Essential role of NHE3 in facilitating formate-dependent NaCl absorption in the proximal tubule

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The absorption of NaCl in the proximal tubule is markedly stimulated by formate (10, 19). Such a process in turn requires H+-formate cotransport. The formate recycling process requires H+ secretion. Although Na+-H+ exchanger isoform NHE3 plays a major role in H+ secretion, but 40–50% of the rates of HCO3− absorption or cellular H+ extrusion persist in NHE3 null mice (7, 21, 26).

The purpose of the present investigation is to use NHE3 null mice to directly test the role of apical membrane NHE3 in mediating NaCl absorption stimulated by formate. We demonstrate that formate stimulates NaCl absorption in the mouse proximal tubule microperfused in vivo, as previously found in the rat (24, 25, 27). Moreover, we find that the component of NaCl absorption stimulated by formate is absent in NHE3 null mice. In contrast, stimulation of NaCl absorption by oxalate is preserved in NHE3 null mice, consistent with the previous proposal that oxalate-stimulated NaCl absorption is independent of Na+-H+ exchange, and is mediated by Na+-sulfate cotransport in parallel with Cl−-oxalate exchange and sulfate-oxalate exchange (3, 12, 24).

MATERIALS AND METHODS

Animals and surgical preparation. Knockout mice deficient in NHE3 were generated by targeted gene disruption (21). Genotype analysis of tail DNA was performed by PCR, using primers derived from the 5′ and 3′ ends of exon 6 and the 5′ end of the neomycin resistance gene. When used in the same reaction, the three primers amplify a 199-bp product from the wild-type gene and a 113-bp product from the mutant gene. Homozygous wild-type (NHE3+/−) and null (NHE3−/−) mice resulting from breeding of heterozygotes were maintained on a regular diet and tap water until the day of the experiment. Ages of mutant animals were matched with their wild-type controls.

During the course of our studies, heterozygotes were progressively back-crossed with Black Swiss mice. Heterozygotes used to generate the wild-type and null mice used in the present experiments were derived from two to four generations of back-crossing with this outbred strain. Wild-type and null mice were matched with respect to genetic background.

Microperfusion of proximal tubules in situ. The details of the methods for surgical preparation and microperfusion of mouse proximal tubules in vivo were described previously (13, 20, 22, 24, 25, 27). This stimulation of NaCl transport is consistent with Cl−-formate and Cl−-oxalate exchange processes in parallel with Cl−-formate cotransport (3). In the case of formate-stimulated NaCl absorption, it has been proposed that there is pH-coupled formate recycling due to nonionic diffusion of formic acid or H+-formate cotransport (10, 19). Such a process in turn requires H+ secretion in parallel with Cl−-formate exchange. In the proximal tubule, Na+-H+ exchanger isoform NHE3 plays a major role in H+ secretion, but 40–50% of the rates of HCO3− absorption or cellular H+ extrusion persist in NHE3 null mice (7, 21, 26).

The purpose of the present investigation is to use NHE3 null mice to directly test the role of apical membrane NHE3 in mediating NaCl absorption stimulated by formate. We demonstrate that formate stimulates NaCl absorption in the mouse proximal tubule microperfused in vivo, as previously found in the rat (24, 25, 27). Moreover, we find that the component of NaCl absorption stimulated by formate is absent in NHE3 null mice. In contrast, stimulation of NaCl absorption by oxalate is preserved in NHE3 null mice, consistent with the previous proposal that oxalate-stimulated NaCl absorption is independent of Na+-H+ exchange, and is mediated by Na+-sulfate cotransport in parallel with Cl−-oxalate exchange and sulfate-oxalate exchange (3, 12, 24).
proximal convoluted tubules with 3–5 loops on the kidney surface were perfused at a rate of 15 nl/min with a perfusion solution containing 20 μCi/ml of low-sodium [3H]methoxyinulin for measuring volume absorption. Tubule fluid collections were made downstream with another micropipette. One collection was made in each perfused tubule, and two to four collections were taken in the experimental kidney of each animal. The perfused segments were marked with Sudan Black heavy mineral oil, and their lengths were determined after filling with high-viscosity microfil (Canton Bio-Medical Products, Boulder, Colorado) and dissection of the silicone rubber casts.

Measurement of rates of Cl\textsuperscript{−} and fluid absorption. The rates of net Cl\textsuperscript{−} (J\textsubscript{Cl}) and fluid (J\textsubscript{v}) absorption were calculated based on changes in the concentrations of [3H]inulin and Cl\textsuperscript{−} as described previously (23, 24). J\textsubscript{Cl} and J\textsubscript{v} were expressed per millimeter tubule length. The composition of the perfusion solution was the same as used previously in the rat (24) (in mM): sodium chloride 140, sodium bicarbonate 5.0, potassium chloride 4.0, calcium chloride 2.0, magnesium sulfate 1.0, dibasic sodium phosphate 1.0, and monobasic sodium phosphate 1.0, pH 6.7. Concentrations of formate and oxalate indicated in the tables and figures were added as sodium salts.

Statistics. Data are presented as means ± SE. Experimental groups were compared with a control group by use of Dunnett’s test. Differences were considered significant if P < 0.05.

RESULTS

In the first series of experiments, we evaluated whether formate and oxalate stimulate NaCl transport in the proximal tubule of the mouse as previously observed in the rat (24, 27). To this end, proximal tubules were microperfused in situ with a relatively high Cl\textsuperscript{−}, low HCO\textsubscript{3}\textsuperscript{−}, pH 6.7 solution mimicking conditions in the late proximal tubule. Under these conditions, the rate of volume reabsorption, J\textsubscript{v}, results pre-

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**Table 1. Effects of formate and oxalate on fluid and chloride absorption in wild-type mice**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>V\textsubscript{o}, nl/min</th>
<th>L, mm</th>
<th>[Cl\textsubscript{o}, mM]</th>
<th>[Cl\textsubscript{L}, mM]</th>
<th>J\textsubscript{v}, nl·min\textsuperscript{-1}·mm\textsuperscript{-1}</th>
<th>J\textsubscript{Cl}, pmol·min\textsuperscript{-1}·mm\textsuperscript{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>15.11 ± 0.05</td>
<td>1.65 ± 0.16</td>
<td>146.6 ± 0.3</td>
<td>145.0 ± 4.2</td>
<td>1.55 ± 0.12</td>
<td>254.3 ± 21.6</td>
</tr>
<tr>
<td>Formate</td>
<td>11</td>
<td>15.52 ± 0.13</td>
<td>1.77 ± 0.16</td>
<td>146.5 ± 0.2</td>
<td>144.0 ± 1.8</td>
<td>2.33 ± 0.09*</td>
<td>348.2 ± 10.5*</td>
</tr>
<tr>
<td>Oxalate</td>
<td>13</td>
<td>15.28 ± 0.14</td>
<td>1.81 ± 0.14</td>
<td>146.2 ± 0.4</td>
<td>154.0 ± 2.6</td>
<td>2.73 ± 0.21*</td>
<td>352.3 ± 23.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of perfused tubules; V\textsubscript{o}, perfusion rates; L, tubular length; [Cl\textsubscript{o}, chloride concentration in the original perfusate; [Cl\textsubscript{L}, chloride concentration in collected fluid; J\textsubscript{v}, fluid absorption; J\textsubscript{Cl}, chloride absorption; formate, 500 μM was added to the luminal perfusate; oxalate, 5 μM was added to the luminal perfusate. *Significant difference from control value (P < 0.05).
stimulation of finding in the previous studies in the rat was that rat proximal tubule (13, 20, 22, 24, 25, 27). A key oxalate exchange processes to transcellular NaCl ab-

sorptions of luminal membrane Cl\(^{2-}\) potential difference.

absorption is paracellular driven by the lumen-positive 1

mate recycling and H\(^{+}\). A: NaCl absorption takes place by Cl\(^{-}\)-formate exchange with pH-coupled formate recycling and H\(^{+}\) secretion by Na\(^{+}\)-H\(^{+}\) exchange. B: NaCl absorption takes place by Cl\(^{-}\)-formate exchange with pH-coupled formate recycling and H\(^{+}\) secretion by H\(^{+}\)-ATPase. Na\(^{+}\) absorption is paracellular driven by the lumen-positive potential difference.

### Table 2. Effects of formate and oxalate on fluid and chloride absorption in NHE3 null mice

<table>
<thead>
<tr>
<th></th>
<th>(V_{o}), nl/min</th>
<th>(L_{s}), mm</th>
<th>[Cl(^{-})](_{o}), mM</th>
<th>[Cl(^{-})](_{l}), mM</th>
<th>(J_{v}), nl/min(^{-1})-mm(^{-1})</th>
<th>(J_{Cl}), pmol(^{-1})-mm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>15.05 (\pm) 0.04</td>
<td>1.68 (\pm) 0.12</td>
<td>146.0 (\pm) 0.9</td>
<td>149.1 (\pm) 3.1</td>
<td>1.03 (\pm) 0.12</td>
</tr>
<tr>
<td>Formate</td>
<td>11</td>
<td>15.36 (\pm) 0.19</td>
<td>1.83 (\pm) 0.18</td>
<td>145.9 (\pm) 0.2</td>
<td>156.7 (\pm) 4.6</td>
<td>1.29 (\pm) 0.25</td>
</tr>
<tr>
<td>Oxalate</td>
<td>12</td>
<td>15.35 (\pm) 0.17</td>
<td>1.64 (\pm) 0.19</td>
<td>146.4 (\pm) 0.5</td>
<td>158.2 (\pm) 3.9</td>
<td>2.08 (\pm) 0.23*</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE. \(n\), No. of perfused tubules; NHE3, Na\(^{+}\)-H\(^{+}\) exchanger isoform-3; formate, 500 μM was added to the luminal perfusate; oxalate, 5 μM was added to the luminal perfusate. *Significant difference from control value (\(P < 0.05\)).
isoform in mediating brush border acid secretion (21, 26). Of the remaining component of HCO3⁻ absorption in NHE3 null mice, ~60% is inhibited by bafilomycin, an inhibitor of the vacuolar H⁺-ATPase (26). Moreover, studies in isolated proximal tubules from NHE3/NHE2 null mice have identified a novel EIPA-sensitive acid extrusion process (7). This process might contribute to the net rates of HCO3⁻ (JHCO3) observed to persist in NHE3 null mice in vivo in the presence of bafilomycin (26).

The models in Fig. 3 illustrate formate-dependent NaCl absorption as occurring by Cl⁻-formate exchange in parallel with H⁺ secretion by either Na⁺-H⁺ exchange or H⁺-ATPase. As shown in Fig. 3A, in which H⁺ secretion takes place by Na⁺-H⁺ exchange, the Na⁺ absorption associated with formate-dependent Cl⁻ transport is transcellular. In contrast, as shown in Fig. 3B, in which H⁺ secretion is mediated by H⁺-ATPase, Cl⁻ absorption is electrogenic, and is accompanied by paracellular Na⁺ absorption. In either case, formate would induce net NaCl absorption.

In microperfused proximal tubules of rabbit and rat, formate markedly stimulates Jv and JC1 (13, 20, 22, 24, 25, 27), consistent with the proposed role of Cl⁻-formate exchange in mediating NaCl absorption according to both of the models in Fig. 3. Moreover, the ability of formate to stimulate proximal NaCl absorption is dependent on luminal acidification, consistent with the necessity for H⁺-coupled formate recycling (20). Despite the evidence that a significant fraction of proximal acidification is independent of NHE3 (7, 21, 26), the Na⁺-H⁺ exchange inhibitor EIPA completely abolishes formate stimulation of Jv and JC1 (24), suggesting a key role for NHE3 or another EIPA-sensitive process.

We now find that the ability of formate to stimulate NaCl transport is virtually abolished in the proximal tubules of NHE3 null mice. This observation indicates that neither another EIPA-sensitive process nor the H⁺-ATPase can sustain the H⁺ secretion needed for appreciable Cl⁻ absorption by Cl⁻-formate exchange to occur. Given that NHE3 accounts for only 50–60% of H⁺ secretion across the brush border membrane and that there exist several alternative mechanisms for acid extrusion, the question arises as to why there is an absolute requirement for NHE3 activity for formate-stimulated NaCl absorption. Indeed, in our studies, the tubules were perfused with a low HCO3⁻ solution (5 mM HCO3⁻, pH 6.7) simulating the maximal acidification achieved along the proximal tubule, so it is not evident why there should be any dependence of formate-stimulated NaCl absorption on the activity of acid secretion processes. Thus the present results raise the possibility that there is an intimate relationship between NHE3 and the formate transporters to permit direct transfer of H⁺ between NHE3 and the formate recycling pathways. Such a mechanism would be analogous to substrate tunneling or channeling in mult-subunit enzyme systems in which substrate is directly transferred from one site to another without diffusion through the bulk phase (15).

It may be noted that the baseline Jv and JC1 measured in the absence of added formate or oxalate are lower in the NHE3 null mice compared with the wild-type controls. One possible explanation is that a component of NaCl absorption dependent on NHE3 activity is present under baseline conditions due to Cl⁻-formate exchange resulting from the presence of formate in peritubular capillary blood. Alternatively, Cl⁻-base exchange independent of formate (e.g., Cl⁻-OH⁻ exchange) might be present, and, in parallel with Na⁺-H⁺ exchange, contribute to NaCl absorption (13, 22, 28). In addition, glomerular filtration rate is chronically and markedly reduced in NHE3 null mice (14), possibly resulting in reduced cell size, membrane surface area, and transporter expression, the opposite of what results from glomerular hyperfiltration (6, 8, 16–18).

Significant stimulation of Jv and JC1 by oxalate is still observed in NHE3 null mice, reflecting the presence of the component of transcellular Cl⁻ absorption taking place by Cl⁻-oxalate exchange. These findings are consistent with the previous observation that EIPA does not inhibit oxalate-stimulated NaCl transport (24). Taken together, the present and previous results indicate that NHE3 has no role in mediating oxalate-stimulated NaCl absorption. In fact, previous evidence strongly suggests that oxalate-stimulated NaCl absorption is mediated by Na⁺-sulfate cotransport in parallel with Cl⁻-oxalate exchange and sulfate-oxalate exchange (3, 12, 24).

In conclusion, we find that NHE3 has a specific role in mediating formate-stimulated NaCl absorption in the proximal tubule. In view of the fact that other secretory pathways contribute to H⁺ secretion in the proximal tubule, the virtually complete dependence of formate-induced NaCl absorption on NHE3 activity raises the possibility that NHE3 and the formate transporters are functionally coupled in the brush border membrane.

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REFERENCES


