Downregulation of AQP1, -2, and -3 after ureteral obstruction is associated with a long-term urine-concentrating defect

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Li, Chunling, Weidong Wang, Tae-Hwan Kwon, Levent Isikay, Jian Guo Wen, David Marples, Jens Christian Djurhuus, Anette Stockwell, Mark A. Knepper, Søren Nielsen, and Jørgen Frokiaer. Downregulation of AQP1, -2, and -3 after ureteral obstruction is associated with a long-term urine-concentrating defect. Am J Physiol Renal Physiol 281: F163–F171, 2001.—Previously, we demonstrated that 24 h of bilateral ureteral obstruction (BUO) and short-term release of BUO was associated with a decrease in the expression of aquaporin-2 (AQP2), polyuria, and a reduced urinary concentrating capacity (10). The purposes of the present study were to examine whether BUO and the long-term release of BUO (BUO-R) for 3, 14, and 30 days were associated with changes in the expression of renal AQP1, AQP2, and AQP3 and whether such changes were associated with parallel changes in urinary output and urinary concentrating capacity. Rats (n = 4–7 in each group) were kept in metabolic cages for measurements of urinary output. Kidneys were removed to determine the expression of aquaporin-1 (AQP1), AQP2, and AQP3 by semiquantitative immunoblotting. AQP2 was downregulated after 24 h of BUO (42 ± 3%), Downregulation of AQP2 persisted 3 (43 ± 14%; P < 0.01) and 15 days after BUO-R (48 ± 11%; P < 0.01) but was normalized 30 days after BUO-R. AQP3 showed a similar pattern. Moreover, AQP1 was downregulated in response to BUO (65 ± 7%) and remained downregulated 3 days after BUO-R (41 ± 5%), 14 days after BUO-R (57 ± 8%), and 30 days after BUO-R (59 ± 5%). BUO-R resulted in a significant polyuria that gradually decreased, although it remained significant at day 30. Urinary concentrating capacity remained significantly impaired when determined 3, 14, and 30 days after BUO-R in response to a 24-h period of thirst (1,712 ± 270 vs. 2,880 ± 91 mosmol/kg H2O at day 30, P < 0.05). In conclusion, the expression of AQP1, AQP2, and AQP3 were long-term downregulated after BUO-R, suggesting that dysregulation of aquaporins located at the proximal tubule, thin descending limb of the loop of Henle, and the collecting duct may contribute to the long-term polyuria and impairment of urinary concentrating capacity associated with obstructive nephropathy.

BILATERAL URINARY TRACT OBSTRUCTION (BUO) is a serious condition both in children and adults and may be associated with long-term impairment in the ability to concentrate urine (4). The pathophysiology behind the loss of urinary concentrating ability is complex and involves both the juxtamedullary, proximal and distal tubules, the loops of Henle, and the collecting ducts (4). Detailed clearance studies showing impaired water reabsorption at the collecting duct level during BUO (43), together with the clinical observations that patients with obstructive nephropathy have massive vasopressin-insensitive polyuria after relief from obstruction, demonstrate that BUO is associated with nephrogenic diabetes insipidus (NDI). Consistent with this, we previously demonstrated that BUO for 24 h in rats is associated with a significant downregulation of the vasopressin-regulated collecting duct water channel aquaporin-2 (AQP2), and this downregulation persists 24 and 48 h after release of obstruction concurrent with a marked postobstructive polyuria (10). Seven days after release of obstruction, AQP2 levels were only partially reversed to normal levels, and consistent with this urinary concentrating capacity remained significantly reduced 7 days after release of BUO, as determined in response to 18 h of thirsting (10). To examine whether this downregulation was caused by

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local effects or systemic changes, rats with unilateral ureteral obstruction (UUO) were examined (8). Similar to the downregulation found in bilateral obstruction, rats with UUO for 24 h also had AQP2 downregulation to the same level as in BUO, suggesting that local/intrarenal factors may be important for downregulation (8). Importantly, separate experiments where AQP2 expression and solute-free water clearance were examined immediately after the release of a 24-h UUO revealed that changes in AQP2 expression are reciprocal to the changes in solute-free water clearance, which indicates a functional association between these two parameters (8). Thus this finding supports the view that the collecting duct is critically involved in the impaired urinary concentrating capacity in urinary tract obstruction.

Recently, a critical role of AQPI in the transcellular water reabsorption of the proximal nephron and in the urinary concentration was demonstrated in transgenic knockout mice lacking AQPI (25). The AQPI-deficient mice were unable to concentrate urine in response to thirsting and became severely dehydrated, strongly suggesting that AQPI is required for the formation of a concentrated urine. Furthermore, defects in fluid reabsorption were demonstrated in these transgenic AQPI knockout mice both at the proximal tubule and the descending limb of Henle (38). In the proximal tubule, transepithelial osmotic water permeability was reduced by 80% (38), and in the descending thin limb it was reduced by 90% (2). Water transport across the basolateral plasma membrane of collecting duct principal cells is thought to be mediated by AQPI and AQP4. Consistent with this view, recent studies by Ma et al. (25) showed a >10-fold greater fluid consumption and a much lower urine osmolality in AQPI null mice compared with wild-type mice. In response to water deprivation and vasopressin administration, renal concentration capacity in AQPI null mice only returned to about 30% of that in wild-type mice (24). Moreover, inner medullary collecting ducts from AQP4-deficient mice have a fourfold reduction in vasopressin-stimulated water permeability (3). This finding suggests that AQP2 in the apical plasma membrane of principal cells, AQP3 and AQP4 located in the basolateral plasma membrane of principal cells in collecting tubules (16), and AQPI in proximal nephron cells are all important for the formation of concentrated urine. In support of this view, we recently demonstrated that AQPI, -2, and -3 abundance are severely reduced in response to experimental acute (12) and chronic renal failure (22, 23), which both are associated with significant polyuria/impaired urinary-concentration capacity, suggesting that AQPI, -2, and -3 may play critical roles in the urinary concentration mechanisms in complex renal disorders.

The purposes of the present study are therefore to examine 1) whether there are long-term changes in the expression levels of aquaporins located in both the proximal tubule and descending limb (AQPI) and the collecting duct (AQP2 and AQP3) after release of BUO; and 2) whether such changes are associated with long-term changes in the urinary concentrating capacity. The results of the present study show that AQPI, -2, and -3 are long-term downregulated and that urinary concentrating capacity is reduced up to 30 days after the release of 24-h bilateral ureteral obstruction, indicating that both the proximal nephron and collecting duct play critical roles in the concentrating defect seen in obstructive nephropathy.

METHODS

Experimental animals. Studies were performed in male Münich Wistar rats initially weighing 250 g (Møllegard Breeding Center, Eiby, Denmark). The rats were maintained on a standard rodent diet (Altromin, Lage, Germany) with free access to water. During the entire experiment, rats were kept in individual metabolic cages with a 12:12-h artificial light-dark cycle, a temperature of 21 ± 2°C, and a humidity of 55 ± 2%. Rats were allowed to acclimatize to the cages for 5 to 7 days before surgery.

The rats were anesthetized with halothane (Halocarbon Laboratories), and during surgery the rats were placed on a heated table to maintain rectal temperature at 37–38°C. Through a midline abdominal incision, both ureters were exposed, and a 5-mm-long piece of bisected polyethylene tubing (PE-50) was placed around the midportion of each ureter. The ureter was then occluded by tightening the tubing with a 5–0 silk ligature. Twenty-four hours later, the obstructed ureters were decompressed by removal of the ligature and the PE tubing. By using this technique, the ureters could be completely occluded for 24 h without evidence of subsequent functional impairment of ureteral function.

Rats were allocated to the protocols indicated below. Age- and time-matched sham-operated controls were prepared, and were observed in parallel with each BUO group in the following protocols (Fig. 1):

Protocol 1. BUO was induced (n = 6) for 24 h or rats were sham operated (n = 6). The two kidneys were removed and separately prepared for semiquantitative immunoblotting.

Protocol 2. BUO was induced for 24 h or rats were sham operated (n = 6), followed by release in which animals were observed during the next 3 days (n = 5). The two kidneys were removed and separately prepared for semiquantitative immunoblotting.

Protocol 3. BUO was induced for 24 h or rats were sham operated (n = 6), followed by release in which animals were observed during the next 14 days (n = 6). The two kidneys were removed and separately prepared for semiquantitative immunoblotting.

Protocol 4. BUO was induced for 24 h or rats were sham operated (n = 6), followed by release in which animals were observed during the next 30 days (n = 6). Kidneys were removed and separately prepared for semiquantitative immunoblotting.

Protocol 5. BUO was induced for 24 h or rats were sham operated (n = 4), followed by release in which animals were observed during the next 3 days (n = 4). Urinary-concentrating capacity was examined in response to a 24-h period of thirst after release of BUO for 2 days.

Protocol 6. BUO was induced for 24 h or rats were sham operated (n = 5), followed by release in which animals were observed for 30 days (n = 7). Urinary-concentrating capacity was examined in response to a 24-h period of thirst at day 11 and 29 after release.
### Protocol 1

**BUO (n=6)**

- **SUCTION**

**SHAM (n=6)**

- **SUCTION**

### Protocol 2

**BUO (n=5)**

- **SUCTION**

**SHAM (n=6)**

- **SUCTION**

### Protocol 3

**BUO (n=6)**

- **SUCTION**

**SHAM (n=6)**

- **SUCTION**

### Protocol 4

**BUO (n=7)**

- **SUCTION**

**SHAM (n=6)**

- **SUCTION**

### Protocol 5

**BUO (n=4)**

- **SUCTION**

**SHAM (n=4)**

- **SUCTION**

### Protocol 6

**BUO (n=7)**

- **SUCTION**

**SHAM (n=6)**

- **SUCTION**

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**Fig. 1. Diagram of the study design.**

- **Protocol 1:** bilateral ureteral obstruction (BUO) was created. Sham-operated control rats matching BUO rats are shown. **Protocol 2:** BUO was created and released after 24 h. Rats are then monitored for the following 3 days, sham-operated control rats matching BUO rats. **Protocol 3:** BUO was created and released after 24 h. Rats were then monitored for the following 14 days, sham-operated control rats matching BUO rats. **Protocol 4:** BUO was created and released after 24 h. Rats were then monitored for the following 30 days, sham-operated control rats matching BUO rats. **Protocol 5:** BUO was created and released after 24 h. Rats were then monitored for the following 3 days, and after 3 days release rats were thirsted for 24 h, sham-operated control rats matching BUO rats. **Protocol 6:** BUO was created and released after 24 h. Rats were then monitored for the following 30 days, and after 11 and 29 days release rats were thirsted for 24 h, sham-operated control rats matching BUO rats. All rats were kept in metabolic cages during the entire study. Urine osmolality, creatinine, sodium, and potassium were measured. Plasma was collected at the time of death for measurement of osmolality and concentration of sodium, potassium, and creatinine.

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### Clearance studies

Urine was collected during 24-h periods throughout the study. Clearance studies were performed during the last 24 h in each protocol. During anaesthesia, at the end of each protocol, 2–3 ml of blood were collected into a heparinized tube for the determination of plasma electrolytes and osmolality before the rat was killed. The plasma and urinary concentrations of creatinine, sodium, and potassium were measured. Plasma was collected at the time of death for measurement of osmolality and concentration of sodium, potassium, and creatinine.

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### Electrophoresis and immunoblotting

Samples of membrane fractions from IM or whole kidney were run on 12% polyacrylamide minigels (Biorad Mini Protein II). For each gel, an identical gel was run in parallel and subjected to Coomassie staining (42). 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 (see below). The other gel was subjected to blotting. After transfer by electroelution to nitrocellulose membranes, blots were blocked with 5% milk in 80 mM Na2HPO4, 20 mM NaH2PO4, 100 mM NaCl, and 0.1% Tween 20, pH 7.5 (PBS-T) for 1 h and incubated with antibody raised against the COOH-terminal 22 amino acids of AQP2 (either serum, diluted 1:1,000, or affinity purified with the immunizing peptide, diluted to 40 ng IgG/ml in PBS-T plus 1% BSA) (29–31), AQP1-immune serum (diluted 1:1,000). All controls were without labeling. After a final wash as above, the blots were incubated with horseradish peroxidase-conjugated secondary antibody (P448, Dako, Glostrup, Denmark) overnight. After being washed as above, antibody binding was visualized using the enhanced chemiluminescence (ECL) system (Amersham International). 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 1:1,000). All controls were without labeling.

### Semi-quantitation of AQP1, AQP2, and AQP3

ECL films were scanned using a Hewlett-Packard Scanjet scanner and Adobe Photoshop software. For AQP1 and AQP2 both the 29-kDa and the 35–50-kDa bands (corresponding to nonglycosylated and glycosylated AQP1 and AQP2) were scanned. For AQP3, the 27-kDa and the 33–40-kDa bands (corresponding to nonglycosylated and glycosylated AQP3) were scanned, as described previously (7, 42). The labeling density was quantitated by using specially written software (available on request). Bands from gels made with serial dilutions of protein from IM, processed as above, were found to be linear over a wide range (26). For quantitation of AQP2 expression, ECL exposures were chosen that gave bands in the control samples that were close to the top of the linear range. To correct for potential minor differences in loading between lanes, Coomassie-stained gels, which were run in parallel to the immunoblotted gels, were subjected to band density scanning.

### Statistics

For densitometry of immunoblots, samples from obstructed kidneys were run on each gel with corresponding sham kidneys. AQP1s labeled in the samples from the experimental animals were calculated as a fraction of the mean sham control value for that gel. Parallel Coomassie-stained gels were subjected to densitometry and used for correction of potential minor differences in loading. Values are presented in the text as means ± SE. Comparisons among groups were made by unpaired t-test. P values < 0.05 were considered significant.
RESULTS

Release of BUO is followed by polyuria and long-term impairment in urinary concentrating capacity. Water intake, urine output, and urine osmolality were determined in rats followed for 30 days after release of a 24-h period of BUO. As previously shown, release of BUO resulted in a highly significant increase in urine output and a parallel reduction in urine osmolality (Table 1). Twenty-four hours after release of BUO, urine output was 127 ± 7 μl-min⁻¹-kg⁻¹ compared with 34 ± 4 μl-min⁻¹-kg⁻¹ before obstruction. Consistent with this, the urine osmolalities were reduced from 1,845 ± 145 to 505 ± 40 mosmol/kgH₂O. During the following 14 days, urine output remained significantly elevated compared with sham-operated controls. The marked increase in urine output after release of BUO was reproduced in separate experiments (protocols 3 and 5) where rats were followed for 3 and 14 days after release of BUO (Table 1). We have previously shown that urine-concentrating capacity was impaired in rats 7 days after release of obstruction. Consistent with this, we found an impaired urinary concentrating capacity in response to 24 h of thirsting at day 3 after release of BUO (see protocol 5, Fig. 3). Next, we tested whether rats have long-term impairment in urinary concentrating capacity by subjecting the animals to a 24-h period of thirst at day 11 and again at day 29 after the release of obstruction. As shown in Figs. 2 and 3, urine-concentrating capacity remained significantly impaired compared with sham-operated control animals. At 3, 12, and 30 days after BUO-R, animals were unable to increase urine osmolalities and reduce urine output to the same level as the sham-operated controls, consistent with a long-term impairment in urine-concentrating capacity after the release of 24 h of BUO.

BUO and release of BUO is associated with long-term downregulation of both AQP2 and AQP3. As previously shown, the affinity-purified anti-AQP2 antibody exclusively recognizes the 29-kDa and the 35- to 50-kDa bands (Fig. 4A), corresponding to nonglycosylated and glycosylated forms of AQP2 (36). Consistent with previous data (26) (Fig. 4, A and E), immunoblotting using whole kidney membrane fractions of obstructed kidneys and sham-operated control animals revealed that AQP2 expression was significantly downregulated 3 days after release of BUO (43 ± 14 vs. 100 ± 7%, P < 0.01; Fig. 4, B and E). Fourteen days after release of obstruction, immunoblotting showed a persistent reduction in AQP2 levels (Fig. 4C). Densitometry demonstrated that AQP2 levels were 48 ± 11% of sham-operated control levels (Fig. 4E). This is consistent with the persistent polyuria and impaired urine-concentrating capacity observed 14 days after release of BUO (Fig. 2).

Although urine output remained elevated in the BUO animals compared with sham-operated animals throughout the experiment, there was a steady decrease from day 10 in all groups (Fig. 2 and Table 1). Immunoblotting and densitometry revealed that AQP2 expression remained downregulated 14 days after release of BUO. Downregulation of both AQP2 and AQP3 was associated with long-term impairment in urinary concentrating capacity by subjecting the animals to a 24-h period of thirst at day 11 and again at day 29 after the release of obstruction. As shown in Figs. 2 and 3, urine-concentrating capacity remained significantly impaired compared with sham-operated control animals. At 3, 12, and 30 days after BUO-R, animals were unable to increase urine osmolalities and reduce urine output to the same level as the sham-operated controls, consistent with a long-term impairment in urine-concentrating capacity after the release of 24 h of BUO.

Table 1. Changes in free water clearance, urine volume, and osmolality in nonthirsted rats subjected to BUO and release of BUO or sham operation

<table>
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<tr>
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<th>24-h BUO (n = 6)</th>
<th>BUO-3daysR (n = 5)</th>
<th>BUO-14daysR (n = 6)</th>
<th>BUO-30daysR (n = 6)</th>
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<tr>
<td></td>
<td>Uvol, μl-min⁻¹-kg bw⁻¹</td>
<td>Uosm, mosmol/kgH₂O</td>
<td>ClH₂O, μl-min⁻¹-kg bw⁻¹</td>
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<td>Sham (n = 6)</td>
<td>30.2 ± 2.2</td>
<td>84.1 ± 8.9</td>
<td>35.4 ± 3.0</td>
<td>77.8 ± 4.5</td>
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Values are means ± SE. n, no. of rats; BUO, bilateral ureteral obstruction; BUO-3daysR, 3 days after release of BUO; BUO-14daysR, 14 days after release of BUO; BUO-30daysR, 30 days after release of BUO; Sham, sham-operated rats. Uvol, urine volume; Uosm, urine osmolality; ClH₂O, free water clearance; bw, body wt. *P < 0.01 compared with Sham control rats.
levels were normalized 30 days after release of BUO (79 ± 11 vs. 100 ± 28%; Fig. 4, D and E).

Another water channel located in collecting tubule, AQP3, also was examined. A dramatic reduction in the expression of IM AQP3 levels was shown in rats with 24-h BUO, from 100 ± 10 to 8 ± 2% (Fig. 5, A and D, P < 0.05). Three days after the release of BUO, AQP3 levels were significantly decreased (50 ± 8% of sham level, Fig. 5, B and D, P < 0.05). This decrease in AQP3 expression was also seen 14 days after BUO-R (36 ± 12% of sham level, Fig. 5, C and D, P < 0.05), whereas AQP3 expression was normalized 30 days after release of BUO (92 ± 22 vs. 100 ± 15%; Fig. 5, D and E).

BUO and release of BUO is associated with persistent downregulation of AQP1. The expression of AQP1 was also examined to test whether this water channel in the proximal tubule and thin descending limb of the loop of Henle contributes to the long-term reduced urinary concentrating capacity seen after the release of BUO. As shown in Fig. 6, densitometric comparison of WK AQP1 expression levels between BUO and sham-operated animals revealed a persistant downregulation of AQP1 up to 30 days after release of obstruction. AQP1 expression decreased markedly in response to BUO from 100 ± 7 to 65 ± 7% (Fig. 6, A and E, *P < 0.05). Three days after BUO-R, there was a marked decrease in total kidney AQP1 expression to 41 ± 5% in BUO rat, of levels in sham-operated rats (100 ± 6%; Fig. 6, B and E, P < 0.05). AQP1 expression levels remained downregulated 14 and 30 days after the release of BUO (BUO-14daysR: 36 ± 10 vs. 100 ± 9%; BUO-30daysR: 59 ± 5 vs. 100 ± 11%, Fig. 6 C, D, and E, P < 0.05).

Because changes in AQP1 expression in the descending limb of Henle’s loop may directly affect urinary concentrating capacity, AQP1 expression levels were examined in the IM. AQP1 was significantly downregulated 14 days after release of BUO (Fig. 7, A and B). In contrast to the expression levels demonstrated in whole kidney samples, no significant changes were detectable at earlier and later stages after release of obstruction.

**DISCUSSION**

Previously, we demonstrated that 24 h of BUO is associated with a significant reduction in the expression of AQP2. After release of BUO, the expression of AQP2 remains downregulated, coinciding with the onset of polyuria. This study confirmed that release of BUO is associated with a dramatic polyuria and im-

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**Fig. 3.** Urine output and urine osmolality were examined in separate experiments (protocols 5 and 6) in BUO rats (solid bars) and compared with matched sham-operated control rats (open bars). All animals were subjected to a 24-h thirst before death to examine urinary concentrating capacity. These experiments reveal a persistent reduction in urinary concentrating capacity during the first 30 days after release of BUO. Three days after release of BUO urine osmolality was significantly reduced (1,051 ± 115 vs. 2,242 ± 156 mosmol/kgH₂O, n = 4, *P < 0.01). Twelve and thirty days after release of BUO another set of rats were subjected to 24 h of thirsting, which demonstrates that urine osmolality remains significantly reduced (1,675 ± 274, n = 7, vs. 3,186 ± 124 mosmol/kgH₂O, P < 0.01 after 12 days, and 1,712 ± 270, n = 5, vs. 2,879 ± 91 mosmol/kgH₂O, *P < 0.01 after 30 days).

**Fig. 4.** Semiquantitative immunoblotting of membrane fractions of inner medulla (IM). A-D: immunoblots were reacted with anti-aquaporin-2 (anti-AQP2) and reveal 28-kDa and the 35- to 45-kDa AQP2 bands, representing nonglycosylated and glycosylated forms of AQP2 after 24 h of BUO (A); 3 days after release of BUO (B); 14 days after release of BUO (C); and 30 days after release of BUO (D). E: densitometric analysis of all samples from BUO and sham-operated rats (corrected according to loading) reveal a persisted decrease in AQP2 expression in rats with BUO and 3 and 14 days after release of BUO (24-hBUO: 42 ± 7 vs. 100 ± 6%; BUO-3daysR: 43 ± 14 vs. 100 ± 7%; BUO-14daysR: 48 ± 11 vs. 100 ± 5%, *P < 0.05), but 30 days after release of BUO, AQP2 levels did not differ significantly from sham operated.
paired urinary concentrating capacity that may last up to 30 days. In parallel, the expression of AQP1, -2, and -3 was significantly downregulated. The results of the present study support the view that AQP1, -2, and -3 play important roles for an intact urinary concentrating capacity and suggest that decreased levels of aquaporins in both the proximal nephron and collecting duct may play a significant role in the impairment of urinary concentrating capacity after release of urinary tract obstruction.

Persistent reduction in urinary concentrating capacity after release of obstruction parallels downregulation of AQP2. The urinary concentrating process is dependent on two fundamental processes: 1) the hypertonic medullary interstitium, which is generated by active NaCl reabsorption as a consequence of countercurrent multiplication 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. Thus defects in any of these mechanisms would be predicted to be associated with urinary concentrating defects. Release of BUO is associated with an immediate significant polyuria (5, 14, 39, 43), which can be attributed to several functional defects in the kidney compromising either generation of a hypertonic medullary interstitium or vasopressin-regulated water transport across the collecting duct epithelium. These defects include 1) a reduction in glomerular filtration rate; 2) an increase in medullary blood flow; 3) a sustained increase in intraluminal and parenchymal pressure, changing transport abilities in the

Fig. 5. Semiquantitative immunoblotting of membrane fractions of IM. A-D: immunoblots were reacted with anti-AQP3 serum and reveal 27-kDa and 33- to 40-kDa AQP3 bands, representing nonglycosylated and glycosylated forms of AQP3 after 24 h of BUO (A); 3 days after release of BUO (B); 14 days after release of BUO (C); and 30 days after release of BUO (D). E: densitometric analysis of all samples from BUO and sham-operated rats (corrected according to loading) reveal a marked decrease in AQP3 expression (8 ± 2% in BUO rats and 100 ± 10% in sham-operated rats, *P < 0.05). AQP3 expression levels remain downregulated 3 and 14 days after release (BUO-3daysR: 50 ± 8 vs. 100 ± 7%, *P < 0.05; BUO-14daysR: 36 ± 12 vs. 100 ± 16%, *P < 0.05). AQP3 expression levels did not differ between BUO-R and sham 30 days after release of BUO (92 ± 22 vs. 100 ± 15%).

Fig. 6. Semiquantitative immunoblotting of membrane fractions of whole kidney from BUO rats and sham (A), and membrane fractions of outer medulla and cortex from rats 3, 14, and 30 days after release of BUO and sham-operated rats (B-D). Immunoblots were reacted with anti-AQP1 serum and reveal 29-kDa and 35- to 50-kDa AQP1 bands, representing nonglycosylated and glycosylated forms of AQP1. E: densitometric analysis of all samples from BUO and sham-operated rats (corrected according to loading) reveal a marked decrease in AQP1 expression in response to 24 h of BUO (65 ± 7 in BUO rats and 100 ± 7% in sham-operated rats, *P < 0.05). AQP1 expression remained reduced 3, 14, and 30 days after release of BUO (BUO-3daysR: 41 ± 5 vs. 100 ± 6%, *P < 0.05; BUO-14daysR: 36 ± 10 vs. 100 ± 9%, *P < 0.05; BUO-30daysR: 59 ± 5 vs. 100 ± 11%, *P < 0.05).
nephrons and collecting ducts; 4) a reduced fractional reabsorption of fluid from the nephron; and 5) a collecting duct dysfunction that caused alterations in sodium and water transport in this segment (4). Indeed, it has been well established that postobstructive polyuria is resistant to vasopressin treatment (43). In the present study, we demonstrated that release of BUO is associated with a persistent impairment of urinary concentrating capacity lasting up to 30 days after release of obstruction. In parallel, AQP2 expression was downregulated after 24 h of BUO, and at 3 and 14 days after release of BUO it was consistent with the development of polyuria, decreased urine osmolality, and increased free water clearance. These results extend our previous findings, where AQP2 remained downregulated up to 7 days after the release of BUO. Thus downregulation of the vasopressin-regulated collecting duct AQP2 may play an important role in polyuria and long-term impairment of urinary concentration after release of BUO.

The mechanisms responsible for the long-term AQP2 downregulation remain incompletely understood. The water permeability of the collecting duct can change acutely in response to vasopressin via V2 receptor-mediated stimulation of cAMP, leading to phosphorylation and translocation of AQP2 to the apical plasma membrane (19, 32). In addition to this acute regulation of collecting duct water permeability, it is now well established that there is a long-term modulation of the water permeability dependent on the total amount of AQP2 present in the principal cells (18, 21, 28). Initially, this was documented in rats with lithium-induced NDI (26, 28) and subsequently in NDI caused by hypokalemia (27) and hypercalcemia (6, 34). In addition, AQP2 downregulation has been described in other situations not generally thought of as acquired forms of NDI. Among these are a protein-deficient diet and aging, which have been shown to cause changes in water reabsorption (35, 41). Thus AQP2 downregulation of AQP2 is a common feature in multiple forms of NDI and non-NDI conditions characterized by urinary concentrating defects. Release of BUO is associated with polyuria, despite high levels of circulating vasopressin. Thus it is possible that BUO causes a change in the number of functional V2 receptors and/or a reduction in the abundance of adenylate cyclase and other intracellular enzymes, playing a key role as an intact second message of the vasopressin signal. However, it should be emphasized that, at this stage, very little is known, and additional studies must be undertaken to address these questions. Furthermore, we have previously shown that AQP2 expression levels are reduced in both the obstructed kidney and the nonobstructed kidney in a rat model with UUO, suggesting that local factors are involved in the dysregulation of AQP2 during obstruction. Such factors could be an increased luminal pressure and/or altered prostaglandin or nitric oxide (NO) production (17). NO is a chemical form of endothelium-derived relaxing factor that has important roles in renal hemodynamics and renal sodium and water metabolism (12, 40). Recently, it was shown directly that NO inhibits vasopressin-stimulated osmotic water permeability in isolated and perfused cortical collecting duct (11). Whether there are long-term alterations in the production of prostaglandins or NO in the kidney after release of 24-h BUO remains to be established. The present findings show that AQP2 downregulation is a prominent finding during and after release of obstruction, and the results suggest that downregulation of AQP2 may at least partially be responsible for the lack in urinary concentrating capacity observed in patients with congenital or acquired disorders of the urinary tract, causing long-term bilateral impairment of renal function (i.e., ureteral valves, high grade reflux, or neurogenic bladder).

***BUO and release of BUO are associated with downregulation of AQP3.*** AQP3 is a water channel predominantly expressed at the basolateral plasma membrane of kidney collecting duct principal cells and is rarely localized in the cytoplasmic vesicles (7). Expression levels of AQP3 were reduced after 24-h BUO and at 3 and 14 days after release of BUO. The mechanisms underlying the regulation of AQP3 expression are presently not well understood. Previous studies have shown that thirsting of normal rats for 48 h (7) or dDAVP treatment of Brattleboro rats for 5 days (42) induced a significant increase in AQP3 expression. Thus there is clear evidence that AQP3 regulation is linked to changes in vasopressin levels and water balance. Importantly, a significant role of AQP3 in urinary concentration was recently demonstrated in AQP3 null mice that manifest a dramatic polyuria and renal concentrating defect (24). In addition, we have recently shown that AQP3 expression levels are dramatically reduced in response to 27 days of lithium treatment, consistent with a significant polyuria and decreased
urinary concentrating capacity. Moreover, we have shown that AQP3 is also downregulated in other complex renal disease models such as chronic renal failure induced by 5/6 nephrectomy (23), and ischemia-induced acute renal failure (22) coinciding with a vasopressin-resistant polyuria and urinary concentrating defect. Thus the reduced levels of AQP3 observed up to 14 days after release of BUO suggest that reduced AQP3 may also play a significant role in the urinary concentrating defects after release of BUO.

**BUO and release of BUO are associated with persisted downregulation of AQP1.** We demonstrated that the expression of the proximal tubule and the descending thin limb water channel AQP1 is significantly decreased in response to 24-h BUO, and AQP1 expression levels remain decreased up to 30 days after release of BUO coinciding with the impaired urinary concentrating capacity. BUO is characterized by a reduction in renal blood flow, glomerular filtration rate (GFR), and oxygen tension in the renal tissue (9, 13, 15). Thus the oxidative metabolism necessary to generate ATP, especially in the proximal tubule, may be very susceptible to hypoxic conditions. The reason for the consistent reduction in AQP1 expression levels in whole kidney and inconsistent reduction in the IM after release of obstruction remains to be characterized. Recently, it was shown that AQP1 is required for the formation of concentrated urine by the kidney, and decreased transepithelial proximal tubule water permeability and defective fluid absorption were found in AQP1 knockout mice, demonstrating that high water permeability in the proximal tubules of wild-type mice is primarily transcellular, is mediated by AQP1 water channels, and is required for efficient near-isosmolar fluid absorption (25).

Water transport in the thin descending limb of Henle’s loop plays a critical role in the countercurrent multiplier mechanism by assuring osmotic equilibration between the descending limb lumen and the surrounding interstitium (20). Studies of AQP1 knockout mice have confirmed this concept by demonstrating that these mice have a virtual absence of concentrating ability (25). Therefore, decreased abundance of AQP1 in the thin descending limb would predict an impairment of urinary concentrating ability. The finding of decreased AQP1 in the IM of BUO kidneys 2 wk after release, reflecting a decrease in thin descending limb AQP1 abundance and a presumed decrease in osmotic water permeability in the descending limb, suggests that reduced AQP1 levels in the IM may participate in the concentrating defect after release of BUO. In addition, it is highly likely that a decrease in the driving force could result from transport defects in the thick ascending limb long after obstruction is relieved, contributing to the concentrating defect in the IM.

AQP1 abundance is also consistently decreased in the proximal tubule. Because the proximal tubule plays no direct role in the urinary concentrating mechanism, the significance of the decrease in AQP1 in the proximal tubules is unclear. Isolated perfused tubule and micropuncture studies in AQP1 knockout mice (38) have demonstrated that the decrease in osmotic water permeability resulting from AQP1 ablation leads to a decrease in NaCl reabsorption in the proximal tubule. In the knockout mice, the decrease in proximal NaCl reabsorption triggers a decrease in GFR, owing to the tubuloglomerular feedback mechanism (37). A similar mechanism in BUO-R rats may provide an explanation for decreased GFR in this model (5). Decreased GFR may be expected to enhance urinary concentrating ability if all other factors were equal (1).

Thus AQP1 plays a key role for normal urinary-concentrating capacity. The concomitant long-term reduction in urinary concentrating capacity and AQP1 expression levels in the present study suggests that reduced AQP1 may play a significant role in the urinary concentrating defect after release of BUO.

**Summary.** In conclusion, the expression of AQP1, AQP2, and AQP3 water channel proteins was long-term downregulated after release of 24-h BUO, suggesting that dysregulation of aquaporins located both at the proximal tubule, the descending thin limb of Henle, and the collecting duct may contribute to the long-term polyuria and impairment in urinary concentrating capacity associated with obstructive nephropathy. The observed downregulation of the collecting duct water channels AQP2 and AQP3 may provide a molecular explanation for the functional defects previously demonstrated in the collecting duct. It remains to be established to what extent these defects contribute to polyuria. Further studies are needed to examine whether BUO is associated with alterations in the expression of sodium transporters that are responsible for the establishment and maintenance of the hypertonic medullary interstitium providing the driving force for water reabsorption in the collecting duct.

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