Early release of neonatal ureteral obstruction preserves renal function

Yimin Shi,1,2 Michael Pedersen,1 Chunling Li,1,2 Jian Guo Wen,1 Klaus Thomsen,5 Hans Stødkilde-Jørgensen,1 Troels Munch Jørgensen,1 Mark A. Knepper,6 Søren Nielsen,2,4 Jens Christian Djurhuus,1 and Jørgen Frøkiær1,2,3

1Institute of Experimental Clinical Research, Aarhus University, and 2Department of Clinical Physiology, Aarhus University Hospital, DK-8200 Aarhus N; 3The Water and Salt Research Center and 4Institute of Anatomy, Aarhus University, DK-8000 Aarhus C; 5Institute for Basic Psychiatric Research, Department of Biological Psychiatry, Aarhus University Hospital, DK-8240 Risskov, Denmark; and 6Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

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Shi, Yimin, Michael Pedersen, Chunling Li, Jian Guo Wen, Klaus Thomsen, Hans Stødkilde-Jørgensen, Troels Munch Jørgensen, Mark A. Knepper, Søren Nielsen, Jens Christian Djurhuus, and Jørgen Frøkiær. Early release of neonatal ureteral obstruction preserves renal function. Am J Physiol Renal Physiol 286: F1087–F1099, 2004. First published January 13, 2004; 10.1152/ajprenal.00201.2003.—The incidence of congenital hydronephrosis is ~1% and is often associated with renal insufficiency. It is unknown whether early release is essential to prevent deterioration of renal function. Rats were subjected to partial unilateral ureteral obstruction (PUUO) on postnatal day 2. The obstruction was left in place or released after 1 or 4 wk. Renal blood flow (RBF) and kidney size were measured sequentially over 24 wk using MRI. In rats in which the obstruction was left in place, RBF of the obstructed kidney was progressively reduced to 0.92 ± 0.17 vs. 1.79 ± 0.12 mL/min·100 g body wt−1 (P < 0.05) after 24 wk. Similarly, glomerular filtration rate of the obstructed kidney was severely reduced at 24 wk: 172 ± 36 vs. 306 ± 42 µL/min·100 g body wt−1 (P < 0.05). These changes were preceded by development of severe hydronephrosis and obstructive nephropathy with a reduction in total protein content: 45 ± 3 vs. 58 ± 4 mg/kidney. Moreover, nonreleased PUUO caused a marked natriuresis (0.32 ± 0.07 vs. 0.11 ± 0.02 µmol/min·100 g body wt−1, P < 0.05) and impaired solute free water reabsorption (0.47 ± 0.16 vs. 2.71 ± 0.67 µL/min·100 g body wt−1, P < 0.05), consistent with a significant downregulation of Na-K-ATPase to 62 ± 7%, aquaporin-1 to 53 ± 3%, and aquaporin-3 to 53 ± 7% of sham levels. Release after 1 wk completely prevented development of hydronephrosis, reduction in RBF and glomerular filtration rate, and downregulation of renal transport proteins, whereas release after 4 wk had no effect. These results suggest that early release of neonatal obstruction provides dramatically better protection of renal function than release of obstruction after the maturation process is completed.

rat; obstructive nephropathy; kidney function; magnetic resonance imaging; aquaporin; sodium transporter

CONGENITAL MALFORMATIONS of the kidney and urinary tract associated with ureteral obstruction account for a major proportion of renal insufficiency in infancy and childhood, but management of antenatally detected hydronephrosis is still debated (25, 42). Whether the treatment should be surgical or nonsurgical is complicated by poor understanding of the natural history of congenital hydronephrosis, as well as unpredictable consequences of surgical intervention. Some advocate for an early surgical intervention (41, 42), whereas others favor a prolonged observation (25). The focus of the controversies still is whether surgical intervention is necessary and, if surgical intervention is unavoidable, the optimal time for such intervention. Clinically, improvement or stabilization of renal function only occurs in the patients treated by surgical intervention before 2 yr of age (32). This observation may be critically related to the continuous maturation of the human kidney during the first 2 yr of life (39). Thus this may raise the hypothesis that surgical intervention before, rather than after, the maturation process is completed may better preserve renal functions of the obstructed kidney.

In the rat, >90% of nephrogenesis has taken place during the first 10 postnatal days and the final maturation of the renal functions occurs thereafter. Cortical and medullary anatomy matures significantly during the critical period of the 3rd wk after birth (39). Previously, it was demonstrated that urinary tract obstruction during this vulnerable period aggravates renal functional development and is associated with disproportionate renal functional impairment (8), and 3-wk-old rats subjected to unilateral ureteral obstruction (UUO) have a highly activated tubuloglomerular feedback mechanism when studied 3–6 wk later (33). Moreover, onset of complete UUO at 14–19 days of age in rat results in more severe impairment of kidney growth than the impairment in response to neonatal UUO at 1–5 days of age or during adulthood (11). Furthermore, 3 mo after release of UUO during days 14–19, renal growth was decreased by 50%, compared with a 30% reduction after release of UUO during days 1–5 (11). The number of glomeruli was reduced by ~50%, regardless of the timing of UUO, but glomerular size was reduced only in rats with UUO during days 14–19 (11). These results demonstrate that, in the period immediately after nephrogenesis, the developing kidney is particularly susceptible to long-term impairment from temporary obstruction, suggesting that a delay in release of severe ureteral obstruction may have a detrimental impact on renal function later in life.

The effect of neonatally induced partial UUO (PUUO) on kidney function during this vulnerable period is not fully understood. Previously, it was demonstrated that glomerular filtration rate (GFR) is reduced in proportion to the severity of the obstruction (21). Recently, using magnetic resonance imaging (MRI), we demonstrated that renal blood flow (RBF)
was progressively reduced in rats with long-term follow-up after neonatal PUUO (51). It has been hypothesized that renal functional deterioration in a hydronephrotic obstructed kidney stimulates the growth and function of the contralateral nonobstructed kidney at a faster-than-normal rate and before functional deterioration of the hydronephrotic kidney is detectable (26). Recently, we demonstrated that compensatory growth of the contralateral nonobstructed kidney during a 24-wk observation period is not detectable before the appearance of RBF deterioration in the neonatally obstructed kidney (51), suggesting that compensatory growth is not a useful predictor of early functional deterioration.

Characteristically, a very important sign of urinary tract obstruction is impairment of urinary concentrating capacity and, eventually, development of nephrogenic diabetes insipidus in severe cases (20). Urinary concentration and dilution depend on a discrete segmental distribution of transport properties along the renal tubule: 1) the hypertonic medullary interstitium, which is generated by active NaCl reabsorption in water-impermeable nephron segments and 2) the high water permeability (constitutive or vasopressin regulated) in other renal tubular segments for osmotic equilibration, which chiefly depends on aquaporins (AQPs) (24). Thus defects in any of these mechanisms would be predicted to be associated with urinary concentrating defects.

AQPs are a family of membrane proteins that function as water channels (37). AQP1 is highly abundant in the proximal tubule and descending thin limb (38), whereas AQP2 is the apical water channel of the principal cells and is the chief target for regulation of collecting duct water permeability by vasopressin (35, 36). Water transport across the basolateral plasma membrane of collecting duct principal cells is mediated by AQP3 (15) and AQP4 (47). Consistent with the roles of AQPs in renal regulation of water balance, we recently demonstrated that UUO and bilateral ureteral obstruction cause severe dysregulation of renal AQPs, which is associated with impaired renal water handling (28, 29). UUO was also demonstrated to alter expression levels of renal sodium transporters associated with deranged urinary sodium excretion (30), supporting the view that dysregulation of these transporters plays an essential role in the impaired urinary concentration and sodium handling in response to obstruction.

The aims of this study were therefore to investigate long-term changes in renal functions in response to neonatal PUUO. In particular, changes in RBF, GFR, and renal sodium and water handling of the obstructed and nonobstructed kidney, together with the renal protein expression of major sodium transporter and water channels, were examined using a proteomic approach. Furthermore, we aimed at addressing whether early, rather than late, release can prevent the renal functional reduction in response to neonatal PUUO.

MATERIALS AND METHODS

Animal Preparation

Studies were performed in female Münch-Wistar rats. Rats were subjected to severe PUUO or sham operation within the first 48 h of life according to a modification of the technique of Ulm and Miller (51). Briefly, newborn rats were anesthetized with ether and placed on a heated table. The left ureter was exposed through a midline incision. A PUUO was created by embedding two-thirds of the left ureter in a psoas muscle tunnel. The sham group was created by laparotomy and mobilization of the left ureter. After surgery, rats were kept in an incubator at 30°C until they had totally awakened; then they were returned to the regular animal units with their mothers. At 1 or 4 wk after onset of obstruction, 18 rats from the PUUO group were subjected to a second operation performed under general anesthesia with ether to release the obstruction by removal of the ligatures and suturing of the underlying psoas muscle. At 4 wk of age, the rats were separated from their mothers and housed two per cage. During the experiments, the rats were maintained at controlled temperature (22–24°C) and moisture (60%) with a 12:12-h artificial light-dark cycle. The rats were fed a standard rodent diet and tap water. After 24 wk, the rats were killed. The study complied with the Danish regulations for care and use of experimental animals. Rats were allocated to the following groups: 1) the PUUO group (n = 7), in which the animals were subjected to PUUO for 24 wk, 2) the PUUO + 1wR group (n = 10), in which PUUO was released 1 wk after onset of obstruction, 3) the PUUO + 4wR group (n = 8), in which PUUO was released 4 wk after onset of obstruction, and 4) the sham group (n = 11), which consisted of matched sham-operated controls.

MRI examinations were carried out under pentobarbital sodium anesthesia (50 mg/kg body wt ip; Pentothal, Abbott Scandinavia, Solna, Sweden) in all rats at 5, 12, and 24 wk of age. Before the animals were killed, single-kidney function was examined. Then the two kidneys were removed, the wet weight was determined, and the kidneys were frozen for later analysis.

MRI

Briefly, the MRI examinations were performed with a small-bore Sisco 7-T system (Varian, Palo Alto, CA). The rat was placed supine in a Helmholz 4-cm-diameter head coil and then subjected to an imaging protocol including measurements of single-kidney RBF and total kidney volume (TKV).

RBF Measurements

RBF measurements were obtained using a phase-contrast technique involving a gradient echo sequence with bipolar flow-sensitive gradients. The strengths of the flow encoding gradients were set according to the values from a previous study (44). Ten 1.2-mm-thick slices were prescribed perpendicular to the renal veins. Each slice had a 7 × 7-cm² field of view and a resolution of 350 × 350 pixels to ensure that blood flow in the renal veins could be derived. Other parameters were as follows: 150-ms repetition time, 5.5-ms echo time, 55° excitation flip angle, and four data averages. Acquired phase images were subtracted, and the vein flow was determined by multiplying by the renal vein area for each available slice. The individual kidney RBF was then calculated as an average of the flow values for all slices.

TKV Measurements

A gradient echo sequence was used to obtain a series of axial slices through the kidney to determine TKV. Depending on the kidney size, 20–30 equidistant 1.0-mm-thick slices were employed to sufficiently cover both kidneys. The field of view and pixel size were the same as those described for RBF measurements, and other parameters were as follows: 125-ms repetition time and 4-ms echo time. Postprocessing included manual identification of each kidney for all slices, and by carefully encompassing regions of interest, TKV was measured by the sum-of-areas principle (50).

Measurement of GFR and Tubular Functions

GFR was measured using renal clearance of 51Cr-EDTA at 24 wk after the onset of PUUO. Seven days before the clearance studies, the left femoral artery and vein were catheterized under pentobarbital sodium anesthesia (50 mg/kg body wt ip). The arterial and venous catheters were fixed as described by Petersen et al. (43), sealed with 50% glucose solution containing heparin (500 U/ml) and streptoki-
nase (10,000 U/ml), and fixed. After instrumentation, 5 ml of saline and 10 μl of analgesic (buprenorphine, Temgesic) were given subcutaneously. After recovery from anesthesia, the rats were returned to the animal units and housed individually (49).

Renal clearance of $^{51}$Cr-EDTA was measured using a constant-infusion clearance technique. Briefly, the rats were anesthetized as described above and then placed on a heating table to maintain rectal temperature at 37°C. Through a midline incision, both ureters were exposed and catheterized (0.762-mm flexible plastic tubing; Tygon, Weyerhaeuser, Cleveland, OH) for the urine collection. The incision was closed to prevent loss of body fluid. A priming 15-min intravenous dose of $^{51}$Cr-EDTA (0.2 MBq) was followed by a sustained infusion (0.005 MBq/min) during a 75-min equilibration period and two 1-h urine collection periods. An intravenous infusion of a 25 mM glucose solution (40 μl/min) was provided simultaneously to maintain an adequate minimum urine flow rate for biochemical analysis of the collected urine. Timed blood samples (150 μl) were taken from the arterial catheter every hour during the urine collection periods and replaced immediately with the same volume of heparinized donor blood. Timed urine samples were gravimetrically collected every hour from both ureters. The plasma and urine samples were diluted, and $^{51}$Cr-EDTA was counted using an Auto-Gamma Counting System (COBRA, Packard Instrument, Meriden, CT).

The osmolality of urine and plasma was determined (Kodak Ektachem 700XRC). The plasma concentration of sodium was determined from samples of kidneys of PUUO, PUUO $\times 1wR$, and sham groups. The labeling density was corrected by densitometry of Coomassie brilliant blue-stained gels (i.e., to control for minor difference in protein loading).

To compare the fractional expressions from whole kidney among the three groups, the labeling density was corrected for difference in total amount of protein in each kidney by multiplication of the density by the total protein content.

**Primary Antibodies**

For semiquantitative immunoblotting, we used previously characterized monoclonal and polyclonal antibodies as follows. For Na-K-ATPase, a monoclonal antibody against the α1-subunit of Na-K-ATPase has been characterized (22). For the bumetanide-sensitive Na-K-2Cl cotransporter (BSC-1, LL320AP), an affinity-purified polyclonal antibody to the apical Na-K-2Cl cotransporter of the thick ascending limb has been characterized (16, 23). For AQP1 (CHIP serum or LL266AP), immune serum or an affinity-purified antibody to AQP1 has been characterized (48). For AQP2 (LL127 serum or LL127AP), immune serum or an affinity-purified antibody to AQP2 has been described (14). For AQP3 (LL178AP), an affinity-purified polyclonal antibody to AQP3 has been characterized (15).

**Calculations and Statistics**

GFR was estimated by calculating the renal clearance of $^{51}$Cr-EDTA ($C_{\text{EDETA}}$) as follows

$$C_{\text{EDETA}} = \frac{(U/P)_{\text{EDETA}} \times U_{\text{vol}}}{P_{\text{Na}}}$$

where $(U/P)_{\text{EDETA}}$ is the urine-to-plasma count ratio of $^{51}$Cr-EDTA and $U_{\text{vol}}$ is the rate of urine flow divided by body weight. The plasma counts correspond to the midpoint of each urine collection period. Filtration fraction (FF) was calculated as follows

$$FF(\%) = \frac{GFR}{100/RPF}$$

where renal plasma flow (RPF) was calculated by multiplying RBF by (1 − hematocrit).

Filtered load of sodium ($F_{\text{Na}}$) was calculated as follows

$$F_{\text{Na}} = GFR \times P_{\text{Na}}$$

where $P_{\text{Na}}$ is plasma sodium.

Excretion rate of sodium ($U_{\text{Na}}V$) was calculated as follows

$$U_{\text{Na}}V = U_{\text{Na}} \times U_{\text{vol}}$$

where $U_{\text{Na}}$ is sodium concentration in urine.

Fractional excretion of sodium ($F_{\text{E}\text{Na}}$) was calculated as follows

$$F_{\text{E}\text{Na}} = \frac{(U/P)_{\text{Na}} \times (P/U)_{\text{EDTA}}}{(U/P)_{\text{Na}}}$$

where $(U/P)_{\text{Na}}$ is the urine-to-plasma concentration ratio of sodium and $(P/U)_{\text{EDTA}}$ is the plasma-to-urine count ratio of $^{51}$Cr-EDTA.

Solute free water reabsorption ($T^{\text{H}_{2}}\text{O}$) was calculated as follows

$$T^{\text{H}_{2}}\text{O} = \left[ \frac{U_{\text{osmol}}}{P_{\text{osmol}}} - 1 \right] \times U_{\text{vol}}$$

where $U_{\text{osmol}}/P_{\text{osmol}}$ is the urine-to-plasma ratio of osmolality.

Values are means ± SE. One-way analysis of variance was performed for statistical analysis. If there was a significant difference,
Bonferroni’s test was performed to ascertain the difference between the groups. \( P < 0.05 \) was considered to be statistically significant.

**RESULTS**

**Early Release of Obstruction Partially Prevents RBF and GFR Reductions in Obstructed Kidneys**

After neonatally induced PUUO, RBF of the obstructed kidney progressively decreased. RBF in the obstructed kidney was reduced to 33% of the sham level at 5 wk, 50% at 12 wk, and 49% at 24 wk \((P < 0.05; \text{Fig. 1A, Table 1})\). Consistent with the progressive RBF reduction, there was a significant reduction in GFR after 24 wk of obstruction to 43% of sham levels \((172 \pm 36 \text{ vs. } 306 \pm 42 \text{ ml/min}^{-1} \text{100 g body wt}^{-1}, P < 0.05; \text{Table 2})\). This progressive reduction in RBF was partially prevented in PUUO + 1wR rats. Examination of the rats at week 5 demonstrated that RBF was significantly higher in PUUO + 1wR than in PUUO rats \((2.79 \pm 0.54 \text{ vs. } 1.64 \pm 0.17 \text{ ml/min}^{-1} \text{100 g body wt}^{-1}, P < 0.05)\), whereas in PUUO + 4wR rats, RBF did not differ significantly from levels in PUUO rats \((1.78 \pm 0.25 \text{ vs. } 1.64 \pm 0.17 \text{ ml/min}^{-1} \text{100 g body wt}^{-1}, P > 0.05; \text{Fig. 1A})\). At 24 wk after onset of obstruction, RBF was significantly reduced in PUUO + 1wR rats compared with sham-operated controls \((1.28 \pm 0.17 \text{ vs. } 1.79 \pm 0.12 \text{ ml/min}^{-1} \text{100 g body wt}^{-1}, P < 0.05)\), but levels were higher than in PUUO rats \((1.28 \pm 0.17 \text{ vs. } 0.92 \pm 0.17 \text{ ml/min}^{-1} \text{100 g body wt}^{-1}, P < 0.05)\) and PUUO + 4wR rats \((1.28 \pm 0.17 \text{ vs. } 0.74 \pm 0.13 \text{ ml/min}^{-1} \text{100 g body wt}^{-1}, P < 0.05; \text{Fig. 1A})\). Consistent with these RBF changes in the released kidneys, the GFR reduction was prevented in PUUO + 1wR rats \((304 \pm 18 \text{ and } 306 \pm 42 \text{ ml/min}^{-1} \text{100 g body wt}^{-1} \text{ in PUUO + 1wR and sham, respectively, } P > 0.05)\), whereas GFR in PUUO + 4wR rats did not differ significantly from that in PUUO rats \((172 \pm 34 \text{ vs. } 172 \pm 36 \text{ ml/min}^{-1} \text{100 g body wt}^{-1}, P > 0.05; \text{Table 2})\).

**Early Release of Obstruction Attenuates Compensatory RBF Increase in the Contralateral Nonobstructed Kidneys**

In the contralateral nonobstructed kidney of rats with PUUO, there was significant compensatory increase in RBF at 5 wk \((5.17 \pm 0.78 \text{ vs. } 2.49 \pm 0.19 \text{ (sham) ml/min}^{-1} \text{100 g body wt}^{-1})\).

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**Table 1. Changes in RBF development in PUUO, PUUO + 1 wR, PUUO + 4 wR, and sham rats at 5, 12, and 24 wk of age**

<table>
<thead>
<tr>
<th></th>
<th>PUUO ((n = 6))</th>
<th>PUUO + 1wR ((n = 7))</th>
<th>PUUO + 4wR ((n = 8))</th>
<th>Sham ((n = 11))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 5 OBS</td>
<td>1.58 ± 0.18*</td>
<td>3.38 ± 0.81</td>
<td>1.79 ± 0.26*</td>
<td>2.48 ± 0.14</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>5.03 ± 0.83*</td>
<td>6.14 ± 1.25*</td>
<td>5.24 ± 0.93*</td>
<td>2.53 ± 0.20</td>
</tr>
<tr>
<td>Week 12 OBS</td>
<td>2.59 ± 0.67*</td>
<td>3.33 ± 0.47*</td>
<td>1.97 ± 0.43*</td>
<td>5.39 ± 0.38</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>6.49 ± 0.50*</td>
<td>6.54 ± 0.58</td>
<td>7.00 ± 0.51*</td>
<td>5.39 ± 0.35</td>
</tr>
<tr>
<td>Week 24 OBS</td>
<td>2.09 ± 0.39*</td>
<td>3.31 ± 0.46</td>
<td>1.89 ± 0.29*</td>
<td>4.29 ± 0.26</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>5.05 ± 0.36*</td>
<td>5.35 ± 0.22*</td>
<td>7.08 ± 0.70*</td>
<td>4.32 ± 0.22</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in ml/min; \( n \), number of rats; RBF, renal blood flow; PUUO, partial unilateral ureteral obstruction; PUUO + 1wR, PUUO released at 1 wk of age; PUUO + 4wR, PUUO released at 4 wk of age; sham, sham-operated controls; OBS, obstructed kidney; non-OBS, nonobstructed kidney. \( *P < 0.05 \) vs. sham.
Table 2. GFR, FF, kidney weight, and body weight in PUUO, PUUO + 1 wR, PUUO + 4 wR, and sham rats at 24 wk of age

<table>
<thead>
<tr>
<th></th>
<th>PUUO (n = 7)</th>
<th>PUUO + 1wR (n = 10)</th>
<th>PUUO + 4wR (n = 8)</th>
<th>Sham (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR, μl·min⁻¹·100 g body wt⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>172±36*</td>
<td>304±18</td>
<td>137±34*</td>
<td>306±42</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>378±32</td>
<td>317±13</td>
<td>296±31</td>
<td>328±40</td>
</tr>
<tr>
<td>FF, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>48±17</td>
<td>48±6</td>
<td>49±15</td>
<td>34±5</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>31±2</td>
<td>30±2</td>
<td>25±5</td>
<td>36±6</td>
</tr>
<tr>
<td>Kidney wt, g/100 g body wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>0.34±0.03</td>
<td>0.28±0.01*</td>
<td>0.34±0.04</td>
<td>0.32±0.01</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>0.37±0.02*</td>
<td>0.35±0.01</td>
<td>0.38±0.03*</td>
<td>0.33±0.01</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>231±7</td>
<td>227±6</td>
<td>243±4</td>
<td>234±4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. GFR, glomerular filtration rate; FF, filtration fraction. *P < 0.05 vs. sham.

Early Release of Obstruction Prevents Progression of Hydronephrosis

PUUO caused a pronounced increase in TKV of the obstructed kidneys in the PUUO group (Fig. 2A). This increase persisted at 5 wk of age [1.49 ± 0.19 vs. 0.50 ± 0.02 (sham) ml/100 g body wt, P < 0.05], 12 wk of age [0.90 ± 0.15 vs. 0.38 ± 0.01 (sham) ml/100 g body wt, P < 0.05], and 24 wk of age [0.98 ± 0.25 vs. 0.38 ± 0.01 (sham) ml/100 g body wt, P < 0.05], whereas the kidney wet weight of the obstructed kidney did not change significantly in the PUUO rats at 24 wk of age [0.34 ± 0.03 vs. 0.32 ± 0.01 (sham) g/100 g body wt, P > 0.05; Table 3]. On the contrary, whole kidney protein content was significantly reduced in the obstructed kidney of the PUUO rats, consistent with the development of a markedly progressive hydronephrosis and obstructive nephropathy (Table 3). In the PUUO + 4wR group, development of TKV in the obstructed kidney did not differ from that in the PUUO group (Fig. 2A). However, release of obstruction after 1 wk (PUUO + 1wR group) completely abolished the increase in TKV. At 5, 12, and 24 wk, TKV of the obstructed kidney was similar to TKV in sham-operated control rats (Fig. 2A). Furthermore, wet kidney weight of the obstructed kidney in the PUUO + 1wR group was slightly reduced, but total protein was identical to that in sham-operated control kidneys, consistent with prevention of the development of obstructive nephropathy (Table 3).
The increase in TKV of the obstructed kidney consistent with progressive hydronephrosis was associated with a compensatory increase in TKV of the contralateral nonobstructed kidney 5 wk after onset of obstruction in the PUUO group [0.64 ± 0.04 vs. 0.52 ± 0.02 (sham) ml/100 g body wt, P < 0.05]. This compensatory increase persisted at 12 wk of age [0.50 ± 0.04 vs. 0.39 ± 0.01 (sham) ml/100 g body wt, P < 0.05] and at 24 wk of age [0.47 ± 0.02 vs. 0.40 ± 0.01 (sham) ml/100 g body wt, P < 0.05; Fig. 2B]. Release of obstruction at 4 wk of age was associated with compensatory changes in the contralateral kidney identical to the PUUO group. In the PUUO + 1wR group, the compensatory increase in TKV of the contralateral kidney was slowly reversed (Fig. 2B). At 5 and 12 wk, TKV remained significantly increased, whereas TKV of the contralateral kidney did not differ from that in sham-operated controls 24 wk after onset of obstruction. There was no difference in kidney weight between nonobstructed kidneys of PUUO + 1wR rats and sham-operated controls after 24 wk (Table 3).

### Early Release of Obstruction Attenuates Natriuresis From the Obstructed Kidney

Neonatal PUUO did not change plasma concentrations of sodium and osmolality, whereas the concentration of sodium was significantly increased in urine from the obstructed kidneys (53.1 ± 12.7, 44.3 ± 8.9, and 21.8 ± 4.9 μmol/ml in PUUO, PUUO + 4wR, and sham, respectively, P < 0.05; Table 4). Consistent with the reduced GFR in obstructed kidneys, the filtered load of sodium decreased in the obstructed kidney in PUUO and PUUO + 4wR rats (Table 4). Furthermore, the fractional excretion of sodium [1.86 ± 0.62 vs. 0.37 ± 0.13% (sham), P < 0.05] and the urinary sodium excretion [0.32 ± 0.07 vs. 0.11 ± 0.02 (sham) μmol/min⁻¹·100 g body wt⁻¹, P < 0.05; Table 4] were significantly increased in the obstructed kidney in PUUO and PUUO + 4wR rats 24 wk after onset of obstruction (Table 4).

Consistent with normalization of GFR in PUUO + 1wR rats, the filtered load of sodium [38.4 ± 3.5 vs. 38.2 ± 5.9 (sham) μmol/min⁻¹·100 g body wt⁻¹, P > 0.05] and the fractional excretion of sodium did not differ from the sham-operated controls (0.47 ± 0.07 vs. 0.37 ± 0.13%, P > 0.05; Table 4). Release of obstruction after 1 wk reduced urinary sodium excretion (0.20 ± 0.03 and 0.32 ± 0.07 μmol/min⁻¹·100 g body wt⁻¹, P < 0.05 vs. sham).

### Table 3. Kidney weight and protein content of kidneys in PUUO, PUUO + 1wR, and sham rats at 24 wk of age

<table>
<thead>
<tr>
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<td>0.37 ± 0.02*</td>
<td>0.35 ± 0.01</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>Protein concentration, mg/g kidney wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>62 ± 3*</td>
<td>87 ± 5</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>84 ± 3</td>
<td>90 ± 3</td>
<td>87 ± 4</td>
</tr>
<tr>
<td>Total protein, mg/kidney wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>45 ± 3*</td>
<td>55 ± 4</td>
<td>58 ± 4</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>66 ± 3</td>
<td>69 ± 3</td>
<td>68 ± 4</td>
</tr>
<tr>
<td>Protein fraction (of sham), %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>77 ± 6*</td>
<td>96 ± 7</td>
<td>100 ± 7</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>98 ± 5</td>
<td>103 ± 4</td>
<td>106 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. *P < 0.05 vs. sham.

### Table 4. Changes in renal tubular function in PUUO, PUUO + 1wR, PUUO + 4wR, and sham rats at 24 wk of age

<table>
<thead>
<tr>
<th></th>
<th>PUUO (n = 5-7)</th>
<th>PUUO + 1wR (n = 7-10)</th>
<th>PUUO + 4wR (n = 6-8)</th>
<th>Sham (n = 9-11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNa, μmol/ml</td>
<td>131 ± 1.3</td>
<td>130.9 ± 1.2</td>
<td>127 ± 1.53</td>
<td>130.3 ± 0.9</td>
</tr>
<tr>
<td>Psmol, mosmol/kg</td>
<td>282.5 ± 1.9</td>
<td>281.5 ± 3.2</td>
<td>277 ± 3.0</td>
<td>281.0 ± 1.5</td>
</tr>
<tr>
<td>FLNa, μmol/min⁻¹·100 g body wt⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>22.4 ± 4.7*</td>
<td>38.4 ± 3.5</td>
<td>17.4 ± 4.3*</td>
<td>38.2 ± 5.9</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>49.2 ± 3.9</td>
<td>39.8 ± 3.1</td>
<td>37.6 ± 4.0</td>
<td>42.7 ± 5.3</td>
</tr>
<tr>
<td>UNa, μmol/ml</td>
<td>5.7 ± 1.5</td>
<td>6.9 ± 1.3</td>
<td>7.4 ± 3.4</td>
<td>6.3 ± 1.4</td>
</tr>
<tr>
<td>OBS</td>
<td>8.3 ± 2.0</td>
<td>4.4 ± 0.8</td>
<td>3.1 ± 0.7</td>
<td>5.4 ± 1.2</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>14.0 ± 0.9*</td>
<td>32.0 ± 6.3</td>
<td>22.9 ± 2.1</td>
<td>24.6 ± 2.8</td>
</tr>
<tr>
<td>USmol, mosmol/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>351 ± 42</td>
<td>377 ± 10</td>
<td>451 ± 46</td>
<td>647 ± 122</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>451 ± 89</td>
<td>450 ± 25</td>
<td>525 ± 113</td>
<td>520 ± 69</td>
</tr>
<tr>
<td>UNaV, μmol/min⁻¹·100 g body wt⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>0.32 ± 0.07*</td>
<td>0.20 ± 0.03*</td>
<td>0.20 ± 0.07*</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>0.15 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>0.06 ± 0.01*</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>FE Na, %</td>
<td>1.86 ± 0.62*</td>
<td>0.47 ± 0.07</td>
<td>1.90 ± 0.94*</td>
<td>0.37 ± 0.13</td>
</tr>
<tr>
<td>OBS</td>
<td>0.32 ± 0.04</td>
<td>0.28 ± 0.03</td>
<td>0.17 ± 0.03</td>
<td>0.30 ± 0.07</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>0.47 ± 0.16*</td>
<td>1.73 ± 0.60</td>
<td>1.59 ± 1.02*</td>
<td>2.71 ± 0.67</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. PNa, plasma sodium; Psmol, plasma osmolality; FLNa, filtered load of sodium; UNa, urine volume; USmol, urine sodium; USmol, urine osmolality; UNaV, excretion rate of sodium; FE Na, fractional excretion of sodium; T³H₂O, solute free water reabsorption. *P < 0.05 vs. sham.
μmol·min⁻¹·100 g body wt⁻¹ in PUUO + 1wR and PUUO, respectively.

Early Release of Obstruction Normalizes Urinary Concentrating Capacity in the Obstructed Kidney

Neonatal PUUO did not change the total urine output. Consistent with the known impairment in renal water handling during obstructive nephropathy, TmH₂O was markedly decreased in the obstructed kidney from PUUO rats (0.47 ± 0.16 and 2.71 ± 0.67 μl·min⁻¹·100 g body wt⁻¹ in PUUO and sham, respectively, P < 0.05; Table 4), demonstrating a reduced ability of these kidneys to reabsorb water in the collecting duct. This was slightly increased in the obstructed kidney in PUUO + 4wR rats (1.59 ± 0.60 and 0.47 ± 0.16 μl·min⁻¹·100 g body wt⁻¹ in PUUO + 1wR and PUUO, respectively, P < 0.05; Table 4), suggesting that early release of obstruction reduced the urinary concentrating defect.

Early Release of Obstruction Prevents the PUUO-Induced Downregulation of Renal Sodium Transporters

To investigate the molecular mechanism involved in the defective sodium reabsorption in the obstructed kidney after neonatal PUUO, expression of the key sodium transporters BSC-1 and Na-K-ATPase was examined in whole kidney samples.

After 24 wk of persistent PUUO, semiquantitative immunoblotting demonstrated a reduced abundance of the α₁-subunit of the Na-K-ATPase (62 ± 7 and 100 ± 11% in PUUO and sham, respectively, P < 0.05; Fig. 3, A and B, Table 5). BSC-1, another key sodium transporter responsible for the secondary active transport of NaCl in the medullary thick ascending limb, was not significantly changed in the obstructed kidney (112 ± 10 and 100 ± 28% in PUUO and sham, respectively, P > 0.05; Fig. 3, C and D, Table 5).

Release of obstruction after 1 wk prevented the decrease in the α₁-subunit of the Na-K-ATPase. Semiquantitative immunoblotting revealed that whole kidney abundance did not differ significantly from that in sham-operated rats (84 ± 8 vs. 100 ± 11%, P > 0.05; Fig. 3, A and B, Table 5), consistent with the reduced natriuresis from the same kidneys. Release of obstruction after 4 wk revealed that obstructed whole kidney abundance of BSC-1 and the α₁-subunit of the Na-K-ATPase did not differ significantly from that in the PUUO group (Fig. 4).

Semi quantitative immunoblotting of the contralateral non-obstructed kidney from PUUO and PUUO + 1wR rats revealed that whole kidney abundance of the same key sodium transporters did not differ from that in sham-operated rats 24 wk after onset of obstruction [Na-K-ATPase: 100 ± 5 vs. 100 ± 11% (sham), P > 0.05; BSC-1: 163 ± 16 vs. 100 ± 3% (sham), P > 0.05; Table 5].

Early Release of Obstruction Prevents the PUUO-Induced Downregulation of Renal AQPs

Acute UUO is associated with a marked reduction in the expression of renal AQPs (18, 28), concurrent with the development of a urinary concentrating defect. To investigate the molecular mechanism involved in the impaired renal water handling in response to neonatal PUUO, expression of AQP1, located at the proximal tubule and descending thin limb of Henle, and the water channels AQP2 and AQP3, located in the collecting duct, was studied using immunoblot analysis. Semi-quantitative immunoblotting using membrane fractions prepared from whole kidney revealed that a 24-wk period of obstruction was associated with markedly reduced AQP1 expression (Fig. 5, A and B, Table 5). Both AQP1 bands (29 and 35–50 kDa) were decreased proportionally. Densitometric analysis revealed a significant decrease in AQP1 expression in
of whole kidney revealed that 24 wk of PUUO marginally increased T\textsubscript{c}
H\textsubscript{2}O\textit{, consistent with the increased T\textsubscript{c}
H\textsubscript{2}O. Release of obstruction after 1 wk prevented the decreased abundance of AQP1 (73
\pm 3\% in PUUO and sham, respectively, P < 0.05) and AQP3 (97
\pm 3\% in PUUO and sham, respectively, P < 0.05) and AQP3 expression in the obstructed kidney
to 53 \pm 7\% of sham levels, consistent with significant reductions in T\textsubscript{c}
H\textsubscript{2}O (Table 4).

Release of obstruction after 1 wk prevented the decreased expression of AQP1 (73
\pm 12 and 100 \pm 10\% in PUUO + 1wR and sham, respectively, P > 0.05) and AQP3 (97
\pm 9 and 100 \pm 11\% in PUUO + 1wR and sham, respectively, P > 0.05), consistent with the increased T\textsubscript{c}
H\textsubscript{2}O. Release of obstruction after 4 wk revealed that obstructed whole kidney abundance of AQP1 did not differ significantly from the level in PUUO rats (Fig. 6, A and B), whereas obstructed whole kidney abundance of AQP2 was marginally increased compared with the level in the obstructed kidney from PUUO rats (Fig. 6, C and D).

Semi quantitative immunoblotting of the contralateral non-obstructed kidney from PUUO rats revealed that the whole kidney abundance of AQP1, AQP2, and AQP3 did not differ from that in sham-operated controls (90 \pm 3 and 100 \pm 10\% for AQP1 and sham, respectively, P > 0.05, 102 \pm 3 and 100 \pm 14\% for AQP2 and sham, respectively, P > 0.05, and 80 \pm 8 and 100 \pm 11\% for AQP3 and sham, respectively, P > 0.05).

DISCUSSION

The main results of this study demonstrated that release of obstruction before the kidney enters the fast neonatal maturation period attenuated the functional impairment of the obstructed kidney in rats with neonatally induced PUUO. Neonatal PUUO for 24 wk resulted in a progressive decrease in RBF and a severe reduction in GFR in the obstructed kidney. The contralateral kidney counterbalanced the impairment of RBF and kidney growth. Obstruction was associated with severe hydrenephrosis and obstructive nephropathy, shown as a marked reduction in total kidney protein content. These changes were associated with a decreased abundance of Na-K-ATPase, consistent with a significant natriuresis from the obstructed kidney. The abundance of AQP1, AQP2, and AQP3 was also reduced, consistent with the reduced GFR and solute free water reabsorption. Importantly, the results demonstrated that release after 4 wk was associated with changes very similar to PUUO without release of obstruction. In contrast, release after 1 wk of obstruction significantly attenuated the progressive reduction in RBF, and GFR was normal at 24 wk of age. The development of hydrenephrosis and obstructive nephropathy was prevented. Moreover, downregulation of renal Na-K-ATPase and AQP1 and AQP3 was prevented, consistent with attenuation of the natriuresis and decreased solute free water reabsorption in kidneys released 1 wk after onset of obstruction.

Table 5. Changes in expression levels of renal AQP\textsubscript{s} and major sodium transporters in PUUO, PUUO + 1wR, and sham rats at 24 wk of age

<table>
<thead>
<tr>
<th>AQP or transporter</th>
<th>PUUO</th>
<th>PUUO + 1wR</th>
<th>Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na-K-ATPase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>62 ± 7*</td>
<td>84 ± 8</td>
<td>100 ± 11</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>100 ± 5</td>
<td>107 ± 13</td>
<td>100 ± 11</td>
</tr>
<tr>
<td>BSC1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>112 ± 10</td>
<td>146 ± 15</td>
<td>100 ± 28</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>163 ± 16</td>
<td>156 ± 19</td>
<td>100 ± 3</td>
</tr>
<tr>
<td>AQP1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>53 ± 3*</td>
<td>73 ± 12</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>90 ± 3</td>
<td>95 ± 13</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>AQP2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>69 ± 9</td>
<td>96 ± 10</td>
<td>100 ± 17</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>102 ± 3</td>
<td>115 ± 11</td>
<td>100 ± 14</td>
</tr>
<tr>
<td>AQP3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>53 ± 7*</td>
<td>97 ± 9</td>
<td>100 ± 11</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>80 ± 8</td>
<td>110 ± 12</td>
<td>100 ± 11</td>
</tr>
</tbody>
</table>

Values are means \pm SE; n = 6, AQP, aquaporin; BSC-1, burmetanidesensitive Na-K-2Cl cotransporter. \*P < 0.05 vs. sham.
neonatal PUUO. Thus the results support the view that obstruction should be released soon after nephrogenesis is completed to prevent progressive deterioration and functional impairment of the developing kidney. Delayed release of obstruction until full maturation of kidney function results in severe impairment of renal functions.

**Early Release of Obstruction Prevents RBF and GFR Reductions in the Obstructed Kidneys**

Congenital urinary tract obstruction may cause profound changes in RBF and GFR of the obstructed kidney. This study

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**Fig. 5.** Immunoblot of membrane fractions of total kidneys from PUUO, PUUO + 1wR, and sham rats. A, C, and E: immunoblots were reacted with anti-AQP1 and anti-AQP2 antibody, respectively. B: densitometric analysis revealed no change in AQP1 expression between PUUO and PUUO rats (113 ± 3 vs. 100 ± 7%). D: expression levels of AQP2 were increased moderately but significantly in PUUO + 4wR compared with PUUO rats (121 ± 3 vs. 100 ± 7%, P < 0.05). *P < 0.05 vs. PULUO.

---

**Fig. 6.** Immunoblot of membrane fractions of total kidneys from PUUO and PUUO + 1wR rats. A and C: immunoblots were reacted with anti-AQP1 and anti-AQP2 antibody, respectively. B: densitometric analysis revealed no change in AQP1 expression between PUUO and PUUO rats (113 ± 3 vs. 100 ± 7%). D: expression levels of AQP2 were increased moderately but significantly in PUUO + 4wR compared with PUUO rats (121 ± 3 vs. 100 ± 7%, P < 0.05). *P < 0.05 vs. PUUO.

---
showed that neonatal PUUO progressively reduced RBF, consistent with previous rat studies using the same model (51). This progressive reduction in ipsilateral RBF is a key manifestation of the development of obstructive nephropathy in response to congenital urinary tract obstruction as demonstrated by others (2, 4, 34). Development of the renal vascularity is delayed by neonatal UUO, and the activity of the intrarenal renin-angiotensin system is enhanced throughout the period of obstruction (4, 6, 9). Previous studies have also demonstrated that blockade of the renin-angiotensin system prevents some of the functional changes associated with neonatal obstruction of the ureter (4, 6, 13).

In this study, GFR was severely reduced by 40% at 24 wk after onset of neonatal PUUO. This finding is consistent with previous studies in guinea pigs in which chronic severe PUUO reduced GFR to a similar degree (7). During neonatal UUO, glomerular maturation is also delayed, and this may play an important pathophysiological role and in part explain the reduction in GFR. This is compatible with observations in the human fetus with obstructive nephropathy, which is associated with reduced numbers of glomeruli (19).

In rats with release of obstruction after 1 wk, GFR was normalized at 24 wk of age, whereas the reduction in RBF was significantly attenuated compared with RBF in the nonreleased kidney of PUUO rats. In contrast, in rats with release of obstruction after 4 wk, reduction in GFR and RBF was similar to the levels observed in nonreleased kidneys of PUUO rats. This demonstrates that early release of the obstruction on completion of nephrogenesis prevents the reduction in GFR. Previous studies have highlighted the significance of the irreversible nature of nephron loss resulting from chronic UUO in the developing kidney (5, 11, 17). This study demonstrated that the filtration fraction was identical and slightly elevated in all obstructed kidneys, whether obstruction was released after 1 wk or 4 wk or was not released. Thus, despite normalization of GFR, the finding that filtration fraction is not changed may indicate ongoing hyperfiltration in the obstructed kidneys, which could be due to a reduction in the total number of glomeruli. The primary concern is that PUUO associated with impaired nephrogenesis and hyperfiltration over time may lead to progressive glomerular sclerosis, which is more marked in immature than in adult rats subjected to unilateral nephrectomy 5−11 mo previously (40).

PUUO was associated with a significant compensatory increase in the contralateral RBF that persisted during the 24 wk of observation. This is consistent with the results of the previous studies demonstrating compensatory changes in RBF of the intact kidney in response to neonatal UUO (50). An important observation of this study was that the compensatory increase in the contralateral RBF was attenuated in rats with release of obstruction after 1 wk, suggesting a blunting of the counterbalance in this group compared with late release and no release of obstruction.

Early Release of Obstruction Prevents Development of Hydronephrosis

Neonatal PUUO was associated with development of severe hydronephrosis and obstructive nephropathy. This study showed that PUUO resulted in a dramatic increase in TKV, consistent with previous findings demonstrating a similar increase in TKV in response to severe neonatal PUUO (50, 51). Furthermore, weight of the obstructed kidney did not increase significantly, suggesting that the volume increase was due to an increased amount of water. In accordance with this, the renal protein content was reduced, consistent with obstructive nephropathy. Similar results were found in the rats with release of obstruction after 4 wk. In contrast, rats with release of obstruction after 1 wk merely manifested mild pelvic dilation at 24 wk of age, and renal protein content was similar to that in sham-operated controls, indicating that early release may prevent progressive structural damage of the renal parenchyma. These results also corresponded with previous findings demonstrating a significant renal atrophy in the postobstructed kidney in rats subjected to even 2 days of neonatally induced complete UUO (12). In this study, release of obstruction at 4 wk did not alter the pattern of RBF, TKV, and GFR changes induced by the neonatal PUUO in the obstructed or nonobstructed kidney. These results support the view that progressive damage of the obstructed kidney is more severe during the period immediately after nephrogenesis when renal maturation takes place than in the late phase of nephrogenesis (11). Thus this study indicates that timing of surgical intervention plays a key role in the outcome of renal function in response to neonatally induced ureteral obstruction. This study supports the previous clinical view that early surgical intervention provides the highest degree of preservation of renal function (41, 42).

After neonatal UUO, growth of the obstructed kidney is impaired. This study showed that although true growth was impaired by obstruction, as demonstrated by a reduction in protein content, a compensatory growth of the contralateral kidney occurred. This is consistent with previous data demonstrating compensatory growth in response to neonatal UUO and is related directly to the duration of obstruction (12, 50). This so-called counterbalance has also been found to be significantly greater in neonatal than in adult animals (46). Consistent with previous observations, this study demonstrated that volume of the contralateral nonobstructed kidney increased persistently during the 24 wk of obstruction. Renal counterbalance was not stimulated in PUUO + 1wR rats, further supporting the view that early release of obstruction protects the kidney from progressive damage.

Although a compensatory increase in kidney mass and RBF was demonstrated in the nonobstructed kidney, there was no compensatory increase in GFR. Indeed, total GFR was decreased. This observation suggests that compensation is not induced exclusively by the functional demands. Various mechanisms may be involved in the functional cross talk between the kidneys. A 1-yr observation study showed that contralateral GFR is not increased in rats subjected to 5 days of neonatal complete UUO (10). The lack of compensatory increase was associated with progressive tubular damage in the nonobstructed kidney, indicating that not only the obstructed kidney, but also the nonobstructed kidney, suffered during severe unilateral obstruction. Our study confirms that severe obstruction early in life is not accompanied by an adaptive increase in function.

Early Release of Obstruction Attenuates PUUO-Induced Natriuresis and Downregulation of Renal Sodium Transporters

Neonatal UUO has profound effects on the developing tubule, with suppression of proliferation and maintenance of an
immatute phenotype by tubular epithelial cells (3). To examine whether the suppressed maturation and the pronounced changes in renal hemodynamics and tubular function are associated with molecular changes of the tubular cells, the expression of various renal transporters was examined. An intact urine concentration is critically dependent on the hypertonic medullar interstitium, which is generated by active NaCl reabsorption as a consequence of countercurrent multiplication and the osmotic equilibration of water across the tubular epithelium via AQPs (24). The active transport of sodium occurs mainly via the key sodium transporters: the basolateral Na-K-ATPase (22), the type 3 Na/H exchanger (NHE3) (1), and the apical BSC-1 (or NKCC2) (16). This study demonstrated that Na-K-ATPase abundance in the obstructed kidney was decreased after 24 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 decreased Na-K-ATPase in the obstructed kidney was decreased via the key sodium transporters: the basolateral Na-K-ATPase (22), the type 3 Na/H exchanger (NHE3) (1), and the apical BSC-1 (or NKCC2) (16). This study demonstrated that Na-K-ATPase abundance in the obstructed kidney was decreased after 24 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 of PUUO rats. The present results support the view that renal sodium transport is critically affected by ureteral obstruction as previously demonstrated (30) 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 release of the obstruction after 1 wk, demonstrating at the molecular level that early release of obstruction is important to protect the developing tubule system from damage. Release of obstruction after 1 wk also attenuated the natriuresis from the released kidney, demonstrating a functional association between the abundance of Na-K-ATPase and epithelial sodium transport. In contrast, release of obstruction after 4 wk demonstrated that abundance of Na-K-ATPase did not change compared with PUUO. This result further underscores that, in this system with severe neonatal PUUO, early release of the obstruction is essential to maintain the normal reabsorptive capacity of sodium.

The Na-K-ATPase is distributed along all nephron segments and functions in basolateral transport of sodium in the kidney tubule (22). This protein is involved in establishing the driving force promoting sodium reabsorption in the kidney tubule (22). The mechanisms involved in downregulation of the Na-K-ATPase are not fully understood. The reduced GFR and associated reduction in the filtered load of sodium may directly regulate the expression of Na-K-ATPase. Alternatively, the progressive hydropnephrosis and development of obstructive nephropathy due to the direct effects of the increased interstitial pressure may also be important factors in the dysregulation of Na-K-ATPase. Because early release of obstruction was associated with prevention of the GFR decrease as well as progression of hydropnephrosis, this study cannot explain which factor(s) is of most significance.

Early Release of Obstruction Prevents PUUO-Induced Downregulation of Renal AQPs

To examine whether dysregulation of renal AQPs is involved in the impaired urinary concentrating capacity, expression of the proximal nephron water channel AQP1 and the collecting duct water channels AQP2 and AQP3 was measured. AQP1, expressed in the proximal tubule and descending thin limb of Henle’s loop, was significantly reduced in the obstructed kidney of PUUO and PUUO + 4wr rats. This finding is consistent with studies demonstrating that ureteral obstruction downregulates AQP1 (28, 29). It was recently demonstrated that AQP1 knockout mice manifest a severe urinary concentrating defect and a decreased transepithelial water permeability, indicating that AQP1 plays a pivotal role in the countercurrent multiplier system (31). Impairment of fluid reabsorption in the proximal tubule and descending thin limb may have significant effects on the urinary concentrating mechanism. Thus downregulation of AQP1 expression in this study may indicate that neonatal PUUO directly impairs water reabsorption in the proximal tubule and descending thin limb. As suggested previously (45), an altered regulation of proximal tubule function and GFR may be a result of resetting of the tubuloglomerular feedback response induced by an increased delivery of NaCl at the macula densa.

Consistent with recent studies, semiquantitative immunoblotting of the obstructed kidneys demonstrated that the protein expression of AQP2 was marginally reduced and AQP3 was significantly reduced in response to neonatal PUUO (18, 28, 29). In parallel, neonatal PUUO was associated with a severe reduction in solute free water reabsorption, demonstrating a functional association between AQP downregulation and water reabsorption at the collecting duct level.

The mechanisms responsible for the downregulation of collecting duct AQPs in response to obstruction remain unclear. It is well established that AQP2 is regulated, in the short and long term, by vasopressin (27, 37). Recent studies from our laboratory have provided evidence for vasopressin-independent regulation of AQP2 and AQP3 in response to acute obstruction in adult rats (28, 29).

An important finding of this study demonstrated that release of obstruction after 1 wk prevented downregulation of AQP2 and AQP3. Furthermore, the functional significance of this finding was demonstrated as a partial prevention of the reduction in solute free water reabsorption. Thus progressive obstruction with a sustained interstitial pressure may significantly dysregulate renal AQPs as a direct consequence of the increased interstitial pressure or an indirect induction of molecular changes in the tubule cells mediated through altered levels of hormone concentration.

Conclusion

In summary, this study confirmed that neonatal PUUO in rats is associated with marked long-term changes in RBF and glomerular and tubular functions. The changes in tubular function could in part be explained by dysregulation of renal sodium transporters and water channels, suggesting that impaired renal handling of sodium and water in response to neonatal PUUO may be a direct consequence of this dysregulation. 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 PUUO and thus protected the kidney from obstructive damage. In conclusion, this study demonstrates that early release of neonatal obstruction before the fast maturation period following completion of nephrogenesis provides a better protection of renal functions than release of obstruction after the maturation process is completed.
REFERENCES