Impaired Renal Sensory Responses after Unilateral Ureteral Obstruction in the Rat

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Abstract. Renal responses to the activation of renal sensory receptors were examined in rats after release of 24-h unilateral ureteral obstruction of the left kidney. The integrity of the renalorenal reflex was examined in both 24-h unilateral ureteral obstruction-treated (UUO) and sham-operated (Sham) rats. Increased ipsilateral afferent renal nerve activity (ARNA) and reflexly decreased efferent renal nerve activity (ERNA) and increased contralateral diuresis and natriuresis produced by increasing the left intrapelvic pressure were observed in Sham rats but not in UUO rats. The lack of responsiveness of the renalorenal reflex in UUO rats was associated with lower release of substance P (SP) and increased neutral endopeptidase (NEP) activity in the renal pelvis in the postobstructive kidney. Compared with Sham rats, urine and sodium excretion after acute saline loading was significantly reduced in the postobstructive kidney. The blunted excretory responses were accompanied by lower activation of ARNA and less reflex inhibition of ERNA. Renal sensory dysfunction in the postobstructive kidney was further examined by stimulation of renal mechanoreceptors and chemoreceptors. Graded increases in intrapelvic pressure or renal pelvic perfusion with hypertonic saline solution elicited, respectively, a pressure- or concentration-dependent increase in ARNA in the control kidney of Sham rats, this response being greatly attenuated in the postobstructive kidney. Western blots showed no quantitative difference in the expression of renal pelvic neurokinin 1 (NK-1) receptors between the two groups. It was concluded that renal sensory function is impaired in the postobstructive kidney of UUO rats and that this defective activation of renal sensory receptors results in an impaired renalorenal reflex, which is associated with enhanced NEP activity and catabolism of SP released in the renal pelvis and is not related to the expression of NK-1 receptor protein.

An impaired renal sensory response associated with various kidney disorders has been demonstrated in rats (1,2), which suggests the important role of renal afferents in the reflex control of renal functions. Two major types of sensory receptors, mechanoreceptors and chemoreceptors, are found on renal afferent fibers in the kidney. These appear to have two major functions, evoking renorenal reflexes by renal mechanostimulation or chemostimulation and pain sensation in renal disease. The majority of renal sensory neurons located in the renal pelvis contain substance P (SP) and calcitonin gene-related peptide (3), which are metabolized by membrane-bound neutral endopeptidase (NEP) (4). Both SP-immunoreactive nerves and an SP receptor, the neurokinin 1 (NK-1) receptor (5), are found in the subepithelial area of the renal pelvis, all factors required for renal sensory transmission therefore being present in this region. In rats, activation of renal sensory neurons by SP-containing neurons (6) elicits an inhibitory renorenal reflex, which plays an important physiologic role in enhancing urine flow and promoting the removal of irritants by tonically inhibiting renal sympathetic nerve activity in the urinary tract (7).

Impairment of urinary flow by obstruction of the urinary tract, referred to as obstructive nephropathy, is a manifestation of a variety of kidney disease (8). When experimental animals undergo 24 h of unilateral ureteral obstruction, a decline in renal hemodynamic and tubular function is seen (9). Harris et al. (10) showed loss of excretory function after release of 24-h occlusion in the postobstructive kidney when it was tested by saline loading. Interruption of renal nerve traffic by kidney denervation before relief of 24-h obstruction results in an improvement in renal hemodynamics and excretory function (11). These results suggest that the impaired diuretic/natriuretic responses seen in the postobstructive kidney might be due to reduced hemodynamics and possibly to increase renal sympathetic nerve activity. However, the factor responsible for the increased renal sympathetic nerve activity in obstructive nephropathy and the functional role of renal afferent nerves are unknown. This study was undertaken to compare differences in the control and 24-h obstructed kidney in rats in terms of the SP-mediated renorenal reflex, renal pelvic expression of NK-1 receptors, NEP activity, renal excretion, and renal efferent and afferent nerve activity after saline loading, by use of stimuli specific for different types of renal receptors.

Materials and Methods

Animal Care and Experimentation

Female Wistar rats weighing 200 to 230 g were used. All animal experiments and animal care were performed in accordance with the Guides for the Care and Use of Laboratory Animals (Washington DC,
Sham-operated (Sham) rats were treated identically but without ligation of the ureter. Unilateral ureteral obstruction-treated (UUO), allowed to recover. Incision, double ligated, and the incision closed under aseptic conditions; the ligation was released after 24 h and the animals, referred to as unilateral ureteral obstruction-treated (UUO), allowed to recover. Sham-operated (Sham) rats were treated identically but without ligation of the left ureter. In the UUO rats, the 24-h ureter-occluded kidney after release is referred to as the postobstructive kidney.

**Induction of Unilateral Ureteral Obstruction**

Under combined ketamine (50 mg kg⁻¹, intraperitoneally) and sodium pentobarbital (15 mg kg⁻¹, intraperitoneally) anesthesia, the left ureter was isolated from the surrounding tissues via an abdominal incision, double ligated, and the incision closed under aseptic conditions; the ligation was released after 24 h and the animals, referred to as unilateral ureteral obstruction-treated (UUO), allowed to recover. Sham-operated (Sham) rats were treated identically but without ligation of the left ureter. In the UUO rats, the 24-h ureter-occluded kidney after release is referred to as the postobstructive kidney.

**General Surgical Procedures**

Twenty-four hours after the above treatment, the rats were anesthetized with sodium pentobarbital (35 mg kg⁻¹, intraperitoneally) and the trachea exposed and intubated for spontaneous ventilation. Catheters (PE-50) were placed in the external jugular vein for saline infusion and in the carotid artery to measure the mean arterial BP (MABP). The rat was placed on its right side and the left kidney exposed via a left flank incision and fixed in position with clips. Both ureters were cannulated with 5 cm PE-50 catheters for urine collection, the catheter in the obstructive kidney being inserted above the obstruction site. Changes in renal intrapelvic pressure (IPP) were recorded with a transducer connected to the left ureteral catheter by a T-tube connector. IPP and MABP were continuously displayed on a Gould polygraph (Quincy, MA). The kidney was then bathed with warmed paraffin oil (38°C) to prevent drying and illuminated with a fiber-optic light source. By use of a stereoscopic dissecting microscope (SZ-STU2, Olympus, Tokyo, Japan), the left renal nerve at the angle between the abdominal aorta and renal artery was carefully isolated from the surrounding tissue to record renal nerve activity.

**Recording of Renal Nerve Activity**

The recording technique has been described elsewhere (12). Briefly, renal efferent and afferent multifiber nerve activities were recorded simultaneously by placing two intact nerve fibers on two pairs of thin bipolar stainless steel electrodes. The electrical signals were amplified 20,000-fold and filtered (high-frequency cutoff, 3000 Hz; low-frequency cutoff, 30 Hz) by a Grass model P511 AC amplifier (Quincy, MA) and continuously displayed on a Gould oscilloscope (Model 1604, Valley View, OH). The amplified signals were fed into a window discriminator (World Precision Instrument 121, Sarasota, FL) and counted on a Gould integrator amplifier (13-4615-70, Valley View, OH). The neural activity was transformed into spike counts or voltage integration. Renal nerve activity was assessed by its pulse-synchronous rhythmicity with the heart beat. After the identification and verification of renal nerve activity, the proximal and distal parts of the nerve fibers were transected so as to record the ipsilateral efferent renal nerve activity (ERNA) and afferent renal nerve activity (ARNA) separately.

**Renorenal Reflex**

The renorenal reflex was studied in 8 Sham and 8 UUO rats. Rats were prepared as described in the “General Surgical Procedures and Recording of Renal Nerve Activity” section. A PE-10 catheter with a heat-pulled tip (<20 μm) was placed inside the 5-cm length of the PE-50 catheter in the left ureter, extending 1 to 2 mm beyond the tip of the PE-50 catheter. The tips of the two catheters were placed together in the left ureter near the renal pelvis, allowing the renal pelvis to be perfused with 150 mM NaCl at 20 μl min⁻¹ via the PE-10 catheter, the effluent being drained away by the PE-50 catheter. The other end of the 5-cm PE-50 catheter was connected to a T-tube connector to record the IPP change. The third end of the T-tube connector was connected to a 50-cm length of PE-50 tubing that could be raised to increase the IPP by ~40 mmHg. Changes in renal IPP, ERNA, and ARNA were recorded as described above in the postobstructive kidney and the urine flow rate (UV) and urinary sodium excretion (Uns,V) measured in the nonobstructed contralateral kidney. A 4-min experimental period, which consisted of a 3-min period of increased renal pelvic pressure and a 1-min collection period, was bracketed by control and recovery periods, each of 10 min.

**Renal Pelvic Substance P Release**

To assay SP release, the renal pelvis was perfused via the PE-10 catheter with 150 mM NaCl that contained 10 μmol l⁻¹ of the endopeptidase inhibitor, thiorphan (Sigma, Saint Louis, MO), to minimize the catabolism of SP (13). The perfusion rate did not affect the ureteral pressure. The effluent from the renal pelvis of the left perfused kidney was collected via the 5-cm PE-50 catheter in the left ureter, placed on ice for the duration of the experiment, and then stored at ~70°C for subsequent analysis for SP content. Samples were collected as above during a 10-min basal period, a 4-min experimental period (3 min of increased renal pelvic pressure and 1 min collection period), and a 10-min recovery period, their content of SP determined by enzyme-linked immunoassay, and the rate of SP release over the basal, test, and recovery periods calculated.

**Acute Saline Loading**

After 1 h of equilibrium, acute saline loading was performed for 10 min in 12 Sham and 12 UUO rats by intravenous infusion of an amount of isotonic saline equal to 5% of the body weight. The MABP and changes in the IPP, ERNA, and ARNA of the left kidney were continuously monitored. Urine samples from the left kidney in both groups were obtained after 10, 20, 30, 45, 60, and 90 min of infusion.

**Renal Mechanoreceptor and Chemoreceptor Stimulation**

The 50-cm catheter connected to the left ureteral catheter was raised to increase the IPP stepwise by 4, 8, 12, 16, or 20 mmHg, with at least a 10 min interval between each pressure increase, each step being maintained for 3 min. Renal mechoreceptors were stimulated by graded increases in the renal pelvic NaCl concentration by administration of saline solutions of 150, 300, 600, or 900 mM via the PE-10 catheter. The experiment consisted of one 10-min basal period, five 10-min consecutive experimental periods at the different tonicities, and one or two 10-min recovery periods.

**Substance P Immunoassay**

Urinary SP was measured with an enzyme immunoassay kit (Cayman, Ann Arbor, MI), as described elsewhere (13). This assay is based on competition between free SP and a SP tracer (SP linked to an acetylcholinesterase) for a limited amount of rabbit anti-SP antibody. Urine samples were added directly to the assay well, then the SP tracer and rabbit anti-SP antisera were added and the assay plate incubated for 18 h at 4°C. The wells were rinsed five times with washing buffer to remove any unbound reagent, then Ellman’s reagent was added for color development and the 410-nm absorbance of each well measured in an enzyme-linked immunosorbent assay Reader (Dynex Technologies, Chantilly, VA). The results for the samples...
were compared with a standard curve to determine the total amount of SP present. All samples were run in duplicate.

**Determination of NEP Activity**

After acute studies of saline loading, both groups of rats were killed by an anesthetic overdose, and the tissues that contained the renal pelvis and the proximal end of the ureter in the left kidney were removed to determine the NEP activity, as described elsewhere (14,15). In brief, the sampled tissues were immediately homogenized on ice with a 20-fold volume of 50 mM Tris buffer (pH 7.4) of tissue weight, then the homogenate was centrifuged at 17,500 \( \times g \) for 15 min at 4°C and the supernatant aspirated for analysis. The incubation mixture consisted of 50 \( \mu l \) of the test sample, 0.4 mM glutaryl-Ala-Ala-Phe-4-MeOH-naphthylamine (Sigma, Saint Louis, MO), 10 \( \mu g \) of leucine aminopeptidase M (Sigma), and 50 mM Tris buffer (pH 7.4), in a final volume of 250 \( \mu l \). After 2 h of incubation at 37°C, 2 ml of 0.025% (wt/vol) fast garnet GBC (Sigma) in 2% Brij 35 solution (Sigma) was added to stop the reaction and to develop the color at room temperature for 10 min. The absorbance of the colored product, 2-naphthylamine, was measured by spectrophotometry (Eclipse, Vitalab, Dieren, Netherlands) at 546 nm. Controls that were blocked by incubation with buffer A (1% normal rabbit serum in buffered saline) were included in each experiment; in addition, another 50 \( \mu l \) of each test sample was analyzed for NEP activity in the presence of 8 \( \mu M \) phosphoramidon (Sigma). The homogenate was centrifuged at 620 \( \times g \) for 60 min. The final pellet was suspended in 0.32 M phosphate–sucrose solution that contained protease inhibitors, as described above. The protein in the membrane preparation was determined by use of the Bradford dye-binding assay (Bio-Rad, Hercules, CA).

**Immunoblotting of NK-1 Receptors in the Renal Pelvis**

After the renal sensory receptor responses were tested, the rats were killed, the left kidney exposed, and the renal pelvis and the proximal end of the ureter removed to prepare membrane fractions. All procedures were performed at 4°C or on ice. The tissues were rinsed in saline and homogenized in 50 mM Tris buffer (pH 7.4) that contained protease inhibitors (10 mM phenylmethylsulfonyl fluoride, 10 \( \mu M \) benzamidine, 10 \( \mu M \) leupeptin, and 1 \( \mu g \) ml \(^{-1}\) of trypsin inhibitor; Sigma). The homogenate was centrifuged at 620 \( \times g \) for 20 min to remove cellular debris and the supernatant centrifuged at 150,000 \( \times g \) for 60 min. The final pellet was suspended in 0.32 M phosphate-buffered saline–sucrose solution that contained protease inhibitors, as described above. The protein in the membrane preparation was determined by use of Bradford’s dye-binding assay (Bio-Rad). A sample of membrane protein (10 \( \mu g \) for the ileum and brain cortex and 80 \( \mu g \) for other samples) was separated on a 12% polyacrylamide gel under denaturing conditions and electrophoretically transferred to a nitrocellulose membrane (Amersham, Buckingham, UK). The membranes were blocked by incubation with buffer A (1% normal rabbit serum in 50 mM Tris buffer [pH 7.4]) then incubated overnight at 4°C with rabbit anti-NK-1 receptor antiserum (Novus Biologicals, Littleton, CO) diluted 1:1000 in buffer A. After being washed, the membrane was incubated for 1 h at room temperature with goat biotinylated anti-rabbit IgG conjugated to horseradish peroxidase diluted 1:200 in buffer A (Vector, Burlingame, CA), washed, and the bound antibody visualized by use of a commercial diaminobenzidine peroxidase substrate kit (Vector). The density of the major band (molecular mass \( \sim 79 \) kD) was determined by densitometry that used an image analytic system (Alpha Innotech, San Leandro, CA).

**Chemical Analyses and Data Treatment**

The urinary excretory volume was determined gravimetrically and the urinary sodium concentration measured by flame photometry (FCM 6341, Eppendorf, Hamburg, Germany). The UV and \( U_{NaV} \) were expressed per gram of kidney weight (gkw).

Systemic or renal excretory functions were averaged over each period. Changes in renal nerve activity were also averaged over each period, and the effects of acute saline loading, increased IPP, and renal pelvic perfusion with hypertonic saline solutions on the ARNA or ERNA calculated by comparison of the experimental value with the average value for the control period. Given that, because of possible differences in the number of nerve fibers and the degree of nerve-electrode contact, it is not possible to reliably compare the absolute values of integrated voltages from multifiber afferent nerve recordings between rats or groups of rats, the data were analyzed as the percentage change from the basal value during the control period.

The amount of 2-naphthylamine released was calculated from the absorbance and the extinction coefficient of 27,000 liters cm \(^{-1}\) mole \(^{-1}\) (16). Specific NEP activity was calculated by subtraction of phosphoramidon-inhibitable NEP activity and the control values and expressed as nmoles of 2-naphthylamine released per milligram of tissue protein per hour.

The data in the text and figures are expressed as the mean ± SEM. Statistical analyses were performed by use of the Newman-Keuls test of ANOVA for multiple comparison. A significance level of 5% was used.

**Results**

**Unilateral Ureteral Obstruction Model**

After 24 h of unilateral ureteral obstruction, the kidney–body weight ratio (%) of the postobstructive kidney (0.44 ± 0.02) in the UUO rats was significantly higher than that of the control kidney (0.34 ± 0.01) in Sham rats. The baseline for the UV (\( \mu l \) min \(^{-1}\) gkw \(^{-1}\)) (upper section of Figure 3B at time point 0) was significantly lower in the postobstructive kidney in UUO rats (2.1 ± 0.4) than in the control kidney in Sham rats (4.0 ± 0.6). The basal value for the \( U_{NaV} \) (\( \mu M \) min \(^{-1}\) gkw \(^{-1}\)) (lower section of Figure 3B at time point 0) was similar in the postobstructive (0.10 ± 0.02) and control (0.10 ± 0.01) kidneys.

**Renorenal Reflex and Substance P Release**

Figure 1 shows that stimulation of renal mechanoreceptors elicited an inhibitory renorenal reflex. The MABP in both Sham and UUO rats was unaffected by renal pelvic mechano-stimulation (data not shown). In Sham rats, an IPP increase to 36.8 ± 3.3 mmHg in the ipsilateral (left) kidney resulted, in the contralateral kidney, in an increase in the UV from 5.1 ± 0.4 to 7.8 ± 0.9 \( \mu l \) min \(^{-1}\) gkw \(^{-1}\) (\( P < 0.05 \)) and an increase in the \( U_{NaV} \) from 0.09 ± 0.03 to 0.16 ± 0.04 \( \mu M \) min \(^{-1}\) gkw \(^{-1}\) (\( P < 0.05 \)) (Figure 1A), accompanied, in the ipsilateral kidney, by an increase of 295.0 ± 54.2% in the ARNA and a decrease of 78.5 ± 36.0% in the ERNA (Figure 1B, top and center). In contrast, contralateral diuretic and natriuretic responses were not seen in UUO rats after a similar IPP increase in the postobstructive kidney, despite slight changes in ipsilateral nerve activity.

As shown in the bottom section of Figure 1B, the basal values for renal pelvic ipsilateral (left) SP release was not significantly different in Sham (3.6 ± 0.5 pg min \(^{-1}\)) and UUO...
An IPP increase to 40 mmHg resulted in a significant increase in SP release in Sham rats to 37.4 ± 3.2 pg min⁻¹ (1018 ± 110%) and in UUO rats to 18.9 ± 1.2 pg min⁻¹ (652 ± 69%), these values being significantly different. The values for the UV, UNaV, and SP release returned to basal levels when the IPP was reduced.

**Acute Saline Loading**

Figure 2 shows original tracings of the response to acute saline loading in (A) one Sham and (B) one UUO rat. Acute saline loading did not significantly alter the systemic MABP in either group. Sham rats displayed a sharp, but transient, increase in IPP from the basal value of 1.0 ± 0.1 to 5.5 ± 0.7 mmHg after 10 min of saline loading, whereas UUO rats only showed an increase from 0.6 ± 0.2 to 2.6 ± 0.4 mmHg, this difference between the groups being significant. In Sham rats, the ERNA decreased from 100.0 ± 3.4% to 58.0 ± 7.5% at the end of saline loading and to 72.0 ± 7.2% at 10 min after the end of loading, whereas the ARNA increased to 154.8 ± 15.0% and 173.0 ± 23.0% at the same time points. In contrast, UUO rats showed markedly blunted ERNA and ARNA responses, the only significant decrease in ERNA (from 100.0 ± 3.4% to 84.1 ± 6.8%) and increase in ARNA (to 34.6 ± 10.4%) being seen in the postobstructive kidney. However, the basal ARNA was similar in both groups, the difference not being statistically significant (0.3 ± 0.2 and 0.5 ± 0.1 mV s⁻¹ in the UUO and Sham rat, respectively).

The grouped data are shown in Figure 3A, in which the 10-min period of saline loading is indicated by “VE.” Saline loading did not significantly alter the systemic MABP in either group. Sham rats displayed a sharp, but transient, increase in IPP from the basal value of 1.0 ± 0.1 to 5.5 ± 0.7 mmHg after 10 min of saline loading, whereas UUO rats only showed an increase from 0.6 ± 0.2 to 2.6 ± 0.4 mmHg, this difference between the groups being significant. In Sham rats, the ERNA decreased from 100.0 ± 3.4% to 58.0 ± 7.5% at the end of saline loading and to 72.0 ± 7.2% at 10 min after the end of loading, whereas the ARNA increased to 154.8 ± 15.0% and 173.0 ± 23.0% at the same time points. In contrast, UUO rats showed markedly blunted ERNA and ARNA responses, the only significant decrease in ERNA (from 100.0 ± 3.4% to 84.1 ± 6.8%) and increase in ARNA (to 34.6 ± 10.4%) being seen in the postobstructive kidney. However, the basal ARNA was similar in both groups, the difference not being statistically significant (0.3 ± 0.2 and 0.5 ± 0.1 mV s⁻¹ in the UUO and Sham rat, respectively).

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at the end of loading. As shown in Figure 3B, the UV and $U_{Na}V$ increased in both groups in response to saline loading. However, the diuretic and natriuretic responses were both attenuated (from the end of loading to either 20 or 10 min after the end of loading for the UV or $U_{Na}V$, respectively) in the postobstructive kidney in UUO rats compared with the control kidney in Sham rats. Cumulative urine and sodium excretion in the postobstructive kidney of the UUO rats were, respectively, $56.2 \pm 5.3\%$ and $58.1 \pm 9.6\%$ of the values seen in the control kidney in Sham rats ($P < 0.05$).

Renal Mechanoreceptor and Chemoreceptor Stimulation

The impaired renal responses to increased ARNA demonstrated in the postobstructive kidney in UUO rats by acute saline loading were further explored to determine whether the receptors involved were renal mechanoreceptors or chemoreceptors. Raising the ureteral catheter above the left kidney to various levels increased the IPP and stimulated mechanosensitive neurons in the renal pelvis; original tracings are shown in Figure 4. Stepwise changes in the IPP from 0 to 20 mmHg did not result in a significant change in the arterial pressure (AP) in either group of animals. However, the IPP increases resulted in a pressure-dependent increase in the ARNA in the Sham rats over the range of 4.5 to 20.5 mmHg, whereas an increase was only seen in UUO rats at 16 and 20 mmHg. The ARNA increase was reversed when the IPP returned to the basal level. The grouped data are shown in Figure 5. The IPP threshold for a significant ARNA increase in the test kidney was $4.5 \pm 0.4$ mmHg in Sham rats and $16.3 \pm 0.3$ mmHg in UUO rats, at which pressures the ARNA was increased by $160.8 \pm 21.3\%$ in control rats and by $16.3 \pm 0.3\%$ in UUO rats.

Intrapelvic perfusion of concentrated saline solutions also activated ARNA. Figure 6 shows typical traces for the specific stimulation of chemoreceptors. Perfusion with increasing NaCl concentrations did not significantly alter the arterial pressure in either Sham or UUO rats. Hypertonic saline caused a graded ARNA increase in Sham rats (Figure 6A) but had only a minor effect in UUO rats (Figure 6B). As with the mechanoreceptors, the increased ARNA was reversible when the concentration of the NaCl solution was reduced to 150 mM. The grouped data are shown in Figure 7. In Sham rats, graded NaCl increases resulted in a concentration-dependent increase in the ARNA, which reached statistical significance when the NaCl concentration were $>600$ mM. In contrast, blunted responses were seen in the postobstructive kidney of the UUO rats. At 900 mM.
NaCl, UUO rats showed a 150.3 ± 39.3% increase in ARNA, whereas Sham rats showed an increase of 370.6 ± 94.7%.

**NEP Activity Measurement**

The mean NEP activity in renal pelvic samples from the postobstructive kidney of UUO rats (n = 8) was 318.9 ± 22.7 nmol mg⁻¹ protein h⁻¹, significantly higher than that of 194.7 ± 20.5 nmol mg⁻¹ protein h⁻¹ in the control kidney of Sham rats (n = 8).

**Immunoblotting of NK-1 Receptors in the Renal Pelvis**

To explore the role of NK-1 receptors in the impaired renal sensory response and reflex function, we quantified NK-1 receptor expression in both groups. As shown in Figure 8A, the anti–NK-1 receptor antiserum recognized the same broad range of protein bands with molecular masses from 70 to >120 kD in positive control membrane preparations from the rat ileum and brain cortex (lanes 11 and 12) and in membranes from the renal pelvis and proximal end of the ureter of Sham rats (lanes 1 to 5) and UUO rats (lanes 6 to 10). Figure 8B shows the semiquantitative data for the major 79-kD band; the integrated digital values were 22,608 ± 1542 and 21,589 ± 1750 for the control and postobstructive renal pelvis, respectively, the difference not being statistically significant.

**Discussion**

This study is the first to demonstrate that all the components required for sensory transmission (SP, NEP activity, NK-1 receptor, and renal afferent nerve activity) are found in the renal pelvis, the site at which the sensory signals transmitted by most renal sensory neurons are generated. The impairment of the renorenal reflex in UUO rats as a result of insufficient activation of mechanoreceptors is explained, in part, by decreased release of SP in the postobstructive kidney but not by altered NK-1 receptor expression in the renal pelvis area. This impairment was also demonstrated by acute saline loading. The decreased SP release was associated with increased renal pelvic NEP activity.

**Renorenal Reflex, SP Release, and Renal Pelvic NEP Activity**

Electrophysiologic and functional studies elsewhere have demonstrated that activation of renal afferents projecting centrally via dorsal root ganglia and vagal nodosal ganglia reflexly increases sympathetic outflow (18,19) and plays an excitatory role in certain renal pathophysiologic states, such as in renovascular hypertensive rats (20), 5/6-nephrectomized induced uremic rats (21), and cyclosporine A–treated rats (22). In contrast, this study demonstrated the operation of an inhibitory renorenal reflex in control rats after the application of ipsilateral renal stimulation. One main difference between this and studies elsewhere is the experimental design. The effectiveness of applied stimuli in eliciting an inhibitory or excitatory renorenal reflex depends on the strength, duration, and frequency, and it also appears that certain renal receptors elicit a broader
reflex involving sympathetic control of cardiovascular function, whereas others act only on the kidney via an unknown mechanism that involves the activation of renal afferent C or A\delta fibers (19). In rats, activation of the renal afferent nerves normally elicits a diuretic signal to the integrative site (7) to reflexly inhibit ERNA via spinal or supraspinal sites (19,23). This renorenal reflex did not operate properly in our UUO rats, which suggests that the impaired inhibitory renorenal reflex may have been unable to reflexly inhibit the ERNA.

SP mediates the mechanoreceptor-induced renorenal reflex, which is abolished by capsaicin pretreatment (6). ARNA is increased in a dose-dependent fashion by renal pelvic perfusion of SP (24), and a SP receptor antagonist attenuates the activation of ARNA elicited by increased pelvic pressure (25). These studies demonstrate the important role of SP and its receptors in ARNA activation of the renal sensory response. We confirmed the presence of this reflex in control rats by renal pelvic mechanostimulation but found that UUO rats showed impairment of the renorenal reflex that is partly due to a lower SP concentration in the postobstructive kidney. The reduced amount of SP released may be responsible for impaired ARNA activation and the subsequent poor inhibitory renorenal reflex. A study elsewhere in humans with ureteropelvic junction obstruction suggested defective renal innervation and loss of neural control around the renal pelvic area (26). Our results, showing similar basal SP release in control and UUO rats, partially rule out the possibility that the impaired ARNA is due to a reduction in sensory neurons in the renal pelvis, although the anatomically defined structures in the two species are quite different.

The lower SP release could be due to inadequate synthesis. We tried to measure prepro-SP mRNA levels in the renal pelvic tissue but did not succeed. Another possibility could be increased SP breakdown. NEP, originally discovered in the kidney, is a major inactivator of SP (4). Renal NEP has been found mainly in the proximal tubule, with a small amount in the distal tubule (27), only the proximal tubule and glomerulus having been shown elsewhere to have measurable NEP activity (28). In this study, measurable NEP activity was also found in the rat renal pelvis. The postobstructive kidney showed 63.8% more renal pelvic NEP activity than the control kidney, which suggests that the increased enzyme activity might reduce the effects of released SP and its role in sensory transmission in the renorenal reflex. However, a clear understanding of the detailed mechanisms of increased NEP activity would require further study.
Another factor that could lead to the impairment of the renorenal reflex could be a reduced number, or altered responsiveness, of SP receptors in the renal pelvis. Using an antisera raised against a 15–amino acid peptide from the COOH-terminus of the rat NK-1 receptor that was confirmed as NK-1 receptor–specific in a study elsewhere (29), we examined NK-1 receptor levels in the postobstructive renal pelvis of UUO and in the control kidney in Sham rats and found no significantly different, which demonstrates that the impaired renorenal reflex was not due to reduced numbers of SP-binding receptors.

Acute Saline Loading

Studies elsewhere have demonstrated decreased renal functions in the postobstructive kidney after 24 h of obstruction (9–11,17,30,31), as well as impaired renal excretory responses to moderate isotonic saline loading (10). The findings of our study here were very similar. In the rat model of complete ureteral obstruction, the intrapelvic pressure displays a phasic change during obstruction, initially increasing to 50 to 70 mmHg, then starting to decline within 2 hr, returning to near-normal levels after 24 h to 3 wk (32). Our direct intrapelvic pressure recording data showed that the basal IPP after 24 h ureteral obstruction was near normal. Genovesi et al. (33) found that the increase in urine flow rates and the corresponding renal pelvic pressure increase closely match the increase in ARNA and concluded that the change in water excretion, via changes in renal pelvic pressure, is a primary factor in the regulation of ARNA. In this study, we showed, for the first time, that the changes in IPP and the increase in, and reflex inhibition of, renal nerve activities occurred simultaneously on saline loading and that the IPP increase in the postobstructive kidney during natriuretic challenge was attenuated (Figure 2B). Because a small increase in IPP cannot stretch the renal pelvic wall, the attenuated IPP increase may also be a mechanism for the impaired responsiveness of the renal pelvic sensory receptors. The possibility that enhanced ERNA or intrarenal vasoconstriction in the postobstructive kidney impedes pressure transduction during acute saline loading, then has a profound effect on changes in IPP and renal afferent activation, cannot be excluded. However, the results from our experiments involving graded IPP increases, which directly stimulated renal pelvic mechanoreceptors, showed lower activation of ARNA over the same pressure range: evidence for a functional defect in the mechanoneurons located in the renal pelvis of UUO rats (Figure 4B).

The use of the alpha-adrenergic blocker, phenoxybenzamine, to deplete systemic catecholamines (30) or acute renal denervation (11) ameliorate the decreased renal functions seen after 24-h obstruction, which suggests that enhanced renal nerve activity results in worsened renal function. Our direct recordings of ERNA also provided support for an increased ERNA involved in the ureteral obstructive injury by showing a high voltage integrative unit and a high spike number when tested by saline loading in the postobstructive kidney (Figure 2B). The factors responsible for the increase in renal tissue catecholamines (30,34) and nerve activity (this study) are not known. This study provides evidence that, on the basis of its role in the neural reflex control of ERNA, attenuated activation of renal sensory neurons might participate in the neurogenic control of renal nerves after one-sided renal obstruction.

Renal Mechanoreceptors and Chemoreceptors

We evaluated the function of renal mechanosensitive and chemosensitive neurons in Sham and UUO rats using specific stimuli, namely graded increases in IPP and NaCl concentration. As reported elsewhere (6,24,35), these stimuli produced dose-dependent ARNA responses in the control kidneys of Sham rats, but, in addition, this study showed, for the first time, that the postobstructive kidney displayed blunted responses.

As in a study elsewhere in normal rats (35) (Sham rats), the threshold for renal pelvic mechanoreceptors, activated by increasing IPP, was found to be within the physiologic range (<5 mmHg); however, this study also showed that, in UUO rats, the threshold was shifted to 16.3 mmHg (Figure 4). These results also support the observation of defective responses in ARNA on saline loading that are not solely due to attenuation of the IPP increase.

Renal chemoreceptors, especially the R2 subtype, responding to the composition of the pelvic contents, are largely responsible for basal renal afferent nerve activity (18). The lower response of renal pelvic chemoreceptors in UUO rats to a raised NaCl concentration (Figure 6) might explain why the sensitivity to changes in urinary composition is reduced in the postobstructive kidney, leading to difficulty in activating renal afferent nerves.

In summary, this study shows that activation of renal mechanoreceptors in the postobstructive kidney failed to fully elicit an inhibitory renorenal reflex. The blunted renal sensory reflex seen in the postobstructive kidney was associated with abnormal excretory functions. The attenuation of sensory receptor responses was due, in part, to increased renal pelvic NEP activity and catabolism of released SP but not to altered NK-1 receptor expression.

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References


