td>584.8 ± 63.5*</td>
</tr>
<tr>
<td>Acidosis</td>
<td>13</td>
<td>20.12 ± 0.03</td>
<td>1.74 ± 0.18</td>
<td>146.9 ± 0.7</td>
<td>140.3 ± 3.7</td>
<td>2.51 ± 0.29</td>
<td>389.2 ± 52.8</td>
</tr>
<tr>
<td>Acidosis + oxalate</td>
<td>14</td>
<td>20.20 ± 0.01</td>
<td>1.58 ± 0.14</td>
<td>150.7 ± 1.9</td>
<td>152.8 ± 2.8</td>
<td>2.57 ± 0.30</td>
<td>395.1 ± 51.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; N, no. of perfused tubules. Oxalate (1 µM) was added to both luminal and capillary perfusates. *Significant difference from control value (\(P < 0.01\)).

![Fig. 4. Mechanisms of transcellular NaHCO\textsubscript{3} and NaCl reabsorption in proximal tubule. See text for details and Ref. 4.](http://aprenal.physiology.org/)
bolic acidosis, the rise in luminal Cl⁻ concentration is reduced, and passive NaCl reabsorption is thereby diminished (5).

Taken together, the results of the present and previous studies indicate that both transcellular and paracellular mechanisms of NaCl reabsorption in the proximal tubule are inhibited in metabolic acidosis. The resulting increased delivery of Na⁺ may facilitate aldosterone and voltage-dependent acidification in the distal nephron. The increased excretion of Cl⁻ secondary to reduced proximal NaCl absorption may be important to allow NH₄⁺ to be excreted with a nonbicarbonate anion.

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