Angiotensin II mediates downregulation of aquaporin water channels and key renal sodium transporters in response to urinary tract obstruction

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1The Water and Salt Research Center, 2Institute of Clinical Medicine, and 3Institute of Anatomy, University of Aarhus, Aarhus, Denmark; and 4Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Angiotensin II mediates downregulation of aquaporin water channels and key renal sodium transporters in response to urinary tract obstruction. Am J Physiol Renal Physiol 291: F1021–F1032, 2006. First published June 6, 2006; doi:10.1152/ajprenal.00387.2005.—The renin-angiotensin system is well known to be involved in the pathophysiological changes in renal function after obstruction of the ureter. Previously, we demonstrated that bilateral ureteral obstruction (BUO) is associated with dramatic changes in the expression of both renal sodium transporters and aquaporin water channels (AQPs). We now examined the effects of the AT1-receptor antagonist candesartan on the dysregulation of AQPs and key renal sodium transporters in rats subjected to 24-h BUO and followed 2 days after release of BUO (BUO-2R). Consistent with previous observations, BUO-2R resulted in a significantly decreased expression of AQP1, -2, and -3 compared with control rats. Concomitantly, the rats developed polyuria and reduced urine osmolality. Moreover, expression of the type 2 Na-phosphate cotransporter (NaPi-2) and type 1 bumetanide-sensitive Na-K-2Cl cotransporter (NKCC2) was markedly reduced, consistent with postobstructive natriuresis. Candesartan treatment from the onset of obstruction attenuated the reduction in GFR (3.1 ± 0.4 vs. 1.7 ± 0.3 ml·min⁻¹·kg⁻¹) and partially prevented the reduction in the expression of AQP2 (66 ± 21 vs. 13 ± 2%, n = 7; P < 0.05), NaPi-2 (84 ± 6 vs. 57 ± 10%, n = 7; P < 0.05), and NKCC2 (89 ± 12 vs. 46% ± 11, n = 7; P < 0.05). Consistent with this, candesartan treatment attenuated the increase in urine output (58 ± 4 vs. 97 ± 5 μl·min⁻¹·kg⁻¹, n = 7; P < 0.01) and the reduction in sodium reabsorption (433 ± 62 vs. 233 ± 45 μmol·min⁻¹·kg⁻¹, n = 7; P < 0.05) normally found in rats subjected to BUO. Moreover, candesartan treatment attenuated induction of cyclooxygenase 2 (COX-2) expression in the inner medulla, suggesting that COX-2 induction in response to obstruction is regulated by ANG II. In conclusion, candesartan prevents dysregulation of AQP2, sodium transporters, and development of polyuria seen in BUO. This strongly supports the view that candesartan protects kidney function in response to urinary tract obstruction.

aquaporins; angiotensin; AT1-receptor blockade

URINARY TRACT OBSTRUCTION is a serious disorder that potentially may result in irreversible kidney damage associated with impaired renal function (31). Release of a bilateral ureteral occlusion is characterized by a reduced urinary concentrating capacity, and patients suffer from natriuresis and polyuria. The natriuresis and vasopressin-resistant polyuria that follow ureteral obstruction in patients have been verified in numerous animal studies using models of bilateral ureteral obstruction (BUO) in various species (15, 38). Moreover, in vitro studies have demonstrated a decreased water and salt reabsorption in the proximal tubule, the distal convoluted tubule, and the collecting duct after release of obstruction (20, 21). Previously, it was demonstrated that the abundance of renal aquaporins (AQPs) located in the collecting duct (AQP2, -3, and -4) was reduced in response to BUO, demonstrating a pivotal role for the impaired urinary concentration capacity of the obstructed kidney (15, 30, 38). Furthermore, the abundance of key renal sodium transporters/cotransporters and urea transporters was reduced throughout the nephron in response to BUO and unilateral ureteral obstruction (UUO) (37, 39, 40), indicating that downregulation of renal sodium and urea transport proteins is part of the molecular mechanisms contributing to the urinary concentrating defect in response to urinary tract obstruction. However, the mediators and signaling pathways responsible for this downregulation of membrane transporters remain to be elucidated.

ANG II plays a central role in the development of obstructive nephropathy (14, 24, 31). ANG II is de novo synthesized in the kidney and may have direct actions on tubular transport function (3, 51). Intraparenal ANG II content is increased in response to urinary tract obstruction (11, 14), and the decrease in glomerular filtration rate (GFR) and renal blood flow (RBF) in ureteral obstruction is mediated by ANG II (13, 58). Angiotensin-converting enzyme (ACE) inhibitors or AT1-receptor antagonists partly prevent reduction in GFR and RBF (13, 58), and recent studies have also demonstrated that blockade of the renin-angiotensin system attenuates the expression of an array of cytokines and growth factors with important roles in the impairment of renal functions in rats with UUO (22).

Moreover, it was recently demonstrated that BUO is associated with COX-2 induction in the renal medullary interstitium (47), which may play a role in the effects of ANG II in obstructive nephropathy. COX-2 is constitutively expressed in the macula densa and inner medulla (IM) (19). Additionally, COX-2 expression in the kidney is inducible by dietary salt intake, vasopressin, and ANG II (19). Previous studies have shown that the angiotensin and prostaglandin systems interact in the obstructed kidney. Inhibition of ANG II prevents the enhanced secretion of eicosanoids by the postobstructive kidney in rats subjected to UUO and reduces renal COX-2 expression in UUO (41).

Both vasopressin and ANG II are importantly involved in the renal conservation of water and sodium. Vasopressin is a peptide hormone that controls body fluid osmolality through

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the regulation of renal tubular water and sodium reabsorption (8, 27, 42, 45). Its main sites of action in kidney tubules are the collecting duct and the thick ascending limb (TAL) of the loop of Henle, where vasopressin binds to the vasopressin V2 receptor and stimulates an increase in intracellular cAMP content via adenylyl cyclase (32, 33, 54, 56). Subsequently, cAMP activates protein kinase A, which phosphorylates various proteins including AQP2 and the type 1 bumetanide-sensitive Na-K-2Cl cotransporter (NKCC2) (7, 16, 46). ANG II also stimulates aldosterone secretion and increases sodium and bicarbonate reabsorption in the kidney proximal tubule via AT1 receptors (18, 35, 52, 53). Several studies have demonstrated that ANG II also has an important effect on the TAL and collecting duct in addition to the proximal tubules (2, 35). Moreover, it was recently demonstrated that ANG II AT1-receptor blockade in DDAVP-treated rats was associated with decreased urine concentration and decreased AQP2 and AQP1 expression. Fractional excretion of sodium was also increased in parallel with decreased expression of NHE3, NCC, and Na-K-ATPase (36), supporting the view that ANG II-mediated AT1-receptor activation plays a significant role in regulating expression of renal AQPs and sodium transporters and thereby modulating urine concentration. The purpose of the present study was therefore to examine whether blockade of the AT1 receptor changed the regulation of renal aquaporins and key sodium transport proteins and whether this was associated with corresponding changes in tubular functions of the postobstructed kidney.

METHODS

Experimental Animals

All procedures conformed with the Danish national guidelines for the care and handling of animals and to the published guidelines from the National Institutes of Health. 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. Studies were performed in male Munich-Wistar rats initially weighing 240 ± 10 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 experimental period, the rats were kept in individual metabolic cages with a 12:12-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.

Surgical Procedures

The rats were anesthetized with halothane (Halocarbon Laboratories, River Edge, NJ). Through a midline abdominal incision, 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 ligature. Twenty-four hours later, the obstructed ureters were decompressed by removal of the ligature and the PE tubing. This technique completelyocludes the ureters for 24 h without evidence of subsequent functional impairment of ureteral function.

Candesartan was dissolved in 1 M Na2CO3 and physiological saline and administered by osmotic minipumps (Alzet, model 2001). The pumps were prepared the night before surgery and kept in physiological saline at 37°C. Following the occlusion of the ureters, the pumps were implanted subcutaneously between the scapulae.

Age- and time-matched sham-operated controls (n = 11) were prepared and observed in parallel with each BUO group (Fig. 1). Animals with BUO for 24 h, followed by release of the obstruction (n = 22), had osmotic minipumps implanted and were divided into two groups: The minipumps in the nontreated animals contained isotonic saline (n = 11), and the minipumps in the treated animals candesartan (CV-11974, 1 mg·kg⁻¹·day⁻¹, AstraZeneca, Möln达尔, Sweden, n = 11). Previous studies have demonstrated that this dose of candesartan effectively blocks the AT1 receptor-induced effects in rats (57). The animals were monitored for another 48 h after the obstruction was released, and the two kidneys were then removed and separately prepared for semiquantitative immunoblotting or immunocytochemistry.

Clearance Studies

When the rats were euthanized, 3–4 ml of blood were collected in a heparinized tube for determination of plasma electrolytes and osmolality. Another 2–3 ml of blood were collected in an EDTA tube for aldosterone determination. For clearance studies, urine was collected over the final 24 h of each protocol. The plasma and urinary concentrations of creatinine and urea, and the plasma concentrations of sodium, potassium, and phosphate, were determined (Vitros 950, Johnson & Johnson). The concentrations of urinary sodium, potassium, and phosphate were determined by standard flame photometry (Eppendorf FCM6341). Urine and plasma osmolality was measured with a vapor pressure osmometer (Osmomat 030, Gonotec, Berlin, Germany).

Membrane Fractions for Immunoblotting

After removal, the right kidney was dissected into outer stripe of outer medulla and cortex (OSOM/C), inner stripe of outer medulla (ISOM), and IM. The left kidney was used as a whole kidney (WK). The tissue was immediately homogenized in 1 (IM), 1.5 (ISOM), 2 (OSOM/C), or 9 ml (WK) dissecting buffer (0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, 8.5 μM leupeptin, and 1 mM phenylmethylsulfonyl fluoride, pH 7.2). In addition, the dissecting buffer used for IM contained the phosphatase inhibitors Na⁺-orthovanadate (0.0184 g/100 ml buffer, Sigma S-6508), NaF (0.1052 g/100 ml buffer, Merck, Whitehouse Station, NJ), and okadaic acid (16.4 μl/100 ml buffer, Calbiochem, San Diego, CA). The tissue was homogenized with five strokes of a motor-driven Ultra-Turrax T8
Table 1. Urine output, urine osmolality, urinary sodium excretion, urinary phosphate excretion, and urinary potassium excretion during the experimental period

<table>
<thead>
<tr>
<th>Day(s) After Release</th>
<th>( U_{\text{osm}} ) (mosmol/kgH2O)</th>
<th>( U_{\text{vol}} ) (μl/min/100 g)</th>
<th>( U_{\text{Na}} + U_{\text{vol}} ) (μmol/min/100 g)</th>
<th>( U_{\text{PO4}} + U_{\text{vol}} ) (μmol/min/100 g)</th>
<th>( U_{K} + U_{\text{vol}} ) (μmol/min/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Sham 34±3</td>
<td>1.662±111</td>
<td>5.1±0.2</td>
<td>0.4±0.1</td>
<td>12.3±0.3</td>
</tr>
<tr>
<td>1</td>
<td>BUO 97±6</td>
<td>634±62†</td>
<td>6.9±0.6†</td>
<td>1.5±0.2†</td>
<td>8.9±0.7</td>
</tr>
<tr>
<td></td>
<td>BUO + CAN 58±4</td>
<td>790±52†</td>
<td>4.3±0.4*</td>
<td>4.2±0.6</td>
<td>7.1±0.8</td>
</tr>
<tr>
<td></td>
<td>Sham 27±3</td>
<td>1,381±199</td>
<td>3.8±0.6</td>
<td>0.6±0.1</td>
<td>9.1±0.6</td>
</tr>
<tr>
<td>2</td>
<td>BUO 92±11</td>
<td>662±96†</td>
<td>3.4±0.8</td>
<td>2.0±0.2‡</td>
<td>7.7±1.1†</td>
</tr>
<tr>
<td></td>
<td>BUO + CAN 55±6</td>
<td>1,099±50†</td>
<td>1.6±0.2†</td>
<td>1.1±0.2‡</td>
<td>8.7±0.6†</td>
</tr>
<tr>
<td></td>
<td>Sham 3±1</td>
<td>1,869±160</td>
<td>3.8±0.6</td>
<td>0.1±0.4</td>
<td>11.2±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. **Statistically significant compared with sham-operated controls. †P < 0.01 compared with sham-operated controls.

Table 2. Changes in plasma concentration of aldosterone, osmolality, and renal functions in sham-operated control rats and rats subjected to 24-h BUO and 48-h release with and without candesartan treatment

<table>
<thead>
<tr>
<th></th>
<th>BUO (n = 7)</th>
<th>BUO + CAN (n = 7)</th>
<th>Sham (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_{\text{aldosterone}} ), nmol/l</td>
<td>4.3±0.1†‡</td>
<td>3.0±0.6*‡</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>( P_{\text{osm}} ), mosmol/kgH2O</td>
<td>338±11‡</td>
<td>324±11‡</td>
<td>308±8</td>
</tr>
<tr>
<td>( C_{\text{Cre}} ), mg/dl</td>
<td>1.7±0.3*‡</td>
<td>3.1±0.4*‡</td>
<td>5.9±0.4</td>
</tr>
<tr>
<td>( T^{3}H_{2}O, ) μl/min/100 g</td>
<td>82±11‡</td>
<td>108±11‡</td>
<td>153±8</td>
</tr>
</tbody>
</table>

Values are means ± SE. ††Statistically significant compared with untreated BUO rats, \( P < 0.05 \). †††Statistically significant compared with sham-operated rats, \( P < 0.01 \).
2) NaPi-2 (no. 143 AP): an affinity-purified polyclonal antibody to the Na-phosphate cotransporter of the proximal tubule. This antibody was raised against the same peptide as the anti-NaPi-2 antibody L697 previously characterized by Kim et al. (28). Labeling with 143 AP antibody revealed an 85-kDa band identical to that seen in rat tissues with the L697 antibody.

3) NKCC2 (LL320 serum): a polyclonal antibody to the apical Na-K-2Cl cotransporter of the TAL (9, 27).

4) NCC (LL573 serum): a polyclonal antibody to the apical Na-Cl cotransporter of the distal convoluted tubule (29).

5) Na\(^{+}\)–K\(^{+}\)-ATPase antibody against the \(\alpha_1\)-subunit of Na\(^{+}\)–K\(^{+}\)-ATPase (25).

6) AQP1 (AP Ra 3391/2353 fr.2 and Ra 3391/2352): an affinity-purified polyclonal antibody to AQP1 (34).

7) AQP2 (human AQP2 An 368 AP frakt.2–5): an affinity-purified polyclonal antibody to human AQP2 (23).

8) AQP3 (AP RA 3040/1592 fr. 2–5): an affinity-purified polyclonal antibody to AQP3 (34).

9) COX-2 (catalog no.160126, Cayman Chemical, Ann Arbor, MI).

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Table 3. Expression of sodium transporters in kidneys subjected to 24-h obstruction and 48-h release with and without candesartan treatment and in kidneys from sham-operated controls

<table>
<thead>
<tr>
<th></th>
<th>NHE3</th>
<th>NaPi-2</th>
<th>NKCC2</th>
<th>NCC</th>
<th>Na(^{+})–K(^{+})-ATPase</th>
<th>Na(^{+})–K(^{+})-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUO (n=7)</td>
<td>71±7% (OSOM+C)†</td>
<td>57±10% (OSOM+C)†</td>
<td>22±8% (OSOM+C)†</td>
<td>64±5% (OSOM+C)†</td>
<td>92±3% (OSOM+C)†</td>
<td>83±4% (WK)†</td>
</tr>
<tr>
<td>BUO + CAN (n=7)</td>
<td>72±4% (OSOM+C)†</td>
<td>84±6% (OSOM+C)*</td>
<td>51±1% (OSOM+C)*†</td>
<td>54±9% (OSOM+C)†</td>
<td>79±5% (OSOM+C)†</td>
<td>79±6% (WK)†</td>
</tr>
<tr>
<td>Sham (n=7)</td>
<td>100±5% (OSOM+C)</td>
<td>100±6% (OSOM+C)</td>
<td>100±2% (OSOM+C)</td>
<td>100±5% (OSOM+C)</td>
<td>100±4% (OSOM+C)</td>
<td>100±4% (WK)</td>
</tr>
<tr>
<td>BUO (n=7)</td>
<td>95±8% (ISOM)</td>
<td>46±11% (ISOM)†</td>
<td>61±1% (ISOM)†</td>
<td>98±18% (IM)</td>
<td>83±4% (WK)†</td>
<td>83±4% (WK)†</td>
</tr>
<tr>
<td>BUO + CAN (n=7)</td>
<td>91±10% (ISOM)</td>
<td>89±12% (ISOM)*</td>
<td>38±5% (ISOM)*</td>
<td>45±9% (IM)*†</td>
<td>79±6% (WK)†</td>
<td>79±6% (WK)†</td>
</tr>
<tr>
<td>Sham (n=7)</td>
<td>100±6% (ISOM)</td>
<td>100±6% (ISOM)</td>
<td>100±8% (ISOM)</td>
<td>100±14% (IM)</td>
<td>100±14% (IM)</td>
<td>100±14% (IM)</td>
</tr>
</tbody>
</table>

Values are means ± SE. \(n\), No. of rats; NHE3, type 3 sodium/proton exchanger; NaPi-2, type 2 sodium-phosphate cotransporter; NKCC2, rat type 1 bumetanide-sensitive Na-K-2Cl cotransporter; TSC, thiazide-sensitive Na-Cl cotransporter; OSOM + C, outer stripe of outer medulla and cortex; ISOM, inner stripe of outer medulla; IM, inner medulla; WK, whole kidney. *\(p\) < 0.05 compared with nontreated BUO rats. †\(p\) < 0.05 compared with sham-operated controls.

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Fig. 3. Immunocytochemical analyses of NaPi-2 in the proximal tubule (PT) of cortex from BUO rats (A, D, G), BUO+CAN rats (B, F, H), and sham-operated rats (C, E, I). C: low-power view of the typical distribution of NaPi-2 in cortex in sham-operated rats. F and I: a high power view, NaPi-2 labeling is seen at the apical plasma membrane domains of the PT in sham-operated rats. A, D, G: NaPi-2 labeling in the obstructed kidney is also seen in the PT but is much weaker compared with sham-operated rats. B, E, H: obstructed kidney from candesartan treated rats shows a stronger labeling of NaPi-2 comparable to that seen in sham-operated rats.
Table 4. Phosphate handling in kidneys subjected to 24-h obstruction and 48-h release with and without candesartan treatment and in kidneys from sham-operated controls

<table>
<thead>
<tr>
<th></th>
<th>BUO (n = 7)</th>
<th>BUO + CAN (n = 7)</th>
<th>Sham (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U_{\text{HPO}_4}$</td>
<td>2.0 ± 0.2‡</td>
<td>1.1 ± 0.2‡‡</td>
<td>0.1 ± 0.04</td>
</tr>
<tr>
<td>$\text{FL}_{\text{HPO}_4}$</td>
<td>4.2 ± 0.5‡</td>
<td>6.6 ± 0.8‡ ‡</td>
<td>13.4 ± 0.1‡</td>
</tr>
<tr>
<td>$\text{NetReab. of HPO}_4$</td>
<td>2.2 ± 0.7‡</td>
<td>5.5 ± 1.3‡ ‡</td>
<td>13.4 ± 2‡</td>
</tr>
<tr>
<td>$\text{FEHPO}_4$ (%)</td>
<td>52 ± 6§</td>
<td>20 ± 4† ‡</td>
<td>1.0 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE, n. no. of rats; FL$_{\text{HPO}_4}$, filtered load of $\text{HPO}_4$; NetReab. of HPO$_4$, net reabsorption of phosphate = ($\text{FL}_{\text{HPO}_4}$ − ($U_{\text{HPO}_4}$×$U_{\text{H}_{2}\text{O}}$)); $\text{FEHPO}_4$, fractional excretion of phosphate. *P < 0.05 compared with nontreated BUO rats. ‡P < 0.01 compared with nontreated BUO rats. §P < 0.05 compared with sham-operated controls.

Immunocytochemistry

Kidneys from all three groups were fixed by retrograde perfusion via the abdominal aorta with 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4. For immunoperoxidase staining, the kidney blocks containing all kidney zones were dehydrated and embedded in paraffin. The paraffin-embedded tissues were cut (2 µm) on a rotary microtome (Leica, Solms, Germany). The sections were deparaffinized and rehydrated. For immunoperoxidase labeling, endogenous peroxidase was blocked by 0.5% H$_2$O$_2$ in absolute methanol for 10 min at room temperature. To expose the antigens, sections were put in 1 mM Tris solution (pH 9.0) supplemented with 0.5 mM EGTA and heated in a microwave oven for 10 min. Nonspecific binding of immunoglobulin was prevented by incubating the sections in 50 mM NH$_4$Cl for 30 min, followed by 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 0.5% 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, followed by incubation with diaminobenzidine.

For double labeling, sections were incubated overnight at 4°C with COX-2 (1:5,000 dilution) followed by incubation with horseradish peroxidase-conjugated secondary antibody (P448, DAKO) and labeling was visualized with 3,3′-diaminobenzidine (brown). Endogenous biotin was then blocked with a commercial kit (X0590, DAKO), and the sections were incubated in 3.5% H$_2$O$_2$ in methanol to remove the remaining peroxidase from the first staining. Sections were rinsed with PBS supplemented with 0.1% BSA and 0.05% saponin and then incubated overnight with bioethyalted AQP2 (1:300 dilution). Finally, avidin peroxidase, which binds to biotin, was added, and Vector SG (Vector Laboratories) was used to visualize the bound avidin peroxidase (blue gray label).

Microscopy was carried out using a Leica DMRE (Leica) light microscope.

Statistics

Values are presented as means ± SE. Comparisons between groups were made by unpaired t-test. As a control for a sufficient obstruction, the BUO rats were compared with sham-operated rats. The candesartan-treated rats with BUO were compared with nontreated BUO rats to evaluate the effects of candesartan. $P$ values < 0.05 were considered significant.

RESULTS

Candesartan Treatment Prevents Postobstructive Polyuria and Reduction in GFR

Urine output was measured during the entire experimental period, and the urine osmolality was determined (Table 1). Release of BUO resulted in a marked polyuria and a reduced urine osmolality, which persisted throughout the experimental period (48 h). Administration of candesartan partly prevented this increase in postobstructive urine production (urine output: (BUO vs. BUO + CAN): 97.5 ± 6 vs. 58.1 ± 3 µl·min$^{-1}$·kg$^{-1}$ (n = 7) 24 h after the release of the obstruction ($P < 0.001$) and 92 ± 11 vs. 55.2 ± 6 µl·min$^{-1}$·kg$^{-1}$ (n = 7) at 48 h after the release ($P < 0.01$; Table 1). Creatinine clearance was used as an estimate of GFR and determined in all groups (Table 2). BUO resulted in a significant reduction in creatinine clearance compared with sham-operated rats ($P < 0.001$), which was partially prevented in rats treated with candesartan (Table 2).

The plasma concentration of aldosterone was used as an indicator of candesartan’s inhibition of the AT$_1$ receptor. ANG II stimulates aldosterone secretion from the adrenal glands via the AT$_1$ receptor. Thus, candesartan would be expected to reduce the plasma concentration of aldosterone if it was given in a sufficient dose. BUO induced a marked increase in plasma aldosterone concentration (4.3 ± 0.1 nmol/l (n = 7)) in BUO rats compared with sham-operated rats.
vs. 1.5 ± 0.1 nmol/l (n = 7) in sham-operated control rats; P < 0.001; Table 2). In the candesartan-treated BUO rats, plasma aldosterone concentration was significantly reduced compared with BUO rats (3.0 ± 0.6 vs. 4.3 ± 0.1 nmol/l (n = 7); P < 0.05; Table 2).

Candesartan Treatment Prevents Downregulation of NaPi-2 and Attenuates Renal Phosphate Loss in Response to BUO

Phosphate reabsorption in the kidney is mainly dependent on an intact expression of the NaPi-2 cotransporter localized in the apical plasma membrane of the proximal tubule. Consistent with previous studies (39, 40), a reduction in NaPi-2 expression in OSOM/C was confirmed in rats subjected to BUO (Fig. 2, Table 3). Rats subjected to BUO for 24 h and released for 48 h had decreased NaPi-2 expression (57 ± 10% of sham levels, P < 0.01, Fig. 2, Table 3). Candesartan partially prevented this downregulation, and the expression of NaPi-2 was significantly higher in BUO rats treated with candesartan compared with nontreated BUO rats (84 ± 6 vs. 57 ± 10%, P < 0.05, Fig. 2, Table 3). Immunocytochemistry confirmed that there was an overall decrease in NaPi-2 expression in the apical plasma membrane domains of the proximal tubule cells from BUO rats without candesartan treatment compared with sham-operated control rats (Fig. 3). In the candesartan-treated BUO rats, NaPi-2 labeling was unchanged compared with sham-operated rats, demonstrating that candesartan prevented NaPi-2 downregulation in response to 24-h BUO and a 48-h release period of BUO (Fig. 3).

Consistent with this, release of BUO was associated with a markedly enhanced urinary excretion of phosphate both at 24 and 48 h (Table 1). Candesartan treatment did not change urinary phosphate excretion during the first 24 h after release of BUO, but the increased urinary phosphate excretion was partially prevented 48 h after the obstruction was released (Table 1). Consistent with the increased GFR in the candesartan-treated rats, there was an increased filtered load of phosphate in BUO rats treated with candesartan compared with nontreated BUO rats (Table 4). Thus candesartan treatment resulted in an increased net reabsorption of phosphate, and candesartan prevented the significant increase in fractional excretion of phosphate observed 48 h after release of BUO (20 ± 4 vs. 52 ± 6%, n = 7, P < 0.01, Table 4).

Candesartan Treatment Prevents Downregulation of NKCC2 Expression and Attenuates Renal Sodium Loss in Response to BUO

NKCC2 in the thick ascending limb is important for generation of the corticomedullary concentration gradient. The expression level of NKCC2 in ISOM and in OSOM/C was reduced by BUO as previously demonstrated (Fig. 4, A and B, Table 3). Immunoblotting revealed that treatment with candesartan almost completely prevented the decrease in NKCC2 expression in ISOM observed in rats subjected to 24-h BUO and a 48-h release period (89 ± 12 vs. 46 ± 11% of sham levels, P < 0.05, Table 3). However, in OSOM/C candesartan...
treatment only partially prevented downregulation of NKCC2 expression (51 ± 1% of sham levels compared with 22 ± 8% in kidneys from nontreated BUO rats; *P < 0.05, Fig. 4, A and B, Table 3). Immunocytochemistry confirmed that there was an overall decrease in NKCC2 expression in the apical plasma membrane domains of cortical and medullary thick ascending limb cells from BUO rats without candesartan treatment compared with candesartan-treated BUO or sham-operated control rats (Fig. 5). In candesartan-treated BUO rats, NKCC2 labeling was unchanged compared with sham-operated rats, demonstrating that candesartan treatment inhibited the decreased expression of NKCC2 in response to 24-h BUO and a 48-h release period (Fig. 5).

Importantly, the decrease in proximal and TAL absorption in BUO rats caused a large decrease in GFR, presumably because of increased tubuloglomerular feedback. Consistent with these changes, urinary sodium excretion was significantly increased compared with sham-operated rats. Candesartan treatment almost prevented this increase in urinary sodium excretion 24 h after release of BUO (Table 1). The same was seen 48 h after release, although the difference between the treated and nontreated BUO rats was not significant. Consistent with the increased creatinine clearance, the filtered load of sodium was higher in the candesartan-treated rats compared with nontreated rats (*P < 0.05; Table 5). Thus in candesartan-treated rats, the net reabsorption of sodium was increased [433 ± 62 vs. 233 ± 45 μmol·min⁻¹·kg⁻¹ (n = 7), *P < 0.05; Table 5].

**Candesartan Treatment Prevents Downregulation of AQP2 Expression**

The vasopressin-regulated water channel AQP2 is responsible for the final water reabsorption in the collecting ducts. Consistent with previous studies (15, 30, 38), immunoblotting showed downregulation of AQP2 expression in the IM and OSOM/C in response to 24-h BUO and a 48-h release period of BUO (Fig. 6, A and B, Table 6). As shown in Table 6, candesartan administration partially prevented this reduction in AQP2 expression in IM (66 ± 21% of sham levels; Fig. 6A, Table 6) and completely prevented downregulation in OSOM/C (101 ± 8% of sham levels; Fig. 6B, Table 6). Immunocytochemistry confirmed that there was an overall decrease in AQP2 expression in the apical plasma membrane domains and intracellular vesicles of the inner medullary collecting duct principal cells from BUO rats compared with sham-operated control rats and that AQP2 downregulation was prevented by candesartan treatment (Fig. 7). Consistent with this, there was a significant reduction in urine output in the candesartan-treated rats both 24 and 48 h after release of BUO.

**Candesartan Treatment Attenuates COX-2 Induction in IM of BUO Kidneys**

COX-2 expression increased markedly to 1,240 ± 203% of sham levels in IM of kidneys subjected to 24-h BUO followed by release for 48 h (*P < 0.05, Fig. 8, Table 6). Candesartan treatment significantly attenuated this COX-2 induction. Im-

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**Table 6. Expression of aquaporins and cyclooxygenase 2 in different zones of kidneys subjected to 24-h obstruction and 48-h release with and without candesartan treatment and in kidneys from sham-operated controls**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>AQP1 (WK)</th>
<th>AQP2 (IM)</th>
<th>AQP2 (OSOM+C)</th>
<th>AQP3 (IM)</th>
<th>COX-2 (IM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUO (n = 7)</td>
<td>67±6%†</td>
<td>13±2%†</td>
<td>57±9†</td>
<td>57±7%†</td>
<td>1,236±203%†</td>
</tr>
<tr>
<td>BUO + CAN (n = 7)</td>
<td>76±9%</td>
<td>66±21%*</td>
<td>101±8%*</td>
<td>72±19%</td>
<td>598±151%*</td>
</tr>
<tr>
<td>Sham (n = 7)</td>
<td>100±8%</td>
<td>100±11%</td>
<td>100±8%</td>
<td>100±12%</td>
<td>100±54%</td>
</tr>
</tbody>
</table>

Values are means ± SE. *No. of rats: AQP1, aquaporin-1; AQP2, aquaporin-2; AQP3, aquaporin-3; COX-2, cyclooxygenase 2; WK, whole kidney; OSOM + C, outer stripe of outer medulla and cortex; IM, inner medulla. †*P < 0.05 compared with nontreated BUO rats. ‡P < 0.01 compared with sham-operated controls.
munoblotting revealed a decrease in COX-2 expression in IM to 500 ± 151% of sham levels in BUO rats treated with candesartan (P < 0.05, Fig. 8, Table 6) compared with non-treated BUO rats. Consistent with previous findings (47), immunocytochemistry demonstrated distinct COX-2 labeling in interstitial cells at the base of the IM in response to BUO (Fig. 9A), whereas no labeling was seen in sham rats (Fig. 9C). Candesartan treatment reduced COX-2 labeling at the base of the IM of BUO rats (Fig. 9B). As expected, we observed localization of COX-2 in interstitial cells at the tip of the IM and there was no difference in the labeling intensity among the three groups (Fig. 9, D–F). Double labeling of the IM showed no colocalization of AQP2 and COX-2 (Fig. 9, G–I). COX-2 was exclusively found in the interstitial cells in the IM, and as expected AQP2 was localized to the collecting ducts. Interestingly, double labeling demonstrated that labeling of AQP2 is absent or very weak in areas with high COX-2 density, whereas AQP2 labeling is stronger in areas without COX-2 expression.

Expression of Na-K-ATPase, NCC, NHE3, AQP1, and AQP3

As previously demonstrated (15, 30, 40), BUO was associated with decreased expression levels of Na-K-ATPase, the thiazide-sensitive Na-C1 cotransporter (NCC), the type 3 Na/H exchanger (NHE3), and AQP1 and AQP3 (Fig. 10, Tables 3 and 6). Administration of candesartan did not prevent down-regulation of these sodium transporters and aquaporins in response to 24-h BUO followed by a 48-h release period.

DISCUSSION

Previously, we have demonstrated that ureteral obstruction reduces expression levels of aquaporins and various key sodium transporters along the nephron, providing an explanation for the polyuria and sodium loss observed after release of ureteral obstruction (15, 40). The main findings in the present study demonstrated that treatment with the AT1-receptor antagonist candesartan partially prevented the decrease in the expression of NaPi-2, NKCC2, and AQP2 in the kidney 2 days after release of BUO. This was paralleled by attenuation of the impairment of phosphate, sodium, and water reabsorption in the tubules of the postobstructed kidney, demonstrating an association between the molecular and functional changes at
these sites of the nephron. The findings indicated that intrarenal ANG II plays an important role in the regulation of sodium transporters and aquaporins in obstructive nephropathy.

**Candesartan Reduces Plasma Aldosterone Levels**

Previously, it was demonstrated that ureteral obstruction induces a substantial increase in plasma aldosterone levels (14). The results of the present study showed that candesartan reduced the increase in plasma aldosterone levels in obstructed animals, consistent with the AT1 receptor-mediated effects of ANG II in the adrenals. The inability to normalize plasma aldosterone by AT1-receptor antagonist treatment have been reported by other groups (5, 13), suggesting that there might be an AT1 receptor-independent pathway involved in the aldosterone release associated with ureteral obstruction.

**Candesartan Prevents Downregulation of NaPi-2 Expression in the Proximal Nephron**

The type II NaPi-2 cotransporter localized in the kidney and in the intestine is the main transporter responsible for inorganic phosphate homeostasis in rats (43). Recently, we demonstrated that BUO and release of BUO are associated with a marked reduction in the expression of NaPi-2 in the proximal tubules. Consistent with this, NaPi-2 expression was significantly reduced in kidneys subjected to 24-h BUO followed by a 48-h release period. In parallel, renal phosphate excretion was increased after release of ureteral obstruction, consistent with previous studies (50). Candesartan treatment almost completely prevented downregulation of NaPi-2 expression in the postobstructed kidney, suggesting that ANG II may play an important role in NaPi-2 downregulation. Importantly and consistent with the prevention of NaPi-2 downregulation after candesartan treatment, the present study also demonstrated that fractional renal phosphate excretion was attenuated and the net renal reabsorption of phosphate was increased compared with untreated BUO rats. These findings demonstrated that there is a functional association between the molecular changes in the NaPi-2 cotransporter and the physiological change in renal phosphate handling. Previously, it was suggested that atrial natriuretic peptide (ANP) is important for regulation of NaPi-2 activity by reducing phosphate transport across the proximal tubule cells. Because it is well known that ANP levels increase in response to BUO (48), it is likely that high ANP levels present immediately after release of the obstruction could add to the dysregulation of phosphate handling in the proximal tubule at that point and may explain that renal phosphate handling was not completely normalized with candesartan treatment despite the prevention of NaPi-2 downregulation.

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**Fig. 9.** Immunocytochemical analyses of COX-2 in the base of inner medulla (IM) and the tip of inner medulla (IM) from BUO rats (A, D), BUO+CAN rats (B, E), and, sham-operated rats (C, F). In sham-operated rats there was no labeling of COX-2 in the base of IM (C) but labeling was observed in the tip of IM (F). In the obstructed kidneys COX-2 labeling were seen in both the base and the tip of inner medulla (A, D). Candesartan treatment attenuated the COX-2 induction in base of inner medulla (B), whereas no difference was found in the tip of IM (E). Double labeling showed no colocalization of COX-2 and AQP2 in IM (G, H, I). COX-2 was found in the interstitial cells whereas AQP2 was only seen in the connecting tubules.
Candesartan Prevents Downregulation of NKCC2 Expression and Reduction in GFR

NKCC2 is expressed throughout the TAL and in the macula densa. This transporter is important for establishing the corticomedullary concentration gradient and is involved in regulation of the tubuloglomerular feedback mechanism. Previously, we demonstrated that the expression level of NKCC2 is reduced as a consequence of ureteral obstruction (39, 40). Consistent with this, the results of the present study demonstrated that expression of NKCC2 in both the ISOM and OSOM/C was markedly reduced in response to 24-h BUO and a 48-h release period. Treatment with candesartan attenuated the reduction in NKCC2 expression in both the ISOM and OSOM/C, suggesting that ANG II plays an important role in regulation of NKCC2 expression during urinary tract obstruction. Importantly and consistent with the attenuation of NKCC2 downregulation after candesartan treatment, the present study also demonstrated that the net renal reabsorption of sodium was increased compared with untreated rats. This finding demonstrated that there is a functional association between the molecular changes in the NKCC2 cotransporter and the physiological change in renal sodium handling.

The decrease in proximal tubule apical sodium transporters in BUO rats likely is a reflection of glomerulotubular balance. The present GFR reduction in BUO rats should then be associated with a decreased tubular reabsorption of water and sodium at these sites (17). Thus downregulation of the abundance of renal tubule apical salt and water transport proteins, which is often rate limiting for transport (59), could be the molecular means through which this is accomplished. The significant increase in GFR in rats treated with candesartan compared with untreated rats supports the view that ANG II is an important mediator of the vasoconstriction associated with BUO. This finding is also consistent with a previous study demonstrating prevention of GFR reduction after treatment using an ACE inhibitor (49). The parallel change in NKCC2 may reflect maintenance of glomerulotubular balance during candesartan treatment. Alternatively, NKCC2 may be directly regulated via AT1 receptors. Recently, it was demonstrated that AT1 receptors are present in medullary TAL cells and it is therefore conceivable that ANG II directly affects NKCC2 expression. Amlal et al. (1) demonstrated a biphasic response of ANG II on NKCC2 activity. Low concentrations of ANG II (10^{-14}-10^{-12} M) inhibited NKCC2 activity, and high concentrations (10^{-11}-10^{-8} M) stimulated it.

Candesartan Prevents Downregulation of AQP2 Expression in the Collecting Duct

The collecting duct water channels are essential for the kidney’s ability to concentrate urine. Previous studies have shown that AQP2 and AQP3 expression is severely reduced in response to ureteral obstruction (15, 30, 38), which was confirmed by the present study. Candesartan treatment prevented the reduction in AQP2 expression levels, whereas AQP3 expression was unchanged. Consistent with this, candesartan treatment was associated with a marked reduction in the development of polyuria, demonstrating a functional association between the molecular changes in AQP2 and the physiological change in collecting duct water handling.

The well-described downregulation of AQP1 expression in response to ureteral obstruction (15, 30, 38) was confirmed in the present study. This effect persisted in candesartan-treated animals, which corresponds to an unchanged reduction in the urinary concentrating capacity after candesartan treatment, consistent with previous findings (55).
Possible Mediators of Effects of Candesartan in Obstructive Nephropathy

Numerous hormones have been considered to be involved in the pathogenesis of obstructive nephropathy, including ANG II, ANP, aldosterone, prostaglandins, and vasopressin (31). In the present study, the role of ANG II has been addressed. Binding of ANG II to the AT₁ receptor activates a complex intracellular signal system. Interestingly, stimulation of the AT₁ receptor in rat glomeruli with ANG II inhibits adenyl cyclase and decreases intracellular cAMP generation (10). Kim and colleagues (30) have demonstrated reduced cAMP generation in response to anginine vasopressin in the inner and outer medulla and cortex in response to BUO. Both AQP2 and NKCC2 are regulated by cAMP, and therefore it is likely that the increased intrarenal ANG II generation in the obstructed kidney could possibly play a role in these changes by blocking the intracellular pathways involved in regulation of the expression of AQP2 and NKCC2. Further evidence for this was provided because candesartan treatment partly prevented these changes. Thus it may be speculated that candesartan treatment increases intracellular CAMP levels in this model.

Recently, it was demonstrated that BUO is associated with a dramatic COX-2 induction in the renal medullary interstitium, which may play a role for the initial inflammatory response in the obstructed kidney. Furthermore, it was demonstrated that COX-2 inhibition prevents downregulation of AQP2 and attenuates the decrease in NKCC2 and NHE3 expression associated with BUO (6, 47). Consistent with this, the present study demonstrated increased COX-2 expression in the IM of BUO kidneys, which was attenuated by candesartan treatment. Immunocytochemistry confirmed this reduction in COX-2 in the IM, or more specifically in the base of the IM, and revealed an association between areas with high COX-2 levels and downregulation of AQP2 in the IM. The present results therefore suggest that blockade of the AT₁ receptor may play a significant role in the regulation of COX-2 expression in the renal medulla of the postobstructed kidney, consistent with previous findings (41). Blockade of the AT₁ receptor as well as COX-2 inhibition had identical effects on the expression of NKCC2 and AQP2 in the postobstructed kidney, so it might be speculated that the effect of candesartan in the present study is at least partially mediated via an inhibition of COX-2 and a reduced synthesis of prostaglandins. The prostaglandin receptors EP₁ and EP3 are localized in the TAL and the collecting duct, respectively, and stimulation of the receptors results in natriuresis and diuresis. The EP₁ receptor acts via an increase in intracellular Ca²⁺, whereas the EP3 receptor mediates its effects via a decreased synthesis of cAMP (4). Thus blockade of COX-2 increases cAMP production similar to AT₁-receptor blockade, which could explain the identical effect of COX-2 blockade and AT₁-receptor blockade on the expression of specific transport proteins in this model. However, the inhibitory effect of COX-2 on vasopressin-stimulated water permeability in inner medullary collecting ducts has been demonstrated to be at a post-CAMP site (44).

In conclusion, candesartan treatment prevented the decreased expression of NKCC2 and AQP2 and partially prevented downregulation of NaPi-2 in response to BUO. Moreover, candesartan treatment attenuated the postobstructive polyuria as well as the renal sodium and phosphate loss in response to release of BUO, demonstrating an association between the molecular and functional changes. Finally, the BUO-induced COX-2 expression was partially prevented by candesartan, suggesting that AT₁-receptor-mediated effects may play an important role in the inflammatory response in the obstructed kidney.

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REFERENCES

14. Froikj J, Knudsen L, Nielsen AS, Pedersen EB, and Djurhuus JC. Enhanced intrarenal angiotensin II generation in response to obstruction of


