Altered expression of epithelial sodium channel in rats with bilateral or unilateral ureteral obstruction

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Li C, Wang W, Norregaard R, Knepper MA, Nielsen S, Frøkiær J. Altered expression of epithelial sodium channel in rats with bilateral or unilateral ureteral obstruction. Am J Physiol Renal Physiol 293: F333–F341, 2007. First published May 2, 2007; doi:10.1152/ajprenal.00372.2006.—The roles of epithelial sodium channel (ENaC) subunits (α, β, and γ) in the impaired renal reabsorption of sodium and water were examined in rat models with bilateral (BUO) or unilateral ureteral obstruction (UUO) for 24 h or with BUO followed by release of obstruction and 3 days of observation (BUO-3dR). In BUO rats, plasma osmolality was increased dramatically, whereas plasma sodium concentration was decreased. Immunoblotting revealed a significantly decreased expression of α-ENaC (57 ± 7%), β-ENaC (19 ± 5%), and γ-ENaC (51 ± 10%) as well as 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) in the cortex and outer medulla (C+OM) compared with sham-operated controls. This was confirmed by immunohistochemistry. BUO-3dR was associated with polyuria and impaired renal sodium handling. The protein abundance and the apical labeling of α-ENaC were significantly increased, whereas β- and γ-ENaC as well as 11β-HSD2 expression remained decreased. In UUO rats, expression of α- and β-ENaC and 11β-HSD2 decreased in the C+OM in the obstructed kidney. In contrast, the abundance and the apical labeling of α-ENaC in the nonobstructed kidneys were markedly increased, suggesting compensatory upregulation in this kidney. In conclusion, α-, β-, and γ-ENaC expression levels are downregulated in the obstructed kidney. The expression and apical labeling of α-ENaC were increased in BUO-3dR rats and in the nonobstructed kidneys from UUO rats. These results suggest that altered expression of α-, β-, and γ-ENaC may contribute to impaired renal sodium and water handling in response to ureteral obstruction.

Recent studies have demonstrated that protein expression of aquaporins 1-4 (AQP1-4), the water channels located at the proximal tubule, descending thin limb, and collecting duct, and the expression of major renal sodium transporters (type 3 Na/H exchanger, type 2 Na-P cotransporter, Na-K-ATPase, Na-K-2Cl cotransporter, Na-Cl cotransporter) located along all renal nephron segments were severely reduced during bilateral (BUO) or unilateral ureteral obstruction (UUO) and release of obstruction (20–23). These findings suggest that the reduction in renal aquaporins and major renal sodium transporters contribute to the impairment of the urinary concentrating capacity and salt wasting in response to urinary tract obstruction (8, 9, 20–23). Furthermore, dysregulation of urea transporters may also participate in the urinary concentrating defect in response to ureteral obstruction (19).

Renal amiloride-sensitive epithelial sodium channels (ENaC) mediate Na+ entry across the apical membrane of cells in the distal convoluted tubule (DCT), the connecting tubule (CNT), and the cortical collecting duct (CCD), which is thought to be the aldosterone-sensitive distal nephron (24, 32, 34). ENaC, in the kidney, is a heteromeric protein made up of three homologous subunits: α, β, and γ (2). It has been shown that α-ENaC is mainly present at the apical domains of the principal cells, whereas β- and γ-ENaC are mainly associated with intracellular vesicles dispersed in the entire cytoplasm (12). The α-, β-, or γ-ENaC knockout mice showed a marked renal loss of sodium despite elevated plasma levels of aldosterone and hyperkalemia, indicating the importance of these three subunits of ENaC in mediating sodium reabsorption and potassium secretion in the distal nephron under the control of aldosterone (14, 27, 33). Several other studies have shown the important roles of ENaC in several physiological and pathophysiological conditions (5, 13, 25, 26, 30). However, the role of ENaC in sodium depletion in response to urinary tract obstruction is still unclear.

In epithelial tissue, glucocorticosteroids bind to and activate the intracellular mineralocorticoid receptor (MR) with the same affinity as mineralocorticoids, and their plasma concentration normally exceeds that of aldosterone by a factor of 10–1,000. Selectivity of aldosterone over glucocorticoids is conferred by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which metabolizes cortisol to cortisone and thereby prevents illicit receptor binding by glucocorticoids.
Methods

Experimental Animals

Studies were performed on male Munich Wistar rats initially weighing 250 g (Møllegaard Breeding Center, 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-h artificial light-dark cycle, a temperature of 21 ± 2°C, and a humidity of 55 ± 2%. Rats were allowed to acclimatize to the cages for 3 days before surgery.

Before surgery, the rats were anesthetized with halothane (Halon Laboratories, River Edge, NJ), and during surgery, they were placed on a heated table to maintain rectal temperature at 37–38°C. BUO and UUO were established as previously described (21, 23). In brief, BUO was established through a midline abdominal incision, where both ureters were 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 suture. Twenty-four hours later, the rats were killed or 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. UUO was established by tightening a 5-0 silk ligature around the midportion of the left ureter. Finally, rats were given 0.1 ml of TemGesic (0.3 mg/ml; Schering-Plough, Farum, Denmark) for analgesia and regained consciousness afterward. The rats were then placed in the metabolic cages again. Twenty-four hours later, urine samples from contralateral kidneys were collected for clearance studies (see below), and the rats were killed. The animal protocols were approved by the board of the Institute of Clinical Medicine, University of Aarhus, according to the licenses for use of experimental animals issued by the Danish Ministry of Justice. Rats were allocated to the protocols indicated below. Age- and time-matched sham-operated controls were prepared and observed in parallel with each BUO group and UUO group in the following protocols.

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

Protocol 2. Rats were subjected to BUO for 24 h, followed by release of the obstruction and observation during the next 3 days (BUO-3Dr; n = 10). The two kidneys were removed and separately prepared for semi-quantitative immunoblotting (n = 7) or for immunocytochemistry (n = 3). For sham-operated rats (n = 12), the two kidneys were removed and separately prepared for semi-quantitative immunoblotting (n = 7) or for immunocytochemistry (n = 5).

Protocol 3. Rats were subjected to UUO for 24 h (n = 11). The two kidneys were removed and separately prepared for semi-quantitative immunoblotting (n = 7) or for immunocytochemistry (n = 4). For sham-operated rats (n = 10), the two kidneys were removed and separately prepared for semi-quantitative immunoblotting (n = 7) or for immunocytochemistry (n = 4) or for immunocytochemistry (n = 3).

Clearance Studies

Urine was collected and clearance studies were performed after the release of BUO or in the nonobstructed kidney during 24-h periods throughout the study of UUO. At the end of each protocol, 4 ml of blood were collected into a heparinized tube for the determination of plasma electrolytes and osmolality before the rat was killed. The plasma concentrations of sodium, potassium, creatinine, and urea as well as urinary concentrations of creatinine and urea were determined (Vitros 950; Johnson & Johnson). In another group of animals, BUO and UUO for 24 h as well as BUO followed by 3 days of release were performed after the above protocols, and plasma aldosterone was measured. The plasma concentration of aldosterone was determined by radioimmunoassay (Active Aldosterone RIA kit, DSL 8600; Diagnostics System Laboratories, Webster, TX). The concentrations of urinary sodium and potassium were determined by standard flame photometry (Eppendorf FC6341). The osmolality of urine and plasma was determined by freezing point depression (Advanced Osmometer, model 3900; Advanced Instruments, Norwood, MA; and Osmomat 030-D; Gonotec, Berlin, Germany).

Membrane Fractionation for Immunoblotting

For removal of kidneys, rats were anesthetized with halothane. In rats with BUO, one total kidney was kept and another kidney was split into cortex plus outer medulla (C+OM) and inner medulla (IM). In rats with UUO, both obstructed and nonobstructed kidneys were split into C+OM and IM. All of them were frozen in liquid nitrogen. Tissue (C+OM) was minced finely and homogenized in 8 ml of dissecting buffer (0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, pH 7.2), containing the following protease inhibitors: 8.5 μM leupeptin and 1 mM phenylmethylsulfonyl fluoride), with five strokes of a motor-driven Potter-Elvehjem homogenizer at 1,250 rpm. This homogenate was centrifuged in a Universal 30RF centrifuge (Hettich, Tuttingen, Germany) at 4,000 g for 15 min at 4°C, and the supernatant was pipetted off, solubilized at 65°C for 15 min in Laemmlli sample buffer containing 2% SDS, and then stored at −20°C.

Electrophoresis and Immunoblotting

Samples of membrane fractions from C+OM were run on 12% polyacrylamide minigels (Bio-Rad Mini Protean II). For each gel, an identical gel was run in parallel and subjected to Coomassie staining. The Coomassie-stained gel was used to verify identical loading or to allow for correction for minor differences in loading after scanning and densitometry of major bands. The other gel was subjected to Western blot analysis. After transfer by electroelution to nitrocellulose membranes, blots were blocked with 5% milk in PBS-T (80 mM Na2HPO4, 20 mM NaH2PO4, 100 mM NaCl, and 0.1% Tween 20, pH 7.5) for 1 h and incubated with primary antibodies (see below) overnight at 4°C. After being washed with PBS-T, the blots were incubated with horseradish peroxidase-conjugated secondary antibody (P448, diluted 1:3,000; DAKO, Glostrup, Denmark). After a final washing as described above, antibody binding was visualized using the enhanced chemiluminescence system (ECL; Amersham International, Little Chalfont, UK).

Primary Antibodies

For semi-quantitative immunoblotting and immunocytochemistry, we used previously characterized affinity-purified polyclonal antibodies to α-, β-, and γ-ENaC (26), 11β-HSD2 (Chemicon), and β-actin (Sigma, A-2066).

Immunocytochemistry

The kidneys from BUO rats, UUO rats, and sham-operated rats were fixed by retrograde perfusion via the abdominal aorta with 3%
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 at 2 μm on a rotary microtome (Leica, Heidelberg, Germany). The sections were depaerfanzined and rehydrated. For immunoperoxidase labeling, endogenous peroxidase was blocked by 0.5% H2O2 in absolute methanol for 10 min. To vented by incubating the sections in 50 mM NH4Cl in 30 min, oven for 10 min. Nonspecific binding of immunoglobulin was pre-

**RESULTS**

**BUO, UUO, and Release of BUO are Associated With Impaired Sodium Handling**

During BUO for 24 h, plasma osmolality increased dramatically, whereas plasma sodium decreased significantly from

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**Table 1. Functional data in rats with 24-h BUO, BUO followed by release for 3 days, and 24-h UUO compared with sham-operated controls**

<table>
<thead>
<tr>
<th></th>
<th>24 hBUO</th>
<th>BUO-3dR</th>
<th>24-h UUO</th>
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<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>[Pren] osmol/kgH2O</td>
<td>343±1*</td>
<td>301±1</td>
<td>308±1*</td>
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<td>[PNa] mM</td>
<td>127±5.1*</td>
<td>140.6±0.3</td>
<td>136.6±2.0</td>
</tr>
<tr>
<td>[PK] mM</td>
<td>6.0±0.3*</td>
<td>3.7±0.1</td>
<td>4.4±0.1</td>
</tr>
<tr>
<td>[Pada, pg/ml]</td>
<td>3.610±246*</td>
<td>250±12</td>
<td>208±30</td>
</tr>
<tr>
<td>[UO, μl/min/kgH2O]</td>
<td>932±66*</td>
<td>2.582±277</td>
<td>1.910±89</td>
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<tr>
<td>[T3H2O], μl/min/kgH2O</td>
<td>113±7*</td>
<td>195±8</td>
<td>114±7*</td>
</tr>
<tr>
<td>[Cl], μM</td>
<td>4.6±0.2*</td>
<td>6.8±0.3</td>
<td>4.3±0.2</td>
</tr>
<tr>
<td>[UNa], μmol/min/kgH2O</td>
<td>2.6±5*</td>
<td>5.6±0.3</td>
<td>3.7±0.4*</td>
</tr>
<tr>
<td>[FENa], μmol/min/kgH2O</td>
<td>604±26*</td>
<td>928±40</td>
<td>591±85</td>
</tr>
<tr>
<td>[NetReab of Na], μmol/min/kgH2O</td>
<td>602±25*</td>
<td>923±40</td>
<td>588±85</td>
</tr>
<tr>
<td>[FNa], %</td>
<td>0.42±0.08*</td>
<td>0.61±0.02</td>
<td>0.62±0.03*</td>
</tr>
<tr>
<td>[UK], μmol/min/kgH2O</td>
<td>11.7±0.6*</td>
<td>15.2±0.5</td>
<td>8.2±0.6*</td>
</tr>
<tr>
<td>Renal K:Na</td>
<td>5.8±1.2*</td>
<td>2.7±0.1</td>
<td>2.5±0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. BUO, bilateral ureteral obstruction; Sham, sham operated; BUO-3dR, release of BUO for 3 days; UUO, unilateral ureteral obstruction; [Pren], [PNa], [PK], [Pada], plasma osmolality, sodium, potassium, and aldosterone; [UO], urine volume; [T3H2O], urine osmolality; T3H2O, solute-free water reabsorption; Cl−, creatinine clearance; [UNa], [FENa], urinary sodium excretion; [FNa], filtered load of sodium. NetReab of Na, net reabsorption of Na = FLNa – UNa·UVol; FENa, fractional excretion of sodium; UK, urinary potassium excretion. Note that during UUO, urinary production was derived exclusively from nonobstructed kidneys and compared with urinary production from 1 kidney in sham-operated rats (i.e., one-half the total urinary production in Sham). *P < 0.05 compared with sham-operated rats.

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**Fig. 1.** Semiquantitative immunoblotting using protein from cortex plus outer medulla (C+OM) in control rats and rats with bilateral ureteral obstruction (BUO) for 24 h. Immunoblots reacted with affinity-purified anti-α-, anti-β-, and anti-γ-ENaC (epithelial sodium channel) antibodies revealed an ~85-kDa band (A–C), anti-11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) antibody revealed an ~41-kDa band (D), and anti-β-actin antibody revealed an ~45-kDa band (E). Densitometric analysis revealed a decrease in α-, β-, and γ-ENaC and 11β-HSD2 in BUO rats compared with sham-operated controls (*P < 0.05). In contrast, there was no significant change in the abundance of β-actin between BUO rats and sham-operated rats.

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**Statistics**

For densitometry of immunoblots, samples from kidneys were run on each gel with corresponding sham kidneys. Renal protein levels in the samples from the experimental animals were calculated as a fraction of the mean sham control value for that gel. Parallel Coomasie stained gels were used for correction of minor differences in loading. Values are means ± SE. Comparisons between groups were made by unpaired t-test. P values <0.05 were considered significant.
140.6 ± 0.3 to 127.1 ± 5.1 mM (Table 1). Consistent with the previous series of studies (21, 22), release of BUO was associated with polyuria and reduced urinary osmolality. Rats with BUO for 24 h and release of BUO showed markedly elevated plasma aldosterone concentration compared with sham-operated controls (Table 1). Solute-free water reabsorption (TcH2O) and creatinine clearance were reduced (Table 1), demonstrating impaired water reabsorption and decreased glomerular filtration rate in response to obstruction. Net reabsorption of sodium and fractional excretion of sodium were markedly decreased at day 3 after release of BUO (Table 1), demonstrating that BUO and release of BUO are associated with impaired renal sodium handling.

Next, we investigated the renal functional changes in the nonobstructed kidney of rats with 24-h UUO. Since the UUO model is characterized by urine production only from the contralateral kidney, this was compared with the urine production generated from one kidney in sham-operated controls. The urine production was anticipated to be equal from the two kidneys in sham-operated controls. Plasma osmolality increased significantly, whereas urine volume and TcH2O were increased compared with sham-operated controls. However, plasma aldosterone concentration was significantly increased compared with sham-operated controls. Fractional excretion of sodium from the nonobstructed kidney in rats with UUO was dramatically increased (Table 1).

Fig. 2. Immunoperoxidase microscopy of α-, β-, and γ-ENaC in the cortex from sham-operated controls (A–C), rats with BUO for 24 h (D–F), and rats with unilateral ureteral obstruction (UUO) for 24 h (G–L). A–C: the labeling of α-ENaC, β-, and γ-ENaC was dispersed in the cytoplasm of principal cells of cortex in sham-operated control rats. D–F: BUO rats showed maintained apical targeting and a much weaker and dispersed cytoplasmic labeling of α-, β-, and γ-ENaC (arrows) in the cortex. G–I: in the obstructed kidneys from UUO rats (24hUUO OBS), the cytoplasmic labeling density of α- and β-ENaC was also markedly decreased (arrows), and the staining of γ-ENaC was unchanged compared with sham controls. In contrast, in the nonobstructed kidneys from UUO rats (24hUUO non-OBS), the apical labeling and the labeling intensity in the cytoplasm of α-ENaC (J) were markedly increased (arrowhead), whereas β- and γ-ENaC labeling intensity and subcellular distribution (K and L) did not change compared with sham controls. Magnification, ×630.
The urinary K/Na ratio was calculated as an index of aldosterone activity in the kidney. At day 3 after release of BUO, the urinary K/Na ratio was increased dramatically (Table 1), indicating increased aldosterone activity in the kidney. In rats with UUO for 24 h, the urinary K/Na ratio did not change (Table 1).

BUO for 24 h is Associated With Reduced Expression Levels of α-, β-, and γ-ENaC and 11β-HSD2

The expression levels of α-, β-, and γ-ENaC in rats with BUO, release of BUO, and UUO were examined by Western blotting and immunohistochemistry. 11β-HSD2, a potent dehydrogenase inactivating glucocorticoids, was also investigated by Western blotting.

In rats with BUO for 24 h, the abundance of α-ENaC (85 kDa), β-ENaC (85 kDa), and γ-ENaC (85 kDa) proteins in the C+OM was decreased significantly (α-ENaC: 0.57 ± 0.07 vs. 1.00 ± 0.02; β-ENaC: 0.19 ± 0.05 vs. 1.00 ± 0.05; γ-ENaC: 0.51 ± 0.10 vs. 1.00 ± 0.10, *P < 0.05, Fig. 1, A, B, and C, respectively). There was also a reduction of 11β-HSD2 proteins in the C+OM in BUO rats to 0.72 ± 0.09 of sham levels (Fig. 1D). In contrast, there was no significant change in the abundance of γ-ENaC (70 kDa, data not shown) and β-actin (Fig. 1E).

Immunohistochemistry confirmed reduced expression of α-, β-, and γ-ENaC proteins. Despite a weaker and dispersed labeling in the cytoplasm of principal cells in the cortex from rats with BUO for 24 h (Fig. 2, D–F) compared with sham-operated controls (Fig. 2, A–C), apical targeting of α-, β-, and γ-ENaC was maintained (Fig. 2, D–F). The tubule identity was confirmed by double labeling of γ-ENaC and calbindin-D28k, which revealed reduced labeling of γ-ENaC in the second half of the DCT (DCT2), the CNT, and the CCD in BUO rats (Fig. 3). (The criteria that distinguished DCT2, CNT, and CCD are from Ref. 36).

Release of BUO for 3 Days Increased α-ENaC Expression and Decreased β- and γ-ENaC and 11β-HSD2 Expression Levels

Release of BUO was associated with polyuria and reduced fractional excretion of sodium (Table 1). Interestingly, after release of BUO for 3 days, α-ENaC was dramatically increased from sham levels of 1.00 ± 0.11 to 1.80 ± 0.25 (Fig. 4A), indicating that upregulation of α-ENaC could contribute to the decreased urinary sodium excretion. The abundance of β- and γ-ENaC (85 kDa) and 11β-HSD2 remained reduced (Fig. 4, B, C, and D, respectively). The 70-kDa band of γ-ENaC did not change after release of BUO.

Immunohistochemistry showed that the labeling of α-ENaC in the cortex was clearly increased in the apical plasma membrane and cytoplasm after release of BUO for 3 days compared with that in the cortex from sham-operated rats (Fig. 5, A and D). Consistent with the reduced protein expression of β- and γ-ENaC, labeling of β- and γ-ENaC was weaker and more dispersed in the cytoplasm of principal cells of cortex (Fig. 5, E and F).

Fig. 3. Immunofluorescence microscopy of γ-ENaC and calbindin-D28k in the cortex from control rats (A–C) and BUO rats (D–F). Calbindin-D28k was used as a marker for the second half of the distal convoluted tubule (DCT2; B, C, E, and F) cells and connecting tubule (CNT; E and F) cells, where it is abundantly expressed. γ-ENaC labeling (green) was seen in the cytoplasm of DCT2, CNT, and cortical collecting duct (CCD) cells in the cortex (A, C, D, and F). With double labeling, the tubule segment with γ-ENaC labeling (green) was also calbindin-D28k-positive (red), thus identifying the tubule as CNT or late DCT (DCT2). BUO rats showed a marked reduction in the labeling density of γ-ENaC (arrow, D and E) in the DCT2, CNT, and CCD compared with control rats (A and C).

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UUO for 24 h is Associated With Downregulation of α- and β-ENaC and 11β-HSD2 in Obstructed Kidneys and Upregulation of α-ENaC in Nonobstructed Kidneys

Furthermore, to test whether changes in the expression of ENaC subunits play a role in renal sodium handling during UUO, immunoblots were performed to examine the expression of α-, β-, and γ-ENaC and 11β-HSD2. As shown in Fig. 6, the level of α-ENaC in the C+OM in the obstructed kidney of UUO rats decreased significantly to 0.45 ± 0.03, that of β-ENaC decreased to 0.56 ± 0.03, and that of 11β-HSD2 decreased to 0.67 ± 0.06 of sham levels. The levels of γ-ENaC (both 85 and 70 kDa) in the obstructed kidneys did not change compared with sham controls. Immunoblot did not show any significant change in the expression levels of β-actin between BUO-3dR rats and control rats.

Fig. 4. Semiquantitative immunoblotting using protein from C+OM in control rats and rats with BUO followed by release of obstruction for 3 days (BUO-3dR). Immunoblots were reacted with anti-α-, anti-β-, and anti-γ-ENaC (A–C), anti-11β-HSD2 (D), and anti-β-actin antibodies (E). The expression of α-ENaC was increased significantly in BUO-3dR rats. In contrast, a persistent decrease in β-ENaC, γ-ENaC, and 11β-HSD2 was revealed in BUO-3dR rats compared with sham-operated controls (*P < 0.05). Immunoblot did not show any significant change in the expression levels of β-actin between BUO-3dR rats and control rats.

Fig. 5. Immunoperoxidase microscopy of α-, β-, and γ-ENaC in the cortex from sham-operated controls (A–C) and BUO-3dR rats (D–F). A–C: the labeling of α-, β-, and γ-ENaC was dispersed in the cytoplasm of principal cells in the cortex in sham-operated control rats. D: the apical labeling and the labeling intensity in the cytoplasm of α-ENaC (arrowhead) in the cortex were dramatically increased in BUO-3dR rats compared with control rats. However, the labeling intensity of β- and γ-ENaC (arrows, E and F) was dispersed and appeared weaker in the BUO-3dR rats compared with control rats. Magnification, ×630.
cytoplasmic labeling of α- and β-ENaC and unchanged γ-ENaC labeling in the cortex in the obstructed kidney of UUO rats compared with that in the sham-operated controls (Fig. 2, A–C and G–I).

In the nonobstructed kidneys of UUO rats, α-ENaC protein expression in the C+OM was increased significantly to 1.42 ± 0.08 of sham levels, whereas β- and γ-ENaC and 11β-HSD2 protein expression did not differ from that in the sham controls (Fig. 7). Immunohistochemical analyses showed that apical α-ENaC labeling in the cortex in the nonobstructed kidneys from UUO rats was much stronger (Fig. 2J) than that in sham controls (Fig. 2A); however, the labeling and subcellular distribution of β- and γ-ENaC in the cortex did not change (Fig. 2, B, C, K, and L). These results suggest that the expression levels of α-, β-, and γ-ENaC in rats with BUO and UUO are regulated specifically.

**DISCUSSION**

Our previous series of studies have demonstrated that urinary tract obstruction is associated with a significant reduction in the expression of water channels, sodium, and urea transporters that are paralleled by water and electrolyte losses. In the present study, we further examined the abundance of three ENaC subunits that are paralleled by water and electrolyte losses. In the expression of water channels, sodium, and urea trans-

Since ENaC mediates ultimate regulation of sodium reabsorption in the proximal tubules, distal tubules, and the collecting duct (15). As early dilation of the tubular system due to pressure increases (predominantly the collecting duct and distal tubular segments) and flattening and atrophy of the cells (18). Thus, in the 24-h obstructed kidney (in both BUO and UUO rats), downregulation of three ENaC subunits and 11β-HSD2 was independent of aldosterone levels. Together with our previous findings, which demonstrated downregulation of renal aquaporins, key renal sodium transporters, and urea transporters in response to obstruction, the present findings suggest that reduced expression levels of β- and γ-ENaC could be additional important molecular determinants for the renal tubular function defect observed in response to obstruction.

Interestingly, release of BUO observed for 3 days was associated with increased expression of α-ENaC in the C+OM, whereas β- and γ-ENaC expression remained low. A selective change in the levels of one or two subunits of ENaC, but not all three, has previously been shown in response to a variety of physiological or pathophysiological stimuli (38). It is known that the ENaC-mediated sodium transport can be regulated by the mineralocorticoid aldosterone (11, 25, 26). The regulation is associated with characteristic alterations in the expression of the individual ENaC subunits (25). Increases in circulating aldosterone induced by either NaCl restriction or aldosterone infusion markedly increased the abundance of α-ENaC protein but not the abundance of the β- and γ-ENaC subunits, suggesting that α-ENaC expression regulation is more sensitive to aldosterone than the other two (26). Consistent with this, previous studies have shown that plasma aldosterone was increased in chronic obstructive uropathy in humans (3) and in rats with a 24-h BUO (31). The urinary K/Na ratio has been widely used to evaluate aldosterone activity in the distal nephron. The urinary K/Na ratio was markedly

![Fig. 7. Semiquantitative immunoblotting using protein in the C+OM from the nonobstructed kidneys in UUO rats (UUO-non-OBS) and control kidneys. Immunoblots were reacted with anti-α-, anti-β-, and anti-γ-ENaC (A–C), anti-11β-HSD2 (D), and anti-β-actin antibodies (E). Densitometric analysis revealed an increase in α-ENaC in the nonobstructed kidneys from UUO rats (*P < 0.05). The expression levels of β- and γ-ENaC, 11β-HSD2, and β-actin did not change.](http://ajprenal.physiology.org/)

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increased in rats after 3 days of release of BUO, indicating an increased aldosterone action in the distal nephron and reflecting a recovery of distal tubular response to aldosterone after release of BUO. Thus it is possible that upregulation of α-ENaC protein and increased apical labeling of α-ENaC in the kidney after release of the obstruction secondary to increased aldosterone levels may be compensatory to prevent sodium loss in the distal tubule and the collecting ducts after release of obstruction.

In addition to these changes in renal channels and transporters, multiple factors may be involved in this process, such as changes in renal metabolism and energy and altered responsiveness to hormones (18). Satlin et al. (35) proposed that ENaC is a flow-regulated ion channel. After the release of obstruction, the fraction of filtered water delivered to the early distal segment is increased. It is therefore possible that increased filtered fluid flow might also be involved in regulation of ENaC expression in the distal tubule and the collecting ducts.

Because of the loss of function of the obstructed kidney during UUO, the contralateral nonobstructed kidney undergoes compensatory changes. Fractional excretion of sodium from the contralateral nonobstructed kidney was increased to maintain plasma osmolality and sodium levels in rats with UUO for 24 h. In our previous studies, a dramatic reduction in the abundance of type 2 Na-Pi cotransporter in the cortex and outer medulla and a moderate decreased expression of Na-K-ATPase in the IM of nonobstructed kidney may help to explain the increased sodium excretion during UUO (23). Interestingly, in the nonobstructed kidney of UUO animals, the abundance and apical labeling of α-ENaC was increased significantly, whereas neither the protein levels nor the staining intensity and subcellular distribution of β- and γ-ENaC and 11β-HSD2 differed from that in the sham controls, indicating that upregulated α-ENaC might correspond to the increased aldosterone levels observed in the UUO animals. Furthermore, α-ENaC could be involved in the regulation of sodium and water in UUO. However, the molecular mechanisms are still unclear. Increased renal filtered fluid flow in the contralateral kidney during UUO may be associated with upregulation of α-ENaC. The present study indicates differential regulation of α-, β-, and γ-ENaC subunits in response to ureteral obstruction and release of obstruction. Further studies are needed to address the mechanisms of regulating different subunits of epithelial sodium channels associated with urinary tract obstruction.

It is also likely that the reduced expression of 11β-HSD2 mediated such an aldosterone-like effect in rats with release of obstruction. In mineralocorticoid target tissues, 11β-HSD2 confers MR selectivity by metabolizing hormonally active cortisol to inactive cortisone, allowing aldosterone access to the receptor (4) and avoiding overactivation of MR by glucocorticoid hormone, whose plasma level is much higher than that of aldosterone. Loss-of-function mutation of 11β-HSD2 or inhibition of 11β-HSD2 activity allows glucocorticoid to promote renal sodium retention and potassium excretion in the cortical collecting tubule (39) by activating MR. In the present studies, there was a significant reduction of 11β-HSD2 abundance in the obstructed kidney in rats with BUO, UUO, or release of BUO. Downregulation of 11β-HSD2 expression may result in occupation and activation of MR by cortisol. Thus coordinated activation of MR by both glucocorticoid, as a consequence of reduced activity of 11β-HSD2, and relatively high plasma aldosterone may stimulate the distal renal tubular sodium reabsorption and potassium loss and hence play an important role for regulation of sodium excretion in distal nephron and the collecting ducts in kidneys from rats subjected to ureteric tract obstruction.

Summary

In conclusion, the expression of α-, β-, and γ-ENaC proteins was decreased in rats with 24 h of obstruction. However, the expression levels and apical levels of α-ENaC were increased in the kidneys after release of obstruction as well as in the nonobstructed kidneys from rats with UUO. The alterations of α-, β-, and γ-ENaC expression may play important roles for the altered reabsorption of water and sodium associated with ureteral obstruction and relief of obstruction. Additional studies are needed to sort out the specific mechanisms of the noncoordinated regulation of ENaC subunits in response to ureteral obstruction.

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