Active Sodium-Urea Counter-transport Is Inducible in the Basolateral Membrane of Rat Renal Initial Inner Medullary Collecting Ducts

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Abstract

Rat inner medullary collecting ducts (IMCDs) possess a luminal Na+-dependent, active urea secretory transport process, which is upregulated by water diuresis. In this study of perfused IMCDs microdissected from base (IMCDb), middle (IMCDm), or tip (IMCDt) of the inner medulla, we tested whether furosemide diuresis alters active urea transport. Rats received furosemide (10 mg/d s.c. for 3–4 d) and were compared with pair-fed control rats. Furosemide significantly decreased urine osmolality and urea clearance, and compared with pair-fed control rats. Furosemide significantly decreased active urea secretion in IMCDb and IMCDm, but not in IMCDt. We also showed that urea is actively secreted by a secondary active, sodium–coupled transport process in the rat terminal IMCD (7) and the medullary tip (8). These findings suggest that an increase in facilitated urea transport and UT-A1 protein abundance is one mechanism that may contribute to a furosemide-induced increase in blood urea nitrogen.

In addition to facilitated urea transport, there is evidence for active urea transport process(es) in the kidney of the rat (9–12), rabbit (13), dog (14, 15), spiny dogfish (16), and human (17). Schmidt-Nielsen and colleagues first demonstrated the existence of active urea reabsorption which is coupled to sodium reabsorption in the kidney of the spiny dogfish, Squalus acanthias (16). We showed that urea is actively reabsorbed via a secondary active, sodium–coupled co-transport process in the rat initial IMCD (IMCDi) from rats fed a low-protein diet for 3 wk (9–11). We also showed that urea is actively secreted via a secondary active, sodium–coupled counter-transport process in the deepest portion of the rat terminal IMCD, the IMCDt, but not in the middle third of the IMCD, the IMCDm, or in the initial IMCD of rats fed a normal diet (12). This active urea secretion is upregulated fivefold by making rats water-diuretic for 3–5 d.

The purpose of this study was to determine whether administering furosemide to rats affects active urea transport. We tested for the presence of active urea transport in each of the three IMCD subsegments using the isolated perfused tubule technique. After demonstrating that urea was actively reabsorbed in the IMCDt, we examined the mechanism for this active urea transport.

Methods

Tissue preparation

All animal protocols were approved by the Emory University Institutional Animal Care and Use Committee. Tubules were obtained from pathogen-free male Sprague–Dawley rats (National Cancer Institute, Frederick, MD). The rats were kept in filter-top cages with autoclaved bedding and received free access to water and a normal protein diet (NIH-31; Ziegler Brothers, Gardner, PA) unless otherwise indicated below. The kidneys were placed into chilled (17°C), isotonic, disecting solution to isolate initial (IMCDi) or terminal (IMCDm or IMCDt) IMCD subsegments (18, 19) as described (12, 20).

Protocols

(A) Furosemide-treated. Rats were implanted with a sustained-release furosemide pellet (pellet number D-141; Innovative Research of America, Sarasota, FL) subcutaneously into their back to administer 10 mg/d of furosemide for 1–7 d. During this period, rats received

Abbreviations used in this paper: BW, body weight; GFR, glomerular filtration rate; IMCD, inner medullary collecting ducts.

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and temperature protocols, since each tubule was used as its own control. Unpaired statistical analysis was used for the protocols comparing untreated and furosemide-treated rats and the time-course protocol.

Results

Clearance studies. The clearance studies compared furosemide-treated rats with pair-fed control rats (n = 5). Administering furosemide for 3 d increased serum urea nitrogen (control: 11±1, furosemide: 22±2 mg/dl, P < 0.01) and urine volume (control: 6±1 ml/d, furosemide: 29±8 ml/d, P < 0.01) but decreased urine osmolality (control: 923±101 mOsm/kg H₂O, furosemide: 388±89 mOsm/kg H₂O, P < 0.01, Table I). Furosemide increased urinary urea excretion (control: 60±6 mg/d per 100 g body weight [BW], furosemide: 162±26 mg/d per 100 g BW, P < 0.01) and decreased urea clearance (control: 6.2±0.7 ml/min per kg BW, furosemide: 4.1±0.1 ml/min per kg BW, P < 0.05). There was no difference in creatinine clearance (control: 5.3±0.2 ml/min per kg BW, furosemide: 4.5±0.2 ml/min per kg BW, P = NS) or urinary corticosterone excretion between the two groups of rats (control: 0.8±0.2 ng/mg creatinine, furosemide: 0.9±0.4 ng/mg creatinine, P = NS).

Net urea flux. Net urea secretion was significantly lower in IMCDs from rats treated with furosemide for 3–4 d (1.9±1.1 pmol/mm per min, n = 6, Fig. 1) than in IMCDs from untreated rats (11.5±1.4 pmol/mm per min, n = 17, P < 0.01). In contrast, there was no significant net urea flux in IMCDs from untreated (2.3±0.6 pmol/mm per min, n = 8) or furosemide-treated rats (3.3±1.4 pmol/mm per min, n = 6). Tubule lengths, perfusate flow rates, and collected/perfusate urea ratios are shown in Table II.

Initial IMCDs (IMCDs) from untreated rats had no significant net urea flux (0.03±0.2 pmol/mm per min, n = 6). However, IMCDs from rats treated with furosemide for 3–4 d had significant net urea reabsorption (14.6±1.9 pmol/mm per min, n = 14, P < 0.01; Fig. 1). Vasopressin (10 nM in the bath) increased net urea reabsorption in IMCDs from furosemide-treated rats from 10.6±2.3 pmol/mm per min to 21.2±1.8 pmol/mm per min (n = 5, P < 0.01, Fig. 2).

Table I. Urine and Serum Parameters after 3 d of Pair-Feeding

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial rat weight (g)</td>
<td>88±4</td>
<td>101±9</td>
</tr>
<tr>
<td>% Change of BW</td>
<td>-13.2±0.6</td>
<td>-14.1±2.3</td>
</tr>
<tr>
<td>Urine volume (ml/d)</td>
<td>6.0±1.2</td>
<td>29.3±8.4*</td>
</tr>
<tr>
<td>Urine osmolality (mOsm/kg H₂O)</td>
<td>923±101</td>
<td>388±89*</td>
</tr>
<tr>
<td>Urea excretion (mg/d per 100 g BW)</td>
<td>60±6</td>
<td>162±26*</td>
</tr>
<tr>
<td>Sodium excretion (mEq/d per 100 g BW)</td>
<td>0.35±0.08</td>
<td>0.18±0.03</td>
</tr>
<tr>
<td>Potassium excretion (mEq/d per 100 g BW)</td>
<td>0.75±0.19</td>
<td>1.18±0.43*</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.4±0.02</td>
<td>0.5±0.04</td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/dl)</td>
<td>11±1</td>
<td>22±2*</td>
</tr>
<tr>
<td>Serum sodium (mEq/liter)</td>
<td>140±1</td>
<td>141±1</td>
</tr>
<tr>
<td>Serum potassium (mEq/liter)</td>
<td>5.4±0.2</td>
<td>5.2±0.2</td>
</tr>
<tr>
<td>Urea clearance (ml/min per kg BW)</td>
<td>6.2±0.7</td>
<td>4.1±0.5*</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min per kg BW)</td>
<td>5.3±0.2</td>
<td>4.5±0.6</td>
</tr>
</tbody>
</table>

Data: mean±SE; n = 5; BW, body weight; *P < 0.05.
for 7 d (917 ± 6 kg H2O; n = 8). Net urea secretion was decreased compared with rats treated with furosemide for 1 d (2.2 ± 0.3 pmol/mm per min; n = 4, P < 0.01 vs. untreated rats). Net urea secretion was increased in IMCD1s from rats treated with furosemide for 3–4 d (4.6 ± 1.9 pmol/mm per min; n = 5, P < 0.01 vs. untreated rats). Net urea secretion was increased in IMCD1s from rats treated with furosemide for 7 d (7.8 ± 1.3 pmol/mm per min, n = 5) compared with untreated rats (P < 0.01) but was decreased compared with rats treated with furosemide for 3–4 d (P < 0.05).

Effect of inhibitors in IMCD1s from rats treated with furosemide for 3–4 d. Phloretin (250 µM in the bath) inhibited net urea reabsorption from 7.3 ± 1.2 pmol/mm per min to 1.5 ± 1.2 pmol/mm per min (n = 5, P < 0.01; Fig. 4). When phloretin was removed, net urea reabsorption returned to 8.5 ± 2.1 pmol/mm per min (n = 4, P = NS vs. control).

Table III. Urine and Serum Parameters after 7 d of Pair-Feeding

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial rat weight (g)</td>
<td>88 ± 4</td>
<td>101 ± 9</td>
</tr>
<tr>
<td>% Change of BW</td>
<td>17.8 ± 2.1</td>
<td>17.2 ± 3.9</td>
</tr>
<tr>
<td>Urine volume (ml/d)</td>
<td>11.5 ± 1.5</td>
<td>11.9 ± 2.3</td>
</tr>
<tr>
<td>Urine osmolality (mOsm/kg H2O)</td>
<td>1695 ± 157</td>
<td>1485 ± 176</td>
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<tr>
<td>Urea excretion (mg/d per 100 g BW)</td>
<td>135 ± 17</td>
<td>142 ± 7</td>
</tr>
<tr>
<td>Sodium excretion (mEq/d per 100 g BW)</td>
<td>0.69 ± 0.11</td>
<td>0.58 ± 0.20</td>
</tr>
<tr>
<td>Potassium excretion (mEq/d per 100 g BW)</td>
<td>1.65 ± 0.28</td>
<td>1.29 ± 0.53</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.4 ± 0.02</td>
<td>0.5 ± 0.04</td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/dl)</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Serum sodium (mEq/liter)</td>
<td>141 ± 1</td>
<td>140 ± 1</td>
</tr>
<tr>
<td>Serum potassium (mEq/liter)</td>
<td>5.1 ± 0.2</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>Urea clearance (ml/min per kg BW)</td>
<td>8.3 ± 1.1</td>
<td>8.3 ± 0.5</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min per kg BW)</td>
<td>3.9 ± 0.4</td>
<td>5.4 ± 0.8</td>
</tr>
</tbody>
</table>

Data: mean ± SE; n = 5; BW, body weight.
Ouabain (1 mM in the bath) decreased net urea reabsorption in IMCD$_3$s from 14.6±2.3 pmol/mm per min to 6.5±3.2 pmol/mm per min (n = 4, P < 0.01; Fig. 5). When ouabain was washed out of the bath, net urea reabsorption returned to 18.7±4.0 pmol/mm per min (n = 4, P < 0.05 vs. basal).

Net urea reabsorption was present when IMCD$_3$s were warmed to 37°C (5.9±1.3 pmol/mm per min; n = 5). Net urea reabsorption disappeared at 23°C (−0.8±1.3 pmol/mm per min; n = 5, P < 0.01 vs. 37°C; Fig. 6).

Effect of ion substitution in IMCD$_3$s from rats treated with furosemide for 3–4 d. Removing Na$^+$ from the bath (and replacing it with N-methyl-D-glucamine$^+$) completely inhibited net urea reabsorption in IMCD$_3$s (control: 8.4±2.8 pmol/mm per min; bath Na$^+$-removal: 0.2±0.6 pmol/mm per min; n = 5, P < 0.01; Fig. 7). When bath Na$^+$ was restored, net urea reabsorption returned to 6.9±0.7 pmol/mm per min (n = 4, P = NS vs. control). In contrast, removing Na$^+$ from the perfusate had no significant effect on net urea reabsorption (control: 5.6±1.4 pmol/mm per min, perfusate Na$^+$-removal: 7.9±1.5 pmol/mm per min; n = 5, P = NS; Fig. 8). Removing Cl$^−$ from the bath (and replacing it with gluconate$^−$) had no effect on net urea reabsorption (control: 9.9±3.0 pmol/mm per min, bath Cl$^−$-removal: 6.5±2.2 pmol/mm per min, restore bath Cl$^−$: 8.5±2.3 pmol/mm per min; n = 5, P = NS; Fig. 9).

Effect of diuretics in IMCD$_3$s from rats treated with furosemide for 3–4 d. Adding bumetanide (10 nM) to the bath to block the basolateral Na$^+$-K$^+$-2Cl$^−$ cotransporter (22, 23) increased net urea reabsorption in IMCD$_3$s from 10.0±2.4 pmol/mm per min (n = 5) to 15.1±2.8 pmol/mm per min (n = 5, P < 0.05; Fig. 10). When bath bumetanide was washed out, net urea reabsorption returned to 8.0±2.4 pmol/mm per min (n = 5, P = NS vs. control). In contrast, adding amiloride (1 mM) to the bath to block basolateral sodium channels and/or Na$^+$/H$^+$ exchange (24, 25) had no effect on net urea reabsorption in
IMCD₁s (control: 12.6±2.6 pmol/mm per min, amiloride: 11.9±1.9 pmol/mm per min; n = 5, P = NS, Fig. 11).

Discussion

The major finding in the present study is that administering furosemide to rats for a few days alters active urea transport processes in two IMCD subsegments: active urea secretion is reduced in the deepest subsegment of the terminal IMCD, the IMCD₃, and active urea reabsorption appears in the initial IMCD (IMCD₁). Both the induction of active urea reabsorption in the IMCD₁ and the decrease in active urea secretion in the IMCD₃ could be mechanisms that contribute to the decrease in urea clearance observed after 3 d of furosemide administration. The response to furosemide administration is transient with significant changes in urinary excretion at 3–4 d that resolve at 7 d (Tables I and III). The time course and response we observed in rats is similar to the response in humans (26–28).

We measured no change in creatinine clearance between the control and furosemide-treated rats (Tables I and III), suggesting that glomerular filtration rate (GFR) was unchanged. However, creatinine clearance is an imperfect measure of GFR. Thus, we cannot exclude the possibility that we missed a small decrease in GFR in the furosemide-treated rats that resulted in a small increase in proximal reabsorption and a decrease in urea excretion. However, it seems unlikely that the entire decrease in urea excretion can be attributed to an increase in proximal reabsorption which resulted from an unmeasurable change in GFR (based upon creatinine clearance). The present study suggests that 16% of the decrease in urea excretion in the furosemide-treated rats is mediated by changes in the active urea transport processes in the IMCD₁ and IMCD₃ (see Ref. 43).²

This interpretation is consistent with the results of a clinical study in which frusemide was administered to humans and showed that the diuretic-induced fall in urea clearance was independent of enhanced proximal urea reabsorption and resulted from enhanced distal reabsorption (2). This study could not identify the site in the distal nephron responsible for enhanced reabsorption, but the authors speculated that it could result from a decrease in active urea secretion in the medullary collecting duct (2). In the present study, we did find a signifi-

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² We estimated the contribution of changes in active urea transport processes to urea excretion as follows. IMCD₁s are found in the outer third (2 mm) of the rat medulla (20). There are ~5,000 IMCD₁s in a rat kidney (Table 5 in Ref. 43). Net urea reabsorption is increased by 15 pmol/mm per min in IMCD₁s from furosemide-treated rats compared with controls. Using these values, 0.4 mmol of urea would be reabsorbed per day. IMCD₃s are found in the deepest third (2 mm) of the rat medulla (20). There are ~500 IMCD₃s in a rat kidney (Table 5 in Ref. 43). Net urea secretion is decreased by 10 pmol/mm per min in IMCD₃s from furosemide-treated rats compared with controls. Using these values, 0.03 mmol of urea would be reabsorbed (not be secreted) each day. Summing these two values and converting the units from millimoles to milligrams yields a value of 26 mg/d of urea being reabsorbed due to changes in the two active urea transport processes. This calculation suggests that urea excretion would increase by an additional 16% in the absence of changes in active urea transport in the IMCD.
Mechanism of active urea reabsorption in the initial IMCD.

The possibility that a loop diuretic could induce active urea transport was initially suggested by a tissue-slice study of dog kidneys that reported that the intrarenal infusion of ethacrynic acid induces active urea reabsorption in the inner medullary tip (29). In the present study, we directly demonstrated that furosemide induces active urea reabsorption in rat initial IMCDs. This active urea transport process differs from previously described active urea transport processes in the rat IMCD because it is completely and reversibly inhibited by removing sodium from the bath but not from the perfusate (Table IV, Figs. 7 and 8). This result suggests that treating rats with furosemide induces a previously unrecognized, sodium-dependent, active urea reabsorptive transport process in the basolateral membrane of the initial IMCD (Fig. 12). In addition, active urea reabsorption is inhibited by ouabain, suggesting that this is a secondary active urea reabsorptive transport process which is dependent upon Na⁺/K⁺-ATPase.

Additional evidence supporting a basolateral localization for this active urea reabsorptive transport process is that it is stimulated by the addition of bumetanide to the bath (Fig. 10). There are two known isoforms of the bumetanide-sensitive Na⁺⁻K⁺⁻2Cl⁻ cotransporter: (i) NKCC2 (BSC1) in the apical membrane of the thick ascending limb (30, 31); and (ii) NKCC1 (BSC2) in the basolateral membrane of the IMCD (23, 32, 33). In isolated IMCDs (34) and cultured IMCD cells (35), NKCC1 (BSC2) is thought to participate in NaCl and/or fluid secretion, as it does in the gastric mucosa (36). We found that adding bumetanide to the bath stimulates active urea reabsorption. A potential mechanism for this effect is that adding bumetanide to the bath could decrease sodium entry across the IMCD basolateral membrane via the Na⁺⁻K⁺⁻2Cl⁻ co-transporter, potentially decreasing intracellular sodium concentration, and could promote urea reabsorption by a basolateral urea–sodium “anti-port” process (Fig. 12).

We also tested the effect of amiloride on active urea transport since an amiloride-sensitive Na⁺⁻H⁺ anti-porter is thought to be present in the basolateral membrane of IMCD cells to mediate cell volume regulation (24, 25). However, we found no effect of amiloride, added to the bath, on active urea transport. Grunewald and colleagues also found that amiloride had no effect on cell volume regulation after hypotonic (300 mOsm/kg H₂O) stress in isolated IMCD cells (37).

The active urea reabsorptive transport process in the initial IMCD of furosemide-treated rats differs in its pharmacologic characteristics (responses to phloretin and vasopressin) from the one induced in the initial IMCD of rats fed a low-protein diet (9–11). However, it has the same pharmacologic characteristics as the active urea secretory transport process in the IMCD3 (Table IV), although phloretin inhibits the former transport process from the bath and the latter from the lumen (12). These results suggest the possibility that these two trans-
port processes may be the same transporter expressed in opposite membranes and oriented in opposite directions in the two different IMCD subsegments.

Changes in urea transport in the IMCD. We showed that urea is actively secreted in the rat IMCD1 and that active urea secretion is upregulated fivefold by water diuresis (12). In the present study, administering furosemide for 1–7 d decreased active urea secretion in the IMCD3. We also showed that administering furosemide to rats for 3–4 d increases facilitated urea transport in rat terminal IMCDs (7). Both a decrease in active urea secretion and an increase in facilitated urea reabsorption could increase overall urea reabsorption from the IMCD, thereby decreasing urea clearance and increasing urea delivery to the inner medullary interstitium. A decrease in active urea secretion has also been proposed as a potential mechanism to explain the etiology of familial azotemia (38).

Physiological role of active urea reabsorption in the initial IMCD. Furosemide decreases urine-concentrating ability, at least in part, by blocking NaCl reabsorption from thick ascending limbs (39, 40). However, mathematical simulations of the urine concentrating mechanism suggest that an increase in urea reabsorption across the initial IMCD would also decrease concentrating ability by decreasing the delivery of urea to the deep inner medullary interstitium (41, 42). Thus, the induction of active urea reabsorption in the initial IMCD of furosemide-treated rats may be a mechanism that contributes to the urine concentrating defect observed at 3 d of furosemide therapy. Consistent with this hypothesis, we found that active urea reabsorption in the initial IMCD is decreased at 7 d of furosemide administration, and that urine osmolality is similar between rats given furosemide for 7 d and pair-fed control rats.

Summary. We found that administering furosemide to rats for a few days decreases active urea secretion in the deepest portion of the IMCD, IMCD3, and induces active urea reabsorption in the initial IMCD (IMCD1). These two changes in active urea transport, along with the increase in facilitated urea transport in the terminal IMCD of furosemide-treated rats (7) could contribute to the decrease in urea clearance (and increase in blood urea nitrogen) observed in these rats. The active reabsorption of urea in the initial IMCD is dependent upon bath sodium, inhibited by phloretin, and stimulated by bumetanide; inhibition of Na+/K+-ATPase by ouabain also reduces active urea reabsorption. We propose that active urea reabsorption is induced by furosemide treatment in the rat initial IMCD and occurs via a previously unrecognized, sodium-dependent, secondary active urea transport process that is located in the basolateral membrane.

Acknowledgments

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References

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