Altered expression of major renal Na transporters in rats with bilateral ureteral obstruction and release of obstruction

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Submitted 1 June 2003; accepted in final form 30 June 2003

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 bilateral ureteral obstruction and release of obstruction. Am J Physiol Renal Physiol 285: F889–F901, 2003. First published July 15, 2003; 10.1152/ajprenal.00170.2003.—Urinary tract obstruction impairs urinary concentrating capacity and reabsorption of sodium. To clarify the molecular mechanisms of these defects, expression levels of renal sodium transporters were examined in rats with 24-h bilateral ureteral obstruction (BUO) or at day 3 or 14 after release of BUO (BUO-R). BUO resulted in downregulation of type 3 Na+/H+ exchanger (NHE3) to 41 ± 14%, type 2 Na-Pi cotransporter (NaPi-2) to 26 ± 6%, Na-K-ATPase to 67 ± 8%, type 1 bumetanide-sensitive Na-K-2Cl cotransporter (BSC-1) to 20 ± 7%, and thiazide-sensitive cotransporter (TSC) to 37 ± 9%. Immunocytochemistry confirmed downregulation of NHE3, NaPi-2, Na-K-ATPase, BSC-1, and TSC. Consistent with this downregulation, BUO-R was associated with polyuria, reduced urinary osmolality, and increased urinary sodium and phosphate excretion. BUO-R for 3 days caused a persistent downregulation of NHE3 to 53 ± 10%, NaPi-2 to 57 ± 9%, Na-K-ATPase to 62 ± 8%, BSC-1 to 50 ± 12%, and TSC to 56 ± 16%, which was associated with a marked reduction in the net renal reabsorption of sodium (616 ± 54 vs. 944 ± 24 μmol·min⁻¹·kg⁻¹; P < 0.05) and phosphate (6.3 ± 0.9 vs. 13.1 ± 0.4 μmol·min⁻¹·kg⁻¹; P < 0.05) demonstrating a defect in renal sodium and phosphate reabsorption capacity. Moreover, downregulation of Na-K-ATPase and TSC persisted in BUO-R for 14 days, whereas NHE3, NaPi-2, and BSC-1 were normalized to control levels. In conclusion, downregulation of renal Na transporters in rats with BUO and release of BUO are likely to contribute to the postobstructive polyuria.

proximal tubule; thick ascending limb of Henle’s loop; distal convoluted tubule; collecting duct; Na-Cl cotransporters; sodium excretion

OBSTRUCTION OF THE URINARY tract is characterized by a significantly reduced ability of the kidney to regulate urinary excretion of water and sodium (7, 17, 18, 32, 36, 43). Obstruction of the urinary tract has marked effects on renal blood flow (10), glomerular filtration rate (GFR), and renal tubular function (33). Tubular abnormalities of the obstructed kidney include altered reabsorption of sodium and water, urinary concentration defect, and impaired excretion of hydrogen and potassium (33). It is therefore known that a marked and sometimes prolonged diuresis may occur after release of bilateral ureteral obstruction (BUO). This diuresis is characterized by massive losses of sodium and water (33a). After release of a 24-h period of BUO, there is a striking increase in renal sodium and water excretion (52). Previous studies using in vivo micropuncture techniques and in vitro isolated, perfused tubules from kidneys subjected to urinary tract obstruction demonstrated a striking impairment of fluid reabsorption in the proximal straight tubule, medullary thick ascending limb (mTAL), and entire collecting duct (8, 21, 60, 62). Consistent with this, we recently demonstrated that ureteral obstruction (both bilateral and unilateral) and release of ureteral obstruction in rats are associated with significant downregulation of aquaporins (AQPs) located at the proximal tubule (PT), thin descending limb of the loop of Henle, and the collecting duct. This demonstrated that a molecular explanation for the urinary concentrating defect may, in part, be due to reduced expression of AQPs associated with obstructive nephropathy (17, 18, 31, 35, 36).

Recently, several major renal Na transporter proteins have been identified, which are chiefly involved in physiological processes of transepithelial sodium transport along the nephron. A series of studies identified that dysregulated Na transporters play important roles in animal models showing disorders of sodium and water balance, including lithium-induced nephrogenic diabetes insipidus (34), vitamin D-induced hypercalcemia (58), and liver cirrhosis (14). In the kidney PT, type 3 Na⁺/H⁺ exchanger (NHE3) is expressed apically and participates in the major fraction of sodium reabsorption in this segment (2). Apical

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type II Na\textsubscript{1},P\textsubscript{i} cotransporter (NaPi-2) is involved in reabsorption of ~80% of the filtered phosphate and 10% of the sodium in PT cells (5, 42). Previously, it was demonstrated that PO\textsubscript{4}\textsuperscript{3-} excretion from the obstructed kidney is markedly reduced, probably because of the striking decrease in GFR (48). The loop of Henle generates a high osmolality in the renal medulla by driving the countercurrent multiplier, which is dependent on NaCl reabsorption by the mTAL. In this segment, the apically located Na-K-2Cl cotransporter (NKCC2) [rat type 1 bumetanide-sensitive cotransporter (BSC-1)] (12, 26, 29, 44, 61) and NHE3 in conjunction with the Na-K-ATPase located in basolateral membrane are mainly responsible for sodium reabsorption by the mTAL (23, 24, 27, 49). In the distal convoluted tubule (DCT), the thiazide-sensitive Na-Cl cotransporter (TSC or NCC) is involved in apical sodium reabsorption (30, 45). Furthermore, in unilateral ureteral obstruction, a significant reduction of NHE3, NaPi-2, Na-K-ATPase, BSC-1, and TSC in the obstructed kidney was demonstrated, consistent with a significant impairment of the tubular reabsorption of filtered sodium (37). Thus it could be hypothesized that altered expression of these renal sodium cotransporters and/or exchangers is likely involved in the impairment of urinary concentrating capacity and increased urinary sodium and water excretion during and after release of BUO. The purpose of the present study was therefore to examine whether BUO and release of BUO are associated with alterations in the expression of major renal sodium transporters to further examine underlying potential molecular mechanisms involved in the impaired renal handling of sodium and water in response to urinary tract obstruction.

METHODS

Experimental animals. Studies were performed in male Munich-Wistar rats initially weighing 250 g (Mellergard 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 light-dark cycle, a temperature of 21°C, a humidity of 55 ± 2%, and a carbon dioxide concentration of less than 0.5%.

Rats were anesthetized with halothane. One total ureter was exposed and a 5-mm-long piece of bisected polyethylene tubing (PE-50) was placed around the midportion of each ureter. The ureter was then occluded by tightening the tubing with a 5–0 silk ligature. Twenty-four hours later, the obstructed ureters were decompressed by removal of the ligature and the PE tubing. With the use of this technique, the ureters could be completely occluded for 24 h without evidence of subsequent functional impairment of ureteral function.

Protocol 1. BUO was induced for 24 h (n = 10). The two kidneys were removed and separately prepared for semiquantitative immunoblotting (n = 6) or for immunocytochemistry (n = 4). For sham-operated rats, n = 9, and the two kidneys were removed and separately prepared for semiquantitative immunoblotting (n = 6) or for immunocytochemistry (n = 3).

Protocol 2. BUO was induced for 24 h, followed by release, and animals were observed during the next 3 days (n = 11). The two kidneys were removed and separately prepared for semiquantitative immunoblotting. For sham-operated rats, n = 13.

Membrane fractionation for immunoblotting. For removal of kidneys, rats were anesthetized with halothane. One total kidney (TK) was kept and another kidney was split into cortex and outer medulla (OM + C) and inner medulla (IM), and all were frozen in liquid nitrogen. Tissue (TK, IM, or OM + C) was minced finely and homogenized in 9 ml (TK), 1 ml (IM), or 8 ml (OM + C) of dissecting buffer (0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, pH 7.2, and containing the following protease inhibitors: 8.5 μM leupeptin, 1 mM phenylmethylsulfonyl fluoride), with five strokes of a motor-driven Potter-Elvehjem homogenizer at 1,250 rpm. This homogenate was centrifuged in a Beckman L8M centrifuge at 4,000 g for 15 min at 4°C. The supernatants were assayed for protein concentration using the method of BCA Protein Assay (Pierce) and measured at 562 nm.

Electrophoresis and immunoblotting. Samples of membrane fractions from TK, IM, and OM + C were run on 12 or 6–16% gradient polyacrylamide minigels (Bio-Rad Mini Protean II). For each gel, an identical gel was run in parallel and subjected to Coomassie staining (54). 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. The other gel was subjected to blotting. After transfer by electroelution to nitrocellulose membranes, blots were blocked with 5% milk in PBS-T (80 mM Na\textsubscript{2}HPO\textsubscript{4}, 20 mM NaH\textsubscript{2}PO\textsubscript{4}, 100 mM NaCl, 0.1% Tween 20, pH 7.5) for 1 h and incubated with primary antibodies (11) overnight. After being washed as above, the blots were incubated with horseradish peroxidase-conjugated secondary antibody (P447 or P448, DAKO A/S, Glostrup Denmark, diluted 1:3,000). After a final washing as above, antibody binding was visualized using the ECL system (Amersham International). Controls were made with exchange of primary antibody to antibody preabsorbed with immunizing peptide (100 ng/40 ng IgG) or with preimmune serum (diluted 1:1,000). All controls were without labeling.

Primary antibodies. For semiquantitative immunoblotting and immunocytochemistry, we used previously characterized monoclonal and polyclonal antibodies as follows: 1) NHE3...
(LL546AP): an affinity-purified polyclonal antibody to NHE3 previously characterized (13, 28); 2) NaPi-2 (LL697AP/LL696AP): an affinity-purified polyclonal antibody to NaPi-2 previously characterized (5); 3) Na-K-ATPase: a monoclonal antibody against the α-1 subunit of Na-K-ATPase previously characterized (27); and 4) BSC-1 (LL320AP): an affinity-purified polyclonal antibody to the apical Na-K-2Cl cotransporter of the DCT previously characterized (30)

Immunocytochemistry. The kidneys from BUO rats and sham-operated rats were fixed by retrograde perfusion via the abdominal aorta with 3% paraformaldehyde, in 0.1 M cacodylate buffer with pH 7.4. For immunoperoxidase microscopy, kidney blocks containing all kidney zones were dehydrated and embedded in paraffin. The paraffin-embedded tissues were cut at 2 μm on a rotary microtome (Leica). The sections were deparaffinized and rehydrated. For immunoperoxidase labeling, endogenous peroxidase was blocked by 0.5% H2O2 in absolute methanol for 10 min at room temperature. To reveal antigens, sections were put in 1 mM Tris solution (pH 9.0) supplemented with 0.5 mM EGTA (3.6-di-oxa-octa-methylen-di-nitrilo-tetraacetic acid) and were heated in a microwave oven for 10 min. Nonspecific binding of immunoglobulin was prevented by incubating the sections in 50 mM NH4Cl for 30 min followed by blocking in 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 for laser confocal microscopy were incubated in Alexa 488-conjugated goat anti-rabbit antibody (Molecular Probes) diluted in PBS supplemented with 0.1% BSA and 0.3% Triton X-100 for 60 min at room temperature. After being rinsed with PBS for 3 × 10 min, the sections were mounted in glycerol supplemented with anti-fade reagent (N-propyl gallat). For immunoperoxidase labeling, the sections were washed, followed by incubation in horseradish peroxidase-conjugated immunoglobulin (DAKO A/S, P448, 1:200) diluted in PBS supplemented with 0.1% BSA and 0.3% Triton X-100. Double labeling was also performed with polyclonal antibodies against rats BSC-1 and mouse monoclonal antibodies against α-1-subunit Na-K-ATPase. The labeling was visualized with Alexa 488- and Alexa 546-conjugated secondary antibodies (Alexa 488 anti-rabbit and Alexa 546 anti-mouse, respectively). Light microscopy was carried out by using a Leica DMRE microscope and by using a Leica TCS-SP2 laser confocal microscope (Leica, Heidelberg, Germany).

Statistics. For densitometry of immunoblots, samples from kidneys were run on each gel with corresponding sham kidneys. Renal Na-Cl transporter labeling in the samples from the experimental animals was calculated as a fraction of the mean sham control value for that gel. Parallel Coomassie-stained gels were subjected to densitometry and used for correction of potential minor differences in loading. Values are presented in the text as means ± SE. Comparisons between groups were made by unpaired t-test. P values <0.05 were considered significant.

RESULTS

BUO and release of BUO are associated with impairment of renal water and sodium balance. As shown in Table 1, rats with BUO for 24 h exhibited higher plasma osmolality (337 ± 3 vs. 308 ± 3 mosmol/kgH2O), plasma potassium (6.9 ± 0.2 vs. 4.5 ± 0.1 mosmol/l), and creatinine (357 ± 11 vs. 32 ± 1.1 μmol/l) levels compared with sham-operated control rats (P < 0.05). After release of BUO for 3 days, plasma creatinine remained significantly higher (51 ± 4.2 vs. 31 ± 0.8 μmol/l; Table 1) and creatinine clearance was lower (4.5 ± 0.4 vs. 6.9 ± 0.2 ml/min·kg⁻¹·g⁻¹; Table 2) than sham-operated controls (P < 0.05), consistent with renal functional impairment. In contrast, at day 14 after release of BUO, plasma potassium, plasma creatinine, and creatinine clearance recovered to control levels (Tables 1 and 2).

Release of BUO was associated with a significant polyuria during the entire experimental period up to 14 days (Table 1 and Figs. 1A and 2A). Urinary osmolality was reduced significantly in rats after release of BUO and persisted to day 14 (Table 1 and Fig. 1B). Consistent with this, solute-free water reabsorption (T(H2O)w) measured at day 3 after release of BUO was markedly decreased (115 ± 11 vs. 196 ± 8 μl·min⁻¹·kg⁻¹; P < 0.05; Table 2), indicating an impairment of urinary concentrating capacity after release of BUO.

Release of BUO was also associated with altered renal sodium handling. Three days after release of BUO, there was a significant decrease in the filtered load of sodium (FLNa+, 620 ± 55 vs. 951 ± 24 μmol·min⁻¹·kg⁻¹, P < 0.05; Table 2) and urinary sodium excretion (3.3 ± 0.9 vs. 7.0 ± 0.5 μmol·min⁻¹·kg⁻¹, P < 0.05; Table 2), partly due to significantly decreased GFR measured by creatinine

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<table>
<thead>
<tr>
<th>Table 1. Changes in plasma concentrations, urinary volume, and urinary osmolality during BUO and after release of BUO</th>
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<tr>
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<tr>
<td>24-h BUO (n = 6)</td>
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<tr>
<td>Sham (n = 6)</td>
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<tr>
<td>BUO-3daysR (n = 11)</td>
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<tr>
<td>Sham (n = 13)</td>
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<tr>
<td>BUO-14daysR (n = 8)</td>
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<td>Sham (n = 8)</td>
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</table>

Values are means ± SE. BUO, bilateral ureteral obstruction; sham, sham-operated rats; BUO-3daysR, release of BUO for 3 days; BUO-14daysR, release of BUO for 14 days; Pemol, plasma osmolality; Pcmmol, plasma sodium; Pcv, plasma potassium; Uvol, urine volume; and Uemm, urine osmolality. * P < 0.05 compared with sham.

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Moreover, the net reabsorption of sodium was reduced (616 ± 54 vs. 944 ± 24 μmol·min⁻¹·kg⁻¹, P < 0.05; Table 2) and fractional excretion of sodium decreased (0.5 ± 0.1 vs. 0.7 ± 0.1%, P < 0.05; Table 2). In contrast, 14 days after release of BUO, urinary sodium excretion and fractional excretion of sodium were significantly increased (Table 2), suggesting that release

Table 2. Changes in renal function after release of BUO

<table>
<thead>
<tr>
<th></th>
<th>BUO-3daysR (n = 11)</th>
<th>Sham (n = 13)</th>
<th>BUO-14daysR (n = 8)</th>
<th>Sham (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T²H₂O, μl·min⁻¹·kg⁻¹</td>
<td>115 ± 11*</td>
<td>196 ± 8</td>
<td>165 ± 7</td>
<td>153 ± 11</td>
</tr>
<tr>
<td>CCr, ml·min⁻¹·kg⁻¹</td>
<td>4.5 ± 0.4*</td>
<td>6.9 ± 0.2</td>
<td>6.2 ± 0.3</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>UNa×UVol, μmol·min⁻¹·kg⁻¹</td>
<td>3.3 ± 0.9*</td>
<td>7.0 ± 0.5</td>
<td>6.2 ± 0.2*</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>FLNa, μmol·min⁻¹·kg⁻¹</td>
<td>620 ± 55*</td>
<td>951 ± 24</td>
<td>834 ± 41</td>
<td>851 ± 60</td>
</tr>
<tr>
<td>NetReab.of Na, μmol·min⁻¹·kg⁻¹</td>
<td>616 ± 54*</td>
<td>944 ± 24</td>
<td>828 ± 41</td>
<td>845 ± 60</td>
</tr>
<tr>
<td>FENa, %</td>
<td>0.5 ± 0.1*</td>
<td>0.7 ± 0.1</td>
<td>0.75 ± 0.02*</td>
<td>0.62 ± 0.03</td>
</tr>
<tr>
<td>UK×UVol, μmol·min⁻¹·kg⁻¹</td>
<td>9.8 ± 0.8*</td>
<td>12.9 ± 0.3</td>
<td>12.6 ± 0.4</td>
<td>11.2 ± 0.7</td>
</tr>
<tr>
<td>FLK, μmol·min⁻¹·kg⁻¹</td>
<td>191 ± 1.4*</td>
<td>290 ± 0.9</td>
<td>27.2 ± 1.6</td>
<td>27.3 ± 2.2</td>
</tr>
<tr>
<td>NetReab.of K, μmol·min⁻¹·kg⁻¹</td>
<td>9.4 ± 1.0*</td>
<td>16.1 ± 1.1</td>
<td>14.6 ± 1.5</td>
<td>16.1 ± 1.5</td>
</tr>
<tr>
<td>FEK, %</td>
<td>53.8 ± 2.6*</td>
<td>45.1 ± 2.0</td>
<td>47.3 ± 2.7</td>
<td>41.5 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; T²H₂O, solute-free water reabsorption; CCr, creatinine clearance; UNa×UVol, sodium excretion; FLNa, filtered load of sodium; NetReab.of Na, net reabsorption of sodium = (FLNa) - (UNa×UVol); FENa, fractional excretion of sodium; UK×UVol, potassium excretion; FLK, filtered load of potassium; NetReab.of K, net reabsorption of potassium = (FLK) - (UK×UVol); FEK, fractional excretion of potassium. *P < 0.05 compared with sham.
Renal phosphate excretion increased significantly at day 1 after release of BUO and recovered to sham levels at day 3 (Fig. 2C). Furthermore, the filtered load of phosphate and net reabsorption of phosphate were calculated at day 3 after release of BUO (Table 3). Consistent with the lower GFR, filtered load of phosphate markedly decreased. Net reabsorption of phosphate also decreased, whereas fractional excretion of phosphate increased (Table 3), demonstrating that release of BUO is associated with altered renal phosphate excretion.

Decreased expression of NHE3, NaPi-2, and Na-K-ATPase in rats with BUO and release of BUO. In rats with BUO and BUO followed by release of obstruction for 3 and 14 days, expression levels of major renal sodium transporters were examined by semiquantitative immunoblotting. NHE3, located apically in the kidney PT, descending thin limb in OM, and TAL, is involved in sodium and bicarbonate reabsorption. Immunoblotting revealed that TK expression of NHE3 was significantly decreased in rats with 24-h BUO and release of BUO for 3 days (BUO-3daysR) (24-h BUO: 41 ± 14 vs. 100 ± 11% and BUO-3daysR: 53 ± 10 vs. 100 ± 7%, P < 0.05; Table 4 and Fig. 3, A, B, and D), whereas no significant changes were observed 14 days after release of BUO (BUO-14daysR) (Table 4 and Fig. 3, C and D).

Immunocytochemical analysis confirmed the reduced expression of NHE3 in rats with BUO for 24 h. In sham-operated rats, anti-NHE-3 antibody labeled the apical plasma membrane domains of proximal convoluted tubule (Fig. 4A) and cortical TAL (not shown) in kidney cortex, as previously described (1). Furthermore, an intense labeling was also seen of the apical plasma membrane domains of mTALs in sham-operated rats (Fig. 4C). In contrast, immunocytochemistry showed that the labeling of NHE3 in the PT (arrows in Fig. 4B) as well as in the mTAL (arrows in Fig. 4D) from the obstructed kidneys was much weaker. NaPi-2 contributes to sodium and phosphate reabsorption in the apical part of kidney PT. As shown in Fig. 5, a marked decrease in TK NaPi-2 expression in rats with both 24-h BUO and release of BUO followed by 3 days were revealed by immunoblotting (24-h BUO: 26 ± 6% of control levels and BUO-3daysR: 57 ± 9% of control levels, P < 0.05; Table 4). In contrast, in kidneys followed by release of BUO for 14 days, there was no significant change compared with sham-operated rats (Fig. 5, C and D). Immunocytochemistry confirmed the reduced expression of NaPi-2 in response to 24-h BUO.

Table 3. Changes in renal phosphate excretion 3 days after release of BUO

<table>
<thead>
<tr>
<th></th>
<th>P_{\text{Phos.}}</th>
<th>U_{\text{Phos.}}</th>
<th>U_{\text{Phos.}} * U_{\text{Vol.}}</th>
<th>FL_{\text{Phos.}}</th>
<th>Net reabsorption of Phos.</th>
<th>FE_{\text{Phos.}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUO-3daysR</td>
<td>1.6 ± 0.1</td>
<td>13.5 ± 2.8</td>
<td>0.8 ± 0.1</td>
<td>7.1 ± 0.8</td>
<td>6.3 ± 0.9</td>
<td>14.1 ± 2.9</td>
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<tr>
<td>Sham (n = 13)</td>
<td>2.0 ± 0.0</td>
<td>23.6 ± 1.3</td>
<td>8.0 ± 0.0</td>
<td>13.9 ± 0.4</td>
<td>13.1 ± 0.4</td>
<td>5.7 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. P_{\text{Phos.}}, plasma phosphate; U_{\text{Phos.}}, urinary phosphate; U_{\text{Phos.}} * U_{\text{Vol.}}, urinary phosphate excretion; FL_{\text{Phos.}}, filtered load of phosphate; Net reabsorption of Phos., net reabsorption of phosphate = (FL_{\text{Phos.}}) - (U_{\text{Phos.}} * U_{\text{Vol.}}); and FE_{\text{Phos.}}, fractional excretion of phosphate. *P < 0.05 compared with sham.
Table 4. Expression of sodium transporters in rats with BUO and release of BUO

<table>
<thead>
<tr>
<th></th>
<th>NHE3</th>
<th>NaPi-2</th>
<th>Na-K ATPase</th>
<th>Na-K ATPase</th>
<th>BSC-1</th>
<th>TSC</th>
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<tr>
<td>24-h BUO</td>
<td>41 ± 14%</td>
<td>26 ± 6%</td>
<td>87 ± 18%</td>
<td>67 ± 8%</td>
<td>20 ± 7%</td>
<td>37 ± 9%</td>
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<tr>
<td>(n = 6)</td>
<td>(TK)*</td>
<td>(TK)*</td>
<td>(IM)</td>
<td>(TK)*</td>
<td>(TK)*</td>
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</tr>
<tr>
<td>Sham</td>
<td>100 ± 11%</td>
<td>100 ± 6%</td>
<td>100 ± 16%</td>
<td>100 ± 4%</td>
<td>100 ± 4%</td>
<td>100 ± 4%</td>
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<tr>
<td>(n = 6)</td>
<td>(TK)</td>
<td>(TK)</td>
<td>(IM)</td>
<td>(TK)</td>
<td>(TK)</td>
<td>(TK)</td>
</tr>
<tr>
<td>BUO-3daysR</td>
<td>53 ± 10%</td>
<td>57 ± 9%</td>
<td>53 ± 10%</td>
<td>62 ± 8%</td>
<td>50 ± 12%</td>
<td>56 ± 16%(TK)*</td>
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<tr>
<td>(n = 5/6)</td>
<td>(TK)*</td>
<td>(TK)*</td>
<td>(IM)*</td>
<td>(TK)*</td>
<td>(TK)*</td>
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</tr>
<tr>
<td>Sham</td>
<td>100 ± 7%</td>
<td>100 ± 8%</td>
<td>100 ± 15%</td>
<td>100 ± 4%</td>
<td>100 ± 6%</td>
<td>100 ± 10%(TK)</td>
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<td>(TK)</td>
<td>(TK)</td>
<td>(IM)</td>
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<td>(TK)</td>
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<tr>
<td>BUO-14daysR</td>
<td>101 ± 25%</td>
<td>88 ± 7%</td>
<td>60 ± 4%</td>
<td>68 ± 3%</td>
<td>68 ± 15%</td>
<td>62 ± 7% (OM + C)*</td>
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<td>Sham</td>
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Values are means ± SE. NHE3, type 3 sodium/proton exchanger; NaPi-2, type 2 sodium-phosphate cotransporter; BSC-1, type 1 bumetanide-sensitive Na-K-2Cl cotransporter; TSC, thiazide-sensitive Na-Cl cotransporter; TK, total kidney; OM + C, outer medulla plus cortex; and IM, inner medulla. For densitometry of immunoblots, sodium transporter labelling was calculated as a fraction of the mean value from sham-operated control rats. *P < 0.05 compared with sham.

As seen on Fig. 6, A and C, the NaPi-2 was located in the apical plasma membrane domains of PT in sham-operated control rats. In contrast, the labeling of NaPi-2 was much weaker in kidneys with BUO for 24 h (Fig. 6, B and D).

Na-K-ATPase is expressed in basolateral membrane along the nephron and participates in the active transport of sodium and potassium across the cell membrane by hydrolysis of ATP. Immunoblotting of the α1-subunit of the Na-K-ATPase demonstrated that the expression was markedly decreased in the IM after release of obstruction (BUO-3daysR: 53 ± 10% vs. 100 ± 15% and BUO-14daysR: 60 ± 4% vs. 100 ± 5%, P < 0.05; Table 4 and Fig. 7, C and E), whereas the expression was unchanged in the obstructed kidneys in response to 24-h BUO (Table 4 and Fig. 7A). Moreover, Na-K-ATPase expression in TK or OM + C was significantly reduced in the obstructed kidneys in response to 24-h BUO and release of BUO for 3 and 14 days (24-h BUO (TK): 67 ± 8% of sham levels; BUO-3daysR (TK): 62 ± 8% of sham levels; BUO-14daysR (OM + C): 68 ± 3% of sham levels, P < 0.05; Table 4 and Fig. 7, B, D, and F).

Double immunofluorescence labeling of Na-K-ATPase together with BSC-1 showed that strong labeling of Na-K-ATPase was seen in the basolateral plasma membrane domains of the cortical and medullary TAL cells in sham-operated controls (see green in Fig. 9, A, C, G, and I). In the obstructed kidney for 24 h, the labeling of Na-K-ATPase in the cortical and medullary TAL cells (see arrowheads in Fig. 9, D, F, J, and L) was weaker compared with that in sham-operated rats.

Reduced expression of BSC-1 and TSC in rats with BUO and release of BUO. The apical Na-K-2Cl cotransporter (BSC-1) is the major transporter for apical sodium reabsorption in the medullary and cortical TAL. The expression of BSC-1 in TK was dramatically decreased (24-h BUO: 20 ± 7 vs. 100 ± 4%; BUO-3daysR: 50 ± 12 vs. 100 ± 6%, P < 0.05; Fig. 8, A and B). This may indicate that reduced BSC-1 expression in mTAL, together with reduced NHE3 and Na-K-ATPase, is involved in increased sodium excretion and thus impairs urinary concentration. In contrast, BSC-1 expression in the OM + C did not change in kidneys at 14 days after release of BUO (Fig. 8C).

Confocal microscopic images with double immunofluorescence labeling of BSC-1 and Na-K-ATPase showed that labeling of BSC-1 in sham-operated controls was seen in the apical plasma membrane domains of the cortical and mTAL cells (Fig. 9, B, C, H, and I). In the obstructed kidney for 24 h, the labeling of BSC-1 in the cortical and mTAL cells was much weaker compared with that in sham-operated rats (arrows in Fig. 9, E, F, K, and L).

TSC, located in the apical domains of DCT cells, was markedly reduced in rats with 24-h BUO and release of BUO for 3 and 14 days (24-h BUO: 37 ± 9 vs. 100 ± 4%; BUO-3daysR: 56 ± 16 vs. 100 ± 10%; BUO-14daysR: 62 ± 7 vs. 100 ± 9%, P < 0.05; Table 4 and Fig. 10).

Fig. 3. Semiquantitative immunoblotting of membrane fractions of total kidney (TK) or outer medulla + cortex (OM + C) from BUO, released BUO, and sham-operated rats. A-C: immunoblots were reacted with affinity-purified antitype 3 Na+/H+ exchange (NHE3) antibody and revealed a single ~87-kDa band. D: densitometric analysis of all samples from rats with BUO and release of BUO and sham-operated controls revealed a persistent decrease in NHE3 levels to 41 ± 14% of sham levels in rats with 24-h BUO and to 53 ± 9% of sham levels in rats with 24-h BUO followed by release for 3 days (BUO-3daysR). However, in rats after release of BUO for 14 days (BUO-14daysR), densitometric analysis revealed no difference from sham controls. *P < 0.05.
Consistent with this, immunocytochemistry revealed that labeling of TSC seen at the apical membrane domains of DCT cells was markedly reduced in kidneys from obstructed animals with BUO for 24 h (arrows in Fig. 11B) compared with sham-operated controls (arrows in Fig. 11A).

DISCUSSION

We demonstrated that BUO and release of BUO are associated with impaired urinary concentration and altered renal sodium and phosphate handling. In parallel, the expression of major renal Na transporters NHE3, NaPi-2, Na-K-ATPase, BSC-1, and TSC was significantly downregulated in the obstructed kidney. The results in the present study therefore suggest that downregulation of these major renal Na transporters plays an important role in the decreased urinary concentrating capacity and increased urinary sodium excretion after release of BUO. The expression of NHE3, NaPi-2, and BSC-1 recovered to control levels at day 14 after release of BUO; however, diuresis and natriuresis persisted, indicating long-term kidney tubule dysfunction, consistent with persistent downregulation of Na-K-ATPase and TSC.

The marked and sometimes prolonged diuresis after release of obstruction is characterized by massive losses of water, sodium, and other solutes (41, 53). Consistent with this, the present study showed that urinary output and sodium excretion were increased in rats with BUO and release of BUO. However, it should be noted that on days 2 and 3 after release of BUO, urinary sodium excretion was significantly lower than controls. Importantly, at day 3, there was also a significant reduction in the net reabsorption of sodium consistent with the marked downregulation of sodium transporters. The reduced FLNa due to the vasoconstriction-induced GFR impairment during the first several days after release of obstruction could, in part,
explain this. Alternatively, the reduced food (sodium) intake observed during the first several days after release of BUO may also add to the lack of natriuresis. These changes may reflect a marked systemic reaction to BUO. Thus several important physiological and pathological factors, including altered expression of renal sodium transporters, changes in renal hemodynamics, and amount of sodium intake, importantly influence the urinary flow rate and sodium excretion following release of obstruction.

An important factor determining the changes in urinary output in obstructive nephropathy is the GFR. After release of BUO, GFR is markedly reduced due to a predominant afferent arteriolar vasoconstriction (10, 40). However, GFR recovers after release of obstruction. In long-term studies of complete 24-h ureteral obstruction, TK GFR was restored to normal levels by 14 and 60 days after release of the obstruction (3, 19). Consistent with this finding, the present study showed that creatinine clearance returned to normal levels 14 days after release of BUO.
days after release of BUO. Despite the continued downregulation of sodium transporters, the recovery of GFR may contribute to the marked polyuria and natriuresis after release of BUO.

A defect in tubular sodium reabsorption during obstruction and release of obstruction is likely to be another important factor for the prolonged postobstructive diuresis. Major renal sodium transporters are importantly involved in tubular reabsorption of sodium along the nephron and collecting ducts, and it has been recently identified that dysregulated sodium transporters play an important role in the altered sodium metabolism in experimental animal models of sodium and water balance disorders (13, 14, 34, 37, 58).

NHE3, which is expressed apically in the PT, descending thin limb in the OM, and TAL cells (1, 6), mediates the large quantities of transcellular sodium and bicarbonate reabsorption in the kidney PT (38), in conjunction with Na-K-ATPase and the electrogenic sodium-bicarbonate cotransporter (kNBC1) in the basolateral plasma membrane. Consistent with this, in situ microperfusion (51, 57) studies of NHE3 knockout mice revealed a 60–70% reduction in fluid absorption and a 50–60% reduction in bicarbonate absorption in the PTs. The present study demonstrated that the expression of NHE3 was significantly downregulated in rats with BUO and release of BUO, suggesting that NHE3 is likely, at least partly, involved in postobstructive natriuresis. In addition to the reduced expression of NHE3, the basolateral Na-K-ATPase protein was also downregulated in rats with BUO and release of BUO, consistent with previous studies that revealed the inhibitory effects of BUO on Na-K-ATPase activity and transcripts (23, 24). NaPi-2 cotransporter located in the renal proximal tubular brush-border membrane is the key player in renal Pi reabsorption and is also responsible for 10% of sodium reabsorption (5). After release of BUO, both absolute and fractional urinary phosphate excretion are increased associated with postobstructive diuresis and natriuresis (4, 48, 53). Consistent with the increased urinary phosphate excretion found in previous studies, the rats with BUO and release of BUO in the present study showed a significantly decreased expression of NaPi-2 in renal PTs, suggesting that downregulation of NaPi-2 is likely involved in the observed phosphaturia and, at least partly, in natriuresis after release of BUO.

Impaired ability to concentrate the urine is a characteristic feature of obstructive nephropathy (50). The urinary concentrating process is largely dependent on the generation of hypertonic medullary interstitium by countercurrent multiplication that is dependent on the active reabsorption of NaCl by the mTAL. The key sodium transporters responsible for the secondary active transport of NaCl in the mTAL are BSC-1 (12, 29), NHE3 (1), and basolateral Na-K-ATPase (27). Our data revealed that the abundance and expression of BSC-1, NHE3, and Na-K-ATPase were markedly downregulated in TAL cells of rats with BUO and release of BUO, indicating that they may all play key roles in the reduced active sodium chloride reabsorption in the TAL. Moreover, the reduced expressions are likely to contribute to loss of the normal medullary solute concentration gradient and thus decreased urinary concentration. Early oxygen consumption studies revealed a marked reduction in the inhibitory effects of both furosemide and ouabain in mTAL cell suspensions from the obstructed kidneys, indicating reduced activities of both the apical Na-K-2Cl cotransporters and the basolateral Na-K-ATPase (23). Saturable [3H]bumetanide binding also showed a reduction in the number of Na-K-2Cl cotransporters (23). The data in the present study demonstrating that the abundance of BSC-1 and Na-K-ATPase protein was significantly decreased in the TAL cells of kidneys in rats with BUO and release of BUO are in agreement with these results. Taken together, not only was the activity of Na transporters reduced, but also the expression level of sodium transporter proteins in TAL was significantly decreased after BUO and release of BUO. Thus it is possible that dysfunction of sodium transporters (BSC-1, NHE3, and Na-K-ATPase) in the TAL may impair both sodium reabsorption in this segment of nephron and the normal medullary interstitial hypertonicity, thereby diminishing urinary concentration as demonstrated in the present study.

How ureteral obstruction downregulates renal transporter expression in the TAL epithelial cells has not been explained in the present study. The expression of Na-K-2Cl cotransporter (BSC-1 or NKCC2) is increased in response to dDAVP (29). Because the vasopressin V2 receptor is coupled to activation of adenyl cyclase, it is possible that the upregulation of BSC-1 by vasopressin is a result of elevated levels of cAMP.

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Fig. 8. Semiquantitative immunoblotting of membrane fractions of TK or OM + C from BUO, released BUO, and sham-operated rats. A–C: immunoblots were reacted with affinity-purified type 1 bumetanide-sensitive Na-K-2Cl cotransporter (BSC-1) antibody and revealed a strong, broad band of molecular weight 146–176 kDa centered at ~161 kDa. Densitometric analysis of all samples from TK in rats with BUO, release of BUO for 3 days, and sham-operated controls revealed a marked reduction (24-h BUO: 20 ± 7 vs. 100 ± 4%; BUO-3daysR: 50 ± 12 vs. 100 ± 6%; *p < 0.05). BSC-1 abundance was not changed in OM + C at day 14 after release of BUO compared with sham-operated controls.
Fig. 9. Immunocytochemical analyses of Na-K-ATPase and BSC-1 in TAL of control rat (A-C, G-I) and BUO rat (D-F, J-L). The whole kidney sections were incubated with monoclonal anti-Na-K-ATPase (α1-subunit) and polyclonal anti-BSC-1. Labeling was visualized with Alexa 546 anti-mouse (green) and Alexa 488 anti-rabbit (red), respectively. In control kidneys, abundant basolateral Na-K-ATPase (green) and apical BSC-1 (red) labeling was seen on plasma membrane domains of TAL cells in cortex (A-C) and ISOM (G-I). In BUO rats, labeling of Na-K-ATPase (arrowheads) and BSC-1 (arrows) in TAL cells were markedly reduced in cortex (D-F) and ISOM (J-L).

Fig. 10. Semiquantitative immunoblotting of membrane fractions of TK or OM from BUO, released BUO, and sham-operated rats. A-C: immunoblots were reacted with affinity-purified anti-thiazide-sensitive cotransporter (TSC) antibody and revealed a broad band centered at ~165 kDa. D: densitometric analysis of all samples from kidney in rats with 24-h BUO and release of BUO and sham-operated controls revealed a marked persistent decrease in the expression of TSC [24-h BUO (TK): 37 ± 9 vs. 100 ± 4%; BUO-3daysR (TK): 56 ± 16 vs. 100 ± 10%; BUO-14daysR (OM + C): 62 ± 7 vs. 100 ± 9%, *P < 0.05].
Consistent with this, a cAMP-regulatory element was identified in the 5'-flanking region of the mouse NKCC2 gene (25). Kim et al. (31) recently demonstrated that medullary cAMP generation in response to AVP was blunted and type VI adenyl cyclase was downregulated in the obstructed kidneys. Therefore, downregulation of BSC-1 may be a result of decreased levels of cAMP or inhibition of adenyl cyclase in rats with BUO and release of BUO. Moreover, several studies emphasized an important role of PGE2 in the impaired renal urinary concentration defect after obstruction (16, 46, 55, 59). These studies revealed ureteral obstruction and release of obstruction induced a striking increase in renal PGE2 production. In contrast to the action of AVP on cAMP accumulation, PGE2, on the other hand, inhibits hormone-dependent cAMP synthesis, via interaction with the PGE2 receptor through a GTP-dependent protein as demonstrated in the mTAL of rats with obstruction (15, 56). Thus increased PGE2 in the obstructed kidney may have an inhibitory effect on TAL cAMP levels. This may be involved in the decreased expression of the Na-K-2Cl cotransporter BSC-1 in the obstructed kidney.

The DCT reabsorbs sodium mainly through the apical thiazide-sensitive NaCl cotransporter, TSC or NCC (30). It has recently been suggested that aldosterone stimulates sodium reabsorption by the kidney, in part, through its action to increase the expression of the TSC in DCT (30). The renin-angiotensin-aldosterone system seems to play a role in the hemodynamic changes and maintaining sodium and water balance following ureteral obstruction (9, 47). The present study showed a significant reduction of TSC in the kidneys of rats with BUO and release of BUO, suggesting a role of diminished sodium reabsorption in the DCT in the increased sodium excretion during postobstructive diuresis, although the mechanism for this remains unclear. In the inner medullary collecting duct, oxygen-dependent transport activities of the Na-K-ATPase were reported to be reduced in cells isolated from obstructed kidneys (24). Our data revealed that the expression of Na-K-ATPase in IM of rats with release of BUO was significantly downregulated, indicating decreased Na-K-ATPase expression may be involved in increased urinary sodium excretion during postobstructive diuresis. Recently, ENaC was shown to be present in the collecting duct principal cells and participate in the fine regulation of sodium reabsorption regulated by aldosterone (20). Studies in gene knockout mice with ENaC subunits have shown that ENaC plays an important role in renal sodium and water metabolism (22, 39). A potential role of ENaC in the development of postobstructive natriuresis and diuresis remains to be established.

In summary, BUO and release of BUO in rats are associated with significant reduction in the expression of major renal sodium transporters along the nephron and collecting ducts. Importantly, the expression of renal sodium transporters was reduced, consistent with a significant impairment of tubular sodium reabsorption and decreased urinary concentration. We conclude that downregulation of major renal sodium transporters located at the PT, TAL, DCT, and IM in rats with BUO and release of BUO may contribute to the impaired renal tubular sodium reabsorption, increased urinary sodium excretion, and decreased urinary concentration.

The authors thank G. Kall, D. Wulff, I. M. Paulsen, M. Vistisen, H. Høyer, Z. Nikrozi, L. V. Holbech, and I. M. Jalk for technical assistance.

DISCLOSURES

The Water and Salt Research Centre at the University of Aarhus is established and supported by the Danish National Research Foundation (Danmarks Grundforskningsfond). Support for this study was provided by The Karen Elise Jensen Foundation, The Human Frontier Science Program, The Novo Nordisk Foundation, The Commission of the European Union (EU-Aquaplugs and EU Action Programs), The Danish Medical Research Council, The University of Aarhus Research Foundation, The Skovgaard Foundation, The Danish Research Academy, The University of Aarhus, The Donguk University, and the intramural budget of the National Heart, Lung, and Blood Institute, National Institutes of Health.

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