COX-2 inhibition prevents downregulation of key renal water and sodium transport proteins in response to bilateral ureteral obstruction

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

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Norregaard, Rikke, Boye L. Jensen, Chunling Li, Weidong Wang, Mark A. Knepper, Søren Nielsen, and Jørgen Frøkiær. COX-2 inhibition prevents downregulation of key renal water and sodium transport proteins in response to bilateral ureteral obstruction. Am J Physiol Renal Physiol 289: F322–F333, 2005. First published April 19, 2005; doi:10.1152/ajprenal.00061.2005.—Bilateral ureteral obstruction (BUO) is a serious clinical condition, which occurs in both children and adults. In children, BUO is usually caused by congenital abnormalities of the kidneys and urinary tract whereas some of the most common explanations among adults are stones, benign enlargement of the prostate, and neoplasms of the urinary tract.

BUO is associated with an increased intraluminal pressure in the ureter, pelvis, and renal tubules and the acute consequence is anuria. Obstruction is associated with ischemia and after relief of an obstruction there is reperfusion, which is associated with parenchymal and functional injury of the kidneys (26). There are extensive changes in the systemic water and sodium balance due to the impaired ability to concentrate urine (5). The pathophysiology behind the loss of the urinary concentrating ability is complex and involves all nephron segments.

Recent studies have demonstrated that aquaporins (AQP1–4), the water channels located at the proximal tubule, descending thin limb, and collecting duct, and major renal sodium transporters located along all renal nephron segments, were significantly reduced during and after BUO or unilateral ureteral obstruction (UUO) (15, 16, 25, 28–30). These findings suggested that reduced expression of the renal aquaporins and sodium transporters contributed to the impairment in urinary concentrating capacity and salt wasting in response to urinary tract obstruction. The mechanisms responsible for the dysregulation of these transport proteins and channels remain incompletely understood.

The mammalian kidney is one of the most active prostaglandin (PG)-producing tissues, and PGE2 is the quantitatively dominant prostanoid. PGE2 regulates and modulates renal hemodynamics and tubular water and salt transport (19). Cyclooxygenase (COX) is the major rate-limiting enzyme in the cascade leading to synthesis of PG from arachidonic acid. Two isoforms of COX have been identified, namely, COX-1 and COX-2. The COX-1 isoform is believed to be constitutively expressed in most cells and thought to be involved in the maintenance of various physiological functions (39), whereas COX-2 is undetectable in most tissues. In contrast, COX-2 is constitutively expressed in the kidney (18). In kidney cortex, COX-2 is expressed in the thick ascending limb (TAL) (6, 14) and macula densa whereas in the medulla, COX-2 is found in medullary interstitial cells (48). COX-2 expression is differentially regulated by salt intake in the kidney zones; dietary salt depletion stimulates COX-2 expression in the cortex, and vice versa in the renal medulla (23, 47). In the renal medulla, COX-2 is enhanced by dehydration and AVP infusion (46). BUO for 24 h enhances prostaglandin production in cortical and medullary tubules of the rat kidney (45), and increased COX-2 expression has been observed in the obstructed kidney in response to both 24-h UUO (8), ureteral obstruction of a solitary kidney (37), and in response to BUO followed by release (7). Importantly, it was demonstrated that administra-
tion of a COX-2 inhibitor reduced tissue transforming growth factor-β, resulting in decreased renal tubular damage and interstitial fibrosis in UUO (33). Moreover, it was recently demonstrated that COX-2 inhibition of rats with a solitary obstructed kidney prevented macrophage infiltration in the interstitium of kidneys 24 h after release of the obstruction (37). Thus there is solid experimental evidence that COX activity is enhanced after obstruction.

The role of PGE2 in vasopressin-mediated water permeability is not fully understood. Recent studies have demonstrated that PGE2 does not affect directly cAMP levels but may have post-cAMP effects rather than actions via cAMP regulation (35). It has been suggested that PGE2 on the basolateral side stimulates calcium mobilization (10, 37). Moreover, recent studies have shown the effect of PGE2 on PKA phosphorylation of AQP2 in kidney papilla associated with retrieval of AQP2 from the plasma membrane but that this appears to be independent of AQP2 phosphorylation by PKA (36, 49). Importantly, it was recently demonstrated that COX-2 inhibition attenuated the polyuria in rats subjected to 24-h BUO followed by release and that this was associated with prevention of AQP2 downregulation in the obstructed kidney (7).

The apparent parallel stimulation of renal COX activity and downregulation of key transport molecules by BUO led us to hypothesize a causal relationship between these events. In the present study, our aim was to examine 1) whether BUO for 24 h is associated with changes in the localization and abundance of cyclooxygenases in kidney, and 2) whether selective COX-2 inhibition during BUO is associated with changes in the expression of both renal aquaporins and key renal sodium transport proteins.

METHODS

Experimental animals. All procedures conformed with the Danish national guidelines for the care and handling of animals and to the published guidelines from the National Institutes of Health. The animal protocols were approved by the board of the Institute of Clinical Medicine, University of Aarhus, according to the licenses for use of experimental animals issued by the Danish Ministry of Justice. Studies were performed in male Munich-Wistar rats initially weighing 200 g (Møllegaard Breeding Centre, Eiby, Denmark). The rats had free access to a standard rodent diet (Altromin, Lage, Germany) and tapwater. During the experiments rats were kept in individual metabolic cages, with a 12:12-h light-dark cycle, a temperature of 21 ± 2°C, and humidity of 55 ± 2%. Rats were allowed to acclimatize to the cages 3–4 days before surgery. The rats were placed on anesthesia with isoflurane (Abbott Scandinavia), and during the operation the rats were reanesthetized with isoflurane (40 mg/ml) were surgically implanted subcutaneously and the dose was given at a rate of 5 mg·kg⁻¹·day⁻¹ during the 24 h of obstruction. Next, sham-operated rats were operated on in parallel. In group 3 (n = 6), osmotic minipumps with saline were implanted subcutaneously. In group 4 (n = 6), osmotic minipumps (Alzet) with parecoxib (Pfizer) dissolved in saline (40 mg/ml) were surgically implanted subcutaneously and the dose was given at a rate of 5 mg·kg⁻¹·day⁻¹ during the 24-h control period. Administration of 5 mg·kg⁻¹·day⁻¹ parecoxib was chosen according to the pharmacological profile of parecoxib previously demonstrated (38).

Blood sampling and removal of kidneys. Twenty-four hours after induction of BUO, rats were reanesthetized with isoflurane. Before death, the aortic bifurcation of the rat was localized, dissected free, and a 5- to 7-ml blood sample was collected for the determination of plasma electrolytes, hormones, and osmolality. Rapidly, the kidneys were removed and dissected into cortex and outer stripe of outer medulla (OM), inner stripe of outer medulla (ISOM), and inner medulla (IM). The plasma concentrations of sodium, potassium, creatinine, and urea were measured (Vitros 950, Johnson & Johnson). The plasma concentration of aldosterone was determined by radioimmunoassay (Active Aldosterone Ria Kit, DSL 8600 Diagnostic System Laboratories, Webster, TX). Plasma renin concentration (PRC) was measured by ulramicroassay of generated ANG I using renin standards as described (31). Five serial dilutions from the same plasma sample were assayed in duplicate for all samples. Only when at least three of the dilutions were linear was the measurement accepted. Renin concentration is expressed in Goldblatt units (GU) compared with renin standards from the National Institute for Biological Standards and Control (Hertfordshire, UK). Finally, osmolality of the plasma was determined with a vapor pressure osmometer (Osmomat 030, Gonotec, Berlin, Germany).

PGE2 measurements in the urine after release of obstruction. To examine the inhibitory effect of parecoxib on prostaglandin production, we performed 24-h BUO followed by release for 24 h with collection of urine. In group 1 (n = 6), osmotic minipumps with saline were implanted subcutaneously. In group 2 (n = 6), osmotic minipumps with parecoxib dissolved in saline (40 mg/ml) were implanted and the dose was given at a rate of 5 mg·kg⁻¹·day⁻¹ during a period of 48 h. Next, sham-operated rats were operated on in parallel. In group 3 (n = 6), osmotic minipumps with saline were implanted subcutaneously. Urine was collected, and PGE2 excretion was measured after 24-h release. A commercial enzyme immunnoassay kit (Amersham Pharmacia Biotech, Little Chalfont, UK) was used to determine PGE2 in the urine.

Measurement of IM osmolality. Kidneys were rapidly removed, and the whole IM was excised on ice from each kidney and weighed. One-half of the IM was thoroughly homogenized with a motor-driven homogenizer, after addition of 300 μl of distilled water. Homogenate osmolality was determined with a vapor pressure osmometer (Osmomat 030, Gonotec).

IM osmolality was calculated, assuming that 80% of IM wet weight is water (2).

Table 1. Biochemical values from plasma samples in protocol 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (n = 6)</th>
<th>24-h BUO (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNa, mmol/kgH₂O</td>
<td>302.7 ± 1.6</td>
<td>345.7 ± 1.8*</td>
</tr>
<tr>
<td>Pk, mM</td>
<td>140.2 ± 0.3</td>
<td>139.3 ± 0.5</td>
</tr>
<tr>
<td>Pk, mM</td>
<td>4.2 ± 0.2</td>
<td>7.0 ± 0.3*</td>
</tr>
<tr>
<td>PCrea, μM</td>
<td>26.3 ± 0.4</td>
<td>273.2 ± 9.7*</td>
</tr>
<tr>
<td>Purea, mM</td>
<td>5.0 ± 0.3</td>
<td>47.6 ± 1.5*</td>
</tr>
<tr>
<td>PAldosterone, pg/ml</td>
<td>267.5 ± 15</td>
<td>3,507 ± 233*</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; Sham, sham-operated control rats; BUO, bilateral ureteral obstruction; PNa, plasma sodium; PK, plasma potassium; PCrea, plasma creatinine; Purea, plasma urea; PAldosterone, plasma aldosterone. *P < 0.05 BUO compared with Sham.
Fig. 1. Semiquantitative immunoblots of cyclooxygenase COX-1 and COX-2 using kidney protein isolated from cortex and outer stripe of outer medulla (Cortex/OSOM) from sham-operated (SHAM) and bilateral ureteral obstructed (BUO) rats. A total of 50 μg protein was used for the COX-1 and COX-2 assay. A: immunoblot was reacted with anti-COX-1 antibody and revealed a single ~71-kDa band. B: densitometric analyses of all the samples from sham-operated and obstructed kidneys of rats with 24-h BUO revealed that there was no difference between control and obstructed kidneys (96 ± 8% vs. 100 ± 8%). C: immunoblot was reacted with anti-COX-2 antibody and revealed a single ~72-kDa band. D: densitometric analyses of all the samples from sham-operated and obstructed kidneys of rats with 24-h BUO revealed that there was no difference between control and obstructed kidneys (91 ± 12 vs. 100 ± 18%).

Quantitative PCR. For quantitative PCR, 100 ng cDNA served as a template for PCR amplification using iQ SYBR-Green supermix according to the manufacturer’s instructions (Bio-Rad). Serial dilution (1 ng 1/10 fold) of plasmid prRenin with exons 1 and 2 inserted in the polylinker site was used as a template for generation of a standard curve. Nested primers were used to amplify standards and kidney cDNA samples (sense CAG ACA CAG CCA GCT TTG; antisense CCA TTC AGC ACT GAT CCT; GenBank accession no. NM012642, covering bases 71–174, 104 bp). Standards and unknown samples were amplified in duplicate in 96-well plates, and PCR was performed for 43 cycles consisting of denaturation for 30 s at 95°C followed by annealing and polymerization at 58°C for 45 s. Emitted fluorescence was detected during the annealing/extension step in each cycle. The samples from sham-operated and obstructed kidneys were amplified in duplicate in 96-well plates, and PCR was performed for 43 cycles consisting of denaturation for 30 s at 95°C followed by annealing and polymerization at 58°C for 45 s. Emitted fluorescence was detected during the annealing/extension step in each cycle. Specificity was assured by postrun melting curve analysis and by agarose gel electrophoresis.

Membrane fractionation for immunoblotting. The tissue (cortex+OM, ISOM, and IM) was homogenized in dissecting buffer (0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, pH 7.2) containing the following protease inhibitors: 8.5 μM leupeptin (serine and cysteine protease inhibitor), 0.4 mM pefabloc (serineprotease inhibitor, Roche). More-
COX-2 (Cayman Chemical, catalog no. 160126): an affinity-purified rabbit polyclonal antibody against COX-2.

AQP2 (LL127 serum or LL127AP): immune serum or an affinity-purified antibody to AQP-2 has previously been described (9).

AQP3 (LL178AP): an affinity-purified polyclonal antibody to AQP3 has previously been described (10).

Type 3 Na⁺/H⁺ exchanger (NHE3; LL546AP): an affinity-purified polyclonal antibody to NHE3 has previously been described (12).

The type II Na-Pi cotransporter (NaPi2; LL697AP/LL696AP): affinity-purified polyclonal antibody to NaPi2 has previously been described (3).

Na-K-ATPase: a monoclonal antibody against the α₁-subunit of Na-K-ATPase has previously been described (24).

The apical Na⁺-K⁺-2Cl⁻ cotransporter (BSC1; LL320AP): an affinity-purified polyclonal antibody to the apical Na-K-2Cl cotransporter of the TAL has previously been described (11).

Immunocytochemistry. The kidneys from BUO rats and sham-operated control rats were fixed by retrograde perfusion via the abdominal aorta with 3% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4. Moreover, the kidneys were immersion fixed for 1 h and washed 3 × 10 min with 0.1 M cacodylate buffer. The kidney blocks were dehydrated and embedded in paraffin. The paraffin-embedded tissues were cut in 2-μm sections on a rotary microtome (Leica Microsystems, Herlev, Denmark).

For immunoperoxidase labeling, the sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked with 5%
with parecoxib-treated BUO. As shown in Fig. 4, parecoxib treatment significantly reduced urinary PGE2 excretion compared with BUO controls. The mean urinary PGE2 excretion was 3.54 ± 0.47 ng/mg creatinine in BUO rats, whereas it was 2.81 ± 0.30 ng/mg creatinine in parecoxib-treated BUO rats (P < 0.05).

We next investigated the effect of COX-2 inhibitor parecoxib on the plasma levels of renin, aldosterone, creatinine, urea, potassium, and osmolality in BUO rats. As shown in Table 2, plasma renin activity and aldosterone levels were significantly increased in BUO rats compared with sham rats (P < 0.05). Parecoxib treatment significantly reduced plasma renin activity and aldosterone levels in BUO rats. The mean plasma renin activity was 1.2 ± 0.1 mU/ml in BUO rats, whereas it was 0.7 ± 0.1 mU/ml in parecoxib-treated BUO rats (P < 0.05). Similarly, the mean plasma aldosterone level was 9.3 ± 0.6 ng/dl in BUO rats, whereas it was 3.1 ± 0.6 ng/dl in parecoxib-treated BUO rats (P < 0.05).

To determine whether the expression of COX-1 and COX-2 was altered in the kidneys of BUO rats, we performed immunohistochemistry and semiquantitative immunoblotting. As shown in Fig. 1, COX-1 expression was unchanged after 24 h of BUO. However, there was a marked increase in the COX-2 labeling of IM interstitial cells at the base of the papillary tip with no apparent difference between sham-operated controls and BUO rats. In contrast, the abundance of COX-2 protein was significantly increased in IM 24 h after BUO.

Increased renal IM COX-2 expression in response to 24-h BUO. To test whether the expression of COX-1 and COX-2 was increased after 24 h of BUO, we performed immunohistochemistry and semiquantitative immunoblotting. In the cortex of BUO rats, COX-2 immunoreactive protein was localized to the macula densa region in kidneys from both obstructed and sham-operated control rats, and in the obstructed group. However, there was a marked increase in the COX-2 labeling of IM interstitial cells at the base of the papillary tip with no apparent difference between sham-operated controls and BUO rats. In contrast, the abundance of COX-2 protein was significantly increased in IM 24 h after BUO.

Immunohistochemical staining of kidney IM sections revealed that COX-2 immunoreactive protein was localized to the medullary interstitial cells in the papillary tip with no apparent difference between sham-operated controls and BUO rats. However, there was a marked increase in the COX-2 labeling of IM interstitial cells at the base of the IM in BUO rats compared with sham-operated control rats. Thus, BUO resulted in recruitment of COX-2-positive cells along the corticomedullary axis. In the cortex, COX-2-immunoreactive protein was detected at the macula densa region in kidneys from both obstructed and sham-operated control rats, and in the obstructed kidneys COX-2 labeling intensity and distribution with sham-operated rats, consistent with impaired renal function (Table 1). Plasma aldosterone levels were significantly increased compared with sham-operated controls (Table 1). This is consistent with previous observations in rats subjected to 24-h BUO (29).

As shown in Table 2, the body weight, kidney weight, and plasma biochemical values from the four groups in protocol 2 are presented as means ± SE. The number of rats in each group is given in parentheses. The Student’s t-test or one-way ANOVA with Bonferroni correction was used to compare the results. The values of brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) were measured using commercially available ELISA kits. The results were expressed as means ± SE. The level of significance was set at P < 0.05.
were comparable to that seen in sham-operated control rats (not shown).

Immunostaining for COX-1 demonstrated an immunopositive signal from IM collecting duct cells and from interstitial cells (not shown). COX-1 immunolabeling in cortex and OM showed that COX-1 was localized to the connecting tubule and cortical collecting ducts (not shown). Furthermore, COX-1 also labeled the glomerular mesangial cells (not shown), as previously described (6). COX-1 labeling in IM and cortex+OM did not differ between the BUO rats and sham-operated control rats.

Effect of the COX-2 inhibitor parecoxib on urinary PGE2 excretion. We examined the effect of parecoxib (dynastat; 5 mg/kg 24 h), a selective COX-2 inhibitor, on urinary PGE2 excretion induced by BUO for 24 h. After release of BUO, PGE2 excretion in urine was significantly stimulated (1.5 ± 0.2 vs. 2.9 ± 0.6 ng·min⁻¹·day⁻¹, P < 0.05, Fig. 4). The augmented PGE2 excretion was abolished by parecoxib (2.9 ± 0.6 vs. 1.4 ± 0.2 ng·min⁻¹·day⁻¹, P < 0.05, Fig. 4), demonstrating that COX-2 was the dominant source of PGE2 synthesis.

Effect of COX-2 inhibition on kidney weight, plasma electrolytes, creatinine, and hormone levels in rats with 24-h BUO. In protocol 2, the effect of a selective COX-2 inhibitor, parecoxib (5 mg·kg⁻¹·day⁻¹), was examined. BUO and sham rats were treated with parecoxib during the entire 24-h obstruction period. There was no significant difference between the untreated and treated sham-operated control rats with regard to body weight, kidney weight, plasma osmolality, and the plasma concentration of sodium, potassium, creatinine, and urea (Table 2). Similar to the results of protocol 1, plasma osmolality, potassium, creatinine, and urea increased significantly in the BUO rats compared with untreated control rats (Table 2). Parecoxib treatment partially inhibited the increase in kidney weight-to-body weight ratio and plasma urea in response to BUO (Table 2). Moreover, the medullary tissue osmolality was significantly decreased in BUO rats compared with sham-operated control rats, and parecoxib had no effect on this parameter (Fig. 5). Plasma renin (Fig. 6B) and aldosterone (Fig. 6C) concentrations diverged as a result of BUO. Similar to the results of protocol 1, plasma aldosterone concentration was markedly elevated in parallel with plasma potassium concentration (Table 2), whereas plasma renin concentration was strongly suppressed (Fig. 6B). Consistent with this, renal cortical renin mRNA level was decreased in BUO rats (Fig. 6C). Parecoxib treatment suppressed basal plasma renin and aldosterone concentrations in sham rats (Fig. 6, B and C), whereas parecoxib had no effect on plasma concentrations in the BUO rats (Fig. 6, B and C). Similarly, parecoxib treatment did not affect the renal renin mRNA level (Fig. 6A).

COX-2 inhibition increased COX-2 expression in renal IM of BUO rats. Consistent with the results in protocol 1, BUO increased COX-2 expression in IM compared with sham-operated control rats (arbitrary units: 7.2 ± 1.3 vs. 0.7 ± 0.2, P < 0.05). Parecoxib treatment caused a further increase in COX-2 expression in BUO rats compared with untreated BUO rats (arbitrary units: 27.1 ± 3.5 vs. 7.2 ± 1.3, P < 0.05). Consistent with this, immunolabeling demonstrated a marked increase in COX-2 labeling of interstitial cells at the base of IM of kidneys from BUO rats (Fig. 7, C and D) compared with sham-operated control rats (Fig. 7, A and B). At the tip of the IM, there was no difference in COX-2 labeling between BUO and sham-operated rats. However, parecoxib treatment markedly enhanced COX-2 labeling in the IM of BUO kidneys, and COX-2 labeling was present in the entire IM from the base to the tip (Fig. 7, E and F). In the ISOM, there was no labeling (Fig. 7G).

Consistent with the results from protocol 1, COX-1 expression in IM from BUO rats was unchanged and maintained at the levels found in control rats (Table 3). Parecoxib

Fig. 6. Effect of COX-2 inhibitor parecoxib on renin parameters and plasma aldosterone in BUO and sham-operated rats. Parecoxib (5 mg·kg⁻¹·day⁻¹) was administrated in osmotic minipumps during the obstruction period for 24 h. A: effect of parecoxib on kidney renin mRNA level. Renin was determined by quantitative PCR (QPCR) using 100 ng COR+OM cDNA. Each bar represents the mean ± SE of 6 determinations. Units are 10^(-11)g. *P < 0.05, compared with sham rats. B: effect of parecoxib on plasma renin concentration (PRC). PRC was determined by RIA of ANG I subsequent to incubation of samples with surplus of purified rat renin substrate. Each bar represents the mean ± SE of 6 determinations. *P < 0.05 BUO compared with sham rats. †P < 0.05 parecoxib-treated, sham-operated compared with untreated sham-operated rats. C: effect of parecoxib on plasma aldosterone concentration. Aldosterone concentration was determined by RIA (Active Aldosterone RIA Kit). Each bar represents the mean ± SE of 6 determinations. *P < 0.05 BUO compared with sham rats. †P < 0.05 parecoxib-treated, sham-operated compared with untreated sham-operated rats.
treatment did not change renal COX-1 expression in BUO rats (Table 3).

**COX-2 inhibition prevents downregulation of AQP2 expression in response to BUO.** AQP2 is localized to the apical plasma membrane and subapical vesicles of the collecting duct principal cells. Consistent with previous studies, we confirmed that AQP2 expression in IM collecting ducts of BUO rats was markedly reduced compared with sham-operated control rats (arbitrary units: 2.0 ± 0.2 vs. 5.1 ± 0.3, P < 0.05) (Fig. 8B). Parecoxib treatment prevented the BUO-induced reduction in AQP2 expression (4.6 ± 0.5 vs. 5.1 ± 0.3, not significant) (Fig. 8B). These findings were confirmed by immunohistochemical analyses. In kidneys from sham-operated control rats, immunoperoxidase microscopy demonstrated that AQP2 labeling was localized in the apical domains of the IM collecting duct principal cells (Fig. 8C). In kidneys from BUO rats, AQP2 labeling was much weaker in the apical plasma membrane domains of the IM collecting duct principal cells (Fig. 8D) compared with sham-operated control rats. In the obstructed kidneys of parecoxib-treated BUO rats, AQP2 labeling was comparable to that seen in sham-operated control rats (Fig. 8E).

AQP3 is localized to the basolateral plasma membranes of the collecting duct principal cells. Consistent with previous studies, we confirmed that AQP3 abundance decreased in IM collecting ducts of BUO rats compared with sham-operated control rats (arbitrary units: 2.8 ± 1.0 vs. 4.5 ± 0.4, P < 0.05) (Table 3). In contrast to AQP2, parecoxib administration did not change AQP3 abundance in BUO rats compared with untreated BUO rats (5.4 ± 1.3 vs. 2.8 ± 1.0, Table 3).

**COX-2 inhibition attenuates downregulation of renal major sodium-transport proteins in response to BUO.** We examined the effect of parecoxib on the expression of renal medullary sodium transporters and cotransporters in response to 24-h BUO. NHE3 was significantly decreased in the cortex and OM
Table 3. Summary of densitometry of immunoblots from the 4 groups in protocol 2

<table>
<thead>
<tr>
<th></th>
<th>Sham + Vehicle</th>
<th>Sham + Parecoxib</th>
<th>24-h BUO + Vehicle</th>
<th>24-h BUO + Parecoxib</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
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<tr>
<td><strong>Cyclooxygenases</strong></td>
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<tr>
<td>COX-1</td>
<td>4.2±0.3</td>
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<tr>
<td>COX-2</td>
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<td><strong>Aquaporins</strong></td>
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<tr>
<td>AQP2 (IM)</td>
<td>5.1±0.3</td>
<td>6</td>
<td>6.0±0.6</td>
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<tr>
<td>AQP3 (IM)</td>
<td>4.5±0.4</td>
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<td>4.8±0.8</td>
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<td><strong>Sodium transporters</strong></td>
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<tr>
<td>NHE3 (COR+OM)</td>
<td>5.0±0.5</td>
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<td>5.9±1.2</td>
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<td>NHE3 (ISOM)</td>
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<td>BSC-1 (ISOM)</td>
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<td>NaPi2 (COR+OM)</td>
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<tr>
<td>Na-K-ATPase (α1-subunit; IM)</td>
<td>4.0±0.4</td>
<td>6</td>
<td>3.9±0.3</td>
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</table>

Values are means ± SE. n, No. of rats. COX-1, cyclooxygenase 1; COX-2, cyclooxygenase 2; AQP2, aquaporin 2; AQP3, aquaporin 3; NHE3, type 3 Na+/H+ exchanger; BSC-1, rat type 1 bumetanide-sensitive cotransporter; NaPi, type II Na-Pi cotransporter; Na-K-ATPase, sodium-potassium-adenosine triphosphatase transporter; IM, inner medulla; ISOM, inner stripe of outer medulla; COR+OM, cortex + outer medulla. *P < 0.05 BUO compared with Sham. †P < 0.05 untreated BUO compared with parecoxib-treated BUO.

after 24-h BUO compared with sham-operated rats (arbitrary units: 1.8 ± 0.5 vs. 5.0 ± 0.5, P < 0.05) (Fig. 9B). Parecoxib treatment of BUO rats attenuated this reduction in NHE3 expression in the obstructed kidneys (cortex+OM: 4.8 ± 0.7 vs. 5.0 ± 0.5, not significant; ISOM: 4.0 ± 0.3 vs. 5.7 ± 0.4, not significant) (Fig. 9B). Consistent with this, immunohistochemistry showed weak labeling of NHE3 in the apical membranes of proximal tubule cells in the cortex (Fig. 9D) and in

Fig. 8. Semiquantitative immunoblot of AQP2 using protein isolated from IM from sham-operated, parecoxib-treated sham-operated, 24-h BUO, and parecoxib-treated 24-h BUO rats. A: immunoblot was reacted with affinity-purified anti-AQP2 antibody and revealed a 29-kDa and 35- to 45-kDa bands, representing nonglycosylated and glycosylated forms of AQP2. B: densitometric analyses revealed that AQP2 expression in IM was marked decreased in the BUO rats compared with sham-operated control rats. Parecoxib treatment of BUO rats prevented this decrease in AQP2 expression, which was comparable to the control kidney. There was no difference between the untreated and parecoxib-treated control rats. *P < 0.05 BUO compared with sham-operated rats. *P < 0.05 parecoxib-treated BUO compared with untreated 24-h BUO rats. C: in sham-operated control rats, immunoperoxidase microscopy demonstrated that AQP2 labeling was associated with apical plasma membrane of principal cells in collecting ducts in the IM. D: in the obstructed kidney of BUO rats, reduced labeling for AQP2 was detected. E: the obstructed kidney from parecoxib-treated rats displayed labeling comparable to that seen in sham-operated rats.
the apical plasma membrane domains of medullary TAL and thin limb (Fig. 9G) of ISOM in BUO compared with sham-operated control rats. In the BUO rats treated with parecoxib, the reduction in NHE3 labeling was attenuated compared with vehicle-treated BUO rats. Immunohistochemistry for NHE3 in COR and ISOM of sham-operated (C and F), 24-h BUO (D and G), and parecoxib-treated 24-h BUO (E and H) rats is shown. C and G: in sham-operated control rats, immunoperoxidase microscopy demonstrated that NHE3 labeling was associated with apical domains of proximal tubule (PT) cells and thick ascending limb (TAL) of the loop of Henle. D and H: in the obstructed kidney of BUO rats, reduced labeling of NHE3 was detected. E and H: obstructed kidney from parecoxib-treated rats shows labeling comparable to that seen in sham-operated rats.

BSC-1 is mainly responsible for the apical sodium reabsorption in the cortical and medullary TAL. Semiquantitative immunoblotting from the ISOM revealed that the expression of BSC-1 was reduced after 24-h BUO compared with sham-operated control rats (1.5 ± 0.4 vs. 7.6 ± 0.8, P < 0.05) (Fig. 10B). Parecoxib treatment of BUO rats resulted in a significant attenuation of BSC-1 downregulation in ISOM (3.7 ± 0.4 vs. 7.6 ± 0.8, P < 0.05) (Fig. 10B). Immunoperoxidase microscopy of kidney cortex, OM, and ISOM demonstrated that BSC-1 staining was associated with the apical plasma membrane domains of cortical (Fig. 10C) and medullary TAL (Fig. 10F) in sham-operated rats. In BUO kidneys, the labeling of BSC-1 in cortical and medullary TAL was much weaker (Fig. 10, D and G), whereas in parecoxib-treated BUO rats BSC-1 labeling (Fig. 10, E and H) was comparable to sham-operated rats, demonstrating a marked parecoxib-induced prevention of BSC-1 downregulation in response to 24-h BUO.

**DISCUSSION**

The main results of the present study were that COX-2, and not COX-1, was markedly and selectively upregulated in IM interstitial cells in response to 24-h BUO despite a significant decrease in medullary tissue osmolality. The abundance of aquaporins AQP2 and AQP3 and sodium transporters NHE-3, BSC-1, and NaPi2 was reduced in response to BUO. Administration of the selective COX-2 inhibitor parecoxib to BUO rats inhibited the increase in PGE2 excretion after release and prevented the downregulation of AQP2 and NHE3 and attenuated BSC-1 decrease. Basal plasma concentration of renin and aldosterone was suppressed by parecoxib in sham rats. BUO led to divergent, parecoxib-insensitive effects on renin and aldosterone.
Increased IM COX-2 expression in response to BUO. The present study demonstrated an increased COX-2 expression in the IM in response to 24-h BUO. This is consistent with previous reports demonstrating an increase in IM COX-2 expression in response to BUO (7), in response to occlusion of a solitary kidney (37), and also in kidney IM of rats subjected to UUO (8). This finding supports the view that COX-2 activity is responsible for the reported increase in renal PGE2 and thromboxane A2 synthesis in UUO (44). Immunohistochemical staining demonstrated that COX-2 was selectively induced in the medullary interstitial cells of BUO kidneys. This is consistent with the results of previous studies demonstrating IM COX-2 expression localized to the interstitial cells of normal kidneys (17, 20, 42). Several conditions induce COX-2 expression, including dehydration (46), AVP (50), and NaCl loading (22) in the IM. Characteristically, these stimuli probably all converge on an increased interstitial osmolality, which is consistent with the observation that COX-2 is induced in cultured interstitial cells by increasing the osmolality of the medium (34). In the present study, IM tissue osmolality decreased in response to 24 h of BUO. Thus it is unlikely that the BUO-induced enhanced medullary COX-2 expression was caused by changes in osmolality. The mechanisms involved in the medullary COX-2 induction in response to 24 h of BUO were not directly addressed in the present study, but several possibilities exist. During the first 24 h after onset of obstruction, the pressure in the urinary tract is highly elevated and this pressure increase may mechanically stimulate interstitial cells and induce increased COX-2 expression. Consistent with this, it was recently demonstrated that mechanical stretch can stimulate COX-2 in the bladder and vasculature (40). Increased COX-2 expression in the IM may also result from inflammatory mediators in the kidney. Consistent with this, previous studies have shown that UUO led to an intense infiltration of inflammatory cells in the renal interstitium (41).

Selective COX-2 inhibition prevents AQP2, NHE3, and BSC-1 downregulation. Our data documented that COX-2 activity contributed importantly to the alterations in water and Na transport proteins in response to 24-h BUO. Thus the well-known downregulation of renal AQP2 and several key sodium transport proteins, previously demonstrated in response to BUO (28, 30), was significantly weakened or prevented by administration of the selective COX-2 inhibitor parecoxib. This finding is consistent with the results of a recent study demonstrating that COX-2 inhibition prevented downregulation of AQP2 after release of 24 h of BUO.

Fig. 10. Semiquantitative immunoblot of the type 1 bumetanide-sensitive Na-K-2Cl cotransporter (BSC-1) using protein isolated from inner stripe of outer medulla from sham-operated, parecoxib-treated sham-operated, 24-h BUO, and parecoxib-treated 24-h BUO rats. A: immunoblot was reacted with affinity-purified anti-BSC-1 antibody and revealed a strong, broad band of molecular mass (146–176 kDa centered at 161 kDa). B: densitometric analyses revealed that BSC-1 expression in inner stripe of outer medulla was markedly decreased in the BUO rats compared with sham-operated control rats. Parecoxib treatment of obstructed kidneys from BUO rats did not significantly change BSC-1 expression compared with untreated BUO rats. There was no difference between the untreated and parecoxib-treated control rats. *P < 0.05 BUO compared with sham-operated rats. #P < 0.05 parecoxib-treated BUO compared with untreated BUO rats. Immunohistochemistry for BSC-1 in kidney cortex and inner stripe of outer medulla of sham-operated (C and F), 24-h BUO (D and G) and parecoxib-treated 24-h BUO (E and H) rats is shown. C and F: in sham-operated control rats, immunoperoxidase microscopy demonstrated that BSC-1 labeling was associated with the apical plasma membrane domains of cortical TAL cells and macula densa cells in the cortex and medullary TAL cells in the inner stripe of outer medulla. D and G: in the obstructed kidney of BUO rats, reduced labeling of BSC-1 was detected. E: in the obstructed kidney from parecoxib-treated rats, there was no difference in labeling intensity of BSC-1 in cortex. H: in the inner stripe of the outer medulla, the reduction in BSC-1 labeling of BUO rats was attenuated by parecoxib treatment.
hance the active NaCl absorption in TAL (13). Diclofenac increase the abundance of BSC-1 and thereby selective cyclooxygenase inhibitors such as indomethacin and interaction with the Gi-coupled EP3 receptor (43), and nonse-
release, which documents effective inhibition of COX-2 and suggests that COX-2 is the dominant source of PGE2 syn-
radation of all proteins. The differential effects of parecoxib on
pert proteins was not mediated indirectly by a change in osmolality. The prevention of the increase in normalized kidney weight after parecoxib treatment indicates that COX-2 activity contributes to the marked swelling of the obstructed kidney.

Not all investigated proteins were equally sensitive to ob-
strucion and/or parecoxib. Thus COX-1 and Na-K-ATPase did not change during the conditions tested, which documents that the observed BUO-induced changes were specific and not related to widespread necrosis or unspcific proteosomal deg-
radation of all proteins. The differential effects of parecoxib on transporters could be related to the localization of the changes in COX-2 activity. Because prostanoids have a short range of action in vivo, PG-mediated effects related to BUO-induced COX-2 activity would be expected to prevail adjacent to the enzyme in the IM and OM. Consistent with this, AQ2P2, NHE3, and BSC-1 are found in these regions.

How are the effects of COX-2 activity mediated? PGE2 is the major prostanooid synthetized in the kidney, and direct effects of PGE2 on tubular epithelium are mediated by at least four distinct receptors with different intracellular coupling (4). In the TAL, PGE2 inhibits reabsorption of NaCl through interaction with the Gi-coupled EP3 receptor (43), and nonselective cyclooxygenase inhibitors such as indomethacin and diclofenac increase the abundance of BSC-1 and thereby en-
hance the active NaCl absorption in TAL (13).

Furthermore, abluminal addition of PGE2 inhibits the AVPinduced increase in water permeability in collecting ducts, probably mediated by the calcium-coupled EP1 receptor (1). PGE2 has been shown to have no effect on the cAMP-induced water permeability of rat terminal IM collecting duct (35) and to counteract vasopressin-induced AQ2P2 translocation to the plasma membrane in slices of IM independently of dephos-
phorylation (49).

Effect of COX-2 inhibition on hormone levels in rats with 24-h BUO. The data indicated that basal COX-2 activity supported renin secretion from the kidneys and thereby also plasma aldosterone. It is generally believed that the renin-
angiotensin-aldosterone system is activated by ureteral ob-
strucion. We detected a strong inhibition of renin secretion and renin expression after obstruction, whereas aldosterone was markedly stimulated. Each of these changes was insensitive to COX-2 inhibition. Because plasma potassium was strongly elevated, this parameter was the factor most likely responsible for stimulating the adrenals to release aldosterone indepen-
dently of renin-mediated angiotensin formation. The suppres-
sion of renin release and mRNA could be mediated by the BUO-associated rise in arterial blood pressure. Moreover, increased pressure in the absence of stretch inhibits renin secretion from isolated cells in vitro, and interstitial pressure rises sharply upon introduction of BUO (21). In the present study, cortical COX-2 expression did not change in response to BUO, which indicated that the macula densa signal was less likely to play a major role in this setting. COX-2 can change rapidly (30 min) at this site in response to macula densamediated signals, e.g., furosemide or renal arterial stenosis (32), and in a previous study where rats were subjected to 24 h of UUO COX-2 expression decreased in the cortex of the obstructed kidney compared with the contralateral kidney (8). However, this model with one unobstructed, hyperfunctioning kidney is not directly comparable to the present model of BUO. The data emphasize that the acute phase of bilateral obstruction is characterized by suppression of renin and, most likely, also angiotensin. This suppression could contribute to downregula-
tion of certain Na transporters, because we have previously shown a stimulation of transporter abundance in response to ANG II (27).

Summary. The present study demonstrated that BUO in rats is associated with induction of COX-2 expression in the renal IM, which is localized to interstitial cells. Consistent with previous studies, BUO is associated with severe downregula-
tion of renal aquaporins and key renal sodium transporters. Importantly, selective COX-2 inhibition prevents downregula-
tion of renal IM AQ2P2 expression and attenuates downregula-
tion of NHE3 and BSC-1 expression at the medullary TAL. Thus the present data suggest that increased PGE2 synthesis in response to urinary tract obstruction may play an important role in the dysregulation of renal aquaporins and sodium transporters, which are crucial for the impaired urinary concentration capacity in obstructive nephrogenic diabetes insipidus.

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