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 ($IMCD_3$s) 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 ($IMCD_1$), middle ($IMCD_2$), or tip ($IMCD_3$) 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 increased blood urea nitrogen. $IMCD_3$s from furosemide-treated rats had significantly lower rates of active urea secretion than $IMCD_3$s from control rats. $IMCD_2$s showed no active urea transport in control or furosemide-treated rats. $IMCD_1$s from control rats had no active urea transport, but $IMCD_1$s from furosemide-treated rats expressed significant rates of active urea reabsorption. In $IMCD_1$s, this active urea reabsorptive transport process was inhibited by: (*i*) 0.25 mM phloretin (bath); (*ii*) 1 mM ouabain (bath); and (*iii*) replacing bath Na^+ with $NMDG^+$; it was stimulated by 10 nM bumetanide (bath). In summary, we found that furosemide decreased active urea secretion in $IMCD_3$s and induced active urea reabsorption in $IMCD_1$s. The new Na^+-dependent, active urea reabsorptive transport process may be a basolateral Na^+-urea antiporter. (*J. Clin. Invest.* 1998. 102: 1008–1015.) Key words: urea • sodium • inner medullary collecting ducts • furosemide • urine-concentrating mechanism

Introduction

The chronic administration of furosemide can decrease urea clearance and increase blood urea nitrogen by increasing urea reabsorption in the distal portion of the nephron (1, 2). The major site of urea reabsorption in the distal nephron is the terminal inner medullary collecting duct (IMCD).[1] Urea is reabsorbed across the terminal IMCD by the vasopressin-regulated, facilitated urea transporter UT-A1 (3–6). We showed that administering furosemide to rats for 3–4 d increases facilitated urea transport in the rat terminal IMCD (7) and the abundance of the UT-A1 urea transporter protein in the inner 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 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 cotransport process in the rat initial IMCD ($IMCD_1$) 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 countertransport process in the deepest portion of the rat terminal IMCD, the $IMCD_3$, but not in the middle third of the IMCD, the $IMCD_2$, nor 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 $IMCD_1$, 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, dissecting solution to isolate initial ($IMCD_1$) or terminal ($IMCD_2$ or $IMCD_3$) 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

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

food and water ad libitum. In some experiments, the quantity of food eaten by the furosemide-treated rats was measured and untreated (control) rats were pair-fed the same amount of food; there were no differences obtained when pair-fed furosemide-treated rats and control rats were compared versus when non–pair-fed furosemide-treated rats and control rats were compared. Therefore, we combined the results from the pair-fed and non–pair-fed furosemide-treated and control rats, since there were no differences in the data obtained.

(B) Untreated (control). Rats were given water ad libitum. Some control rats were given food ad libitum while others were pair-fed to furosemide-treated rats.

The dissecting solution was gassed with 95% O_2 and 5% CO_2 and contained: 118 mM NaCl, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 2.5 mM K_2HPO_4, 1.2 mM $MgSO_4$, 5.5 mM glucose, and 4 mM creatinine. Tubules were transferred into a bath that was continuously exchanged and bubbled with 95% O_2/5% CO_2 gas and perfused using standard techniques (10, 12, 20).

Urea measurement. The urea concentration in perfusate, bath, and collected fluid was measured using a continuous-flow ultramicrofluorometer as described (10, 12, 20). This assay is capable of resolving differences of 4% or greater in urea concentration (20). Urea flux (J_{urea}) was calculated as: $J_{urea} = C_oV_o\text{-}C_lV_l$, where C_o is the urea concentration in the perfusate, C_l is the urea concentration in the collected fluid, V_o is the perfusion rate per unit length of tubule, and V_l is the collection rate per unit length of tubule. Based upon this formula, a positive value for J_{urea} indicates urea reabsorption, while a negative value indicates urea secretion.

To study active urea transport, tubules were perfused with the same perfusate and bath solution (except during the ion substitution studies described below) whose composition was identical to the dissection solution (described above) except that 3 mM urea was added to the solution (9, 10, 12). To calculate J_{urea}, V_o is assumed to be equal to V_l, because there is no osmotic gradient across the tubule and hence, no driving force for water reabsorption. We showed that the measured volume flux is 0, both with and without vasopressin added to the bath solution, under these experimental conditions (9, 12).

Effect of vasopressin or inhibitors on active urea transport. The urea concentration of three to four collections was measured, after which the following compounds (Sigma Chemical Co., St. Louis, MO) were added to the bath (and vehicle added to the perfusate): (*i*) 10 nM arginine vasopressin (10, 12); (*ii*) 250 μM phloretin (9, 10, 12); (*iii*) 1 mM ouabain (10, 12); (*iv*) 1 mM amiloride; or (*v*) 10 nM bumetanide; or the tubule was cooled to 23°C (10, 12), and three to four additional collections were obtained. Next, the inhibitor was washed out and three to four additional collections were obtained.

A 250-mM stock solution of phloretin was prepared in absolute ethanol and added to perfusate to achieve a final concentration of 250 μM phloretin and 0.1% ethanol (10, 12). Control collections were obtained with 0.1% ethanol added to the perfusate (10, 12).

Effect of ion substitution on net urea transport. The urea concentration of three to four collections was measured. Next, Na^+ was removed from either the perfusate or bath and replaced by *N*-methyl-D-glucamine$^+$ (in equimolar concentrations) and three to four collections were obtained. After the solution was changed to return Na^+, three to four additional collections were obtained (10, 12). In separate tubules, Cl^- was removed from the bath and replaced with equimolar gluconate$^-$ (12); three to four collections were obtained for each experimental condition. To ensure nearly equal osmolality of the perfusate and bath solutions, their osmolality was measured by vapor pressure osmometry (model 5500; Wescor, Logan, UT).

Statistics. All data are presented as mean±SE and n = number of rats. Data from three to four collections were averaged to obtain a single value from each experimental phase in each tubule. To test for statistical significance between two groups, Student's t test was used. To test more than 2 groups, an ANOVA was used, followed by Tukey's protected t test (21) to determine which groups were significantly different. The criterion for statistical significance was $P < 0.05$. Paired statistical analysis was used for the vasopressin, inhibitor, ion substitution, 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 H_2O, furosemide: 388±89 mOsm/kg H_2O, $P < 0.01$, 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 $IMCD_3$s from rats treated with furosemide for 3–4 d (−1.9±1.1 pmol/mm per min, $n = 6$, Fig. 1) than in $IMCD_3$s 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 $IMCD_2$s 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 ($IMCD_1$s) from untreated rats had no significant net urea flux (0.03±2.2 pmol/mm per min, $n = 6$). However, $IMCD_1$s 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 $IMCD_1$s 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

	Untreated	Furosemide
Initial rat weight (g)	88±4	101±9
% Change of BW	−13.2±0.6	−14.1±2.3
Urine volume (ml/d)	6.0±1.2	29.3±8.4*
Urine osmolality (mOsm/kg H_2O)	923±101	388±89*
Urea excretion (mg/d per 100 g BW)	60±6	162±26*
Sodium excretion (mEq/d per 100 g BW)	0.35±0.08	0.18±0.03
Potassium excretion (mEq/d per 100 g BW)	0.75±0.19	1.88±0.43*
Serum creatinine (mg/dl)	0.4±0.02	0.5±0.04
Serum urea nitrogen (mg/dl)	11±1	22±2*
Serum sodium (mEq/liter)	140±1	141±1
Serum potassium (mEq/liter)	5.4±0.2	5.2±0.2
Urea clearance (ml/min per kg BW)	6.2±0.7	4.1±0.5*
Creatinine clearance (ml/min per kg BW)	5.3±0.2	4.5±0.6
Urinary corticosterone (ng/mg creatinine)	0.78±0.15	0.91±0.35

Data: mean±SE; $n = 5$; BW, body weight; *$P < 0.05$.

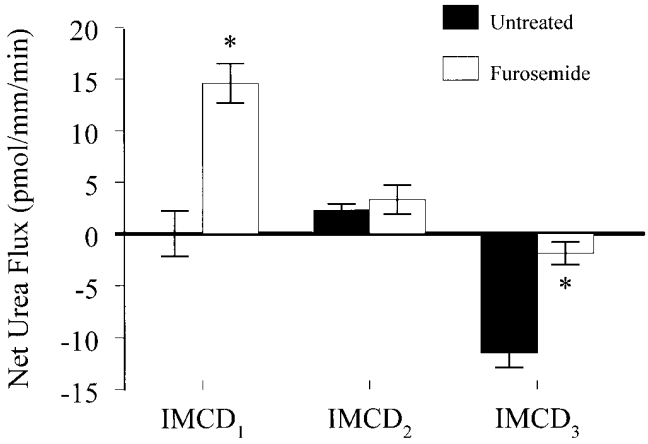

Figure 1. Net urea flux in IMCD subsegments. In untreated rats (*solid bars*), a significant rate of net urea secretion was present only in the $IMCD_3$. In rats treated with furosemide for 3–4 d, net urea secretion was significantly inhibited in $IMCD_3$s and net urea reabsorption was significantly stimulated in $IMCD_1$s. A positive net urea flux indicates reabsorption, while a negative flux indicates secretion. Data are mean±SE. For untreated rats: $n = 5$ in $IMCD_1$; $n = 4$ in $IMCD_2$; and $n = 17$ in $IMCD_3$. For furosemide-treated rats: $n = 14$ in $IMCD_1$; $n = 5$ in $IMCD_2$; and $n = 7$ in $IMCD_3$. $^*P < 0.01$.

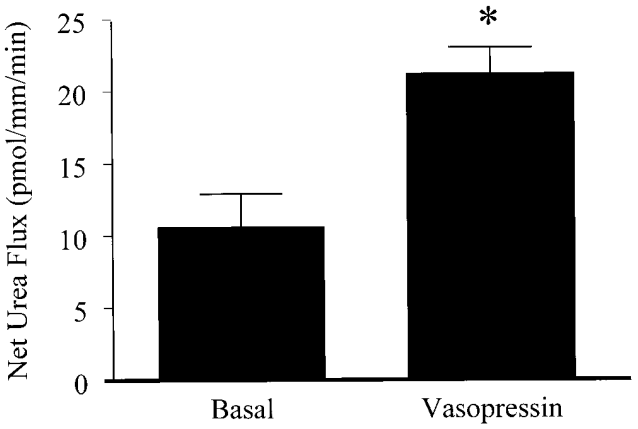

Figure 2. Effect of vasopressin (10 nM added to the bath) on net urea reabsorption in $IMCD_1$s from furosemide-treated rats. Vasopressin significantly increased net urea reabsorption. Data are mean±SE; $n = 5$; $^*P < 0.01$.

Time course for changes in net urea flux in IMCD subsegments. In untreated rats, urine osmolality was 943±83 mOsm/kg H_2O ($n = 9$). Urine osmolality was reduced in rats treated with furosemide for 1 d (288±45 mOsm/kg H_2O; $n = 4$, $P < 0.01$) and for 3–4 d (247±43 mOsm/kg H_2O; $n = 13$, $P < 0.01$), but returned to control values in rats treated with furosemide for 7 d (917±118 mOsm/kg H_2O; $n = 6$, P = NS vs. untreated rats). Clearance studies comparing rats treated with furosemide for 7 d with pair-fed control rats showed no significant differences in serum urea nitrogen, urine volume, urinary urea excretion, urea clearance, or creatinine clearance (Table III).

In $IMCD_3$s from untreated rats, net urea secretion was present (−15.2±1.7 pmol/mm per min, $n = 8$). Net urea secretion was decreased in $IMCD_3$s from rats treated with furosemide for: 1 d (−3.9±1.8 pmol/mm per min; $n = 5$, $P < 0.01$ vs. untreated rats; Fig. 3, *solid line*); 3–4 d (−1.9±1.1 pmol/mm per min; $n = 6$, $P < 0.01$ vs. untreated rats); and 7 d (1.9±1.3 pmol/mm per min, $n = 4$, $P < 0.01$ vs. untreated rats).

In $IMCD_1$s, there was no difference in net urea reabsorption between untreated rats (0.1±0.5 pmol/mm per min, $n = 5$) and rats treated with furosemide for 1 d (2.2±2.3 pmol/mm per min; $n = 4$, P = NS, Fig. 3, *dashed line*). Net urea reabsorption was increased in $IMCD_1$s from rats treated with furosemide for 3–4 d (14.6±1.9 pmol/mm per min; $n = 6$, $P < 0.01$ vs. untreated rats). Net urea reabsorption was increased in $IMCD_1$s 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 $IMCD_1$s 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 II. Perfusate Flow Rate and Collected/Perfusate Urea Ratio

IMCD subsegment	*n*	Tubule length (mm)	Perf. flow rate (nl/min)	Perf.–Coll. urea concentration (mM)	Coll./Perf. urea ratio
(A) Untreated (control) rats					
$IMCD_1$	6	0.50±0.05	22.8±1.2	0.01±0.01	1.00±0.02
$IMCD_2$	8	0.59±0.04	24.8±2.8	0.04±0.01	0.99±0.01
$IMCD_3$	14	0.54±0.04	26.3±2.5	−0.35±0.03	1.12±0.01
(B) Rats treated with furosemide for 3–4 d					
$IMCD_1$	14	0.51±0.03	24.2±1.3	0.37±0.02*	0.88±0.01*
$IMCD_2$	6	0.68±0.06	16.1±0.9	0.08±0.06	0.98±0.02
$IMCD_3$	6	0.61±0.02	19.4±1.5	−0.08±0.04*	1.03±0.01*

Data: mean±SE; Coll., collected; Perf., perfusate; $^*P < 0.01$ vs. untreated rats.

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

	Untreated	Furosemide
Initial rat weight (g)	88±4	101±9
% Change of BW	17.8±2.1	17.2±3.9
Urine volume (ml/d)	11.5±1.5	11.9±2.3
Urine osmolality (mOsm/kg H_2O)	1695±157	1485±176
Urea excretion (mg/d per 100 g BW)	135±17	142±7
Sodium excretion (mEq/d per 100 g BW)	0.69±0.11	0.58±0.20
Potassium excretion (mEq/d per 100 g BW)	1.65±0.28	1.29±0.53
Serum creatinine (mg/dl)	0.4±0.02	0.5±0.04
Serum urea nitrogen (mg/dl)	12±1	12±1
Serum sodium (mEq/liter)	141±1	140±1
Serum potassium (mEq/liter)	5.1±0.2	5.5±0.2
Urea clearance (ml/min per kg BW)	8.3±1.1	8.3±0.5
Creatinine clearance (ml/min per kg BW)	3.9±0.4	5.4±0.8

Data: mean±SE; $n = 5$; BW, body weight.

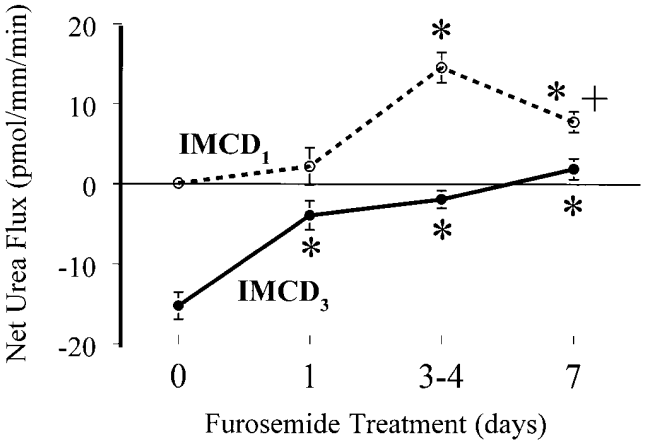

Figure 3. Time course for changes in net urea transport. Compared with $IMCD_3$s (*solid line*, *closed circles*) from untreated rats (0 d, n = 8), net urea secretion was significantly decreased in $IMCD_3$s from rats treated with furosemide for 1 d (n = 5), 3–4 d (n = 6), and 7 d (n = 4). In contrast, there was no difference in net urea reabsorption between $IMCD_1$s (*dashed line*, *open circles*) from untreated rats (0 d, n = 5) and rats treated with furosemide for 1 d (n = 4). Net urea reabsorption was significantly increased in $IMCD_1$s from rats treated with furosemide for 3–4 d (n = 6) and 7 d (n = 5), but was significantly lower in rats treated for 7 d than in rats treated for 3–4 d. Data are mean±SE; $^*P < 0.01$ vs. same IMCD subsegment from untreated rats, $^+P < 0.05$ vs. same IMCD subsegment from rats treated for 3–4 d.

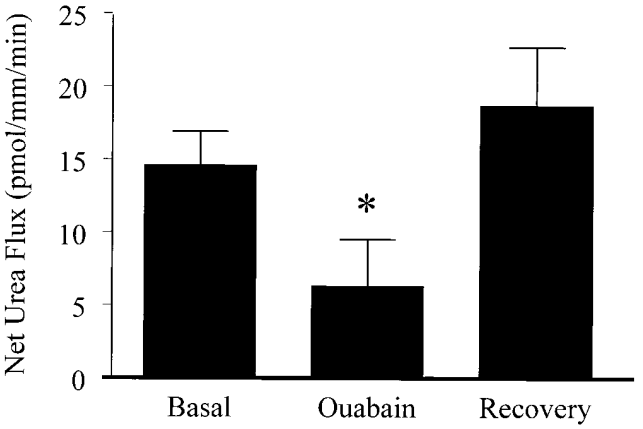

Figure 5. Effect of ouabain (1 mM added to the bath) on net urea reabsorption in $IMCD_1$s from furosemide-treated rats. Ouabain significantly and reversibly inhibited net urea reabsorption. Data are mean±SE; n = 4; $^*P < 0.01$.

Ouabain (1 mM in the bath) decreased net urea reabsorption in $IMCD_1$s from 14.6±2.3 pmol/mm per min to 6.3±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 $IMCD_1$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 $IMCD_1$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 $IMCD_1$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 $IMCD_1$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 $IMCD_1$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

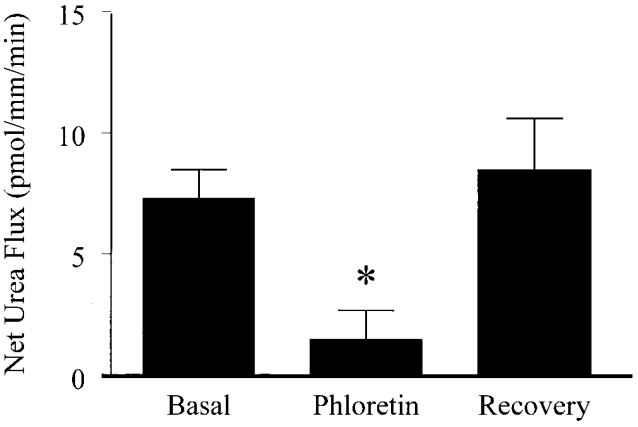

Figure 4. Effect of phloretin (0.25 mM added to the bath) on net urea reabsorption in $IMCD_1$s from furosemide-treated rats. Phloretin significantly and reversibly inhibited net urea reabsorption. Data are mean±SE; n = 5; $^*P < 0.01$.

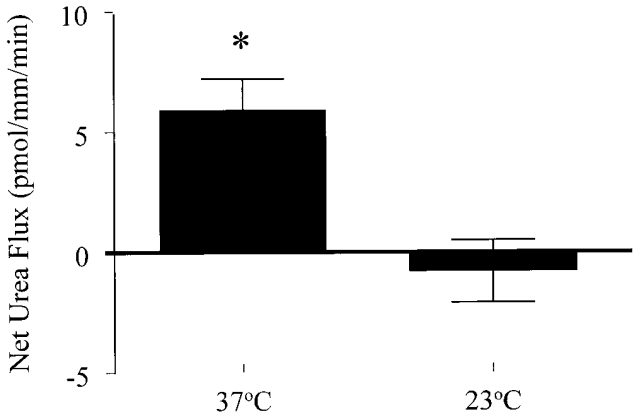

Figure 6. Effect of temperature on net urea reabsorption in $IMCD_1$s from furosemide-treated rats. Cooling the tubule to room temperature significantly inhibited net urea reabsorption. Data are mean±SE; n = 5; $^*P < 0.01$.

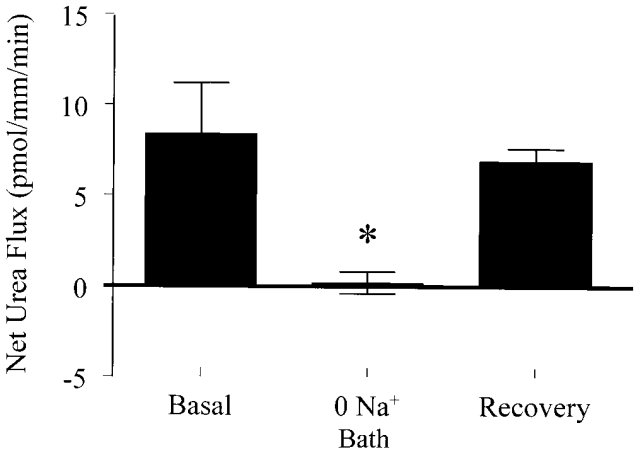

Figure 7. Effect of Na^+ removal from the bath on net urea reabsorption in $IMCD_1$s from furosemide-treated rats. Removing Na^+ from the bath (and replacing it with *N*-methyl-D-glucamine$^+$) significantly and reversibly inhibited net urea reabsorption. Data are mean±SE; $n = 5$; $^*P < 0.01$.

$IMCD_1$s (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 $IMCD_3$; and active urea reabsorption appears in the initial IMCD ($IMCD_1$). Both the induction of active urea reabsorption in the $IMCD_1$ and the decrease in active urea secretion in the $IMCD_3$ 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).

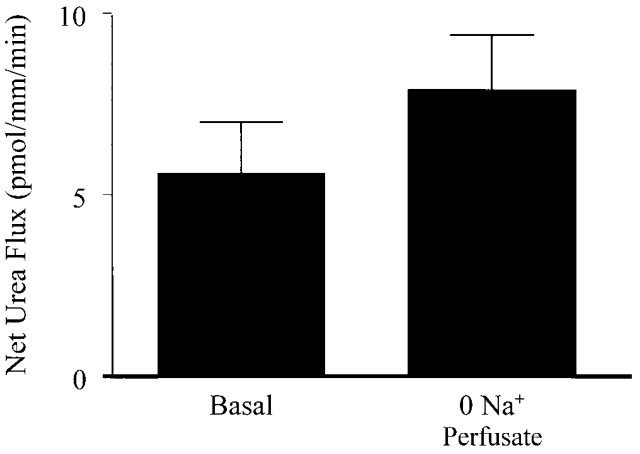

Figure 8. Effect of Na^+ removal from the perfusate on net urea reabsorption in $IMCD_1$s from furosemide-treated rats. Perfusate Na^+ removal did not change net urea reabsorption. Data are mean±SE; $n = 5$.

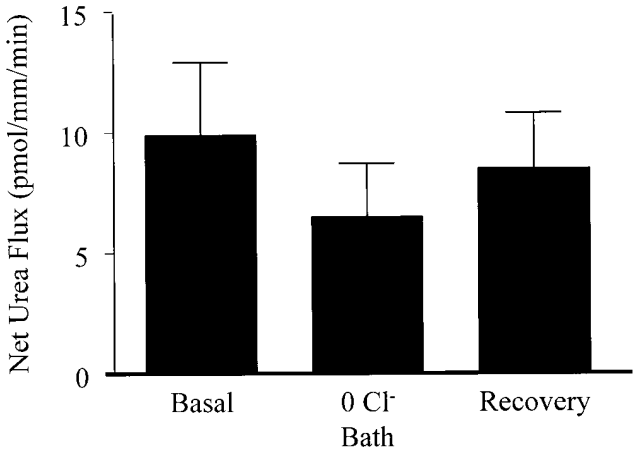

Figure 9. Effect of Cl^- removal from the bath on net urea reabsorption in $IMCD_1$s from furosemide-treated rats. Bath Cl^- removal did not change net urea reabsorption. Data are mean±SE; $n = 5$.

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 $IMCD_1$ and $IMCD_3$ (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-

2. We estimated the contribution of changes in active urea transport processes to urea excretion as follows. $IMCD_1$s are found in the outer third (2 mm) of the rat medulla (20). There are ~ 5,000 $IMCD_1$s in a rat kidney (Table 5 in Ref. 43). Net urea reabsorption is increased by 15 pmol/mm per min in $IMCD_1$s from furosemide-treated rats compared with controls. Using these values, 0.4 mmol of urea would be reabsorbed per day. $IMCD_3$s are found in the deepest third (2 mm) of the rat medulla (20). There are ~ 500 $IMCD_3$s in a rat kidney (Table 5 in Ref. 43). Net urea secretion is decreased by 10 pmol/mm per min in $IMCD_3$s 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.

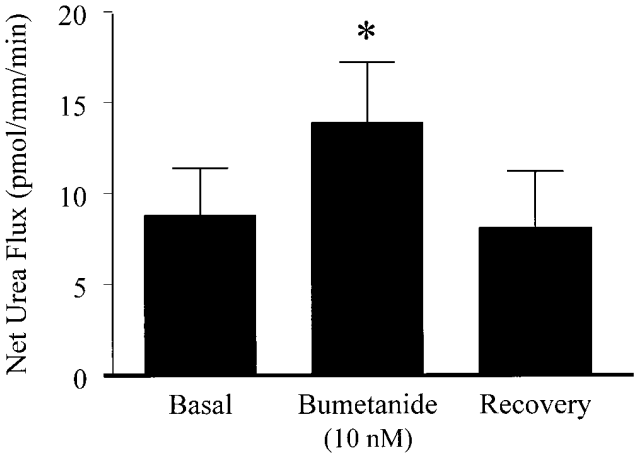

Figure 10. Effect of bumetanide (10 nM added to the bath) on net urea reabsorption in $IMCD_1$s from furosemide-treated rats. Bumetanide significantly and reversibly stimulated net urea reabsorption. Data are mean±SE; $n = 5$; $^*P < 0.05$.

cant decrease in active urea secretion in the $IMCD_3$ of furosemide-treated rats.

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.

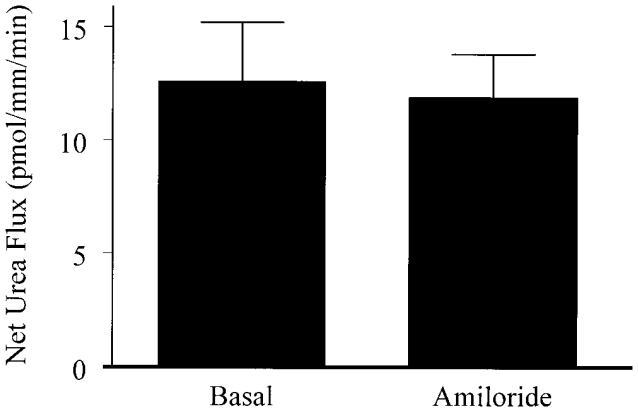

Figure 11. Effect of amiloride (1 mM added to the bath) on net urea reabsorption in $IMCD_1$s from furosemide-treated rats. Amiloride did not change net urea reabsorption. Data are mean±SE; $n = 5$.

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^+-$2Cl^-$ cotransporter, 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 H_2O) 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 $IMCD_3$ (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-

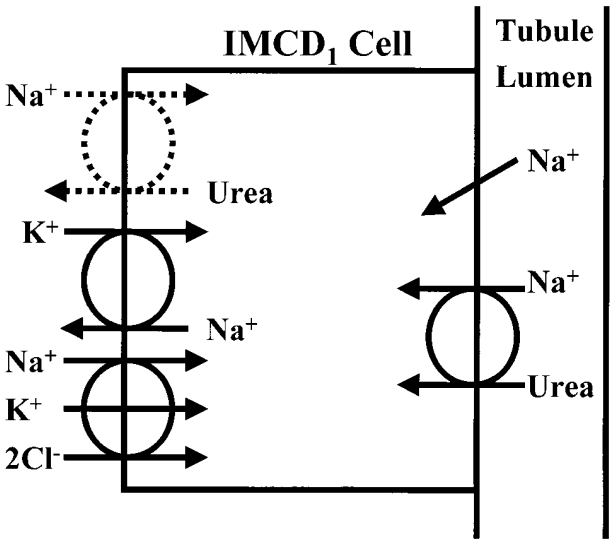

Figure 12. Model of initial IMCD ($IMCD_1$) cell. Two Na^+-dependent active urea transport processes can be induced in the initial IMCD: a Na^+-urea "anti-porter" in the basolateral membrane of furosemide-treated rats (this study) and a Na^+-urea "co-transporter" in the apical membrane of rats fed a low-protein diet (10). Also shown is an amiloride-sensitive Na^+ channel (ENaC) in the apical membrane and an Na^+/K^+-ATPase and a bumetanide-sensitive Na^+-K^+-$2Cl^-$ cotransporter (NKCC1, BSC2) in the basolateral membrane.

Table IV. Comparison of Sodium-dependent Active Urea Transport Mechanisms in the Rat IMCD

	$IMCD_1$ Na^+-Urea "co-transport"	$IMCD_3$ Na^+-Urea "counter-transport"	$IMCD_1$ Na^+-Urea "counter-transport"
Expression in normal rats	Not expressed	Expressed	Not expressed
Induced or upregulated by	Low-protein diet	Water diuresis	Furosemide treatment
Na^+ removal–perfusate	Inhibits	Inhibits	No effect
Na^+ removal–bath	No effect	No effect	Inhibits
Cl^- removal	Not tested	No effect	No effect
Phloretin sensitive	No	Yes	Yes
Ouabain sensitive	Yes	Yes	Yes
Vasopressin sensitive	No	Yes	Yes
Temperature sensitive	Yes	Yes	Yes
Transport inhib. or stim. by	Not tested	Amiloride (inhib.)	Bumetanide (stim.)
Presumed localization*	Apical membrane	Apical membrane	Basolateral membrane
References	(9, 10)	(12)	This study

*Localization is based upon whether transport is inhibited by perfusate (apical) or bath (basolateral) Na^+ removal; inhib., inhibited; stim., stimulated.

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 $IMCD_3$. We showed that urea is actively secreted in the rat $IMCD_3$ 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 $IMCD_3$. 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, $IMCD_3$, and induces active urea reabsorption in the initial IMCD ($IMCD_1$). 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.

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