Neonatal ureteral obstruction alters expression of renal sodium transporters and aquaporin water channels

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Neonatal ureteral obstruction alters expression of renal sodium transporters and aquaporin water channels.

Background. Congenital urinary tract obstruction is a common cause of renal insufficiency in the neonate and during infancy. Recently, we demonstrated that ureteral obstruction in adult rats was associated with a reduction in the abundance of renal aquaporins (AQPs) and renal sodium transporters, which paralleled an impaired urinary concentrating capacity.

Methods. In the present study, renal handling of sodium and water, together with the expression of renal aquaporins and major renal sodium transporters, was examined in rats with neonatally induced partial unilateral ureteral obstruction (PUUO) within the first 48 hours of life to clarify the molecular mechanisms involved in the tubular functional defects in response to congenital obstruction. Rats were then followed for 12 or 24 weeks.

Results. Neonatal PUUO caused a progressive reduction in single kidney glomerular filtration rate (SKGFR) on the obstructed side to 43% of controls at 12 weeks (115 ± 28 vs. 267 ± 36 μL/min/100g bw, P < 0.05), and 31% of controls at 24 weeks (106 ± 24 vs. 343 ± 41 μL/min/100g bw, P < 0.05). Na-K-ATPase abundance was decreased in the obstructed kidney compared with the nonobstructed kidney at 24 weeks (79 ± 6%, P < 0.05), and the abundance of bumetanide-sensitive Na-K-2Cl cotransporter (BSC-1) located to the medullary thick ascending limb (mTAL) was significantly reduced both at 12 weeks (42 ± 10%, P < 0.05) and 24 weeks (50 ± 10%, P < 0.05). Immunohistochemistry confirmed down-regulation of BSC-1 both at 12 and 24 weeks after onset of obstruction. Consistent with this, sodium excretion from the obstructed kidney was increased at 12 weeks (0.13 ± 0.03 vs. 0.04 ± 0.01 μmol/min/100g bw, P < 0.05), and persisted 24 weeks after onset of PUUO (0.15 ± 0.02 vs. 0.06 ± 0.01 μmol/min/100g bw, P < 0.05). AQP2 abundance in the collecting duct was also reduced both at 12 weeks (68 ± 5%, P < 0.05) and at 24 weeks (69 ± 13%, P < 0.05). Consistent with this, solute-free water reabsorption was decreased in the obstructed kidney at 12 weeks (0.61 ± 0.42 vs. 1.97 ± 0.63 μL/min/100g bw, P < 0.05) and remained decreased after 24 weeks of PUUO (0.42 ± 0.04 vs. 1.56 ± 0.39 μL/min/100g bw, P < 0.05).

Conclusion. Major sodium transporters and aquaporins in the obstructed kidney are down-regulated in response to neonatally induced PUUO, which indicates that these transporters may play a crucial role for the persistent reduction in renal handling of sodium and water in response to PUUO.

Congenital urinary tract obstruction is a frequent manifestation in neonates with malformations of the urinary tract, and it is characterized by profound impairment of kidney function involving both glomerular filtration rate (GFR) and tubular handling of water and solutes [1]. Characteristically, urinary tract obstruction increases impedance in the renal pelvis and ureter, which causes an increase in the renal pelvic pressure. Subsequently, a reduction of renal blood flow (RBF) and GFR is induced by active vasoconstrictor mechanisms within the kidney vasculature due to an increased renal abundance of vasoconstrictive compounds [2, 3]. In parallel, tubular sodium reabsorption and urinary concentration become impaired [1]. Previous studies have demonstrated the segmental localization of sodium transporters along the nephron [4–6]. The key sodium transporters responsible for transport of NaCl across the apical membrane are type 3 Na/H exchanger (NHE3) in the proximal tubule [5] and the apical bumetanide-sensitive Na-K-2Cl cotransporter (BSC-1 or NKCC2) in the medullary thick ascending limb (mTAL) [4]. In the basolateral membrane, sodium is transported actively out of the cell by the Na-K-ATPase [6]. Previous studies have demonstrated that urinary tract obstruction is associated with a reduced
Na-K-ATPase activity [7, 8] and abundance of Na-K-2Cl cotransporter measured by a reduction in the saturable 
[^H] bumetanide binding to the Na-K-2Cl cotransporter 
[7]. Recently, we found that acute unilateral ureteral oc-
closure (UUO) for 24 hours in adult rats is associated 
with a reduced abundance of several major renal sodium 
transporters demonstrating that transporters located to 
all segments of the nephron and collecting duct may be af-
fected by acute urinary tract occlusion [9]. Thus, it is spec-
ulated that impaired sodium reabsorption in response to 
neonatally induced partial unilateral ureteral obstruction 
(PUUO) may be associated with altered expression of 
these major renal sodium transporters.

It was previously demonstrated that release of obstruc-
tion in rats with 24 hours of bilateral ureteral obstruction 
(BUO) is associated with a postobstructive diuresis [1]. 
Importantly, long-term PUUO in neonatal rats was asso-
ciated with an increased water excretion when examined 
at 1 year of age, suggesting a persisting defect in urine 
concentrating capacity [10]. Recently, we demonstrated 
that ureteral obstruction (BUO and UUO) is associated 
with a decreased abundance of aquaporin1 (AQP1), -2 
(AQP2), and -3 (AQP3) [11, 12]. Aquaporins are a fam-
ily of membrane water channels that mediate rapid water 
transport across the cell membrane [13]. Recent stud-
yes have identified the role of aquaporins in both short-
term and long-term regulation of body water balance 
and have elucidated their critical roles in multiple wa-
ter balance disorders [13, 14]. AQP1 is highly abundant 
in the proximal tubule and descending thin limb, and the 
critical role of AQP1 in urinary concentration was con-
firmed in transgenic knockout mice lacking AQP1 [15]. 
At least three aquaporins (AQP2, AQP3, and AQP4) 
are expressed in kidney collecting duct principal cells. 
AQP2 is the apical water channel of collecting duct prin-
cipal cells, and is the chief target for vasopressin regu-
lation of collecting duct water permeability [16]. A series 
of studies have shown that altered expression and apic-
cal targeting of AQP2 play a significant role in multiple 
hereditary and acquired water balance disorders (recent 
review [17]). Water transport across the basolateral 
plasma membrane of collecting duct principal cells is me-
diated by AQP3 [18] and AQP4 [19]. Consistent with 
this, transgenic mice lacking AQP3 are markedly polyuric 
[20], and inner medullary collecting ducts (IMCD) from 
AQP4-deficient mice demonstrated a significant reduc-
tion in vasopressin-induced water permeability [21].

Thus, we hypothesize that changes in the abundance of 
renal aquaporins may play an important role for the urin-
ary concentrating defect in PUUO.

In the present study, we therefore aimed to examine 
whether long-term PUUO induced at birth affects the 
abundance of key renal sodium transporters and aqua-
porins; and whether such changes are associated with im-
paired renal handling of sodium and water.

METHODS

Animal preparation

Experiments were performed using male Wistar rats. 
Rats were subjected to PUUO or sham operation within 
the first 48 hours of life. PUUO was created using a mod-
ification of the Ulm and Miller’s technique, which results 
in development of severe hydronephrosis [22–24]. Briefly, 
the 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 muscle tunnel of the underlying psoas muscle created 
PUUO. 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 awake, and then 
returned to regular animal units with their mother. Af-
ter 4 weeks, the rats were separated from their mother 
and housed 2 per cage. During the experiments the rats 
were maintained at controlled temperature (22–24°C) 
and moisture (60%) with a 12-hour artificial light-dark 
cycle. The rats were fed a standard rodent diet and tap 
water.

The rats were divided into 4 groups: (1) PUUO–12 
weeks; (2) SHAM–12 weeks; (3) PUUO–24 weeks; and 
(4) SHAM–24 weeks. GFR, urinary sodium excretion, 
and solute-free water reabsorption were examined at 12 
or 24 weeks of age, respectively, and then the rats were 
terminated. The harvested kidneys were rapidly frozen 
in the liquid nitrogen and kept at −80°C until assayed or 
rapidly put into formalin for immunohistochemistry.

Catheterizations and renal clearance of 51Cr-EDTA

GFR was measured using renal clearance of 
chromium-51 ethylenediaminetetraacetic acid (51Cr-
EDTA) at 12 or 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 bw; Abbott Scandi-
navia, Solna, Sweden). The arterial and venous catheters 
were sealed with 50% glucose solution containing 
500 U/mL of heparin and 10,000 U/mL of streptokinase 
and fixed. After instrumentation, 5 mL saline and 10 μL 
analgesic (Temgesic®) were given subcutaneously. After 
recovery from anesthesia, the rats were returned to the 
animal units and housed individually.

Renal clearance of 51Cr-EDTA was measured using a 
constant infusion technique. Briefly, the rats were 
anesthetized as described above, and then placed on 
a heating table to maintain the rectal temperature at 37°C. 
Through a midline incision, both ureters were ex-
posed and catheterized using a flexible plastic tubing 
(0.76 plastic tubing: TYGON®, Weyerhaeuser, Cleve-
land, OH, USA) as a catheter with the tip placed in 
the renal pelvis for direct urine collection. The incision 
was closed in order to prevent the 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) during a 75-minute equilibration period and two 1-hour urine collection periods. An intravenous infusion of 25 mmol/L glucose solution (40 µL/min) was provided simultaneously in order to keep an adequate minimum urine flow rate necessary for the analysis. 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).

The osmolality of urine and plasma was determined by freezing point depression (The Advanced Osmometer, Model 3900; Advanced Instruments, Norwood, MA, USA, and Osmomat 030-D, Gonotec, Berlin, Germany). Plasma concentrations of sodium, potassium, creatinine, and urea, and urinary concentration of creatinine and urea were determined (Vitros 950; Johnson & Johnson, Rochester, NY, USA). The concentrations of urinary sodium and potassium were determined by standard flame photometry (Eppendorf FCM6341).

**Primary antibodies**

For semiquantitative immunoblotting and immunohistochemistry, we used previously characterized monoclonal and polyclonal antibodies, summarized as follows: (1) β-actin (Sigma, A-2066); (2) AQP2 (LL127 serum, 1:3000); immune serum to AQP2 has previously been described [25]; (3) AQP3 (LL178, 1:300): an affinity-purified rabbit polyclonal antibody to AQP3 has previously been described [18]; (4) AQP1 (LL266 serum, 1:3000); immune serum to AQP1 has previously been described [26]; (5) NHE3 (LL546, 1:300): an affinity-purified polyclonal antibody to NHE3 was previously characterized [27]; (6) Na-K-ATPase (1:5000): a monoclonal antibody against the α1-subunit of Na-K-ATPase was previously characterized [6]; (7) BSC-1 (LL320, 1:300): an affinity-purified polyclonal antibody to the apical Na-K-2Cl cotransporter of the thick ascending limb (TAL) has previously been characterized [28].

**Membrane fractionation for immunoblotting**

On the day of the analysis, the kidneys were minced finely and homogenized in 9 mL dissecting buffer (0.3 mol/L sucrose, 25 mmol/L imidazole, 1 mmol/L EDTA, pH 7.2, containing protease inhibitors: 8.5 µmol/L leupeptin and 1 mmol/L phenylmethylsulfonyl fluoride) with five strokes of a motor-driven Potter-Elvehjem homogenizer at 1250 rpm. This homogenate was centrifuged in a Universal 30RF centrifuge (Hettich, Tuttingen, Germany) at 4000g for 15 minutes at 4°C. Gel samples (in 8% Laemmli sample buffer containing 2% SDS) were made from the supernatant.

**Electrophoresis and immunoblotting**

Samples of membrane fractions from whole kidney were run on 9% or 12% polyacrylamide minigels (Bio-Rad Mini Protein II; Bio-Rad). For each gel, an identical gel was run in parallel and subjected to Coomassie staining [26]. 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 80 mmol/L Na$_2$HPO$_4$, 20 mmol/L NaH$_2$PO$_4$, 100 mmol/L NaCl, and 0.1% Tween 20, pH 7.5 (PBS-T) for 1 hour, and incubated with the primary antibodies overnight at 4°C. After being washed as above, the blots were incubated with horseradish peroxidase–conjugated secondary antibody (P448, diluted 1:3000; Dako, Glostrup, Denmark). After a final wash as above, antibody binding was visualized using the enhanced chemiluminescence (ECL) system (Amersham International, Buckinghamshire, UK). Controls were made with the exchange of primary antibody to antibody preabsorbed with immunizing peptide (100 ng/40 ng IgG) or with preimmune serum (diluted 1:1000). All controls were without labeling.

**Semiquantitation of AQPs and sodium transporters**

ECL films were scanned using a Fluor-S Multi Imager (Bio-Rad Laboratories, Hercules, CA, USA) and Adobe Photoshop software (San Jose, CA, USA). The labeling density was determined of blots where samples of the obstructed and nonobstructed kidneys from either 12 or 24 weeks of PUUO 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).

**Immunohistochemistry**

Kidneys from PUUO-12W and PUUO-24W rats were immersion-fixed by formalin. For immunoperoxidase microscopy, kidney blocks were dehydrated and embedded in paraffin. The paraffin-embedded tissues were cut at 2 µm on a rotary microtome (Leica, Wetzlar, Germany). The sections were deparaffinated and rehydrated. For immunoperoxidase labeling, endogenous peroxidase was blocked by 0.5% H$_2$O$_2$ in absolute methanol for 10 minutes at room temperature. To reveal antigens, sections were put in 1 mmol/L Tris solution (pH 9.0) supplemented with 0.5 mmol/L EGTA (3.6-di-oxa-octyl-methylendi-nitrito-tetra-acetic-acid), and were heated using a microwave oven for 10 minutes. Nonspecific binding of immunoglobulin was prevented by incubating the sections in 50 mmol/L NH$_4$Cl for 30 minutes, followed by blocking in PBS supplemented with 1% BSA,
Filtered load of sodium ($FL_{Na}$) was calculated by using the following formula:

$$ FL_{Na} = GFR \times P_{Na} $$

where $P_{Na}$ denotes plasma sodium.

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

$$ U_{Na}V = U_{Na} \times (V_u/bw) $$

where $U_{Na}$ denotes sodium concentration in urine.

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

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

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

Solute-free water reabsorption ($TCH_{2O}$) was calculated by using the following formula:

$$ TCH_{2O} = [(Uosmolality/Posmolality) - 1] \times (V_u/bw) $$

in which $Uosmolality/Posmolality$ denotes the urine to plasma ratio of osmolality.

All values are presented as means ± SEM. Unpaired Student $t$ tests were used for the comparisons. $P$ values less than 0.05 were considered to be statistically significant.

**RESULTS**

**Neonatal PUUO is associated with a progressive reduction in GFR**

Glomerular filtration rate was impaired on the obstructed side. After 12 weeks, GFR had decreased to 43% of sham levels (115 ± 28 vs. 267 ± 36 $\mu$L/min/100g bw, $P < 0.05$) (Fig. 1A). To further examine the long-term effect of PUUO on renal function, rats were followed to 24 weeks of age, where measurement of renal function demonstrated a persistent and progressive GFR reduction to 31% of sham levels (106 ± 24 vs. 343 ± 41 $\mu$L/min/100g bw, $P < 0.05$) (Fig. 1B). In the nonobstructed kidney, GFR did not show compensatory increase either at 12 or at 24 weeks after onset of PUUO. Thus, total GFR in the PUUO animals were reduced compared with sham-operated control animals at both 12 weeks ($418 \pm 75$ vs. $555 \pm 53$ $\mu$L/min/100g bw, $P < 0.05$) and at 24 weeks ($403 \pm 50$ vs. $679 \pm 67$ $\mu$L/min/100g bw, $P < 0.05$).

**PUUO is associated with sodium loss and impaired urinary concentration of the obstructed kidney**

To examine the effects of PUUO on renal handling of sodium and the urinary concentrating mechanisms, urinary sodium excretion was measured from both the obstructed and the nonobstructed kidney. Importantly, plasma sodium levels were identical in all groups...
To examine renal handling of water, the solute-free water reabsorption was measured. The solute-free water reabsorption of the obstructed kidney was markedly reduced at 12 weeks compared with the sham-operated controls (0.61 ± 0.42 vs. 1.97 ± 0.63 μL/min/100g bw, \( P < 0.05 \)), and remained decreased after 24 weeks of PUUO (0.42 ± 0.04 vs. 1.56 ± 0.39 μL/min/100g bw, \( P < 0.05 \)). Solute-free water reabsorption did not increase in the nonobstructed kidney either at 12 or at 24 weeks of PUUO.

**Table 1.** Changes in renal function and plasma electrolyte values at 12 and 24 weeks after onset of obstruction

<table>
<thead>
<tr>
<th></th>
<th>12 weeks</th>
<th>24 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PUUO</td>
<td>SHAM</td>
</tr>
<tr>
<td>Number</td>
<td>8(^a)</td>
<td>6</td>
</tr>
<tr>
<td>Body weight g</td>
<td>353 ± 10</td>
<td>343 ± 33</td>
</tr>
<tr>
<td>Total GFR µL/min/100g bw</td>
<td>418 ± 75(^a)</td>
<td>555 ± 53</td>
</tr>
<tr>
<td>( P_{Na} ) µmol/mL</td>
<td>131.6 ± 1.1</td>
<td>130.3 ± 1.8</td>
</tr>
<tr>
<td>( P_{K} ) µmol/mL</td>
<td>4.8 ± 0.4</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>( P_{Cr} ) µmol/mL</td>
<td>58 ± 5</td>
<td>47 ± 3</td>
</tr>
<tr>
<td>( P_{UA} ) µmol/mL</td>
<td>9.6 ± 0.6</td>
<td>7.3 ± 0.8</td>
</tr>
<tr>
<td>( F_{Na} ) mOsmol/kgH₂O</td>
<td>295.9 ± 2.3</td>
<td>292.2 ± 2.3</td>
</tr>
<tr>
<td>( F_{Na} ) µmol/min/100g bw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS</td>
<td>10.6 ± 4.7(^a)</td>
<td>35.0 ± 4.9</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>39.9 ± 11.4</td>
<td>37.6 ± 3.0</td>
</tr>
<tr>
<td>( U_{Na} ) µmol/mL</td>
<td>2.28 ± 0.67</td>
<td>1.63 ± 0.57</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>2.17 ± 0.70</td>
<td>2.09 ± 0.75</td>
</tr>
<tr>
<td>( U_{Na} ) mOsmol/kgH₂O</td>
<td>78.2 ± 11.7(^b)</td>
<td>29.6 ± 5.1</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>37.6 ± 10.6</td>
<td>27.0 ± 3.4</td>
</tr>
<tr>
<td>( U_{Osm} ) mOsmol/kgH₂O</td>
<td>403 ± 39(^a)</td>
<td>809 ± 196</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>662 ± 128</td>
<td>907 ± 235</td>
</tr>
<tr>
<td>( U_{Na} V ) µmol/min/100g bw</td>
<td>0.13 ± 0.03(^b)</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>0.06 ± 0.02</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>( F_{Na} % )</td>
<td>1.20 ± 0.25(^a)</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>Non-OBS</td>
<td>0.19 ± 0.09</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>( T^{3} H_{2}O ) µmol/min/100g bw</td>
<td>0.61 ± 0.42(^a)</td>
<td>1.97 ± 0.63</td>
</tr>
<tr>
<td>OBS</td>
<td>1.48 ± 0.46</td>
<td>2.07 ± 0.78</td>
</tr>
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Abbreviations are: PUUO, partial unilateral ureteral obstruction; OBS, obstructed kidney; Non-OBS, non-obstructed kidney; Total GFR, glomerular filtration rate measured by clearance of \( ^{14} \text{C}-\text{EDTA} \); \( P_{Na} \), plasma sodium; \( P_{K} \), plasma potassium; \( P_{Cr} \), plasma creatinine; \( P_{UA} \), plasma urea; \( P_{Osm} \), plasma osmolality; \( F_{Na} \), filtered load of sodium; \( U_{Na} \), urine sodium; \( U_{Osm} \), urine osmolality; \( U_{Na} V \), excretion rate of sodium; \( F_{Na} \), fractional excretion of sodium; \( T^{3} H_{2}O \), solute-free water reabsorption; bw, body weight. Mean values ± SEM are shown.

\(^a\) \( P < 0.05 \) vs. SHAM group.
\(^b\) \( P < 0.05 \) vs. Non-OBS. There is no significant difference between 12 and 24 weeks group.

\(^\text{In 3 rats plasma electrolyte determination failed.}\)
response to either 12 weeks (42 ± 10% vs. 100 ± 7%, \( P < 0.05 \)) or 24 weeks (50 ± 10% vs. 100 ± 14%, \( P < 0.05 \)) (Fig. 3F) of PUUO. Immunohistochemistry showed that labeling of BSC-1 in the TAL cells of the obstructed kidney was markedly decreased (Figs. 5C, D, G, and H) compared with that in the nonobstructed kidney in rats with PUUO for 12 weeks (Fig. 5A and B) and 24 weeks (Fig. 5E and F).

After 12 weeks of PUUO, Na-K-ATPase levels in the obstructed kidney did not differ from that in the nonobstructed kidney (Fig. 3D). However, Na-K-ATPase levels decreased moderately to 79 ± 6% of the levels of the nonobstructed kidney as examined at 24 weeks after onset of PUUO (\( P < 0.05 \)) (Fig. 4D). Consistent with this, immunohistochemistry demonstrated lack of major down-regulation of Na,K-ATPase (data not shown).

**PUUO reduces AQP2 expression**

Immunoblotting using membrane fractions prepared from the obstructed and the nonobstructed kidneys revealed that PUUO for both 12 and 24 weeks was associated with a marked down-regulation of AQP2 expression in the obstructed kidney (Figs. 6C and 7C). Both AQP2 bands (the 29-kD and 35- to 50-kD bands) were decreased proportionately. Densitometry of these bands (combined) revealed a decrease in AQP2 expression after 12 weeks of PUUO to 68 ± 5% of the levels of the nonobstructed kidney (\( P < 0.05 \)) (Fig. 6D). This down-regulation persisted at 24 weeks after onset of PUUO (69 ± 13 vs. 100 ± 7%, \( P < 0.05 \)) (Fig. 7D). Consistent with the impaired water reabsorption, this result indicates that neonatally induced PUUO caused a substantial and persistent reduction in AQP2 protein levels of the obstructed kidney. Consistent with the moderate down-regulation of AQP2, immunohistochemistry demonstrated lack of major down-regulation of AQP2 and lack of major changes in the subcellular localization of AQP2 (data not shown).

To examine whether other aquaporins were involved in the impaired water handling in the neonatally induced PUUO, the expression of AQP3 and AQP1 were examined. Immunoblotting using whole kidney membrane fractions revealed an unchanged abundance of these 2 aquaporins (Figs. 6A and E, and 7A and E).

**DISCUSSION**

The main results of the present study demonstrated that neonatal PUUO in rats caused a significant decrease in GFR of the obstructed kidney both at 12 and 24 weeks after onset of PUUO. Moreover, PUUO was associated with a marked down-regulation of AQP2 expression in the obstructed kidney. Consistent with this, sodium excretion was increased and solute-free water reabsorption decreased from the obstructed kidney. These results support our previous findings that both sodium transporters and aquaporins are down-regulated in response to obstruction, and indicate that these transporters play a crucial role for the persistent reduction in renal handling of sodium and water in response to neonatally induced PUUO.

**PUUO is associated with a persistent GFR reduction**

Neonatally induced ureteral obstruction results in severe injury of the obstructed kidney. Consistent with this, the results of the present study demonstrated that PUUO induced within 48 hours of life was associated with a marked GFR reduction both at 12 and 24 weeks after onset of obstruction. Although total GFR was reduced, the rats were not azotemic. Moreover, the systemic solute balance was unchanged. This finding is consistent with previous results demonstrating a similar reduction in SKGFR of the obstructed kidney from guinea pigs, which also was associated with an arrested growth of the obstructed kidney after 3 weeks of age [29]. Using
Neonatal PUUO is associated with an increased sodium excretion and a reduced abundance of key sodium transporters

The urinary concentrating capacity 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 [34]. The active transport of sodium occurs mainly via the key sodium transporters, which are the apical bumetanide-sensitive Na-K-2Cl cotransporter (BSC-1 or NKCC2) [4], the basolateral Na-K-ATPase [6], and the type 3 Na/H exchanger (NHE3) [5].

The present study demonstrated that PUUO markedly reduced the abundance of BSC-1 in the obstructed kidney following both 12 and 24 weeks of PUUO by immunoblotting and immunohistochemistry. Importantly, this down-regulation of BSC-1 was associated with an
increased urinary excretion of sodium from the obstructed kidney. This is consistent with our previous finding in rats with UUO, where we demonstrated down-regulation of BSC-1 and increased sodium excretion from the obstructed kidney in response to 24 hours of UUO [9]. BSC-1 mediates sodium transport across the apical membrane of the mTAL [4]. The results of the present study therefore demonstrate that down-regulation of BSC-1 may play a major role for the renal handling of sodium in this model of long-term PUUO. In addition, PUUO reduced the abundance of Na-K-ATPase after 24 weeks of partial obstruction. In a previous study, reduction of Na-K-ATPase abundance was also found in rats subjected to 24 hours of UUO [9]. The demonstration of a reduced abundance of Na-K-ATPase in response to partial neonatal ureteral obstruction indicates that reduced Na-K-ATPase is involved in the long-term impairment of sodium handling in obstructive nephropathy. This finding is consistent with previous studies demonstrating a reduced activity and amount of Na-K-ATPase from the mTAL and the IMCD of rabbit kidneys with 24 hours of UUO [7, 8]. Thus, the results of the present study demonstrated that long-term PUUO reduces the abundance of both BSC-1 and Na-K-ATPase. The long-term effect is a chronic decrease in sodium entry into the mTAL cells. This finding supports the view that the reduction in sodium entry caused by reduced abundance of the apical BSC-1 and the reduced delivery of sodium to the mTAL in the obstructed kidney as a result of a reduced FLNa may contribute to the reduced abundance of Na-K-ATPase in the basolateral membrane [8].

NHE3 is expressed at the apical plasma membrane of the proximal tubule and the TAL, and it is the predominant transporter for active secretion of H⁺ and reabsorption of Na⁺ and HCO₃⁻ [5]. It has been demonstrated that down-regulation of NHE3 is associated with proximal tubule defects in sodium reabsorption, and increased renal sodium excretion in several conditions [35]. Recently, we demonstrated that acute UUO was associated with a reduced abundance of NHE3 in parallel with natriuresis [9]. However, NHE3 abundance of the obstructed kidney was maintained in this study. This finding may indicate that PUUO does not directly affect transporters located at the proximal tubule of the nephron.

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**Fig. 4.** Immunoblot of membrane fractions of total kidneys from PUUO for 24 weeks (N = 6). (A, C, E) Immunoblot was reacted with anti-NHE3, anti-Na-K-ATPase, and anti-BSC-1 antibody. (B) Densitometric analysis revealed no significant difference in total kidney NHE3 expression between the obstructed and nonobstructed kidneys (96 ± 10 vs. 100 ± 13%, P > 0.05). (D) Total kidney Na-K-ATPase expression of the obstructed kidney was significantly lower than that of the nonobstructed kidney (79 ± 6 vs. 100 ± 5%, P < 0.05). (F) Total kidney BSC-1 expression in the obstructed kidney was significantly lower than that in the nonobstructed kidney (50 ± 10 vs. 100 ± 14%, P < 0.05).
Fig. 5. Immunohistochemical analyses of BSC-1 in the TAL cells in obstructed and nonobstructed kidneys from rats with PUUO for 12 and 24 weeks. (A, B, E, F) Abundant BSC-1 labeling is seen of apical plasma membrane domains of TAL cells in the cortex (A and E) and the inner stripe of outer medulla (ISOM) (B and F) from the nonobstructed kidney in rats with PUUO for 12 weeks (A and B) and 24 week (E and F). (C, D, G, H) In the obstructed kidney of rats with PUUO for 12 and 24 weeks, labeling of BSC-1 in the TAL cells in the cortex (C and G) and the ISOM (D and H) is weaker compared with nonobstructed kidneys. 12w PUUO: PUUO for 12 weeks; 24w PUUO: PUUO for 24 weeks. Magnification ×250.
Alternatively, the finding may indicate a compensatory phenomenon in response to prolonged PUUO.

**Neonatal PUUO is associated with a reduced abundance of AQP2 in the obstructed kidney, but maintenance of AQP3 and AQP1 abundance**

In the present study, we demonstrated that the abundance of AQP2 is persistently reduced in the obstructed kidney. Furthermore, the present study showed that solute-free water reabsorption was reduced simultaneously with the reduced AQP2 abundance, demonstrating a functional association between the expression of AQP2 and the ability to reabsorb water at the collecting duct. Thus, these findings are consistent with an important role of reduced AQP2 expression in the impairment of urinary concentration and support the results from a previous study in adult rats with acute UUO, which demonstrated that both AQP2 protein and mRNA levels were reduced in the obstructed kidney [11]. Similar to the previous study [11], the parallel changes in AQP2 expression and solute-free water reabsorption supports the view that AQP2 levels are closely correlated with defects in water reabsorption in the collecting ducts in the obstructed kidney.

To address whether the defect in renal water handling is due to dysregulation of other aquaporins, the abundance of the proximal nephron water channel AQP1 and another collecting duct water channel, AQP3, was examined. The results demonstrated that AQP1 abundance was unchanged both at 12 and 24 weeks after onset of PUUO. Previously, we demonstrated that 24 hours of BUO caused a dramatic down-regulation of AQP1, which persisted up to at least 4 weeks after release of BUO [12]. The present results indicate that PUUO does not affect the abundance of the proximal tubule transporters to the same proportion. Alternatively, PUUO may induce a temporary down-regulation in AQP1, which is not detectable at 12 and 24 weeks after onset of PUUO. Previously, we demonstrated that BUO was associated with dysregulation of AQP3, which persisted up to 2 weeks after release of obstruction [12]. The results of the present study demonstrated that AQP3 abundance was unchanged at 12 and 24 weeks after onset of PUUO.

**Fig. 6. Immunoblot of membrane fractions of total kidneys from PUUO for 12 weeks (N = 6). (A, C, E) Immunoblot was reacted with anti-AQP1, anti-AQP2, and anti-AQP3 antibody. (B) Densitometric analysis revealed no significant difference in total kidney AQP1 expression between the obstructed and nonobstructed kidneys (89 ± 7 vs. 100 ± 9%, P > 0.05). (D) Total kidney AQP2 expression of the obstructed kidney was significantly lower than that in the nonobstructed kidney (68 ± 5 vs. 100 ± 5%, P < 0.05). (F) There was no significant difference in total kidney AQP3 expression between the obstructed and nonobstructed kidneys (127 ± 20 vs. 100 ± 17%, P > 0.05).**
Mechanisms involved in reduced abundance of BSC-1, Na-K-ATPase, and AQP2 in response to neonatal ureteral obstruction

By using a model with UUO there are no major changes in plasma solute concentrations because the contralateral intact kidney in part compensates for the loss of function in the obstructed kidney. Thus, it is possible in this model to distinguish between the roles of local intrarenal factors and the role of systemic factors. Indeed, the persistent reduction in BSC-1, Na-K-ATPase, and AQP2 abundance indicates that local factors may play a key role. Both BSC-1 and AQP2 are specifically regulated via the vasopressin/cAMP pathway [28], and it is well accepted that urinary tract obstruction is associated with a vasopressin-resistant urinary concentrating defect [36]. Another study using isolated collecting ducts from rabbits subjected to 4 hours of UUO revealed a marked impairment in the ability of the tubule to increase water permeability in response to both vasopressin and cyclic AMP [37]. Thus, the present study supports the view from previous studies that an impaired response to vasopressin may be a key factor for the long-term decreased abundance of BSC-1 and AQP2 in response to PUUO. Urinary tract obstruction also significantly increases the renal medullary PGE2 content [38, 39], and it is well known that PGE2 stimulates natriuresis in the mTAL. Importantly, it was demonstrated that blockade of PGE2 synthesis using indomethacin restores the collecting duct water permeability in the obstructed kidney [40], suggesting that an enhanced PGE2 generation in the obstructed kidney may participate in the down-regulation of BSC-1, Na-K-ATPase, and AQP2 demonstrated in the present study.

It is well established that congenital ureteral obstruction is associated with development of a pronounced interstitial inflammatory response, which subsequently leads to progressive renal interstitial fibrosis [41]. Thus, it is likely that the inflammatory response in the obstructed kidney may activate mechanisms in the tubular cells, which interferes with the regulation of expression of epithelial transporters and channels. Importantly, it has been demonstrated that TGF-β1, which is a cytokine that stimulates fibrosis and apoptosis, is expressed in tubular epithelial cells [42]. Recently, it was demonstrated that

Fig. 7. Immunoblot of membrane fractions of total kidneys from PUUO for 24 weeks (N = 6). (A, C, E) Immunoblot was reacted with anti-AQP1, anti-AQP2, and anti-AQP3 antibody. (B) Densitometric analysis revealed no significant difference in total kidney AQP1 expression between the obstructed and nonobstructed kidneys (71 ± 5 vs. 100 ± 12%, P > 0.05). (D) Total kidney AQP2 expression of the obstructed kidney was significantly lower than that of the nonobstructed kidney (69 ± 13 vs. 100 ± 7%, P < 0.05). (F) Total kidney AQP3 expression of the obstructed kidney did not differ from that of the nonobstructed kidneys (123 ± 19 vs. 100 ± 29%, P > 0.05).
persistent UUO in the neonatal rat increases TGF-β1 expression linearly with age during the first month of life, suggesting that an increased expression of this cytokine during obstruction may have detrimental effects on renal function. Furthermore, previous studies demonstrated that neonatally, obstruction induces phenotypic transformation of renal tubular cells, which may be associated with loss of normal epithelial cell polarity [abstract; Yoo KH et al, J Am Soc Nephrol 7:1768, 1996].

The potential mechanisms responsible for dysregulation of the renal sodium transporters and aquaporins may show a dynamic change with time. Thus, the observed changes at 12 and 24 weeks after onset of obstruction may not reveal the immediate response to PUUO.

Surprisingly, total GFR in the obstructed animals did not reach the same level as GFR in the sham-operated animals at 12 and 24 weeks after onset of obstruction due to the lack of contralateral compensation. This indicates that systemic factors may play an important role. It is well known that the renal renin-angiotensin system plays a crucial role for some of the pathophysiological characteristics observed during obstruction [1, 41], and possibly the renin-angiotensin system may participate in a vasoconstrictor response in the contralateral nonobstructed kidney. Also, the demonstrated reduction in the contralateral GFR may be due to long-term hyperfiltration, supporting the view that immature kidneys are more susceptible to glomerulosclerosis than adult kidneys [43].

The combined effects of a reduced GFR and the reduced abundance of sodium transporters in the mTAL and AQP2 in the collecting duct are a reduction in the active sodium reabsorption by the mTAL. This impairs the generation of medullary hypertonicity and the osmotic equilibration across the collecting duct, contributing to the impairment of the obstructed kidney to concentrate urine.

CONCLUSION

The present study demonstrated that major sodium transporters and aquaporins in the obstructed kidney are down-regulated in response to neonatally induced PUUO. These changes were paralleled by increased sodium excretion and a significant reduction in the solute-free water reabsorption from the obstructed kidney, demonstrating a functional association between the molecular changes and the ability of the obstructed kidney to handle sodium and water. Thus, the study indicates that down-regulation of these transporters and channels plays a crucial role for the persistent reduction in renal handling of sodium and water in response to PUUO.

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REFERENCES

10. JOSEPHSON S, JACOBSSON E, LARSSON E: Experimental partial ureteric obstruction -2, and -3 levels in kidneys of rats with CRF induced by surgical intervention of the renal sodium transporters and aquaporins may show a dynamic change with time. Thus, the observed changes at 12 and 24 weeks after onset of obstruction may not reveal the immediate response to PUUO.