Altered expression of major renal Na transporters in rats with unilateral ureteral obstruction

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Li, Chunling, Weidong Wang, Tae-Hwan Kwon, Mark A. Knepper, Søren Nielsen, and Jørgen Frøkiær. Altered expression of major renal Na transporters in rats with unilateral ureteral obstruction. Am J Physiol Renal Physiol 284: F155–F166, 2003. First published August 21, 2002; 10.1152/ajprenal.00272.2002.—It has been demonstrated previously that ureteral obstruction was associated with downregulation of renal AQP2 expression and an impaired urinary concentrating capacity (Li C, Wang W, Kwon TH, Isikay L, Wen JG, Marples D, Djurhuus JC, Stockwell A, Knepper MA, Nielsen S, and Frøkiær J. Am J Physiol Renal Physiol 281: F163–F171, 2001). In the present study, changes in the expression of major renal Na transporters were examined in a rat model with 24 h of unilateral ureteral obstruction (UUO) to clarify the molecular mechanisms of the marked natriuresis seen after release of UUO. Urine collection for 2 h after release of UUO revealed a significant reduction in urinary osmolality, solute-free water reabsorption, and a marked natriuresis (0.29 ± 0.03 vs. 0.17 ± 0.03 μmol/min, P < 0.05). Consistent with this, immunoblots revealed significant reductions in the abundance of major renal Na transporters: type 3 Na+/H+ exchanger (NHE3; 24 ± 4% of sham-operated control levels), type 2 Na-Pi cotransporter (NaPi-2; 21 ± 4%), Na-K-ATPase (37 ± 4%), type 1 bumetanide-sensitive Na-K-2Cl cotransporter (BSC-1; 15 ± 3%), and thiazide-sensitive Na-Cl cotransporter (TSC; 15 ± 4%). Immunocytochemistry confirmed the downregulation of NHE3, BSC-1, and TSC in response to obstruction. In nonobstructed contralateral kidneys, a significant reduction in the abundance of inner medullary Na-K-ATPase and cortical NaPi-2 was found. This may contribute to the compensatory increase in urinary production (23 ± 2 vs. 13 ± 1 μl·min⁻¹·kg⁻¹) and increased fractional excretion of urinary Na (0.62 ± 0.03 vs. 0.44 ± 0.03%, P < 0.05). In conclusion, downregulation of major renal Na transporters in rats with UUO may contribute to the impairment in urinary concentrating capacity and natriuresis after release of obstruction, and reduced levels of Na-K-ATPase and NaPi-2 in the contralateral nonobstructed kidney may contribute to the compensatory increase in water and Na excretion from that kidney during UUO and after release of obstruction.

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Recently, we demonstrated that altered expression of major renal sodium transporters is associated with deranged urinary concentration and urinary sodium excretion in several conditions with water and sodium balance disorders (4, 29, 46, 47). The proximal tubule is the main site for renal tubular sodium reabsorption, and the type 3 Na+/H+ exchanger (NHE3) is mainly responsible for apical sodium reabsorption (2), and the type 2 Na-Pi cotransporter (NaPi-2) is also involved (3, 31). Downregulation of NHE3 and NaPi-2 has been demonstrated in several conditions known to have proximal tubule defects in sodium reabsorption and increased urinary sodium excretion (2, 29, 46). In addition, unilateral ureteral obstruction (UUO) is associated with significant changes in renal phosphate excretion (37). Moreover, the Na-K-ATPase is heavily expressed in the basolateral plasma membrane of the renal tubule cells and is responsible for sodium reabsorption (24). In animals with acute UUO for 18–24 h, Na-K-ATPase activity was previously shown to be markedly decreased in all nephron segments (21, 22, 39). Thus reduced expression levels of NHE3, NaPi-2, and Na-K-ATPase in the obstructed kidney could be speculated to be associated with the marked natriuresis in response to obstructive nephropathy.

The key sodium transporters responsible for the active transport of NaCl in the mTAL are the apical bumetanide-sensitive Na-K-2Cl cotransporter (BSC-1 or NKCC2) (11, 23, 26, 33, 49), NHE3, and basolateral Na-K-ATPase. Particularly, the apically expressed Na-K-2Cl cotransporter in the TAL is known to be regulated by vasopressin and involved in the long-term regulation of the countercurrent multiplication system. Previously, measurement of the saturable [3H]bumetanide binding to the Na-K-2Cl cotransporter indicated that the abundance of Na-K-2Cl is reduced in response to UUO (21). In the DCT, the thiazide-sensitive Na-cl cotransporter (TSC or NCC) is chiefly involved in apical sodium reabsorption (27, 34). Thus dysregulation of TSC is also hypothesized to participate in the deranged renal sodium and water excretion in response to UUO.

The purposes of the present study are therefore to characterize the mechanisms responsible for salt wasting in obstructive nephropathy 1) to examine the changes in renal water and electrolyte handling in rats with a 24-h period of UUO and 2) to examine whether these changes are associated with altered expression of major renal sodium transporters in both the obstructed and the nonobstructed kidneys of rats with UUO.

**METHODS**

**Experimental Animals**

Studies were performed in male Munich-Wistar rats initially weighing 250 g (Mellegard Breeding Centre, Eiby, Denmark). The rats were maintained on a standard rodent diet (Altromin, Lage, Germany) with free access to water. During the entire experiment, rats were kept in individual metabolic cages, with a 12:12-h artificial light-dark cycle, a temperature of 21 ± 2°C, and humidity of 55 ± 2%. The rats were allowed to acclimatize to the cages for 5–7 days before surgery. The rats were anesthetized with halothane (Halo- carbon Laboratories), and during surgery they were placed on a heated table to maintain rectal temperature at 37–38°C. Through a midline abdominal incision, the left ureter was exposed and the midportion of ureter was occluded by a 5-0 silk ligature. Twenty-four hours later, the rats were either killed (protocol 1) or released by inserting polyethylene tubing (PE-35) into the proximal ureters to collect urine from the left and right ureter (protocol 2), respectively. After urine collection for at least 2 h, the rats were killed. Age- and time-matched sham-operated controls were prepared and observed in parallel with each UUO group (Fig. 1).

**Protocols**

The rats were allocated to the following protocols.

**Protocol 1.** Rats underwent UUO for 24 h (n = 11). Both kidneys were removed and separately prepared for semiquantitative immunoblotting (n = 7) or immunocytochemistry (n = 4). A sham-operated group was also used (n = 10).

**Protocol 2.** Rats underwent UUO for 24 h followed by release, and animals were observed during the next 2 h (n = 8). Urine was collected for 2 h. A sham-operated group was also used (n = 8).

**Table 1. Changes in renal function in rats subjected to 24-h UUO**

<table>
<thead>
<tr>
<th></th>
<th>UUO (n = 7)</th>
<th>Sham (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_fconv, mmol/kgH2O</td>
<td>308 ± 0.5*</td>
<td>304 ± 1.1</td>
</tr>
<tr>
<td>P_Na, mmol/l</td>
<td>137 ± 2.0</td>
<td>138 ± 0.8</td>
</tr>
<tr>
<td>P_K, mmol/l</td>
<td>4.4 ± 0.1</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>P_HCO3, mmol/l</td>
<td>47 ± 1.9*</td>
<td>30 ± 1.1</td>
</tr>
<tr>
<td>P_Ca, mmol/l</td>
<td>7.4 ± 0.3*</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>P_Pi, mmol/l</td>
<td>2.1 ± 0.04</td>
<td>2.2 ± 0.08</td>
</tr>
<tr>
<td>U_V, μl/min kg⁻¹kg⁻¹</td>
<td>23 ± 1.9†</td>
<td>25 ± 2.4</td>
</tr>
<tr>
<td>P_oconv, mmol/kgH2O</td>
<td>1,910 ± 89</td>
<td>1,916 ± 121</td>
</tr>
<tr>
<td>U_Na, mmol/l</td>
<td>165 ± 17</td>
<td>177 ± 10</td>
</tr>
<tr>
<td>U_K, mmol/l</td>
<td>399 ± 22</td>
<td>422 ± 30</td>
</tr>
<tr>
<td>U_Ca, mmol/l</td>
<td>9.2 ± 0.7</td>
<td>9.0 ± 0.7</td>
</tr>
<tr>
<td>U_Pi, mmol/l</td>
<td>915 ± 78</td>
<td>880 ± 77</td>
</tr>
<tr>
<td>U_Poconv, mmol/l</td>
<td>47 ± 6.3*</td>
<td>25 ± 4.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; UUO, unilateral ureteral obstruction; Sham, Sham-operated; P_fconv, plasma osmolality; P_Na, plasma sodium; P_K, plasma potassium; P_HCO3, plasma bicarbonate; P_Ca, plasma calcium; P_Pi, plasma phosphate; U_V, urinary volume; U_Na, urinary sodium; U_K, urinary potassium; U_Ca, urinary calcium; U_Pi, urinary phosphate; *P < 0.05 compared with sham-operated control rats. †Urine volume represents urine output exclusively from the contralateral nonobstructed kidney during UUO.
Table 2. Changes in renal function in nonobstructed kidneys subjected to 24-h UUO and in right kidneys in sham-operated controls

<table>
<thead>
<tr>
<th></th>
<th>UUO (n = 7)</th>
<th>Sham (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ultrasonogram</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>UO, μl·min⁻¹·kg⁻¹</strong></td>
<td>23 ± 2*</td>
<td>13 ± 1</td>
</tr>
<tr>
<td><strong>UNa, UNa, μmol/min</strong></td>
<td>3.69 ± 0.44*</td>
<td>2.20 ± 0.13</td>
</tr>
<tr>
<td><strong>UK, UNa, μmol/min</strong></td>
<td>8.24 ± 0.60*</td>
<td>5.19 ± 0.29</td>
</tr>
<tr>
<td><strong>UP, UNa, μmol/min</strong></td>
<td>0.97 ± 0.09*</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td><strong>FENa %</strong></td>
<td>0.62 ± 0.03*</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td><strong>FEP %</strong></td>
<td>47 ± 2.1*</td>
<td>32 ± 9.0</td>
</tr>
<tr>
<td><strong>FEK %</strong></td>
<td>10.8 ± 0.8*</td>
<td>3.4 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; UNa, UNa, urinary sodium excretion; UK, UNa, urinary potassium excretion; UP, UNa, urinary phosphate excretion; FENa, fractional excretion of sodium; FEP, fractional excretion of potassium; FEK, fractional excretion of phosphate. For the first 4 parameters, urinary production during UUO is derived exclusively from the nonobstructed kidney and compared with urine production from 1 kidney (half of the total urine production) in sham-operated rats. *P < 0.05 compared with sham-operated control kidney.

Clearance Studies

Urinary was collected during 24-h periods throughout the study or for 2 h after the release of obstruction. Clearance studies were performed over the last 24 h in protocol 1 or for 2 h after release in protocol 2. At the end of each protocol, under anesthesia, 2–3 ml of blood were collected into a heparinized tube for determination of plasma electrolytes and osmolality before the rats were killed. The plasma concentrations of sodium, potassium, creatinine, urea, and phosphate and the urinary concentration of creatinine and urea were determined (Vitros 950, Johnson & Johnson). The concentrations of urinary sodium and potassium were determined by standard flame photometry (Eppendorf FCMM6341). Urinary phosphate was determined (Vitros 250, Johnson & Johnson). The osmolality of urine and plasma was determined by freezing-point depression (Advanced Osmometer, model 3900, Advanced Instruments, Norwood, MA, and Osmonat 030-D, Gonotec, Berlin, Germany).

Membrane Fractionation for Immunoblotting

For removal of kidneys, rats were anesthetized with halothane. The kidney was split into cortex plus outer medulla and inner medulla and frozen in liquid nitrogen. Tissue (inner medulla or cortex plus outer medulla) was finely minced and then homogenized in 1 ml (inner medulla) or 8 ml (cortex plus outer medulla) of dissecting buffer (0.3 M sucrose, 25 mM imidazole, and 1 mM EDTA, pH 7.2, containing the protease inhibitors 8.5 μM leupeptin and 1 mM phenylmethylsulfonyl fluoride) with 5 strokes of a motor-driven IKA homogenizer at 1,250 rpm. The homogenate was centrifuged in a Universal 30 RF centrifuge at 4,000 g for 15 min at 4°C. Gel samples (in Laemml sample buffer containing 2% SDS) were made from this membrane preparation.

Electrophoresis and Immunoblotting

Samples of membrane fractions from inner medulla or cortex plus outer medulla were run on 12 or 6–16% gradient polyacrylamide minigels (Bio-Rad Mini Protein II). For each gel, an identical gel was run in parallel and subjected to Coomassie staining (43). The Coomassie-stained gel was used to ascertain identical loading or to allow for potential correction for minor differences in loading after scanning and densitometry of major bands (see below). The other gel was subjected to blotting. After transfer by electroelution to nitrocellulose membranes, blots were blocked with 5% milk in 80 mM NaHPO₄, 20 mM NaH₂PO₄, 100 mM NaCl, 0.1% Tween 20, pH 7.5, for 1 h and incubated with primary antibodies (see below) (10) overnight at 4°C. After being washed as above, the blots were incubated with horseradish peroxidase-conjugated secondary antibody (P447 or P448, diluted 1:3,000, DAKO, Glostrup, Denmark), and bands were visualized using the enhanced chemiluminescence system (ECL; Amersham International). Controls were made with an exchange of the primary antibody for an antibody preabsorbed with immunizing peptide (100 ng/40 ng IgG) or with preimmune serum (diluted 1:1,000). All controls were not labeled.

Primary Antibodies

For semiquantitative immunoblotting and immunocytochemistry, we used previously characterized monoclonal and polyclonal antibodies as summarized below:

- NHE3 (LL546AP). An affinity-purified polyclonal antibody to NHE3 was previously characterized (13, 25).
- NaPi-2 (LL697AP/LL696AP). An affinity-purified polyclonal antibody to type NaPi-2 has previously been characterized (3).
- Na-K-ATPase. A monoclonal antibody against the α₁ subunit of Na-K-ATPase has previously been characterized (24).
- BSC-1 (LL320AP). An affinity-purified polyclonal antibody to the apical Na-K-Cl cotransporter of the thick ascending limb has previously been characterized (11, 26).
- TSC (LL573AP). An affinity-purified polyclonal antibody to the apical Na-CI cotransporter of the distal convoluted tubule has previously been characterized (27).

Immunocytochemistry

The kidneys from UUO rats and sham-operated rats were fixed by retrograde perfusion via the abdominal aorta with 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4. For immunoperoxidase microscopy, kidney blocks containing all kidney zones were dehydrated and embedded in paraffin. The paraffin-embedded tissues were cut (2 μm) on a rotary micr-

Table 3. Changes in renal function in rats subjected to 24-h UUO followed by release for 2 h or sham operation

<table>
<thead>
<tr>
<th></th>
<th><strong>Un</strong></th>
<th><strong>CLNa</strong></th>
<th><strong>FLNa</strong></th>
<th><strong>FLK</strong></th>
<th><strong>UNa</strong></th>
<th><strong>UK</strong></th>
<th><strong>UP</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µl·min⁻¹·kg⁻¹</td>
<td>ml·min⁻¹</td>
<td>µmol·min⁻¹·kg⁻¹</td>
<td>µmol·min⁻¹·kg⁻¹</td>
<td>µmol·min⁻¹·kg⁻¹</td>
<td>µmol·min⁻¹·kg⁻¹</td>
<td>µmol·min⁻¹·kg⁻¹</td>
</tr>
<tr>
<td>Obs</td>
<td>2.6 ± 0.71</td>
<td>0.31 ± 0.16*</td>
<td>42 ± 22*</td>
<td>19 ± 1.0*</td>
<td>0.29 ± 0.03*</td>
<td>0.20 ± 0.06*</td>
<td>6.4 ± 5.5*</td>
</tr>
<tr>
<td>Non-Obs</td>
<td>7.75 ± 0.91†</td>
<td>2.42 ± 0.33†</td>
<td>314 ± 57†</td>
<td>14.6 ± 2.9†</td>
<td>0.36 ± 0.08*</td>
<td>1.27 ± 0.20†</td>
<td>1.1 ± 0.2†</td>
</tr>
<tr>
<td>Sham</td>
<td>3.16 ± 0.26</td>
<td>1.94 ± 0.24</td>
<td>264 ± 32</td>
<td>10.2 ± 1.4</td>
<td>0.17 ± 0.03</td>
<td>0.62 ± 0.08</td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Values for sham-operated control animals are mean of left and right kidney. n, No. of rats; CLNa, creatinine clearance; FLNa, filtered load of sodium; FLK, filtered load of potassium; Obs, obstructed; Non-Obs, nonobstructed. *P < 0.05 compared with sham-operated control kidneys. †P < 0.05 compared with obstructed kidneys.
rotome (Leica). The sections were deparaffinized and rehydrated. For immunoperoxidase labeling, endogenous peroxidase were blocked by 0.5% H₂O₂ in absolute methanol for 10 min at room temperature. To reveal antigens, sections were put in 1 mmol/l Tris solution (pH 9.0) supplemented with 0.5 mM EGTA and heated using a microwave oven for 10 min. Nonspecific binding of Ig was prevented by incubating the sections in 50 mM NH₄Cl for 30 min, followed by blocking in

Fig. 2. Semiquantitative immunoblotting of membrane fractions of outer medulla and cortex from UUO and sham-operated rats. A and C: immunoblots were reacted with affinity-purified antitype 3 Na/H exchanger (NHE3) antibody and revealed a single ~87-kDa band. OBS, obstructed. B: densitometric analysis (corrected according to densitometry of Coomassie-stained gels and loading fraction of total kidney mass; see METHODS) of all samples from obstructed kidneys (OBS) of rats with 24-h UUO and sham-operated controls revealed a significant decrease in NHE3 levels from 100 ± 11% in sham-operated controls to 24 ± 4% in obstructed kidneys. D: in nonobstructed kidneys (non-OBS), densitometric analysis revealed no difference from sham controls (81 ± 14 vs. 100 ± 10%).

Fig. 3. Immunocytochemical analyses of NHE3 in proximal tubules and thick ascending limb (TAL) of the loop of Henle from sham-operated and UUO rats (obstructed and nonobstructed kidney). A and B: in the obstructed kidneys of UUO rats, NHE3 labeling (arrows) is seen at apical domains of proximal tubule cells (A) and at apical membrane domains in TAL cells (B), but labeling is much weaker compared with that seen in kidneys from sham-operated rats. C and D: in the nonobstructed kidneys of UUO rats, NHE3 labeling is comparable to that seen in sham-operated controls. E: in sham-operated rats, NHE3 labeling is seen at apical domains of proximal tubule cells but is absent in brush-border and basolateral membrane domains. F: in sham-operated rats, NHE3 labeling is seen at apical membrane domains of the thick ascending limb in the inner stripe of the outer medulla (ISOM). P, proximal tubule; T, thick ascending limb; CD, collecting duct. Magnification: ×650.
PBS supplemented with 1% BSA, 0.05% saponin, and 0.2% gelatin. Sections were incubated overnight at 4°C with primary antibodies diluted in PBS supplemented with 0.1% BSA and 0.3% Triton X-100. After being rinsed with PBS supplemented with 0.1% BSA, 0.05% saponin, and 0.2% gelatin for 3 × 10 min, the sections were incubated in horseradish peroxidase-conjugated secondary antibodies (P448, DAKO) diluted 1:200 in PBS supplemented with 0.1% BSA and 0.3% Triton X-100. After being rinsed with PBS, sections were incubated in horse-radish peroxidase-conjugated secondary antibodies (P448, DAKO) diluted 1:200 in PBS supplemented with 0.1% BSA, 0.05% saponin, and 0.2% gelatin. The microscopy was carried out using a Leica DMRE light microscope.

Statistics

Values are presented as means ± SE. Comparisons between groups were made by unpaired t-test. P values < 0.05 were considered significant.

RESULTS

UUO Impairs Urinary Concentrating Capacity

Plasma from UUO rats and urine collected from nonobstructed kidneys in the same animals were sampled at the end of the 24-h period of UUO, and osmolality and concentration of potassium, creatinine, urea, and phosphate were determined (Table 1). Twenty-four hours of UUO resulted in a highly significant increase in the concentration of plasma creatinine (47 ± 1.9 vs. 30 ± 1.1 μmol, P < 0.05) and urea (7.4 ± 0.3 vs. 4.8 ± 0.3 mmol, P < 0.05) and a parallel increase in plasma osmolality (308 ± 0.5 vs. 304 ± 1.1 mosmol/kg H2O, P < 0.05; Table 1). Urinary volume from nonobstructed kidneys was increased significantly during UUO compared with that in sham-operated control rats (23 ± 2 vs. 13 ± 1 μl·min⁻¹·kg⁻¹; Table 2). Furthermore, we examined plasma and urine samples from both kidneys during a 2-h sampling period after release of 24-h UUO for changes in clearance and urinary excretion of electrolytes from both the obstructed and nonobstructed kidneys. Compared with sham-operated rats, urine from obstructed kidneys had significantly lower creatinine clearance [0.31 ± 0.16 vs. 1.94 ± 0.24 ml·min⁻¹·kg⁻¹; Table 3]. Thus, glomerular filtration rate (GFR) and urinary concentrating capacity were significantly decreased in the obstructed kidneys. In contrast, in the nonobstructed kidneys there was a significant increase in urinary

Fig. 4. Immunoblot of membrane fractions of outer medulla and cortex from UUO and sham-operated rats. A and C: immunoblots were reacted with affinity-purified anti-type 2 NaPi-transporter (NaPi-2) antibody and revealed a single ~85-kDa band. B: densitometric analysis of all samples from obstructed kidneys of rats with 24-h UUO and sham-operated controls revealed a marked decrease in NaPi-2 levels in obstructed kidneys to 21 ± 4% compared with sham-operated rats (100 ± 12%, *P < 0.05). D: densitometric analysis of all samples from nonobstructed kidneys of rats with 24-h UUO and sham-operated controls also revealed a marked decrease in NaPi-2 levels in nonobstructed kidneys to 22 ± 4% compared with sham-operated controls (100 ± 27%, *P < 0.05).

Fig. 5. Semiquantitative immunoblotting of membrane fractions of outer medulla and cortex from UUO and sham-operated rats. A and C: immunoblots were reacted with α-isofrom-specific Na-K-ATPase. B: densitometric analysis of all samples from the obstructed kidneys of rats with 24-h UUO and sham-operated controls revealed a marked decrease in expression from 100 ± 6% in sham-operated controls to 37 ± 4% in obstructed kidneys (*P < 0.05). D: densitometric analysis revealed that Na-K-ATPase expression (~90 ± 12%) in nonobstructed kidneys did not differ from levels in sham-operated controls (100 ± 15%).
of UUO, with urine sampling from both kidneys. Consistent with lower levels of creatinine clearance in obstructed kidneys, the filtered load of sodium and potassium was decreased markedly compared with in sham-operated control kidneys and contralateral nonobstructed kidneys. There was a significant increase in urinary sodium excretion (0.29 ± 0.03 vs. 0.17 ± 0.03 µmol/min) and fractional sodium excretion (6.4 ± 5.5 vs. 0.7 ± 0.1%, \( P < 0.05 \)) from obstructed kidneys. Urinary excretion of potassium was markedly decreased (0.20 ± 0.06 vs. 0.62 ± 0.08 µmol/min), whereas fractional potassium excretion was increased markedly (158.8 ± 35.7 vs. 70.1 ± 10%, Table 3). Increased urinary sodium and potassium excretion from nonobstructed kidneys was observed during 2-h sampling from both kidneys after release of UUO (Table 3). These findings show the compensatory increase in the excretion of sodium, potassium, and phosphate from nonobstructed kidneys during obstruction.

**Decreased Abundance of NHE3 and NaPi-2 in Obstructed Kidneys of Rats With UUO**

The expression levels of major renal sodium transporters were examined in both obstructed and nonobstructed kidneys in response to 24-h UUO and compared with those in age- and time-matched sham-operated control rats. NHE3 is expressed in renal proximal tubules and TAL and is involved in sodium and bicarbonate reabsorption. Immunoblotting revealed that the abundance of NHE3 was significantly decreased in the obstructed kidneys of UUO rats (24 ± 4 vs. 100 ± 11%, \( P < 0.05 \); Fig. 2, A and B), whereas no significant changes were observed in the nonobstructed kidneys (Fig. 2, C and D).

Immunocytochemical analysis confirmed these results. In sham-operated rats, anti-NHE3 antibody labeled the apical plasma membrane domains of proximal convoluted tubules (Fig. 3E), as previously described (1). Furthermore, intense labeling of the apical plasma membrane domains of mTAL cells in sham-operated rats was also seen (Fig. 3F). In contrast,
immunocytochemistry showed that the labeling of NHE3 in the proximal tubule cells as well as of TAL cells from the obstructed kidneys was reduced (Fig. 3, A and B). The labeling density did not change in the nonobstructed kidneys (Fig. 3, C and D), consistent with the immunoblotting data. These findings indicate that reduced abundance of NHE3 in the obstructed kidney may contribute to increased sodium excretion observed in response to release of UUO.

In the apical part of the renal proximal tubules, NaPi-2 contributes to sodium and phosphate reabsorption. As shown in Fig. 4, immunoblotting revealed a marked decrease in NaPi-2 levels in both obstructed and nonobstructed kidneys (21 ± 4 vs. 22 ± 4% of control levels, P < 0.05). Thus the reduced abundance of NaPi-2 may also contribute to the increased urinary sodium excretion and is also likely to play a major role in increased urinary phosphate excretion.

**Abundance of Na-K-ATPase Is Reduced in Obstructed Kidneys of Rats With UUO**

Immunoblotting of the α-subunit of the Na-K-ATPase shows that the abundance is markedly decreased in the outer medulla and cortex of obstructed kidneys (37 ± 4 vs. 100 ± 6%, P < 0.05; Fig. 5, Table 4), whereas the abundance is unchanged in the inner medulla (Fig. 6, Table 4). In contrast, in nonobstructed kidneys the abundance in the inner medulla was significantly reduced (46 ± 10 vs. 100 ± 12%, P < 0.05; Fig. 6, Table 5), whereas there was no change in the outer medulla and cortex (90 ± 12 vs. 100 ± 15%, not significant; Fig. 5, Table 5).

**Abundance of BSC-1 and TSC Is Reduced in Obstructed Kidneys of Rats With UUO**

The abundance of BSC-1 in the outer medulla and cortex was dramatically decreased (15 ± 3 vs. 100 ± 13%, P < 0.05, Fig. 7, A and B). In contrast, BSC-1 abundance was unchanged in nonobstructed kidneys (Figs. 7, C and D) compared with sham-operated rats. Immunocytochemistry showed that the labeling of BSC-1 in the mTAL of obstructed kidneys was markedly decreased (Fig. 8A). In nonobstructed kidneys, labeling of BSC-1 in the mTAL was unchanged compared with that in sham-operated rats (Fig. 8, B and C).

TSC abundance was markedly reduced in obstructed (15 ± 4 vs. 100 ± 8%, P < 0.05; Fig. 9, A and B) but not in nonobstructed kidneys (Fig. 9, C and D). Consistent with this, immunocytochemistry revealed that labeling of TSC seen at the apical membrane domains of DCT cells was markedly reduced in obstructed kidneys (Fig. 10A), whereas no change in labeling was found in nonobstructed kidneys (Fig. 10B). These results suggest that BSC-1 and TSC may also play significant roles in the changes in sodium and water handling in kidneys with urinary tract obstruction.

**DISCUSSION**

This study demonstrated that UUO is associated with an impaired urinary concentrating capacity and salt wasting in the obstructed kidney. In parallel, the expression of several major renal sodium transporters, i.e., NHE3, NaPi-2, Na-K-ATPase, BSC-1, and TSC, were significantly downregulated in the obstructed kidney, suggesting that reductions in these major renal sodium transporters contribute to the impaired urinary concentrating capacity and salt wasting after UUO.
release of UUO. In the contralateral nonobstructed kidney, immunoblotting revealed a reduced expression of Na-K-ATPase in the inner medulla and a reduced expression of NaPi-2 in the proximal tubule. This was associated with an increased excretion of sodium and phosphate during and after release of UUO. These findings demonstrate segmental changes at the molecular level in sodium and phosphate transporters in both the obstructed and nonobstructed kidneys during UUO.

UUO Is Associated With Salt Wasting

Urinary tract obstruction results in profound changes in renal hemodynamics. The present study showed that UUO resulted in a dramatic reduction in GFR in the obstructed kidney when creatinine clearance was measured 2 h after release, consistent with previous studies demonstrating a marked GFR reduction due to severe vasoconstriction (17, 20). This reduction in GFR in the postobstructed kidney reduces the filtered load of sodium, which is consistent with the finding of the present study that the filtered load of sodium was severely reduced in the obstructed kidney. During UUO, these hemodynamic changes are associated with disruption of tubule function, which ultimately results in salt wasting and an impaired urinary concentrating capacity of the obstructed kidney (6). Despite the reduced load of sodium entering the tubules, UUO was associated with a significant increase in urinary sodium excretion. Thus sodium reabsorption was severely impaired during the passage of the ultrafiltrate through the tubules and collecting ducts. Previous studies have indicated that the major defects in renal tubular sodium reabsorption are located in the distal segments of the nephron. Micropuncture studies have shown a diminished net reabsorption of both sodium and water between the loop of Henle and the beginning of the papillary CD (41, 48, 50), demonstrating that these segments of the nephron are critically affected by ureteral obstruction. The results of the present study together with previous findings (Li, Wang, Knepper, Nielsen, and Frøkiær, unpublished observations) support the view that the distal segments of the nephron and collecting ducts play a significant role in the impairment in urinary concentrating capacity of the obstructed kidney.

In the contralateral nonobstructed kidney, GFR increased during 24 h of UUO. This resulted in an increased filtered load of sodium and a dramatic increase in urinary sodium excretion from the nonobstructed kidney. Importantly, analysis of urine collected during UUO from the contralateral nonobstructed kidney demonstrated an increased excretion of both sodium and phosphate. Thus UUO is associated with sodium and phosphate loss caused by dysregulation of sodium transporters in both the obstructed and contralateral nonobstructed kidney.
Reduced Abundance of Sodium Transporters in the Proximal Tubule in UUO

The present study demonstrated that the abundance of NHE3 and NaPi-2 in the obstructed kidney were severely decreased 24 h after the onset of obstruction. Downregulation of NHE3 in the proximal tubule was confirmed by immunocytochemistry. The observation that the aforementioned two major proximal sodium transporters are reduced in the obstructed kidney supports the view that the epithelial transport ability of the proximal tubule is impaired in the obstructed kidney in response to UUO. Furthermore, the abundance of Na-K-ATPase in the cortex and outer medulla of the obstructed kidney was reduced after 24-h UUO. This finding is consistent with previous studies demonstrating markedly reduced Na-K-ATPase activity in basolateral membrane vesicles prepared from the cortex of the obstructed kidneys of rats with 24-h UUO (5).

It is evident that functional adaptation of the proximal tubule may play a critical role in the changes in reabsorption and secretion of sodium. In vitro experiments have previously demonstrated that volume reabsorption in proximal straight tubules was diminished in obstruction (19). Regulated apical membrane Na/H exchange is a major pathway for proximal tubule sodium reabsorption. NHE3 has been shown to be responsible for this (1). The reduced abundance of NaPi-2 in the obstructed kidney may contribute to the impairment of sodium reabsorption at the proximal tubule. Furthermore, downregulation of NaPi-2 may affect renal proximal tubular handling of phosphate in the obstructed kidney. Consistent with the increased urinary excretion and fractional excretion of sodium, UUO was associated with a marked reduction in proximal tubular sodium transporter abundance (i.e., NHE3, NaPi-2, and Na-K-ATPase). Thus it is likely that the reduction in expression of these proximal tubule sodium transporters plays a significant role in the increased urinary sodium excretion after UUO. During UUO, renal function of the obstructed kidney is gradually impaired due to severe vasoconstriction, which leads to reduced blood supply; the obstructed kidney then becomes gradually ischemic in response to obstruction (28). It may be of interest to note that the reduction in proximal tubular sodium transporters in the obstructed kidney (NHE3, NaPi-2, and Na-K-ATPase) is similar to that demonstrated in response to acute ischemia-induced renal failure (29). The present findings demonstrate that 1) the proximal tubule is susceptible to injury in response to ureteral obstruction, consistent with previous observations (19); and 2) reabsorption of sodium and phosphate at the proximal tubular level may be severely compromised due to the significant reduction in the expression of proximal tubular sodium transporters.

Reduced Abundance of Sodium Transporters in the TAL and DCT During UUO

The mTAL of Henle is known to be susceptible to ischemia-induced injury (28). Thus the progressive ischemia induced by ureteral obstruction may change the function of the mTAL. BSC-1, which is localized at the apical plasma membrane domains of mTAL and cortical TAL (cTAL) segments (11, 26), and the basolaterally located Na-K-ATPase (24) are the key sodium transporters that mediate the NaCl transport in these water-impermeable segments. The countercurrent multiplication process is dependent on the active reabsorption of NaCl in mTAL (7, 38). Thus these pathways are critical for the generation of the hypertonic medullary interstitium. In particular, the apically expressed Na-K-2Cl cotransporter in TAL is known to be regulated by vasopressin (26), and this regulation may be involved in the long-term regulation of the countercurrent multiplication system (7). Importantly, the present study demonstrated both by immunoblotting and by immunocytochemistry that the abundance of BSC-1 and Na-K-ATPase was significantly decreased in obstructed kidneys of rats with UUO. This finding is consistent with previous studies indirectly demonstrating reduced activities of both the Na-K-2Cl cotransporter and Na-K-ATPase by reductions in the
inhibitory effects of both furosemide and ouabain in suspensions of mTAL cells from obstructed kidneys (21). This finding strongly indicates that the reduced expression of BSC-1 plays a key role in the reduced reabsorption of sodium and chloride in the TAL in response to UUO. Moreover, the reduced expression is likely to contribute to the decreased urinary concentration and increased urinary sodium excretion from the obstructed kidney.

Several studies have demonstrated that vasopressin increases the rate of net sodium reabsorption in microperfused TAL segments from mice and rats (18, 40) and that the abundance of Na-K-2Cl cotransporter in the TAL is increased in response to dDAVP (26). Vasopressin administration also stimulates cAMP production. However, in the postobstructed kidney vasopressin administration does not stimulate cAMP (28), which supports the view that the impaired sodium reabsorption in the TAL is caused in part by a reduced vasopressin sensitivity in the obstructed kidney. In contrast, PGE2 decreases the rate of vasopressin-stimulated NaCl reabsorption in TAL (8, 14, 42). Previously, it was demonstrated that the accumulation of volume and osmotic substances during obstruction may stimulate renal secretion of PGE2 (35), which may have an inhibitory effect on the TAL cAMP levels, and hence this may influence the abundance of Na-K-2Cl cotransporter. Therefore, it is possible that both the impaired sensitivity to vasopressin and potential PGE2-induced cAMP inhibition may contribute to the reduction in BSC-1 abundance in the obstructed kidney after UUO.

In the DCT, TSC is expressed and responsible for a large proportion of the apical sodium reabsorption that takes place in this segment of the nephron (27, 34). In the present study, we demonstrated that the abundance of TSC was severely reduced in the obstructed kidney. This was confirmed by immunocytochemistry, demonstrating much weaker labeling at the apical membrane of the DCT. The abundance of TSC protein appears to be highly regulated. TSC abundance in the rat kidney is increased in response to aldosterone infusion or use of a low-salt diet (27), by nitric oxide synthase inhibition (44), by estrogen infusion (45), and during vasopressin escape (9). The renin-angiotensin-aldosterone system plays a pivotal role in the pathophysiology of obstructive nephropathy. Although we did not measure aldosterone levels in the present study, 24-h UUO has been demonstrated to increase plasma aldosterone levels (12). Consequently, the downregulation of TSC in response to obstruction may be speculated to be caused by an insensitivity to the mineralocorticoid receptor or due to disruption of intracellular pathways regulating TSC expression. The discovery that both BSC1 and TSC are reduced in the obstructed kidney demonstrates that 1) the TAL and DCT are highly susceptible to injury induced by obstruction; and 2) sodium reabsorption may be severely compromised, which contributes to the impairment of the urinary concentrating capacity and to increased urinary sodium loss.

**Fig. 10.** Immunocytochemical analyses of TSC in DCT of sham-operated and UUO rats (obstructed and nonobstructed kidney). A: in the obstructed kidneys of UUO rats, labeling of TSC (arrows) in DCT cells is much weaker compared with sham-operated kidneys (C). B: in the nonobstructed kidneys of UUO rats, labeling of TSC in DCT cells is similar to the labeling seen in sham-operated controls. C: TSC labeling is seen at apical plasma membrane domains of DCT cells in sham-operated rats. Magnification: \( \times 1,100 \).
The demonstration of reduced abundance of sodium transporters in both proximal and distal nephron segments suggests that the mechanism responsible for the downregulation is complex and cannot be ascribed to a single mediator. The increased pressure associated with 24 h of UUO may be of crucial importance, because it is well known that numerous hormonal and inflammatory pathways are activated in response to UUO (28).

**Abundance of Sodium Transporters in the Contralateral Nonobstructed Kidney**

It is well known that during UUO the contralateral kidney undergoes adaptational changes due to the loss of function of the obstructed kidney. In the present study, there was a dramatic reduction in the abundance of NaPi-2 in the cortex and outer medulla and a moderate reduction in the abundance of Na-K-ATPase in the inner medulla of nonobstructed kidneys. Consistent with these findings, the excretion of both phosphate and sodium was markedly increased from nonobstructed kidney during UUO. This is a new finding, demonstrating changes at the molecular level in both the proximal tubule and the collecting ducts of the contralateral nonobstructed kidney, indicating that regulation of sodium transporters in these segments plays an important role in the compensatory response to UUO. It is well known that the intact kidney compensates for the reduction in renal function in response to uninephrectomy and UUO (28). However, the present results are the first to demonstrate compensation of renal sodium transport at the molecular level in response to UUO. Additional studies in response to uninephrectomy are required to identify whether these changes are of general significance as a compensatory response to unilateral kidney disease.

**Summary**

UUO in rats is associated with significant reductions in the abundance of major renal sodium transporters along the nephron. Importantly, the abundance of both apically and basolaterally located sodium transporters was reduced, consistent with a significant impairment of tubular reabsorption of filtered sodium. We conclude that 1) downregulation of major renal sodium transporters in rats with UUO may contribute to the impairment in urinary concentrating capacity and natriuresis if the release of obstruction and 2) reduced levels of NaPi-2 and Na-K-ATPase in the contralateral nonobstructed kidney may contribute to the compensatory increase in water and sodium excretion from this kidney during UUO.

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