EARLY RELEASE OF NEONATAL URETERAL OBSTRUCTION PRESERVES RENAL FUNCTION

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Abstract

The incidence of congenital hydronephrosis is about 1% and often associated with renal insufficiency. It is currently unknown if early release is essential to prevent deterioration of renal function. Partial unilateral ureteral obstruction (PUUO) was performed in rat at postnatal day 2. The obstruction was either unreleased or released after 1 or 4 weeks of obstruction. Renal blood flow (RBF) and kidney size were measured sequentially during 24 weeks using magnetic resonance imaging (MRI). In unreleased rats, RBF of the obstructed kidney was progressively reduced to $0.92 \pm 0.17$ vs. $1.79 \pm 0.12 \text{ml} \cdot \text{min}^{-1} \cdot 100 \text{g bw}^{-1}$, $P<0.05$ after 24 weeks. Likewise GFR of the obstructed kidney was severely reduced at 24 weeks ($172 \pm 36$ vs $306 \pm 42 \mu\text{l} \cdot \text{min}^{-1} \cdot 100 \text{g bw}^{-1}$, $P<0.05$). These changes were preceded by development of severe hydronephrosis and obstructive nephropathy with a reduction in total protein content ($45 \pm 3$ vs. $58 \pm 4 \text{mg} \cdot \text{kidney}^{-1}$). Moreover, non-released PUUO caused a marked natriuresis ($0.32\pm0.07$ vs. $0.11\pm0.02 \mu\text{mol/min/100g bwt}$, $P<0.05$) and impairment in solute free water reabsorption ($TcH_2O; 0.47\pm0.16$ vs. $2.71\pm0.74 \mu\text{l/min/100g bwt}$, $P<0.05$) consistent with a significant downregulation of Na-K-ATPase to $62\pm7\%$, AQP1 to $53\pm3\%$ and AQP3 to $53\pm7\%$ of sham levels. Release after 1 week completely prevented the development of hydronephrosis, the reduction in RBF and GFR, and the downregulation of renal transport proteins whereas release after 4 weeks had no effect. This suggests that early release of neonatal obstruction provides a dramatically better protection of renal function than release of obstruction after the maturation process is completed.

Key words: rat, obstructive nephropathy, kidney function, magnetic resonance imaging, aquaporin, sodium transporter.
Introduction

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 antenatal detected hydronephrosis is still debated (25,42). The poor understanding of the natural history of congenital hydronephrosis as well as the unpredictable consequences of surgical intervention complicates the decision of whether the treatment should be surgical or non-surgical. Some advocate for an early surgical intervention (41,42), whereas others favor a prolonged observation (25). The focus of the controversies still is whether there is a necessity of surgical intervention and what the optimal time is if surgical intervention is unavoidable. Clinically, improvement or stabilization of renal function only occurs in the patients operated upon before 2 years of life (32). This observation may be critically related to the continuous maturation of the human kidney during the first 2 years 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.

More than 90% of nephrogenesis in the rat has taken place during the first 10 postnatal days. Subsequently the final maturation of the renal functions takes place. Cortical and medullary anatomy undergoes significant maturation during the critical period of the third week after birth (39). Previously it was demonstrated that the presence of urinary tract obstruction during this vulnerable period aggravates renal functional development and is associated with disproportionate renal functional impairment (8), and 3 week-old rats subjected to unilateral ureteral obstruction (UUO) have a highly activated tubuloglomerular feedback mechanism when studied 3 to 6 weeks later (33). Moreover, onset of complete UUO during the period from 14 to 19 days of age in rat results in more severe impairment of kidney growth than the impairment that takes place in response to neonatal UUO at 1 to 5 days of age or during adulthood (11). Furthermore, 3 months following release of UUO during days 14 to 19, renal growth was decreased by 50%, compared to a 30% reduction following release of UUO during days 1 to 5 (11). The number of glomeruli was reduced by approximately 50% regardless of the timing of UUO, but glomerular size was reduced only in rats with UUO during days 14 to 19 (11). These results demonstrate that in the period immediately following 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 detrimental impact on renal function later in life.

The effect of neonatally induced partial unilateral ureteral obstruction (PUUU) on kidney function during this vulnerable period is yet not fully understood. Previously it was demonstrated that GFR is reduced in proportion to on the severity of the obstruction (21). Recently we demonstrated that renal blood flow (RBF) was progressively reduced in rats with long-term follow up after neonatal PUUU using magnetic resonance imaging (MRI) (51). It has been hypothesized that renal functional deterioration in a hydronephrotic obstructed kidney stimulates the growth and function of the contralateral non-obstructed kidney at a faster rate than normal and before functional deterioration is detectable of the hydronephrotic kidney (26). Recently, we demonstrated that compensatory growth of the contralateral non-obstructed kidney during 24 week 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 (NDI) in severe cases (20). Urinary concentration and dilution depend on the presence of 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). Aquaporin-1 (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 have recently demonstrated that UUO and bilateral ureteral obstruction (BUO) cause severe dysregulation of renal AQPs which is associated with impaired renal water handling (28,29). UUO was also demonstrated to have altered expression levels of renal sodium transporters associated with deranged urinary sodium excretion (30), supporting the view that
dysregulation of these transporters play an essential role for 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, renal sodium and water handling of both the obstructed and non-obstructed kidney were examined together with the renal protein expression of major sodium transporter and water channels using a proteomic approach. Furthermore, we aimed at addressing whether early release rather than late release can prevent the renal functional reduction in response to neonatal PUUO.
Materials and Methods

Animal preparation

Studies were performed on female Münich Wistar rats. Rats were subjected to severe PUUO or SHAM operation within the first 48 hours of life using the modified Ulm and Miller technique previously described (51). Briefly, newborn rats were anesthetized with ether and placed on a heated table. The left ureter was exposed through a midline incision. Embedding two thirds of the left ureter in a psoas muscle tunnel created a PUUO. The SHAM group was prepared by laparotomy and mobilization of the left ureter. After surgery, rats were kept in an incubator at 30°C until totally awakened and then returned to the regular animal units with their mothers. One week or 4 weeks, respectively, after onset of obstruction 18 rats from the PUUO group were subjected to a second operation performed in general anesthesia with ether and release of the obstruction was achieved by removal of the ligatures and suturing the underlying psoas muscle. At 4 weeks of age, the rats were separated from their mothers and housed 2 per cage. During the experiments the rats were maintained at controlled temperature (22-24°C) and moisture (60%) with a 12 hours artificial light-dark cycle. The rats were fed a standard rodent diet and tap water. After 24 weeks the rats were sacrificed. The study complied with the Danish regulations for care and use of experimental animals. Rats were allocated to the protocols described below:

Protocol 1: PUUO group (n=7): PUUO for 24 weeks.

Protocol 2: PUUO+1wR group (n=10): PUUO was released 1 week after onset of obstruction.

Protocol 3: PUUO+4wR group (n=8): PUUO was released 4 weeks after onset of obstruction


MRI examinations were performed using intraperitoneal anesthesia with Pentothal® (50 mg·kg⁻¹ body-weight) (Abbott Scandinavia, Solna, Sweden) in all rats at 5, 12, and 24 weeks of age (see details below). Before sacrifice single kidney function were examined (see details below). Afterwards the two kidneys were removed, the wet weight determined and frozen for later analysis.
Magnetic Resonance imaging (MRI)

Briefly, the MRI examinations were performed with a smallbore SISCO 7 Tesla system (Varian Inc., Palo Alto, CA). The rat was placed supine in a Helmholtz head-coil of 4 cm in diameter and then subjected to an imaging protocol including measurements of single-kidney renal blood flow (RBF) and total kidney volume (TKV).

RBF Measurements

RBF measurements were obtained using a phase contrast technique involving a gradient echo sequence employed with bipolar flow sensitive gradients. The strengths of the flow encoding gradients were set according to the values from a previous study (44). Ten slices of 1.2mm thickness were prescribed perpendicular to the renal veins. Each slice had a 7x7cm² field of view and a resolution of 350x350 pixels to ensure appropriate pixels to find and derive the blood flow in the renal veins. Other parameters were repetition time (TR)=150 ms, echo time (TE)=5.5 ms, excitation flip angle=55°, and the number of data averages was four. Acquired phase images were subtracted and the vein flow was determined by multiplication with 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. Dependent on the kidney size, 20 to 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 RBF measurements, and other parameters were: TR=125 ms and TE=4 ms. Post-processing included manual identification of each kidney for all slices, and by careful encompassing regions of interest, the total kidney volume was measured by the sum of areas principle (50).

Measurement of GFR and tubular functions

GFR was measured using renal clearance of chromium-51 ethylenediaminetetraacetic acid (⁵¹Cr-EDTA) at 24 weeks after the onset of PUUO. Seven days before the clearance studies, the left femoral artery and vein were catheterized under intraperitoneal anesthesia with Pentothal® (50 mg · kg⁻¹ body-weight) (Abbott Scandinavia, Solna, Sweden). The arterial and venous catheters were fixed as described by Petersen et al. (43) and sealed with 50% glucose solution containing 500 units·ml⁻¹ of heparin and 10,000 units · ml⁻¹ of streptokinase and fixed.
After instrumentation, 5 ml saline and 10 µl analgesic (Temgesic®) were given subcutaneous. 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 37ºC. Through a midline incision, both ureters were exposed and catheterized (0.762 mm, Flexible plastic tubing, TYGON®, Weyerhaeuser, Cleveland, OH, USA) for the urine collection. The incision was closed to prevent loss of body fluid. A priming dose of $^{51}$Cr-EDTA (0.2 MBq) was given intravenously during 15 minutes followed by a sustained infusion (0.005 MBq ⋅ min⁻¹) during a 75 minutes equilibration period followed by two 1-hour urine collection periods. An intravenous infusion of 25 mM glucose solution (40 µl ⋅ min⁻¹) was provided simultaneously in order to keep an adequate minimum urine flow rate necessary 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 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 in an Auto-Gamma Counting System (COBRA™, PACKARD Instrument Company, Meriden, CT 06450).

The osmolality of urine and plasma was determined by freezing point depression (The Advanced Osmometer, Model 3900, Advanced Instruments, Norwood, MA and Osmomat 030-D, Gonotec, Berlin, Germany). The plasma concentration of sodium was determined (Kodak Ektachem 700XRC). The concentration of urinary sodium was determined by standard flame photometry (Eppendorf FCM6341).

The rats were sacrificed after the study. The harvested kidneys were rapidly frozen in liquid nitrogen and kept at –80ºC until assayed.

**Analysis of Renal Aquaporins and Sodium Transporters**

**Membrane fractionation for immunoblotting**

Kidneys were minced finely and homogenized in 9 ml dissecting buffer (0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, pH 7.2, containing protease inhibitors: 8.5 µM leupeptin and 1 mM phenylmethylsulfonfyl fluoride) with five strokes of a motor-driven Potter-Elvehjem homogenizer at 1,250 rpm. One microliter of this homogenate was used to measure the total protein concentration as previously described (see
below) (28). This homogenate was centrifuged in a Beckman L8M centrifuge at 4,000g for 15 minutes at 4°C. Gel samples (Laemmli sample buffer containing 2% SDS) were made from this membrane preparation.

**Total protein concentration.**

A BCA protein assay kit from Pierce® was used. A fresh set of protein standards (Bovine Serum Albumin, BSA, 2mg·ml⁻¹, 0, 1.5, 5, 10, 20, 30µl) containing the same component was prepared and used to determine the protein concentration for each sample. Distilled water was then added to all standards and samples to assure the same total volume in each tube (50 µl). One ml of BCA protein assay reagent mix solutions (reagent A: reagent B = 50:1) was added and all tubes were incubated at 37°C for 30 min. All samples were measured at 562 nm using a Helios Gamma spectrophotometer (Thermo Spectronic, UK) with the Protein Analysis Pack installed and choosing the BCA method. The protein standards were determined before all the other samples in order to make a standard curve. Measurement of each sample was made in duplicate and the mean values were used.

**Electrophoresis and immunoblotting**

Samples of membrane fractions from whole kidney were run on 9% or 12% polyacrylamide minigels (BioRad Mini Protean II). For each gel, an identical gel was run in parallel and subjected to Coomassie staining (48). The Coomassie-stained gel was used to ascertain identical loading or to allow for potential correction for minor differences in loading after scanning and densitometry of major bands. The other gel was subjected to blotting. After transfer by electroelution to nitrocellulose membranes, blots were blocked with 5% milk in PBS-T (80 mM Na₂HPO₄, 20 mM NaH₂PO₄, 100 mM NaCl, 0.1% Tween 20, pH 7.5) for 1 hour, and incubated with primary antibodies (see below) overnight at 4°C. After washing as above, the blots were incubated with horseradish peroxidase-conjugated secondary antibody (P448, Dako A/S, Glostrup Denmark, diluted 1:3000). After a final washing as above, antibody binding was visualized using enhanced chemiluminescence (ECL) system (Amersham International, U.K.). ECL films were scanned using a Hewlett-Packard Scanjet scanner and Adobe Photoshop software. The labeling density was determined of blots where samples of kidneys from PUUO, PUUO+1wR, and SHAM groups were run. The labeling density was corrected by densitometry of Coomassie brilliant blue-stained gels (i.e., to control for minor difference in protein loading).
In order to compare the fractional expressions from whole kidney between the 3 groups, the labeling density was then corrected for difference in total amount of protein present in each kidney by multiplying the density with the total protein content.

**Primary antibodies**

For semiquantitative immunoblotting, we used previously characterized monoclonal and polyclonal antibodies summarized in the following.

1) Na-K-ATPase: A monoclonal antibody against the alpha-1 subunit of Na-K-ATPase has previously been characterized (22);

2) BSC-1 (LL320AP): An affinity purified polyclonal antibody to the apical Na-K-2Cl cotransporter of the thick ascending limb has previously been characterized (16,23);

3) AQP1 (CHIP serum or LL266AP). Immune serum or an affinity-purified antibody to AQP1 has previously been characterized (48);

4) AQP2 (LL127 serum or LL127AP). Immune serum or affinity-purified antibody to AQP2 has previously been described (14);

5) AQP3 (LL178AP). An affinity-purified polyclonal antibody to AQP3 has previously been characterized (15).

**Calculations and Statistics**

GFR was estimated by calculating the renal clearance of $^{51}$Cr-EDTA ($\text{Cl}_{\text{EDTA}}$) using the following formula:

$$\text{Cl}_{\text{EDTA}} = \left[\frac{U}{P}\right]_{\text{EDTA}} \times U_{\text{vol}}$$

where $[U/P]_{\text{EDTA}}$ denotes the urine-to-plasma count ratio of $^{51}$Cr-EDTA, and $U_{\text{vol}}$ denotes the rate of urine flow divided by the body weight. The plasma counts corresponding to the midpoint of each urine collection period.

Filtration Fraction (FF) was calculated using the following formula:

$$\text{FF} \, (\%) = \frac{\text{GFR} \times 100}{\text{RPF}}$$

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

Filtered load of sodium (FLNa) was calculated by using the following formula:

$$\text{FL}_{\text{Na}} = \text{GFR} \times P_{\text{Na}}$$
where $P_{Na}$ denotes plasma sodium.

Excretion rate of sodium ($U_{Na}V$) was calculated by using the following formula:

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

where $U_{Na}$ denotes sodium concentration in urine, and $U_{vol}$ the rate of urine flow divided by the body weight.

Fractional excretion of sodium ($FE_{Na}$) was calculated by using the following formula:

$$FE_{Na} = \frac{[U/P]_{Na} \times [P/U]_{EDTA}}{}$$

in which $[U/P]_{Na}$ denotes the urine-to-plasma concentration ratio of sodium, and $[P/U]_{EDTA}$ the plasma-to-urine count ratio of $^{51}$Cr-EDTA.

Solute free water reabsorption ($T^{2}H_{2}O$) was calculated by using the following formula:

$$T^{2}H_{2}O = \frac{[(U_{osmolality}/P_{osmolality})-1] \times U_{vol}}{}$$

in which $U_{osmolality}/P_{osmolality}$ denotes the urine to plasma ratio of osmolality, and $U_{vol}$ the rate of urine flow divided by the body weight.

All values are presented as means ± SEM. One way analysis was performed for statistical analysis. If there was a significant difference, a Bonferroni test was performed to ascertain the difference between the groups. $P$ values less than 0.05 were considered to be statistically significant.
Results

**Early release of obstruction partially prevents RBF and GFR reductions in the obstructed kidneys**

Following neonatal induced PUUO, RBF of the obstructed kidney progressively decreased. RBF in the obstructed kidney was reduced by 33% of the sham level at 5 weeks of age, by 50% at 12 weeks, and by 49% at 24 weeks ($P<0.05$) (Fig. 1A; Table 1). Consistent with the progressive RBF reduction, there was a significant reduction in GFR after 24 weeks of obstruction to 43% of sham levels ($172 \pm 36 \text{ vs. } 306 \pm 42 \mu l \cdot \text{min}^{-1} \cdot 100 \text{g bw}^{-1}, P<0.05$) (Table 2). This progressive reduction in RBF was partially prevented in rats subjected to release of obstruction after 1 week. Examination of the rats at week 5 demonstrated that RBF was significantly higher in rats with PUUO+1wR than in rats with non-released PUUO ($2.79 \pm 0.54 \text{ vs. } 1.64 \pm 0.17 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{g bw}^{-1}, P<0.05$), whereas in rats with release of obstruction after 4 weeks RBF did not differ significantly from levels in non-released PUUO rats ($1.78 \pm 0.25 \text{ vs. } 1.64 \pm 0.17 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{g bw}^{-1}, P>0.05$) (Fig. 1A). Twenty-four weeks after onset of obstruction RBF in kidneys released after 1 week was significantly reduced compared with SHAM operated controls ($1.28 \pm 0.17 \text{ vs. } 1.79 \pm 0.12 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{g bw}^{-1}, P<0.05$), but levels were higher than in the non-released kidneys ($1.28 \pm 0.17 \text{ vs. } 0.92 \pm 0.17 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{g bw}^{-1}, P<0.05$) and in kidneys released after 4 weeks ($1.28 \pm 0.17 \text{ vs. } 0.74 \pm 0.13 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{g bw}^{-1}, P<0.05$) (Fig 1A). Consistent with these RBF changes in the released kidneys, the GFR reduction was prevented in rats where obstruction was released after 1 week ($304 \pm 18 \text{ vs. SHAM } 306 \pm 42 \mu l \cdot \text{min}^{-1} \cdot 100 \text{g bw}^{-1}, P>0.05$), whereas in rats where obstruction was released after 4 weeks GFR did not differ significantly from rats with non-released kidney ($137 \pm 34 \text{ vs. } 172 \pm 36 \mu l \cdot \text{min}^{-1} \cdot 100 \text{g bw}^{-1}, P>0.05$) (Table 2).

**Early release of obstruction attenuates compensatory RBF increase in the contralateral non-obstructed kidneys**

In the contralateral non-obstructed kidney of rats with PUUO there was significant compensatory increase in RBF at 5 weeks ($5.17 \pm 0.78 \text{ vs. SHAM } 2.49 \pm 0.19 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{g bw}^{-1}, P<0.05$) (Fig.1B; Table 1). This compensatory increase in RBF persisted at 12 weeks ($3.12 \pm 0.32 \text{ vs. SHAM } 2.49 \pm 0.19 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{g bw}^{-1}, P<0.05$) and at
24 weeks of age (2.20 ± 0.18 vs. SHAM 1.80 ± 0.10 ml/min⋅100g bw⁻¹, \( P < 0.05 \)). However, in the non-obstructed kidney of rats with PUUO+4wR a similar compensatory increase in RBF was demonstrated at 5, 12 and 24 weeks of age (Fig. 1B; Table 1). This compensatory increase in RBF was attenuated in the non-obstructed kidney of rats with PUUO+1wR compared with the PUUO+4wR (Fig. 1B; Table 1). Although being significantly elevated at 5 weeks (4.77 ± 0.54 vs. SHAM 2.49 ± 0.19 ml ⋅ min⁻¹ ⋅ 100g bw⁻¹, \( P < 0.05 \)), RBF normalized to SHAM levels at 12 weeks (2.99 ± 0.26 vs. 2.49 ± 0.19 ml ⋅ min⁻¹ ⋅ 100g bw⁻¹, \( P > 0.05 \)) and at 24 weeks after onset of obstruction (2.09 ± 0.12 vs. 1.80 ± 0.10 ml ⋅ min⁻¹ ⋅ 100g bw⁻¹, \( P > 0.05 \)).

In contrast to the RBF increase in the non-obstructed kidneys, GFR did not compensate when examined at 24 weeks after onset of obstruction. GFR in the non-obstructed kidney was identical in all 4 groups (Table 2).

**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 weeks (1.49 ± 0.19 vs. SHAM 0.50 ± 0.02 ml ⋅ 100g bw⁻¹, \( P < 0.05 \)), at 12 weeks (0.90 ± 0.15 vs. SHAM 0.38 ± 0.01 ml ⋅ 100g bw⁻¹, \( P < 0.05 \)) and at 24 weeks of age (0.98 ± 0.25 vs. SHAM 0.38 ± 0.01 ml ⋅ 100g bw⁻¹, \( P < 0.05 \)), whereas the kidney wet weight of the obstructed kidney did not change significantly in the PUUO rats at 24 weeks of age (0.34 ± 0.03 vs. SHAM 0.32 ± 0.01 g ⋅ 100g bw⁻¹, \( P > 0.05 \)) (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 the development in TKV of the obstructed kidney did not differ from the PUUO group (Fig. 2A). However, release of obstruction after 1 week (PUUO+1wR group) demonstrated that the increase in TKV was completely abolished. At 5, 12 and 24 weeks 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 PUUO+1wR group was slightly reduced, but total protein was identical to 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 non-obstructed kidney already at week 5 after onset of obstruction (0.64 ± 0.04 vs. SHAM 0.52 ± 0.02 ml ⋅ 100g bw⁻¹, \( P < 0.05 \)). This compensatory increase
persisted at 12 weeks (0.50 ± 0.04 vs. SHAM 0.39 ± 0.01 ml ∙ 100g bw⁻¹, P<0.05) and at 24 weeks of age (0.47 ± 0.02 vs. SHAM 0.40 ± 0.01 ml ∙ 100g bw⁻¹, P<0.05)(Fig. 2B). Release of obstruction at 4 weeks of age was associated with compensatory changes of 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 weeks TKV remained significantly increased, whereas TKV of the contralateral kidney did not differ from SHAM operated controls 24 weeks after onset of obstruction. There was no difference in kidney weight between non-obstructed kidneys of PUUO+1wR and SHAM operated controls after 24 weeks (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 from (PUUO: 53.1 ± 12.7 and PUUO+4wR: 44.3 ± 8.9 vs. SHAM: 21.8 ± 4.9 µmol ∙ ml⁻¹, P<0.05) (Table 4). Consistent with the reduced GFR in obstructed kidneys, the filtered load of sodium decreased in the obstructed kidney in both PUUO and PUUO+4wR (Table 4). Furthermore, the fractional excretion of sodium (1.86 ± 0.62 vs. SHAM 0.37 ± 0.13 %, P<0.05) and the urinary sodium excretion (0.32 ± 0.07 vs. SHAM 0.11 ± 0.02 µmol ∙ min⁻¹ ∙ 100g bw⁻¹, P<0.05) (Table 4) were significantly increased in the obstructed kidney in PUUO and PUUO+4wR rats 24 weeks 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. SHAM 38.2 ± 5.9 µmol ∙ min⁻¹ ∙ 100g bw⁻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 week completely reduced urinary sodium excretion (0.20 ± 0.03 vs. PUUO 0.11 ± 0.02 µmol ∙ min⁻¹ ∙ 100g bw⁻¹, P>0.05).

**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 T²H₂O was
markedly decreased in the obstructed kidney from PUUO rats (0.47 ± 0.16 vs. SHAM 2.71 ± 0.67 µl · min⁻¹ · 100g bw⁻¹, 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 (1.59 ± 1.02 µl · min⁻¹ · 100g bw⁻¹), whereas release of the obstruction after 1 week demonstrated that TcH₂O increased significantly compared with PUUO (1.73 ± 0.60 in PUUO+1wR vs. 0.47 ± 0.16 in PUUO µl · min⁻¹ · 100g bw⁻¹, 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 following neonatal PUUO, the expression of key sodium transporters BSC-1 and Na-K-ATPase was examined in whole kidney samples.

Following 24 weeks of persistent PUUO, semiquantitative immunoblotting demonstrated a reduced abundance of the α-subunit of the Na-K-ATPase (62 ± 7 vs. SHAM 100 ± 11%, 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 mTAL, was not significantly changed in the obstructed kidney (112 ± 10 vs. SHAM 100 ± 28%, P>0.05) (Fig. 3, C and D; Table 5).

Release of obstruction after 1 week prevented the decrease in the α-subunit of the Na-K-ATPase. Semiquantitative immunoblotting revealed that whole kidney abundance did not differ significantly from SHAM level (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 weeks revealed that obstructed whole kidney abundance of BSC-1 and the the α-subunit of the Na-K-ATPase did not differ significantly from PUUO level (Fig. 4, A-D).

Semiquantitative immunoblotting of the contralateral non-obstructed kidney from PUUO and PUUO+1wR revealed that whole kidney abundance of the same key sodium transporters did not differ from SHAM level 24 weeks after onset of obstruction (Na-K-ATPase: 100 ± 5 vs. SHAM 100 ± 11%, P>0.05; BSC1: 163 ± 16 vs. SHAM 100 ± 3%, P>0.05) (Table 5).
**Early release of obstruction prevents the PUUO-induced downregulation of renal aquaporins**

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 the expression of AQP1 located at the proximal tubule and descending thin limb of Henle, and the collecting duct located water channels AQP2 and AQP3 were studied using immunoblotting analysis. Semiquantitative immunoblotting using membrane fractions prepared from whole kidney revealed that 24 weeks of obstruction was associated with markedly reduced AQP1 expression (Fig. 5, A and B; Table 5). Both AQP1 bands (29 kDa and 35-50 kDa) were decreased proportionally. Densitometric analysis revealed a significant decrease in AQP1 expression in rats with PUUO (53 ± 3% vs. SHAM 100 ± 10%, *P* < 0.05).

Semiquantitative immunoblotting using membrane fraction of whole kidney revealed that 24 weeks of PUUO marginally reduced AQP2 (Fig. 4, C and D; Table 6) and significantly reduced AQP3 (Fig. 5, E and F; Table 5). Both AQP3 bands (29 kDa and 35-50 kDa) were decreased proportionally. Densitometric analysis revealed that AQP2 expression in the obstructed kidney of rats with PUUO was decreased to 69 ± 9% of SHAM levels and AQP3 expression in the obstructed kidney to 53 ± 7% of SHAM levels, consistent with significant reductions in TcH2O (Table 4).

Release of obstruction after 1 week prevented the decreased expression of both AQP1 (73 ± 12% vs. SHAM 100 ± 10%, *P* >0.05) and AQP3 (97 ± 9% vs. SHAM 100 ±11%, *P* >0.05), consistent with the increased TcH2O. Release of obstruction after 4 weeks revealed that obstructed whole kidney abundance of AQP1 did not differ significantly from PUUO level (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).

Semiquantitative immunoblotting of the contralateral non-obstructed kidney revealed that the whole kidney abundance of AQP1, -2 and -3 did not differ from that in SHAM operated controls (AQP1: 90 ± 3% vs. SHAM 100 ± 10%, *P* >0.05; AQP2: 102 ± 3% vs. SHAM 100 ± 14%, *P* >0.05; AQP3: 80 ± 8% vs. SHAM 100 ± 11%, *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 weeks resulted in a progressive decrease in RBF and a severe reduction in GFR of the obstructed kidney. The contralateral kidney counterbalanced the impairment of RBF and kidney growth. Obstruction was associated with severe hydronephrosis and obstructive nephropathy evidenced 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, -2 and -3 were also reduced consistent with the reduced GFR and solute free water reabsorption. Importantly, the results demonstrated that release after 4 weeks was associated with changes very similar to PUUO without release of obstruction. In contrast release after 1 week of obstruction significantly attenuated the progressive reduction in RBF and GFR was normal at 24 weeks of age. The development of hydronephrosis and obstructive nephropathy was prevented. Moreover, downregulation of renal Na-K-ATPase and AQP1 and -3 was prevented consistent with attenuation of the natriuresis and decreased solute free water reabsorption in kidneys released 1 week after onset of neonatal PUUO. Thus, the results support the view that release of obstruction should be performed early after nephrogenesis is completed to prevent progressive deterioration and functional impairment of the developing kidney. Delayed release of obstruction until full maturation of kidney functions is completed 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 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 vasculature is delayed by neonatal UUO, and the activity of the intrarenal renin-angiotensin system is enhanced throughout the period of obstruction (4,6,9). Consistent with this, previous
studies have demonstrated that blockade of the renin-angiotensin system prevents some of the functional changes associated neonatal obstruction of the ureter (4,6,13).

In this study GFR was severely reduced by approximately 40% twenty-four weeks after onset of neonatal PUUO. This 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 which 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 week, GFR was normalized at 24 weeks of age, whereas the reduction in RBF was significantly attenuated compared with RBF in the non-released kidney of PUUO rats. In contrast, rats with release of obstruction after 4 weeks showed that reduction in GFR and RBF was similar to the levels observed in non-released kidneys of PUUO rats. This demonstrates that early release of the obstruction at the time where nephrogenesis is completed 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 regardless whether obstruction was released after 1 week, 4 weeks or 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 months previously (40).

PUUO was associated with a significant compensatory increase in the contralateral RBF which persisted during the 24 weeks 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 week suggesting that there is a blunting of the counterbalance in this group compared to 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, it was demonstrated that the obstructed kidney weight 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 compatible with obstructive nephropathy. Similar results were found in the rats with release after 4 weeks. In contrast, rats with release of obstruction after 1 week merely manifested mild pelvic dilation at 24 weeks of age and renal protein content was similar to 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 rat subjected to even 2 days of neonatally induced complete UUO (12). In this study, release of obstruction at 4 weeks did not alter the pattern of RBF, TKV, and GFR changes induced by the neonatal PUUO in either the obstructed or the non-obstructed kidney. These results supports the view that progressive damage of the obstructed kidney is more severe during the period immediately following nephrogenesis where 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 on the outcome of renal function in response to neonatal induced ureteral obstruction. This study supports the previous clinical view that early surgical intervention provides the highest degree of renal functional preservation (41,42).

Following neonatal UUO, growth of the obstructed kidney is impaired. Consistent with this, this study showed that despite a true growth was impaired by obstruction demonstrated by a reduction in protein content; on the contrary, a compensatory growth of the contralateral kidney took place. This is consistent with previous data demonstrating that compensatory growth takes place 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 kidney volume of the contralateral non-obstructed kidney increased persistently during the 24 weeks of obstruction. Importantly, the results demonstrated that renal counterbalance was not stimulated in the rats where the obstruction was released 1
week after onset of PUUO, further supporting the view that that early release of obstruction protects the kidney from progressive damage.

Although a compensatory increase in kidney mass and RBF was demonstrated in the non-obstructed kidney, GFR was not compensatory increased. 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 one-year 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 and tubular damage in the non-obstructed kidney, indicating that not only the obstructed kidney but also the non-obstructed 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 immature 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 were examined. An intact urine concentration is critically dependent on the hypertonic medullary 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 aquaporins (24). The active transport of sodium occurs mainly via the key sodium transporters, which are the basolateral Na-K-ATPase (22), the type 3 Na/H exchanger (NHE3) (1) and the apical bumetanide-sensitive Na-K-2Cl cotransporter (BSC-1 or NKCC2) (16). This study demonstrated that Na-K-ATPase abundance in the obstructed kidney was decreased following 24 weeks of obstruction. Consistent with this, 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 (30) and underscore the role of an
intact expression of renal sodium transporters for 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 week, demonstrating at the molecular level that early release of obstruction is important to protect the developing tubule system from damage. Consistent with this release of obstruction after 1 week 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 weeks 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 normal reabsorptive capacity of sodium.

The Na-K-ATPase is distributed along all nephron segments and is functioning on basolateral transport of sodium in kidney tubule (22). This protein is involved in establishing the driving force to promote sodium reabsorption in 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 hydronephrosis and development of obstructive nephropathy due to the direct effects of the increased interstitial pressure may also be an important factor for the dysregulation of Na-K-ATPase. Since early release of obstruction was associated with prevention of the GFR decrease as well as progression of hydronephrosis, this study cannot provide an explanation for which factor/factors are of most significance.

**Early release of obstruction prevents PUUO-induced downregulation of renal aquaporins**

To examine whether dysregulation of renal AQP5s are involved in the impaired urinary concentrating capacity, the expression of the proximal nephron water channel AQP1 and the collecting duct water channels AQP2 and AQP3 were measured. The results demonstrated that 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 knock-out mice manifest a severe urinary concentrating defect and a decreased transepithelial water permeability indicating the AQP1 plays a pivotal role for the countercurrent multiplier system (31). Impairment of fluid reabsorption in
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, both short term and long term, by vasopressin (27,37). Recent studies from our laboratory have provided evidence for vasopressin independent regulation 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 week 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 either as a direct consequence of the increased interstitial pressure or due to 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 renal blood flow, 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 week but not after 4 weeks 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.
Acknowledgements

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References


Legends

Fig. 1. Changes in renal blood flow (RBF) development with the age in the 4 groups. A) Ipsilateral kidney: PUUO caused a persistent reduction in RBF. Release of obstruction at 1 week of age attenuated the RBF reduction, but RBF was significantly lower at 24 weeks of age as compared with SHAM operated controls. Release of obstruction at 4 weeks did not significantly change the progressive RBF reduction compared with the non-released kidney. B) Contralateral kidney: Contralateral RBF in all 3 obstructed groups increased compensatory at 5 weeks of age. The increased RBF after release of obstruction at 1 week of age was normalized at 12 weeks of age. The compensatory increase in contralateral RBF persisted in both PUUO rats and rats where obstruction was released after 4 weeks.

Fig. 2. Changes in total kidney volume (TKV) with the age in the 4 groups. A) Ipsilateral kidney: PUUO caused a persistent increase in TKV. Release of obstruction at 1 week of age prevented the TKV increase. TKV remained identical to the unobstructed kidney in SHAM. Release of obstruction at 4 weeks did not significantly change the progressive TKV increase compared with the non-released kidney. B) Contralateral kidney: TKV in all 3 obstructed groups increased compensatory at 5 weeks of age, however release of obstruction at 1 week attenuated this increase. At 24 weeks TKV in the PUUO and PUUO+1wR were identical, whereas TKV in the PUUO and PUUO+4wR was increased.

Fig. 3. Immunoblot of membrane fractions of total kidneys from rats at 24 weeks of age subjected to neonatal PUUO, PUUO released at 1 week of age, and SHAM operation (n = 6). A and C: Immunoblots were reacted with anti-Na-K-ATPase and anti-BSC-1 antibody. B: densitometric analysis revealed that total kidney \( \alpha \)-subunit of Na-K-ATPase expression of the obstructed kidney of PUUO group was significantly lower than that of SHAM group. Release of obstruction at 1 week of age prevented the decrease in the \( \alpha \)-subunit of Na-K-ATPase. D: total kidney BSC-1 expression in the obstructed kidney of PUUO group was not significantly changed in the obstructed kidney. Release of obstruction at 1 week of age did not change total kidney BSC-1 expression in the obstructed kidney compared with SHAM operated controls. * \( P<0.05 \) vs. SHAM.

Fig. 4. Immunoblot of membrane fractions of total kidneys from rats at 24 weeks of age subjected to neonatal PUUO, PUUO released at 4 weeks of age. A and C: Immunoblots were reacted with anti-Na-K-ATPase and anti-BSC-1 antibody. B and
C: densitometric analysis revealed that total kidney α-subunit of Na-K-ATPase and BSC-1 expression in the obstructed kidney of PUUO group released at 4 weeks of age did not change compared with 24 weeks of PUUO group (Na-K-ATPase: 97 ± 3% in PUUO-4wR vs. 100 ± 3% in PUUO; BSC-1: 71 ± 17% in PUUO-4wR vs. 100 ± 13% in PUUO).

Fig. 5. Immunoblot of membrane fractions of total kidneys from rats at 24 weeks of age subjected to neonatal PUUO, PUUO released at 1 week of age, and SHAM operation (n = 6). A, C, and E: Immunoblots were reacted with anti-AQP1, anti-AQP2, and anti-AQP3 antibody. B: Densitometric analysis revealed a significant decrease in AQP1 expression in rats with PUUO. Release of obstruction at 1 week of age prevented the decrease in AQP1 expression of the obstructed kidney. D: 24 weeks of PUUO marginally reduced AQP2 expression of the obstructed kidney. Release of obstruction at 1 week of age prevented the decrease in AQP2 expression of the obstructed kidney; F: 24 weeks of PUUO significantly decreased total kidney AQP3 expression of the obstructed kidney. Release of obstruction at 1 week of age prevented the decrease in AQP3 expression of the obstructed kidney. * P<0.05 vs. SHAM.

Fig. 6. Immunoblot of membrane fractions of total kidneys from rats at 24 weeks of age subjected to neonatal PUUO, PUUO released at 4 weeks of age. A, and C: Immunoblots were reacted with anti-AQP1 and anti-AQP2 antibody. B: Densitometric analysis revealed AQP1 expression in rats with PUUO released at 4 weeks of age did not change compared with PUOO group (113 ± 3% vs. 100 ± 7%). D: Release of obstruction at 4 weeks of age increased moderately but significantly the expression levels of AQP2 in the obstructed kidney compared with PUOO group (121 ± 5% vs. 100 ± 7%, p<0.05). * P<0.05 vs. PUUO.
Table 1. Changes in renal blood flow (RBF) development (ml · min⁻¹) in rats at 5, 12 and 24 weeks of age subjected to neonatal PUUO, PUUO released at 1 week or 4 weeks of age, and SHAM operation

<table>
<thead>
<tr>
<th></th>
<th>PUUO</th>
<th>PUUO+1wR</th>
<th>PUUO+4wR</th>
<th>SHAM</th>
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<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>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>
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<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>
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<tr>
<td>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>
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<tr>
<td>Non-OBS</td>
<td>6.49 ± 0.50 *</td>
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<td>7.00 ± 0.51 *</td>
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<tr>
<td>OBS</td>
<td>2.09 ± 0.39 *</td>
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<td>4.29 ± 0.26</td>
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<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>
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</table>

Mean values ± SEM are shown. PUUO, partial unilateral ureteral obstruction; PUUO+1wR, PUUO released at 1 week of age; PUUO+4wR, PUUO released at 4 weeks of age; SHAM, SHAM operated controls; n, number of rats; OBS, obstructed kidney; Non-OBS, non-obstructed kidney. * P<0.05 vs. SHAM group.
Table 2. Glomerular filtration rate (GFR), Filtration Fraction (FF), Kidney weight (KW), and Body weight (BW) in rats at 24 weeks of age subjected to neonatal PUUO, PUUO released at 1 week or 4 weeks of age, and SHAM operation

<table>
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<tr>
<th></th>
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<th>PUUO+4wR</th>
<th>SHAM</th>
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<tr>
<td>n</td>
<td>7</td>
<td>10</td>
<td>8</td>
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<tr>
<td>GFR (µl⋅min⁻¹⋅100g bw⁻¹)</td>
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<tr>
<td>OBS</td>
<td>172 ±36 *</td>
<td>304 ±18</td>
<td>137 ±34 *</td>
<td>306 ±42</td>
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<tr>
<td>Non-OBS</td>
<td>378 ±32</td>
<td>317 ±13</td>
<td>296 ±31</td>
<td>328 ±40</td>
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<tr>
<td>FF (%)</td>
<td></td>
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<tr>
<td>OBS</td>
<td>48 ±17</td>
<td>48 ±6</td>
<td>49 ±15</td>
<td>34 ±5</td>
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<tr>
<td>Non-OBS</td>
<td>31 ±2</td>
<td>30 ±2</td>
<td>25 ±5</td>
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<td>KW (g⋅100g bw⁻¹)</td>
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<tr>
<td>OBS</td>
<td>0.34 ±0.03</td>
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<td>0.34 ±0.04</td>
<td>0.32 ±0.01</td>
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<td>0.37 ±0.02*</td>
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<td>BW (g)</td>
<td>231 ±7</td>
<td>227 ±6</td>
<td>243 ±4</td>
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Mean values ± SEM are shown. PUUO, partial unilateral ureteral obstruction; PUUO+1wR, PUUO released at 1 week of age; PUUO+4wR, PUUO released at 4 weeks of age; SHAM, SHAM operated controls; n, number of rats; OBS, obstructed kidney; Non-OBS, non-obstructed kidney. * P<0.05 vs. SHAM group
Table 3. Kidney weight and protein content of kidneys in rats at 24 weeks of age subjected to neonatal PUUO, PUUO released at 1 week of age, and SHAM operation

<table>
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<th>SHAM</th>
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<td>n</td>
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<td>10</td>
<td>11</td>
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<tr>
<td>KW (g · 100g bw&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
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<tr>
<td>OBS</td>
<td>0.34 ± 0.03</td>
<td>0.28 ± 0.01*</td>
<td>0.32 ± 0.01</td>
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<tr>
<td>Non-OBS</td>
<td>0.37 ± 0.02*</td>
<td>0.35 ± 0.01</td>
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<tr>
<td>PC (mg · g kw&lt;sup&gt;-1&lt;/sup&gt;)</td>
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<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>
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<tr>
<td>TP (mg · kidney&lt;sup&gt;-1&lt;/sup&gt;)</td>
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<tr>
<td>OBS</td>
<td>45 ± 3*</td>
<td>55 ± 4</td>
<td>58 ± 4</td>
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<tr>
<td>Non-OBS</td>
<td>66 ± 3</td>
<td>69 ± 3</td>
<td>68 ± 4</td>
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<tr>
<td>PF (%)</td>
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<tr>
<td>OBS</td>
<td>77 ± 6*</td>
<td>96 ± 7</td>
<td>100 ± 7</td>
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<tr>
<td>Non-OBS</td>
<td>98 ± 5</td>
<td>103 ± 4</td>
<td>100 ± 6</td>
</tr>
</tbody>
</table>

Mean values ± SEM are shown. PUUO, partial unilateral ureteral obstruction; PUUO+1wR, PUUO released at 1 week of age; SHAM, SHAM operated controls; OBS, obstructed kidney; OBS, obstructed kidney; Non-OBS, non-obstructed kidney; KW, kidney weight; BW, body weight; PC, protein concentration; TP, total protein; PF, protein fraction of SHAM; * P<0.05 vs. SHAM group.
Table 4. Changes in renal tubular function in rats at 24 weeks of age subjected to neonatal PUUO, PUUO released at 1 week of age, and SHAM operation

<table>
<thead>
<tr>
<th></th>
<th>PUUO</th>
<th>PUUO+1wR</th>
<th>PUUO+4wR</th>
<th>SHAM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>5 - 7</td>
<td>7 - 10</td>
<td>6 - 8</td>
<td>9 - 11</td>
</tr>
<tr>
<td><strong>PNa</strong> (µmol ⋅ ml⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obs</td>
<td>131.2 ± 1.3</td>
<td>130.9 ± 1.2</td>
<td>127 ± 1.53</td>
<td>130.3 ± 0.9</td>
</tr>
<tr>
<td>Non-Obs</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><strong>POsm</strong> (mOsmol ⋅ kg⁻¹)</td>
<td>OBS</td>
<td>22.4 ± 4.7 *</td>
<td>38.4 ± 3.5</td>
<td>17.4 ± 4.3 *</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><strong>FLNa</strong> (µmol-min⁻¹·100g bw⁻¹)</td>
<td>OBS</td>
<td>5.7 ± 1.5</td>
<td>6.9 ± 1.3</td>
<td>7.4 ± 3.4</td>
</tr>
<tr>
<td>Non-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><strong>Uvol</strong> (µl-min⁻¹·100g bw⁻¹)</td>
<td>OBS</td>
<td>53.1 ± 12.7 *</td>
<td>27.6 ± 3.1</td>
<td>44.3 ± 8.9 *</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><strong>UNa</strong> (µmol ⋅ ml⁻¹)</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><strong>Uosm</strong> (mOsmol ⋅ kg⁻¹)</td>
<td>OBS</td>
<td>0.32 ± 0.07 *</td>
<td>0.20 ± 0.03*</td>
<td>0.20 ± 0.07*</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><strong>UNa V</strong> (µmol-min⁻¹·100g bw⁻¹)</td>
<td>OBS</td>
<td>1.86 ± 0.62 *</td>
<td>0.47 ± 0.07</td>
<td>1.90 ± 0.94 *</td>
</tr>
<tr>
<td>Non-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><strong>FENa</strong> (%)</td>
<td></td>
<td></td>
<td></td>
<td></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>
<tr>
<td><strong>TcH₂O</strong> (µl-min⁻¹·100g bw⁻¹)</td>
<td>OBS</td>
<td>3.24 ± 1.84</td>
<td>3.11 ± 0.82</td>
<td>3.37 ± 1.21</td>
</tr>
</tbody>
</table>

Mean values ± SEM are shown. * P<0.05 vs. SHAM group. n, no. of rats; PUUO, partial unilateral ureteral obstruction; PUUO+1wR, PUUO released at 1 week of age; SHAM, SHAM operated controls; OBS, obstructed kidney; Non-OBS, non-obstructed kidney; PNa, plasma sodium; POsm, plasma osmolality; FLNa, filtered load of sodium; Uvol, urine volume; UNa, Urine sodium; Uosm, urine osmolality; UNa V, excretion rate of sodium; FENa, fractional excretion of sodium; TcH₂O, solute free water reabsorption; bw, body weight.
Table 5. Changes in the expression levels of renal aquaporins and major sodium transporters in rats at 24 weeks of age subjected to neonatal PUUO, PUUO released at 1 week of age, and SHAM operation

<table>
<thead>
<tr>
<th></th>
<th>PUUO</th>
<th>PUUO+1wR</th>
<th>SHAM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><strong>Na-K-ATPase</strong></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><strong>BSC1</strong></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><strong>AQP1</strong></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><strong>AQP2</strong></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><strong>AQP3</strong></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>

Mean values ± SEM are shown. * P<0.05 vs. SHAM group. PUUO, partial unilateral ureteral obstruction; PUUO+1wR, PUUO released at 1 week of age; SHAM, SHAM operated controls; OBS, obstructed kidney; OBS, obstructed kidney; Non-OBS, non-obstructed kidney.
Fig. 1.
Fig. 2.

A Obstructed Kidney

B Non-obstructed Kidney
Fig. 3.
A Na-K-ATPase in obstructed kidney

B BSC-1 in obstructed kidney

Fig. 4
AQP1 in obstructed kidney

B

AQP1 Expression (Fraction of Sham)

PUUO n=6
PUUO+1wR n=6
SHAM n=6

C AQP2 in obstructed kidney

D

AQP2 Expression (Fraction of Sham)

PUUO n=6
PUUO+1wR n=6
SHAM n=6

E AQP3 in obstructed kidney

F

AQP3 Expression (Fraction of Sham)

PUUO n=6
PUUO+1wR n=6
SHAM n=6

Fig. 5.
Fig. 6