Candesartan prevents long-term impairment of renal function in response to neonatal partial unilateral ureteral obstruction

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1The Water and Salt Research Center, 2Institute of Clinical Medicine, 3Institute of Anatomy, University of Aarhus, 4Department of Clinical Physiology, 5The MR Research Centre, and 6Department of Urology, Aarhus University Hospital-Skejby, Aarhus, Denmark; and 4Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Submitted 27 June 2006; accepted in final form 2 October 2006

Topcu SO, Pedersen M, Nørregaard R, Wang G, Knepper M, Djurhuus JC, Nielsen S, Jørgensen TM, Frøkiaer J. Candesartan prevents long-term impairment of renal function in response to neonatal partial unilateral ureteral obstruction. Am J Physiol Renal Physiol 292: F736–F748, 2007. First published October 10, 2006; doi:10.1152/ajprenal.00241.2006.—Angiotensin II (ANG II) plays an important role in the development of obstructive nephropathy here, we examined the effects of the ANG II receptor type 1 (AT1R) blockade using candesartan on long-term renal molecular and functional changes in response to partial unilateral ureteral obstruction (PUUO). Newborn rats were subjected to severe PUUO or sham operation (Sham) within the first 48 h of life. Candesartan was provided in the drinking water (10 mg/kg · day−1) from day 21 of life until 10 wk of age. Renal blood flow (RBF) was evaluated by MRI, glomerular filtration rate (GFR) was measured using the renal clearance of 51Cr-EDTA, and the renal expression of Na-K-ATPase in response to congenital PUUO in rats, suggesting that AT1R blockade may protect the neonatally obstructed kidney against development of obstructive nephropathy.

CONGENITAL OBSTRUCTIVE NEPHROPATHY is the primary cause for end-stage renal disease (ESRD) in children (2). As many as 1% of newborn infants have a prenatal diagnosis of hydropnephrosis or significant renal pelvic dilation (11). The management of these patients remains controversial, some advocating for an early surgical repair whereas others favoring observation unless renal function decreases (21).

Several animal models have been developed to understand the development and pathophysiology of congenital obstructive uropathy. More than 90% of nephrogenesis in the rat takes place postnatally in the first 10 days of life, resembling the midtrimester in the human fetus (5), which makes neonatal ureteral obstruction (UUO) attractive as an experimental model for congenital obstructive nephropathy. Recently, Wen et al. (48) developed a newborn rat model with severe partial obstruction, which was associated with the development of progressive hydropnephrosis.

ANG II, the primary product of the renin-angiotensin system (RAS), is thought to play a crucial pathophysiological role in the functional changes in response to urinary tract obstruction (10). Furthermore, ANG II has also been demonstrated to play a critical role in kidney development (39). There are two major types of ANG II receptors, type 1 (AT1) and type 2 (AT2), but the AT1 receptor is much better characterized than the AT2 receptor. The AT1 receptor is localized in proximal tubules, cortical and medullary collecting duct, and glomeruli, where it is primarily present on the mesangial cells. Furthermore, the AT1 receptor is found on vascular smooth muscle cells in the arteriolar, interlobular, and arcuate arteries (16).

Inhibition of the RAS by both AT1 blockade and angiotensin I-converting enzyme (ACE) inhibitors shows renoprotective effects in rats with chronic renal failure (38). In addition, Moriyama et al. (31) found that the ANG II receptor antagonist TCV-116 ameliorates the interstitial fibrosis and the progression of morphological changes in rat kidneys in response to complete UUO. Other studies have also shown that AT1 receptor blockade improved tubulointerstitial fibrosis in rats subjected to UUO (18, 47).

Inflammatory cell infiltration plays a key role in the onset and progression of renal injury in response to obstruction. Nuclear factor-κB participates in the inflammatory response, regulating many proinflammatory genes. In a recent study, it was demonstrated that ANG II, both via AT1 and AT2 receptors, activates NF-κB in response to obstruction in a mouse model (13). Pharmacological blockade of the RAS prevented NF-κB activation and upregulation of NF-κB-related proinflammatory genes (32), and inhibition of NF-κB activation...
prevented inflammatory injury in UUO (30). Thus NF-κB may play a pivotal role in the kidney injury observed in response to congenital induced ureteral obstruction.

Aquaporins (AQPs) are a family of membrane water channels that mediate rapid water transport across the cell membrane. Recent studies have demonstrated that AQP1–4 were reduced in response to both bilateral ureteral obstruction (BUO) and UUO (14, 23, 35, 37). Additionally, Li et al. (24, 25) demonstrated that BUO and UUO in adult rats were associated with a reduced abundance of several major renal sodium transporters. Thus it is highly likely that dysregulation of renal AQPs and sodium transporters is responsible for the altered renal handling of water and sodium in response to ureteral obstruction. Importantly, this view was supported by studies in rats subjected to congenital UUO which demonstrated significant downregulation of AQP1, AQP3, and Na-K-ATPase and, consistent with these molecular changes, the obstructed kidneys had reduced solute-free water reabsorption and natriuresis (42). Importantly, this study demonstrated that release of obstruction after 1 wk, but not after 4 wk, prevented the majority of the changes in kidney function induced by the partial UUO, suggesting that early release of an obstruction protected the kidney from obstructive damage. Moreover, we have recently demonstrated in adult rats that treatment with candesartan partially prevented the decrease in AQP2 and Na-K-2Cl cotransporter expression in the kidney 2 days after release of BUO (19). This was paralleled by prevention of an 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 regulation of sodium transporters and AQPs in obstructive nephropathy (19).

The aims of the present study were therefore to examine whether blockade of the AT1 receptor after completion of nephrogenesis in newborn rats with severe partial obstruction prevented the reduction in renal function as well as dysregulation of renal AQPs and sodium transporters and to explore potential pathways involved in this.

**MATERIALS AND METHODS**

**Experimental Protocol**

This study included 44 (2-day-old) female Munich-Wistar rats (Mollegaard, Aarhus, Denmark), weighing 6–8 g, randomized into two groups: 1) severe PUUO (n = 21), and 2) sham operation (n = 23). Newborn rats are poikilothermic, and adult thermoregulatory capabilities do not develop until week 3 of life. Because of their small body mass, rapid core cooling can be achieved by surface cooling (40). Accordingly, the newborn rat was placed on crushed ice for about 8–10 min. When the newborn rat was deeply asleep, the rat was removed to the operating table. If needed, additional crushed ice was placed around the body and neck region during the operation. Normally, placement of a newborn rat on crushed ice for 8–10 min is sufficient to maintain anesthesia for 30–40 min.

Severe partial obstruction of the left ureter was produced according to a modification of the technique of Ulm and Miller (46). The left ureter was exposed via an abdominal transperitoneal incision using a microscope with ×25 magnification. The underlying psoas muscle was split longitudinally to form a groove, into which the upper two-thirds of the left ureter was embedded. The muscle edges were closed by ethilon sutures (9-0). In the sham group, a laparotomy was performed and the left ureter was exposed. Afterward, the rats were placed in an incubator with a temperature of 28°C until they totally awakened.

The mother rats had free access to a standard rodent diet and were kept in an animal facility with a controlled room temperature of 21 ± 2°C and 12:12-h dark-light cycle.

Two rats from the PUUO group and two rats from the sham group were eaten by mother rats during the preweaning term (before day 21 of life). Two rats from the PUUO group had a wound infection, so they were excluded from the study at day 10 of life. At day 21 of life, the rats were separated from their mother and divided randomly into the following four protocols.

Protocol 1 (n = 8): severe PUUO.

Protocol 2 (n = 9): severe PUUO treated with the AT1 receptor antagonist candesartan (PUUO+CAN).

Protocol 3 (n = 10): sham rats treated with candesartan (Sham+CAN).

Protocol 4 (n = 11): sham-operated rats (Sham).

All procedures conformed with the Danish National Guidelines for care and handling of animals and to the published guidelines from the National Institutes of Health. The experimental animal protocols were approved by the board of the Institute of Clinical Medicine, University of Aarhus, according to rules issued by the Danish Ministry of Justice. Candesartan cilexetil (10 mg kg⁻¹day⁻¹, AT1 receptor antagonist, batch no: 300087–05, AstraZeneca, Mölndal, Sweden) was suspended in 5% gum arabic (Sigma). From day 21 to week 10, candesartan cilexetil was provided in the drinking water daily at a dosage of 10 mg·kg⁻¹·day⁻¹, which complies with the pharmacological profile previously reported (15, 31, 33, 34).

At 10 wk of age, each rat was placed in an individual metabolic cage and allowed 2 days of acclimatization followed by three consecutive 24-h urine collections. Moreover, the intake of water and food was measured daily.

**MRI**

MRI was performed with a small-bore 7-T system (Varian, Palo Alto, CA). The rat was placed supine in a Helmholtz head-coil of 4 cm in diameter and subjected to an imaging protocol including measurements of single-kidney renal blood flow (RBF) and total kidney volume (TKV). Rats were anesthetized with 50 mg/kg body wt pentobarbital sodium (ip), and body temperature was maintained at 37°C during the studies.

RBF measurements were performed using a velocity-sensitive technique, involving a gradient echo-pulse sequence employed with bipolar gradients. The strengths of the velocity-encoding gradients were set according to the values from previous studies (42, 43, 49, 50). In brief, 10 slices of 1.2-mm thickness were prescribed perpendicularly to the renal veins. Each slice had a 7 × 7-cm² field of view and a resolution of 350 × 350 pixels to ensure appropriate pixels to find and derive the blood velocity in the renal veins. Other parameters included repetition time (TR) = 150 ms, echo time (TE) = 5.5 ms, flip angle = 55°, and no. of data averages = 4. Acquired phase images were subtracted, and the vein flow was determined by multiplication with the renal vein area in each available slice. The single-kidney RBF was then calculated as the average of the flow values found in all slices (43).

A gradient echo sequence was used to obtain a series of axial slices through both kidneys to determine TKV. Dependent on the kidney size, 20–30 equidistant slices of 1.0-mm thickness were employed to sufficiently cover both kidneys. The field of view and pixel size were identical as described for the RBF measurements, and other parameters were: TR = 125 ms and TE = 4 ms. Postprocessing included manual identification of each kidney for all slices, and, by careful encompassing of the regions of interest, TKV was measured by the sum-of-areas principle (43, 50).
Measurement of GFR

GFR was evaluated from renal clearance of \( ^{51} \text{Cr}-\text{EDTA} \) as previously described (43). In brief, the left femoral artery and vein were catheterized under inhalation anesthesia with isoflurane (Abbott Scandinavia). The anesthetized rats were placed on a heating table to maintain a rectal temperature of 37°C. The arterial and venous catheters were fixed as described by Shi et al. (43). To collect urine, both ureters were exposed and catheterized (0.8-mm flexible plastic tubing, Tygon, Weyerhauser, Cleveland, OH) through a midline incision. Afterward, a priming dose of \( ^{51} \text{Cr}-\text{EDTA} \) (0.2 MBq) was given (iv) for 15 min, followed by a sustained infusion (0.005 MBq/min) during a 75-min equilibration period, which was then followed by two 1-h urine collection periods. An intravenous infusion of 25 mM glucose (40 \( \mu \text{mol/min} \)) was provided simultaneously to keep an adequate minimum urine flow rate necessary for biochemical analysis of the collected urine. Timed blood samples (150 \( \mu \text{l} \)) were withdrawn from the arterial catheter every hour during the urine collection periods and replaced immediately with a similar volume of heparinized donor blood without changing hematocrit. Timed urine samples were gravimetrically collected every hour from both ureters. During the experiment, the incision was closed to prevent loss of body fluid. The plasma and urine samples were diluted, and \( ^{51} \text{Cr}-\text{EDTA} \) was counted in a scintillation system (Cobra, Packard Instrument, Meriden, CT) (42, 43).

Before the rats were killed, 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. The plasma and urinary concentrations of creatine, urea, and phosphate and the plasma concentrations of sodium and potassium were determined (Vitros 950, Johnson & Johnson). The concentrations of urinary sodium and potassium were determined by standard flame photometry (Eppendorf FCM6341). The urine and plasma osmolalities were measured with a vapor pressure osmometer (Osmomat 030-D, Gonotec, Berlin, Germany). The harvested kidneys were rapidly frozen in liquid nitrogen and kept at \(-80^\circ \text{C} \) until assayed.

Analysis of Renal AQP5 and Key Sodium Transport Proteins

Membrane fractionation for immunoblotting. Kidneys were minced finely and homogenized in 9 ml of dissecting buffer (0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, pH 7.2, containing protease inhibitors 8.5 \( \mu \text{M} \) leupeptin and 1 mM phenylmethylsulfonyl fluoride) with five strokes of a motor-driven Ultra-Turrax homogenizer (IKA Labortechnik, Staufen, Germany) at 1,250 rpm. This homogenate was centrifuged in a Beckman L8M centrifuge at 4,000 \( g \) for 15 min at 4°C, and gel samples (Laemmli sample buffer containing 2% SDS) were made of this pellet. The total protein concentration of the homogenate was measured using a Pierce BCA protein assay kit (Roche).

Primary antibodies. For semiquantitative immunoblotting and immunocytochemistry, we used the characterized monoclonal and polyclonal antibodies as summarized below.

- AQP1 (CHIP serum or LL266AP): immune serum or an affinity-purified antibody to AQP1 (44).
- AQP2 (LL127 serum or LL127AP): immune serum or an affinity-purified antibody to AQP2 (7).
- AQP3 (LL178AP): an affinity-purified polyclonal antibody to AQP3 has previously been described (8).
- Na-K-ATPase: a monoclonal antibody against the \( \alpha_1 \)-subunit of Na-K-ATPase has previously been described (20).

Electrophoresis and immunoblotting. Samples of membrane fractions from whole kidney were run on 9 or 12% polyacrylamide mini-gels (Bio-Rad Mini Protein II). For each gel an identical gel was run in parallel and subjected to Coomassie staining. 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 being transferred by electroelution to nitrocellulose membranes, blots were blocked with 5% milk in PBS-T (80 mM Na2HPO4, 20 mM NaH2PO4, 100 mM NaCl, 0.1% Tween 20, pH 7.5) for 1 h and incubated with primary antibodies overnight at 4°C. After being washed, the blots were incubated with horseradish peroxidase-conjugated secondary antibody (Dako, Glostrup, Denmark).

For a final washing, antibody binding was visualized using the enhanced chemiluminescence (ECL) system (Amersham International). ECL films were scanned using a Hewlett-Packard Scanjet scanner and Adobe Photoshop software. The labeling density was determined from blots, where samples of kidneys from each group were run. The labeling density was corrected by densitometry of Coomassie brilliant blue-stained gels to detect minor differences in protein loading.

Immunocytochemistry

The kidneys from experimental 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, the blocks of the kidneys containing all zones were dehydrated and embedded in paraffin. The paraffin-embedded tissues were cut on a rotary microtome (Leica, Heidelberg, Germany).

The sections were subsequently deparaffinized and rehydrated. For immunoperoxidase labeling, endogenous peroxidase was blocked by 0.5% \( \text{H}_2\text{O}_2 \) in absolute methanol for 10 min at room temperature. To reveal antigens, the sections were put in a 1 mM Tris solution (pH 9.0) supplemented with 0.5 mM EDTA and 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. Finally, sections were incubated overnight at 4°C with primary antibodies diluted in PBS-T supplemented with 0.1% BSA and 0.3% Triton X-100. The following day, the sections were washed 3 \( \times \) 10 min in PBS-T with 1% BSA and 0.3% Triton X-100 before being incubated in horseradish peroxidase-conjugated secondary antibodies (Dako) diluted in PBS-T supplemented with 0.1% BSA and 0.3% Triton X-100. Afterward, the sections were incubated in diaminobenzidine. Microscopy was carried out using a Leica DMRE light microscope (Leica).

Statistical Analysis

Values are expressed as means \( \pm \) SE. Statistical analyses were carried out by one-way ANOVA followed by appropriate post hoc tests with the Bonferroni test, and \( P < 0.05 \) was considered statistically significant.

RESULTS

Candesartan Prevents a Significant Reduction in GFR

Kidney GFR was measured using renal clearance of \( ^{51} \text{Cr}-\text{EDTA} \) at 10 wk after the onset of PUUO. In the PUUO group, total GFR was significantly reduced compared with sham-operated rats (\( P < 0.05 \), Fig. 1A). Candesartan treatment partially prevented this GFR reduction. To examine the single-kidney GFR (SKGFR) the renal clearance of \( ^{51} \text{Cr}-\text{EDTA} \) was obtained from each kidney. Neonatally induced PUUO caused a significant reduction of SKGFR in the obstructed kidney compared with the left kidney in sham-operated rats (\( P < 0.05 \), Fig. 1B). In contrast, candesartan treatment of PUUO rats...
demonstrated a partial prevention of the SKGFR reduction in the obstructed kidney \((P < 0.05, \text{Fig. 1B})\). SKGFR in candesartan-treated sham rats did not differ compared with untreated sham-operated rats (Fig. 1B). Additionally, SKGFR values in the contralateral nonobstructed kidneys were similar in all four groups (Fig. 1B).

**Candesartan Prevents PUUO-Induced RBF Reduction**

RBF measurements were obtained using a phase-contrast MRI technique involving a gradient echo sequence with bipolar flow-sensitive gradients. RBF was markedly decreased in the obstructed kidney of PUUO compared with sham-operated rats \((P < 0.05, \text{Fig. 2A})\). Candesartan treatment prevented this RBF reduction completely \((P < 0.05, \text{Fig. 2A})\). Candesartan treatment did not change RBF in sham-operated rats. Interestingly, a significant compensatory RBF increase in the contralateral nonobstructed kidneys was detected in the PUUO+CAN group at 10 wk of age \((P < 0.05, \text{Fig. 2A})\). Candesartan treatment significantly reduced mean arterial blood pressure in both PUUO and sham-operated rats (see Table 2).

**Long-Term Candesartan Administration Prevents Progression of Hydronephrosis**

The average TKV was also measured using MRI. This demonstrated a significant increase in TKV in the obstructed kidney of PUUO compared with sham-operated rats \((P < 0.05, \text{Fig. 2B})\). Candesartan treatment (from day 21 to week 10) markedly attenuated the pronounced increase in TKV of the obstructed kidney in PUUO animals \((2.2 \pm 0.2 \text{ vs. } 5.3 \pm 0.6 \text{ ml/g kidney wt in PUUO+CAN and PUUO, respectively, } P < 0.05)\). Figure 3 demonstrates representative MRI images from the different groups. As shown in Fig. 3A, PUUO of the left kidney was associated with a marked enlargement of the left kidney due to severe hydrenephrosis. Candesartan treatment significantly reduced this enlargement; as can be seen, there was only moderate hydrenephrosis of the left kidney in this group (Fig. 3B). To examine the associated protein changes each kidney protein concentration (PC) and total kidney protein (TP) were measured in both the obstructed and nonobstructed kidney. Consistent with severe hydrenephrosis and obstructive nephropathy, TP was significantly reduced in the obstructed kidney of PUUO rats compared with sham-operated rats \((33 \pm 3 \text{ vs. } 126 \pm 8 \text{ mg/kidney, } P < 0.05, \text{Table 1})\). However, candesartan treatment of PUUO rats attenuated the decrease in TP \((91 \pm 5 \text{ vs. } 126 \pm 8 \text{ mg/kidney, } P < 0.05)\) after 10 wk PUUO. Interestingly, candesartan treatment increased contralateral kidney weight but did not change TP either in the contralateral nonobstructed kidney of PUUO rats or in sham-operated rats (Table 1). This may be caused by as yet undefined mechanisms potentially involving AT2-mediated effects, which should be addressed in a future study.

**Candesartan Prevents PUUO-Induced Downregulation of AQP2 and Alters Renal Water Handling**

AQP2 was expressed in the apical plasma membrane and subapical vesicles of collecting duct principal cells. The affinity-purified anti-AQP2 antibody exclusively recognizes 29-
and 35- to 50-kDa bands, corresponding to nonglycosylated and glycosylated forms of AQP2. Semiquantitative immunoblotting using membrane fractions of whole kidney revealed that the abundance of AQP2 in the obstructed kidney of rats with PUUO was decreased compared with sham levels (52 ± 15 vs. 100 ± 4%, P < 0.05, Fig. 4, A and B). Candesartan treatment partly prevented the reduced abundance of AQP2 (P < 0.05, Fig. 4A). These findings were confirmed by immunocytochemical analysis demonstrating very weak AQP2 labeling in the apical plasma membrane of the collecting duct principal cells of the untreated PUUO group compared with sham-operated rats (Fig. 4C). In the obstructed kidney of candesartan-treated PUUO rats, AQP2 labeling was comparable to that seen in sham-operated control rats (Fig. 4, D and E).

To evaluate whether this was associated with changes in renal water handling, plasma osmolality, urine osmolality, and urine output were analyzed. Plasma osmolality did not change among groups (Table 2). Total urine output was significantly increased in PUUO rats compared with sham-operated rats (84 ± 11 vs. 52 ± 6 μmol·min⁻¹·kg⁻¹, P < 0.05, Table 3). In parallel, the increased urine output was associated with decreased urine osmolality (801 ± 119 vs. 1,316 ± 96 mosmol/kgH₂O, P < 0.05, Table 3), indicating a decreased urinary concentrating capacity. Furthermore, the total solute-free water reabsorption was reduced in the PUUO group compared with the sham-operated group (0.3 ± 0.12 vs. 1.2 ± 0.62 μl·min⁻¹·100 g⁻¹, P < 0.05, Table 4). Candesartan treatment reduced polyuria (63 ± 7 vs. 52 ± 6 μmol·min⁻¹·kg⁻¹, P < 0.05, Table 3) and normalized the reduced solute-free water reabsorption in PUUO rats compared with untreated PUUO rats (1.11 ± 0.35 vs. 0.3 ± 0.12 μl·min⁻¹·100 g⁻¹, P < 0.05, Table 4). To address whether the defect in renal water handling was due to dysregulation of other aquaporins, another water channel located in the proximal nephron, AQP1, was also examined. Immunoblotting demonstrated that AQP1 abundance was unchanged after the onset of PUUO (90 ± 24 vs. 100 ± 13%, P > 0.05, Table 5), and candesartan treatment did not significantly change this (85 ± 14 vs. 100 ± 13%).

Water transport across the basolateral membrane of the collecting duct principal cells is mediated by AQP3. Consistent with the findings on AQP1 abundance, total kidney AQP3 abundance did change significantly in either PUUO or PUUO+CAN rats (Table 5).

**Candesartan Prevents PUUO-Induced Downregulation of Na-K-ATPase and Decreases Fractional Sodium Excretion**

The active transport of sodium occurs mainly via the key sodium transporters and cotransporters in the nephron, and we therefore examined the expression of key sodium transporters in whole kidney samples by semiquantitative immunoblotting. The abundance of Na-K-ATPase in the obstructed kidney of
The kidneys are clearly identified. Enlarged obstructed pelvis is clearly visible.

Table 1. Body weight, kidney weight, protein concentration, and total protein in rats at 10 wk of age subjected to neonatal PUUO, PUUO + CAN, Sham + CAN, and sham operation

<table>
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<tr>
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<th>PUUO</th>
<th>PUUO + CAN</th>
<th>Sham + CAN</th>
<th>Sham</th>
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<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>6–7</td>
<td>8–10</td>
<td>11</td>
</tr>
<tr>
<td>Body wt, g</td>
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<td>172±9</td>
<td>189±6</td>
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<td>0.34±0.01</td>
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<tr>
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<td>PC, mg/g kidney wt</td>
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<td>115±10*</td>
<td>149±8</td>
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<tr>
<td>Non-OBS</td>
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<td>113±7*</td>
<td>140±7</td>
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<tr>
<td>TP, mg/kidney</td>
<td>OBS</td>
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<td>91±5*</td>
<td>124±10</td>
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<td>113±9</td>
<td>103±8</td>
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Values are means ± SE; n, no. of rats. OBS, obstruction. PC, protein concentration; TP, total protein; PUUO, partial unilateral ureteral obstruction; PUUO + CAN, partial unilateral ureteral obstruction with candesartan treatment; Sham + CAN, sham-operated rats with candesartan treatment. *P < 0.05 PUUO compared with sham-operated rats. †P < 0.05 untreated PUUO compared with candesartan-treated PUUO.

ANG II Activates NF-κB in Response to PUUO

To evaluate the contribution of the NF-κB pathway of renal changes in this model, we examined whole kidney NF-κB expression in the different groups. NF-κB expression in the obstructed kidney of PUUO rats was markedly elevated compared with sham-operated control rats (192±38 vs. 192±38). Candesartan treatment of PUUO rats was associated with complete normalization of Na-K-ATPase expression in the obstructed kidney compared with sham-operated rats. These findings were confirmed by immunocytochemistry. In kidneys from sham rats, immunocytochemistry showed abundant Na-K-ATPase labeling of the basolateral plasma membrane of the renal tubule cells. In the obstructed kidney from PUUO rats, Na-K-ATPase labeling was much weaker compared with sham-operated rats (Fig. 5C). This reduction in basolateral labeling was markedly attenuated in response to candesartan treatment (Fig. 5, D and E).

To investigate the molecular mechanisms involved in the impaired sodium reabsorption in the obstructed kidney after neonatal PUUO, expression of NKCC2 was examined in whole kidney samples. NKCC2 did not significantly change in the obstructed kidney, and candesartan treatment of PUUO rats revealed that NKCC2 did not differ from that in PUUO rats (Table 5).

Renal sodium handling was calculated from both obstructed and nonobstructed kidneys. Importantly, neonatally induced PUUO did not change plasma concentrations of sodium (Table 2). In accordance with the reduced GFR, the filtered load of sodium was severely reduced in the obstructed kidney in PUUO rats compared with sham-operated rats (5.8±1.0 vs. 29.3±7.6 μmol·min⁻¹·100 g⁻¹, P < 0.05, Table 4), and this was attenuated by candesartan. PUUO was also associated with increased fractional excretion of sodium compared with sham-operated rats (3.6±1.0 vs. 0.3±0.1%, P < 0.05, Table 4), demonstrating a reduced sodium reabsorption in the obstructed kidney. Administration of candesartan to the PUUO group reduced this increased fractional sodium excretion (3.6±1.0 vs. 1.3±0.6%, P < 0.05; Table 4). No compensatory changes in the fractional excretion of sodium in the contralateral kidneys were observed (Table 4). Overall, these findings suggest that PUUO-induced natriuresis can be prevented by long-term candesartan treatment.

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<th>PUUO + CAN</th>
<th>Sham + CAN</th>
<th>Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>6–7</td>
<td>8–10</td>
<td>11</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>192±8</td>
<td>172±9</td>
<td>189±6</td>
<td>189±5</td>
</tr>
<tr>
<td>Kidney wt, g/100 g</td>
<td>0.32±0.04</td>
<td>0.41±0.03</td>
<td>0.39±0.01</td>
<td>0.34±0.01</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>0.45±0.03*</td>
<td>0.47±0.02*</td>
<td>0.42±0.01*</td>
<td>0.35±0.01*</td>
</tr>
<tr>
<td>PC, mg/g kidney wt</td>
<td>OBS</td>
<td>50±3*</td>
<td>115±10*</td>
<td>149±8</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>113±9</td>
<td>113±7*</td>
<td>140±7</td>
<td>155±8</td>
</tr>
<tr>
<td>TP, mg/kidney</td>
<td>OBS</td>
<td>33±3*</td>
<td>91±5*</td>
<td>124±10</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>113±9</td>
<td>103±8</td>
<td>123±8</td>
<td>122±7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. OBS, obstruction. PC, protein concentration; TP, total protein; PUUO, partial unilateral ureteral obstruction; PUUO + CAN, partial unilateral ureteral obstruction with candesartan treatment; Sham + CAN, sham-operated rats with candesartan treatment. *P < 0.05 PUUO compared with sham-operated rats. †P < 0.05 untreated PUUO compared with candesartan-treated PUUO.
11%, P < 0.05, Fig. 6). Importantly, candesartan treatment prevented the PUUO-induced increase in NF-κB expression, suggesting that the beneficial effects of candesartan on kidney function in response to PUUO may be mediated via blockade of NF-κB.

**Plasma Aldosterone Levels Are Reduced in Response to Candesartan Treatment**

ANG II stimulates aldosterone release from the adrenal gland via AT1 receptors. The plasma concentration of aldosterone was used to verify inhibition of the AT1 receptor by candesartan. In the present study plasma aldosterone levels were reduced in the candesartan-treated PUUO group compared with sham-operated rats (Table 2). The candesartan-treated sham group showed a statistically significant decrease in aldosterone levels compared with those observed in the sham group (Table 2), demonstrating that ANG II via the AT1 receptors stimulates aldosterone release.

**DISCUSSION**

The main results of the present study demonstrated that neonatally induced PUUO for 10 wk resulted in a decrease in GFR and RBF in the obstructed kidney, paralleled by a marked increase in TKV in the obstructed kidney of PUUO rats. Consistent with these findings, AQP2 and Na-K-ATPase expression was downregulated in response to PUUO. Administration of the AT1 receptor antagonist candesartan attenuated the decrease in ipsilateral RBF, GFR, and prevented the in-
crease in TKV. Importantly, candesartan partly prevented the decrease in AQP2 and Na-K-ATPase expression in newborn rats subjected to PUUO. These findings indicate that ANG II is an important mediator of the hemodynamic changes during PUUO. The progression of hemodynamic and tubular changes was at least partly mediated via the angiotensin AT1 receptor, and thus candesartan appears to be useful in the prevention of these changes.

**Candesartan Prevents PUUO-Induced GFR and RBF Reduction**

The present study demonstrated that PUUO induced within 2 days of life was associated with a marked GFR reduction. This is consistent with our previous studies demonstrating that GFR is markedly reduced at week 24 after onset of neonatal PUUO (42, 43). As suggested by a recent study using a model of variable PUUO in the neonatal rat in which GFR was also reduced by 80% and a reduced number of nephrons (45), this indicates that neonatal PUUO is associated with a dramatic impact on glomerulogenesis, which has also been demonstrated in pigs subjected to PUUO (12).

Candesartan attenuated the GFR reduction in the obstructed kidney of PUUO rats. This finding is also consistent with a previous study from our lab demonstrating prevention of the GFR reduction after treatment with candesartan in adult rats subjected to BUO for 24 h followed by a 2-day release (19). Moreover, Pimentel et al. (18, 41) also administered an ACE inhibitor or an AT1 receptor inhibitor to rats before UUO and showed that both drugs improved the hemodynamics of the postobstructed kidney. The marked reduction in the mean arterial pressure by candesartan is also consistent with its ability to inhibit the vasoconstrictor effect of ANG II. Candesartan-induced augmentation of renal function is most likely related to antagonism of the renal AT1 receptors. Consistent with previous studies indicating progressive RBF reduction in chronic PUUO, the present study confirmed that PUUO resulted in a dramatic decrease in RBF and candesartan treatment attenuated the reduction in RBF. Thus the results of the present paper support the view that ANG II is an important mediator of the vasoconstriction associated with ureteral obstruction of the kidney. Despite the profound reduction in mean arterial blood pressure in response to candesartan, the parallel increase in both RBF and GFR suggests a preponderant ANG II-dependent afferent arteriolar vasoconstriction of the partially obstructed kidney.

Recently, it was demonstrated that both mild and severe PUUO induced a significant decrease in RBF and the magnitude of the decrease was dependent on both the severity and duration of the PUUO (50). Additionally, MRI measurements in a 24-wk-old rat PUUO model revealed that both TKV and RBF increased significantly in the contralateral nonobstructed kidney. Consistent with this, Shi and co-workers (43) also demonstrated compensatory changes in RBF of the intact kidney in response to neonatal UUO, which persisted up to 24 wk of age. Interestingly, in the present study RBF of the contralateral kidney did not increase at 10 wk, suggesting that the compensatory increase in RBF is detectable at a later stage. However, our study demonstrated that RBF in the contralateral kidney of PUUO + CAN rats increased, supporting the view that there is ANG II-dependent vasoconstriction of the contralateral kidney in response to PUUO, as suggested in a previous study where the effect of an ACE inhibitor was evaluated in a guinea pig model of partial ureteral obstruction (4). The effect of candesartan cilexetil treatment on RBF is

Table 3. Functional data in rats at 10 wk of age subjected to neonatal PUUO, PUUO + CAN, Sham + CAN, and sham operation

<table>
<thead>
<tr>
<th></th>
<th>PUUO</th>
<th>PUUO + CAN</th>
<th>Sham + CAN</th>
<th>Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Water intake, μl·min⁻¹·kg⁻¹</td>
<td>146 ± 15</td>
<td>124 ± 10</td>
<td>98 ± 4</td>
<td>116 ± 10</td>
</tr>
<tr>
<td>Urine output, μl·min⁻¹·kg⁻¹</td>
<td>84 ± 29</td>
<td>63 ± 7</td>
<td>40 ± 3</td>
<td>52 ± 6</td>
</tr>
<tr>
<td>Uosm, mosmol/kgH₂O</td>
<td>801 ± 119*</td>
<td>1,014 ± 102†</td>
<td>1,664 ± 96</td>
<td>1,316 ± 96</td>
</tr>
<tr>
<td>Ucre, mmol/l</td>
<td>60 ± 8*</td>
<td>72 ± 7</td>
<td>120 ± 6</td>
<td>87 ± 6</td>
</tr>
<tr>
<td>Uα, mmol/l</td>
<td>185 ± 26*</td>
<td>245 ± 23</td>
<td>403 ± 26</td>
<td>302 ± 20</td>
</tr>
<tr>
<td>Ucre, mmol/l</td>
<td>398 ± 70*</td>
<td>488 ± 58</td>
<td>816 ± 47</td>
<td>675 ± 56</td>
</tr>
<tr>
<td>Clcre, ml·min⁻¹·kg⁻¹</td>
<td>3.2 ± 0.5</td>
<td>2.9 ± 0.3</td>
<td>2.9 ± 0.2</td>
<td>3.6 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; Uosm, urine osmolality; Ucre, creatinine clearance. *P < 0.05 PUUO compared with sham-operated rats. †P < 0.05 untreated PUUO compared with candesartan-treated PUUO.
likely due to antagonism of AT1 receptors located on the afferent and efferent arterioles. Consistent with this, previous studies have demonstrated similar renoprotective effects of AT1 receptor antagonist treatment in several animal models (28, 31, 33). Despite a significant reduction in mean arterial blood pressure, renal functions were unchanged in the sham-operated rats, suggesting that blood pressure remained within the limits of renal autoregulation. This is consistent with previous data demonstrating that inhibition of AT1 receptors with candesartan cilexetil provides protection against ANG II-mediated increases in arterial pressure and prevents the associated deterioration of renal autoregulatory responsiveness (17).

### Long-Term Candesartan Administration Prevents Progression of Hydronephrosis

Partial ureteral obstruction is associated with pelvic dilatation, and the degree of dilatation may reflect the severity of the obstruction. To examine the effect of obstruction on pelvic dilatation, total kidney volume was determined by MRI. Consistent with previous in vivo and in vitro studies, measurements of kidney volume correlated significantly, demonstrating that MRI is a reliable technique for evaluation of the degree of hydronephrosis (6, 50). Thus the present study revealed a significant hydronephrosis in obstructed PUUO kidneys which is consistent with previous findings demonstrating a similar increase in response to severe neonatal PUUO (43, 49, 50). In sham-operated rats, the TKV of the left and right kidneys did not differ. However, an important and novel finding of the present study was that long-term candesartan treatment markedly prevented the increase in TKV. In the candesartan-treated rats, TKV of the obstructed kidney was diminished by 50%. This result indicates that candesartan cilexetil may play an important role in reduction in TKV of the obstructed kidney in rats with PUUO, most likely by blocking detrimental ANG II-induced cellular effects in the obstructed kidney. Moreover, candesartan treatment prevented the reduction in PC and TP of the obstructed kidney, supporting the view from previous studies that sustained enhanced intrarenal ANG II generation is detrimental for renal functional development in response to unilateral ureteral obstruction and that blockade of the RAS may prevent the progression of proteinuria (38) and fibrosis (26, 29). Consistent with this, a recent study by Bahrir and co-workers (3) demonstrated that enalapril treatment affords protection by attenuating proteinuria, promoting uricosuria, and diverting solute diuresis. Thus there is now evidence that angiotensin blockade during chronic obstruction protects kidney function in the young rat. In a number of studies, it has been demonstrated that AT1 receptor blockade ameliorates the obstruction-induced fibrosis, most likely via inhibition of the NF-κB pathway, which participates in the regulation of renal monocyte recruitment (15, 27). At present, it is unclear whether the same pathways are of similar importance in response to congenital obstruction in the rat, and this needs to be further addressed in a future study. However, the obstruction-induced tubulointerstitial fibrosis is a complex renal disease, where multiple hormonal systems are activated (2).

### Table 5. Summary of densitometric changes in the expression of renal aquaporins and major sodium transporters in rats subjected to PUUO, PUUO + CAN, Sham + CAN, and sham operation

<table>
<thead>
<tr>
<th></th>
<th>PUUO</th>
<th>PUUO + CAN</th>
<th>Sham + CAN</th>
<th>Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquaporins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AQPI</td>
<td>90 ± 24</td>
<td>85 ± 14</td>
<td>98 ± 9</td>
<td>100 ± 13</td>
</tr>
<tr>
<td>AQPII</td>
<td>113 ± 11</td>
<td>104 ± 9</td>
<td>98 ± 6</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>AQPIII</td>
<td>52 ± 15*</td>
<td>74 ± 13†</td>
<td>97 ± 7</td>
<td>100 ± 4</td>
</tr>
<tr>
<td>Sodium transporters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NKCC2</td>
<td>97 ± 10</td>
<td>121 ± 20</td>
<td>128 ± 20</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>Na-K-ATPase</td>
<td>75 ± 12*</td>
<td>103 ± 8†</td>
<td>93 ± 4</td>
<td>100 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; AQPI, aquaporin; NKCC2, bumetanide-sensitive Na-CI cotransporter. *P < 0.05 PUUO compared with sham-operated rats. †P < 0.05 untreated PUUO compared with candesartan-treated PUUO.

### Candesartan Partly Prevents PUUO-Induced Downregulation of AQ2 and Alters Renal Water Handling

AQP2 is expressed in the apical plasma membrane and subapical vesicles of the collecting duct principal cells, and this protein is responsible for water transport across the apical membrane in the collecting duct (35, 36). The present study demonstrated that AQP2 expression is downregulated in the obstructed kidneys of the rats with neonatally induced PUUO. In parallel, solute-free water reabsorption was severely reduced in obstructed kidneys, demonstrating a functional association between AQP2 downregulation and water reabsorption at the collecting duct level. This finding is consistent with previous...
studies demonstrating that unilateral ureteral obstruction down-regulates several AQPs in the kidney (22, 23, 42, 43).

Candesartan treatment partially prevented the reduction in AQP2 expression in PUUO rats. Consistent with this, candesartan treatment was associated with an increase in solute-free water reabsorption, demonstrating a functional association between the molecular changes in AQP2 and the physiological change in collecting duct water handling. Thus the present findings support the view that ANG II may play an important role in the regulation of collecting duct function, as recently demonstrated by Jensen et al. (19), who showed that candesartan administration to adult rats subjected to 24-h BUO followed by a 2-day release partially prevented the reduction in AQP2 expression levels in the obstructed kidney.

**Candesartan Prevents PUUO-Induced Downregulation of Na-K-ATPase and Decreased Fractional Sodium Excretion**

The active transport of sodium occurs mainly via the key sodium transporters: the basolateral Na-K-ATPase (20), the type 3 Na/H exchanger (NHE3) (1), and the apical BSC-1 (or NKCC2) (9). This study demonstrated that Na-K-ATPase abundance in the obstructed kidney was decreased after 10 wk of obstruction. There was a defective reabsorption of sodium in
the obstructed kidney, which was evidenced by the increase in sodium excretion. Thus it is likely that the reduced abundance of Na-K-ATPase plays a significant role in the increased urinary excretion of sodium from the obstructed kidney in PUUO rats. The present results support the view that renal sodium transport is critically affected by ureteral obstruction, as previously demonstrated in a similar model (42, 43), and underscore the role of an intact expression of renal sodium transporters in maintaining an intact renal epithelial sodium transport in response to neonatal PUUO. Downregulation of Na-K-ATPase was prevented by candesartan treatment, demonstrating at the molecular level that blockade of ANG II-induced effects in the obstructed kidney is important in protecting the developing tubule system from damage. Candesartan treatment also attenuated the natriuresis from the obstructed kidney, demonstrating a functional association between the abundance of Na-K-ATPase and epithelial sodium transport in response to neonatal PUUO. Downregulation of Na-K-ATPase was prevented by candesartan treatment, demonstrating the beneficial effects of candesartan on kidney function in response to PUUO may be mediated via blockade of the NF-κB pathway.

Conclusion

In summary, this study confirmed that neonatal PUUO in rats is associated with marked long-term changes in renal functions. AT1 receptor blockade prevented the decrease in GFR and RBF in the obstructed kidney in neonatally induced PUUO rats. Moreover, it also effectively prevented the kidney protein reduction, reduced hydropnephrosis, and prevented downregulation of Na-K-ATPase and partially prevented AQP2 downregulation. Consistent with this, AT1 receptor blockade prevented impairment of tubular transport of water and sodium from the obstructed kidney. Candesartan also attenuated the increase in NF-κB expression, suggesting that NF-κB may play a role in the renal injury in response to congenital ureteral obstruction.

ACKNOWLEDGMENTS

The expert technical assistance of Gitte Kall, Inger Merete Paulsen, and Dorte Wulff is highly appreciated.

GRANTS

The Water and Salt Research Centre at the University of Aarhus was established and supported by the Danish National Research Foundation (Danmarks Grundforskningsfond). Support for this study was also provided by The Danish Medical Research Council, The Karen Elise Jensen Foundation, The Novo Nordisk Foundation, WIRED program (Nordic Council and the Nordic Centre of Excellence Program in Molecular Medicine), and The Commission of the European Union (EU-Aquaplugs and EU-Action Programs), The Danish Research Academy, and the intramural budget of the National Heart, Lung, and Blood Institute, National Institutes of Health.

Candesartan Attenuates the PUUO-Induced Increase in NF-κB Expression

Previous studies have demonstrated that ANG II activates NF-κB in the kidney, via stimulation of both AT1 and AT2 receptors. Consistent with previous studies, we confirmed that NF-κB expression in the obstructed kidney of PUUO rats was markedly elevated compared with sham-operated control rats. Importantly, candesartan treatment prevented the PUUO-induced increase in NF-κB expression, suggesting that the beneficial effects of candesartan on kidney function in response to PUUO may be mediated via blockade of the NF-κB pathway.
REFERENCES


