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Increased expression of atrial natriuretic peptide in the kidney of rats with bilateral ureteral obstruction

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Increased expression of atrial natriuretic peptide in the kidney of rats with bilateral ureteral obstruction.

Background. Whether the postobstructive diuresis can be related to an altered regulation of local atrial natriuretic peptide (ANP) in the kidney was investigated.

Methods. Three groups of rats had both of their ureters obstructed for 48 hours. The kidneys were taken without releasing the obstruction in one group [bilateral ureteral obstruction (BUO)]. The obstruction was released in the other two groups and the animals were kept for 4 and 24 hours thereafter to collect urinary data (BUR-4 and BUR-24, respectively). Plasma and urine ANP levels were measured by radioimmunoassay. The mRNA expression of ANP, natriuretic peptide receptor-A (NPR-A), and NPR-C was determined by reverse transcription-polymerase chain reaction. ANP receptors were also quantitated by in vitro autoradiography. The activity of guanylyl cyclase was determined by the amount of cGMP generated in response to ANP.

Results. Urinary volume and sodium excretion increased in BUR-4, along with the ANP mRNA expression in the kidney and the urinary ANP excretion. The ANP excretion positively correlated with the urinary volume and sodium excretion. The mRNA expression of both NPR-A and NPR-C was decreased by BUO, the latter being far more prominently affected. The maximal binding capacity of radiolabeled ANP was decreased in the glomerulus and papilla in BUO. Not only the urinary parameters but also the mRNA expression of ANP, NPR-A, and NPR-C were comparable between BUR-24 and control rats. ANP-stimulated cGMP generation was reduced in the glomerulus and papilla in BUO animals, which was rapidly resumed following the release of the obstruction.

Conclusions. Postobstructive diuresis may be due partially to an increased ANP activity in the kidney.

Obstruction of the urinary tract is a common cause of loss of renal function. A marked and sometimes prolonged diuresis may follow the relief of severe obstruction of both kidneys. Various factors contributing to the postobstructive diuresis have been suggested, including decreases of tubular sodium reabsorption [1, 2], retention of urea [3, 4], and expansion of extracellular fluid volume [5]. Although a role of atrial natriuretic peptide (ANP) in mediating the postobstructive diuresis has been also suggested [6], the detailed mechanisms remain to be determined.

Recent studies have shown that the kidney per se is also a site of production and release of ANP. ANP immunoreactive signals are localized to the distal tubule and intercalated cells of the collecting duct [7, 8]. In addition, in vitro autoradiography and in situ hybridization demonstrated natriuretic peptide receptors (NPRs)-A, -B, and -C, and their respective mRNAs in the kidney [9–11]. The locally synthesized ANP may act in a paracrine manner to increase the urinary excretion of sodium and water. In this context, an altered regulation of ANP in the kidney may result in an altered urinary excretion.

The present study was aimed to examine whether the postobstructive diuresis can be related to an altered regulation of ANP in the kidney. Rats were made of experimental bilateral ureteral obstruction (BUO), and the mRNA expression of ANP, NPR-A, and NPR-C, and the activity of particulate cyclase stimulated by ANP were determined in the kidney.

METHODS

Animals

Male Sprague-Dawley rats weighing 200 to 250 g were used. They were kept in accordance with the Institutional Guidelines of Experimental Animal Care and Use. The abdominal cavity was opened, and 2-0 silk ligature was placed at both proximal ureters under anesthesia with ketamine (50 mg/kg, intraperitoneally). After closure of the abdomen, the animals were kept for 48 hours while they were given food and water ad libitum. The rats were considered to have a successful ureteral obstruction when the ureteral diameter was >2 mm and hydronephrosis was evident.

Key words: postobstructive diuresis, atrial natriuretic peptide, kidney obstruction, natriuretic peptide receptors, cyclase.

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On the experimental day, among the three groups of ureteral obstructed rats, one group was designated as BUO because the kidneys were taken without releasing the obstruction. The ureteral obstruction was released in the other two groups, and the rats were kept alive for 4 and 24 hours thereafter; they were designated as groups BUR-4 and BUR-24, respectively. Corresponding control groups were treated the same, except that no ureteral obstruction was performed.

Urine collection

In BUR-4, BUR-24, and their respective control rats, the bladder was cannulated under anesthesia with ketamine (50 mg/kg, intraperitoneally) to collect urine samples. Urine was collected during four-hour periods of three to seven hours after releasing the ureteral obstruction in BUR-4 and of 23 to 27 hours in BUR-24. Urine volume, osmolality, creatinine, sodium, and ANP excretion measurements were determined. The trunk blood was collected at the end of the experiment by the animal's decapitation to determine serum creatinine, sodium, and osmolality.

Radioimmunoassay of ANP

To determine plasma ANP, the trunk blood was collected by the animal's decapitation while in a conscious state. The plasma and urine were extracted with Sep-Pak C_{18} cartridge (Waters Associates, Milford, MA, USA), and the concentrations of ANP were determined using a commercial radioimmunoassay kit (Research & Diagnostic Antibodies, Berkeley, CA, USA).

Isolation of total RNA

Following decapitation, the kidney was quickly removed and stored at −70°C until use. Total RNA was isolated according to the protocols of Ultraspec™ RNA isolation system (Biotecx Laboratories, Houston, TX, USA). The RNA concentration was determined by the absorbance read at 260 nm (Ultraspec 2000; Pharmacia Biotech, Cambridge, UK). Total RNA (1 μg) was incubated with reverse transcriptase (200 U; GIBCO BRL, Grand Island, NY, USA), Rnasin (10 U), dNTP mix (10 mmol/L), dithiothreitol (DTT; 100 mmol/L), $MgCl_2$ (25 mmol/L), oligo-dT (0.5 μg/μL), and reaction buffer (200 mmol/L Tris-HCl, pH 8.4, 500 mmol/L KCl) in a final volume of 20 μL at 42°C for 50 minutes. The cDNA was stored at −70°C.

Competitive polymerase chain reaction

DNA competitors of ANP, NPR-A, and NPR-C were made using a Competitive DNA Construction kit according to the manufacturer's instructions (Competitive RNA Transcription Kit; Takara Shuzo, Shiga, Japan). The competitive reference standard (CRS)-DNA was designed to be identical to the target template at both ends to maximize the possibility that it would be amplified with the same efficiency and specificity as the target template and be affected in the same way by variations in reaction conditions. Constant amounts of wild-type cDNA and decreasing amounts of DNA competitors (1:2.5 dilution scheme) were added to polymerase chain reaction (PCR) tubes. All tubes were subjected to PCR using specific primers for ANP, NPR-A, and NPR-C, prepared as described previously [12]. They consistently produced two bands of predicted sizes (ANP 332 bp, ANP competitor 580 bp; NPR-A 451 bp, NPR-A competitor 350 bp; NPR-C 573 bp, NPR-C competitor 380 bp). The PCR profile for ANP was: 27 cycles at 93°C for one minute, 63.5°C for 30 seconds, 72°C for one minute, and final extension at 72°C for five minutes. The PCR profile for NPR-A and NPR-C was: 27 cycles at 93°C for one minute, 56°C for 30 seconds, 72°C for one minute and 30 seconds, and final extension at 72°C for five minutes. PCR was performed in a thermal cycler (MJ Research, Watertown, MA, USA) in a total volume of 20 μL containing 10 pmol of each primer, dNTP mix (250 μmol/L), $MgCl_2$ (1.5 mmol/L), 1 × reaction buffer, and *Taq* DNA polymerase (1 U; Perkin Elmer, New Jersey, USA). For quantitation of G3PDH, a competitive reverse transcription (RT)-PCR was performed using DNA competitor of G3PDH. Decreasing amounts of DNA competitors of G3PDH and wild-type cDNA representing 2 ng of total RNA were used. The PCR products were size fractionated by 1.2% agarose gel electrophoresis, visualized with an ultraviolet transluminator with ethidium bromide staining, and photographed. All images were quantitated by IMAGER™ and 1D MAIN (Bioneer, Cheongwon, Korea).

Results were plotted as the copy number of CRS-DNAs (x axis) versus the corrected CRS-DNA band to wild-type band density ratios (y axis) in a full logarithmic chart. The point in which the ratio was 1.0 was regarded as the concentration of wild-type cDNA (Fig. 1). Tissue concentrations of ANP, NPR-A, and NPR-C mRNA were normalized to G3PDH mRNA concentrations and were reported in micromoles per mole of G3PDH.

Binding studies

The kidney was snap frozen in isopentane cooled by dry ice and stored in a sealed box at −70°C until section. Serial 20 μm sections were made on a cryostat at −20°C, thaw mounted onto gelatin-coated slides, and dried in a desiccator at 4°C overnight before immediate incubation. The binding of ^{125}I-labeled rat (r)ANP to the kidney sections was studied as described previously [13]. The sections were washed with 150 mmol/L NaCl-0.5% acetic acid (pH 5.0) at room temperature for 10 minutes in order to remove the endogenous ANP [14]. The sections were preincubated with 30 mmol/L phosphate buffer (pH 7.2) containing 120 mmol/L NaCl and 1 mmol/L phenanthro-

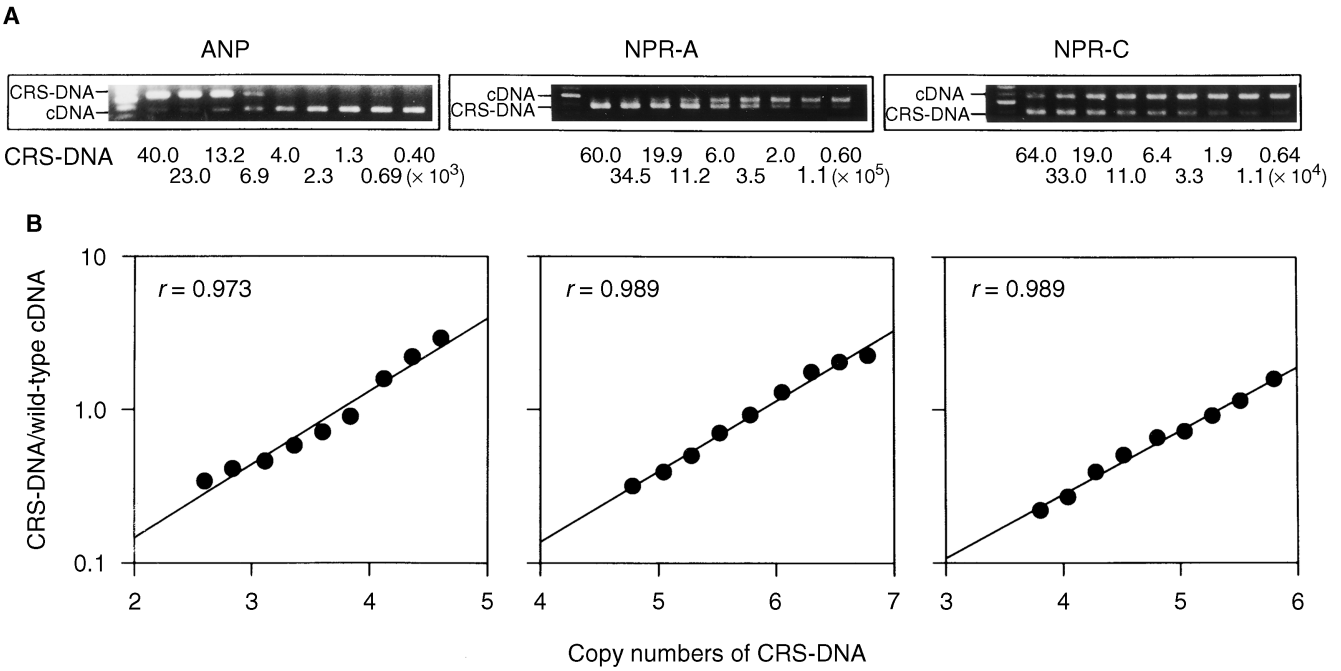

Fig. 1. Representative fluorographs showing ethidium bromide-stained agarose gels containing reverse transcription-polymerase chain reaction (RT-PCR) products of atrial natriuretic peptide (ANP), natriuretic peptide receptor-A (NPR-A), and NPR-C. (*A*) RT-PCR products using decreasing amounts of competitive reference standard (CRS)-DNA. (*B*) Examples determining cDNA concentration. The point of equal density (ratio = 1) was regarded as the concentration of wild-type cDNA.

line at room temperature for 10 minutes. They were then incubated with ^{125}I-rANP in fresh preincubation buffer containing 40 μg/mL bacitracin, 100 μg/mL leupeptin, and 0.5% bovine serum albumin (BSA) at room temperature for 60 minutes. To characterize ANP receptors, the competitive inhibition of ^{125}I-rANP binding was examined in consecutive sections by coincubation with various concentrations (0 to 10 μmol/L) of unlabeled rANP. After incubation, the sections were rinsed in cold distilled water at 4°C and quickly dried under a stream of cold air.

Autoradiographic images were generated by exposing the slides with dried ^{125}I-rANP–labeled kidney sections to Hyperfilm-^{3}H (Amersham, Buckinghamshire, UK) in x-ray cassettes together with 20 μmol/L thick ^{125}I-labeled polymer standard strips at room temperature for seven days. The image was viewed with a Leica Wild M 420 macroscope and captured using a Sony video camera with CCD iris and a Hamamatsu AC adaptor connected to a Power Macintosh 8100/80 AV computer. Regional bindings of ^{125}I-rANP in the glomerulus and papilla were analyzed for a mean gray scale value using PRISM image program (Version 3.6-1; Improve Vision, Coventry, UK). Optical densities were measured as disintegrations per min (dpm) per mm^2, based on a comparison with a calibration curve from the autoradiograms of ^{125}I standard microscale included in each x-ray cassette. Data were converted into fmol of ^{125}I-rANP per mm^2, as described previously [15]. The densities of 10 different glomerular or papillary regions in each individual section were captured and averaged.

Membrane preparation and guanylyl cyclase activity

The glomerulus was isolated from cortex by graded sieve methods [16]. In brief, the kidney was decapsulated, and the cortex was consecutively filtered through standard sieves (250, 150, 125, and 75 μm). The glomeruli on the 75 μm sieve were collected by centrifugation (1000 × *g* for 15 min at 4°C). The glomerulus or papilla was homogenized in an ice-cold homogenization buffer [50 mmol/L Tris-HCl, pH 8.0, containing 1 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.2 mmol/L phenylmethylsulfonyl fluoride (PMSF), and 250 mmol/L sucrose]. The homogenate was centrifuged at 1000 × *g* for 10 minutes at 4°C, and the supernatant was recentrifuged at 100,000 × *g* for 60 minutes at 4°C. The membrane pellet was washed thrice with 50 mmol/L Tris-HCl (pH 7.4) and resuspended. Protein concentrations were determined using bicinchonic acid assay kit (Bio-Rad, Hercules, CA, USA).

Particulate guanylyl cyclase activity was measured in the glomerular and papillary membrane aliquots by the method of Winquist et al [17] with a slight modification. The aliquots were incubated for 15 minutes at 37°C in 50 mmol/L Tris-HCl (pH 7.6) containing 1 mmol/L 3-isobutyl-1-methylxanthine, 1 mmol/L guanosine triphosphate, 1 mmol/L adenosine triphosphate, 15 mmol/L phosphocre-

Table 1. Renal functional parameters following bilateral ureteral obstruction

	Control ($N = 7$)	BUO ($N = 7$)
BUN *mg/dL*	23 ± 3	231 ± 4^b
S_{Cr} *mg/dL*	0.35 ± 0.03	8.28 ± 0.25^b
P_{ANP} *pg/mL*	134.6 ± 23.8	261.1 ± 32.8^a

Values are mean ± SEM. Abbreviations are: *N*, number of rats; BUO, bilateral ureteral obstruction; BUN, blood urea nitrogen; S_{Cr}, serum creatinine; P_{ANP}, plasma ANP.
[a] $P < 0.05$, [b] $P < 0.001$ vs. control

atinine, 80 μg/mL creatine phosphokinase, and 4 mmol/L $MgCl_2$ in the presence of 0 to 10 μmol/L ANP. Incubations were stopped by adding ice-cold 50 mmol/L sodium acetate (pH 5.0) and boiling for five minutes. Samples were then centrifuged (10,000 × *g* for 10 min at 4°C).

cGMP was measured in the supernatant by equilibrated radioimmunoassay. In brief, standards and samples were introduced in a final volume of 100 μL of 50 mmol/L sodium acetate buffer (pH 4.8), and then 100 μL of dilute cGMP antiserum (Calbiochem-Novabiochem, San Diego, CA, USA) and iodinated cGMP (10,000 cpm/100 μL, specific activity = 2,200 Ci/mmol; DuPont-New England Nuclear, Wilmington, DE, USA) were added and incubated for 24 hours at 4°C. The bound form was separated from the free form by charcoal suspension. Results were expressed as picomoles of cGMP generated per mg protein per minute. cGMP levels were also determined in urine samples.

Statistical analysis

The numbers of ligand binding sites of different affinities and their dissociation constant (K_d) and maximal binding capacity (B_{max}) were derived by Scatchard analysis using the LIGAND iterative model-fitting computer program [18]. Data are presented as means ± SEM. Comparisons between the groups were performed by nonpaired *t* test or one-way analysis of variance (ANOVA).

RESULTS

Functional parameters

Following the 48-hour ureteral obstruction, not only blood urea nitrogen and serum creatinine but also the plasma ANP was significantly increased (Table 1). Table 2 shows the urinary excretion and renal function data following the release of obstruction. The serum creatinine was kept increased in BUR-4 and BUR-24, along the concomitant decrease of its renal clearance. The urinary flow and sodium excretion were threefold to sevenfold increased in BUR-4, which was, furthermore, positively correlated with the urinary ANP excretion (Fig. 2). The urinary volume and ANP excretion in BUR-24 did not differ significantly from the controls. Accordingly, the plasma ANP was normalized in BUR-24, although it was decreased in BUR-4. Neither in BUR-4 nor in BUR-24 did the urinary cGMP excretion differ significantly from the control.

Expression of ANP and NPR mRNA

Figure 3 shows the expression of ANP mRNA in the kidney. The abundance of ANP mRNA was significantly increased in BUO and BUR-4, but was not in BUR-24. The mRNA expressions of NPR-A and NPR-C are shown in Figures 4 and 5, respectively. The ureteral obstruction decreased both NPR-A and NPR-C expression, the magnitude of which, however, was far more prominent for the latter. BUR-24 showed no significant differences of NPR-A and NPR-C expression from the control.

ANP binding study

Figure 6 shows the distribution of ^{125}I-rANP binding in the kidney in control and BUO. The radiolabeled ANP binding was shown in the cortical as well as in the medullary regions. In the cortex, the ANP binding was primarily localized to the glomerulus. In the outer medulla, the binding was found in longitudinal bands in the inner stripe, corresponding to medullary vascular bundles. The binding was minimal in the interbundle area, whereas it was highly detected in the papilla.

Unlabeled ANP displaced the radiolabeled ANP binding. Nonspecific binding was less than 10%. Increasing concentrations of unlabeled ANP resulted in a monophasic displacement of radiolabeled ANP binding. In the glomerulus, B_{max} was significantly reduced in BUO compared with that in control (4539 ± 583 vs. 9661 ± 772 amol/mm^2, $N = 6$ each, $P < 0.05$), while the K_d was not significantly affected (1.3 ± 0.4 vs. 2.1 ± 0.3 nmol/L, $N = 6$ each; Fig. 7). In the papilla, B_{max} was also significantly decreased in BUO compared with that in the control (2794 ± 749 vs. 5297 ± 912 amol/mm^2, $N = 6$ each, $P < 0.05$) with no significant differences in K_d (4.1 ± 1.2 vs. 6.3 ± 0.8 nmol/L, $N = 6$ each; Fig. 7).

Guanylyl cyclase activity

Figures 8 and 9 show the particulate guanylyl cyclase activity evoked by ANP in the glomerulus and papilla, respectively. The degree of maximal increase was significantly reduced in both the glomerulus and the papilla in BUO, with no significant differences in EC_{50}. The decrease of guanylyl cyclase activity was rapidly reversible with the release of the ureteral obstruction, showing a partial recovery in BUR-4 and complete normalization in BUR-24.

Table 2. Renal functional parameters in BUR-4 and BUR-24 rats

	BUR-4		BUR-24	
	Control ($N = 7$)	Experimental ($N = 7$)	Control ($N = 8$)	Experimental ($N = 6$)
S_{Cr} *mg/dL*	0.44 ± 0.02	4.30 ± 1.25[b]	0.57 ± 0.02	1.25 ± 0.35
C_{Cr} *mL/min*	1.23 ± 0.16	0.38 ± 0.12[c]	1.07 ± 0.09	0.61 ± 0.17[a]
Urinary flow *μL/h*	221 ± 35	1626 ± 375[b]	214 ± 47	294 ± 51
FE_{Na} %	0.26 ± 0.03	11.9 ± 4.2[a]	0.31 ± 0.05	0.53 ± 0.39
$U_{Na}V$ *μEq/h*	37.9 ± 9.6	156.7 ± 27.5[b]	30.8 ± 7.1	34.2 ± 8.6
P_{ANP} *pg/mL*	145.4 ± 33.4	36.5 ± 0.6[b]	116.7 ± 26.2	117.3 ± 12.9
U_{ANP} *pg/h*	2.35 ± 0.4	69.6 ± 6.5[c]	2.0 ± 0.6	1.3 ± 0.5
U_{cGMP} *pg/h*	210.4 ± 12.2	222.7 ± 24.3	198.3 ± 24.4	168.2 ± 18.3

Values are mean ± SEM. Abbreviations are: *N*, number of rats; S_{Cr}, serum creatinine; C_{Cr}, creatinine clearance; FE_{Na}, fractional Na excretion; $U_{Na}V$, urinary Na excretion; P_{ANP}, plasma ANP; U_{ANP}, urinary ANP excretion.
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ vs. control

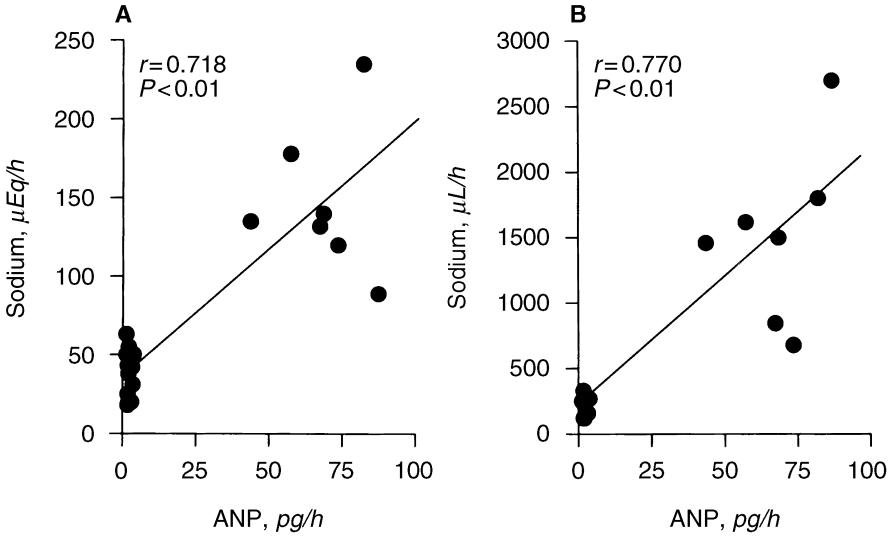

Fig. 2. Correlation between urinary ANP and (*A*) sodium or (*B*) volume excretion in control and experimental rats.

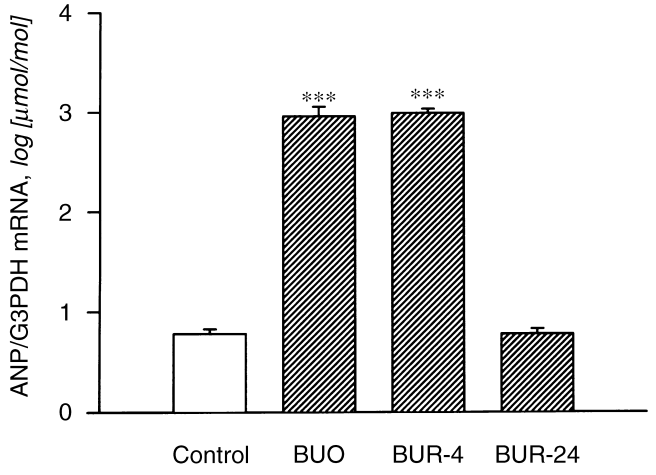

Fig. 3. Expression of ANP mRNA in rats with bilateral ureteral obstruction (BUO), and BUO rats with their obstructions released and then measurements taken four (BUR-4), and 24 hours (BUR-24) later. Each column represents the mean ± SEM of six rats. ***$P < 0.001$ vs. control.

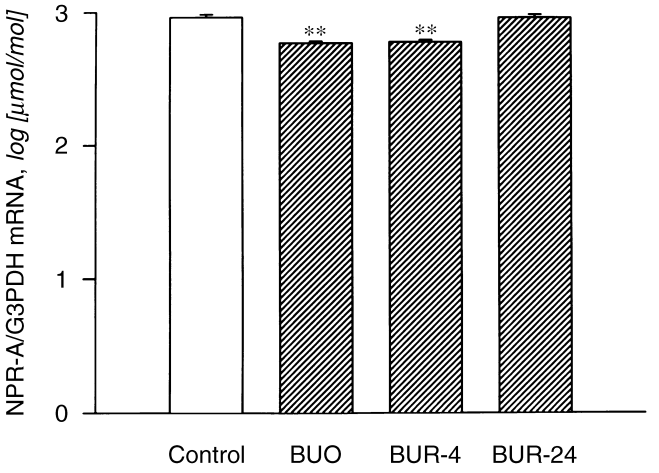

Fig. 4. Expression of NPR-A mRNA in BUO, BUR-4, and BUR-24 rats. Each column represents the mean ± SEM of six rats. **$P < 0.01$ vs. control.

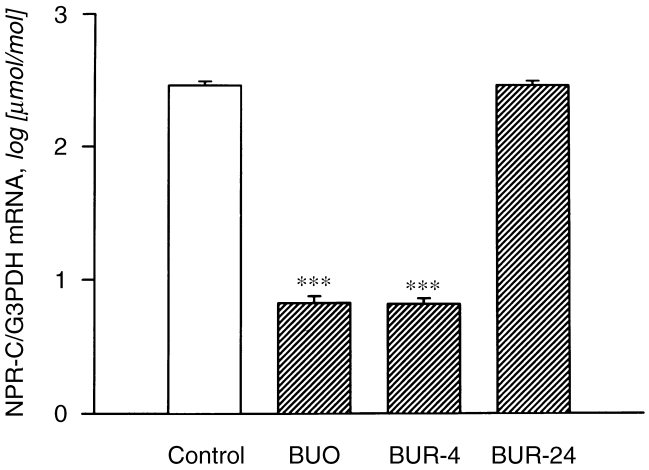

Fig. 5. Expression of NPR-C mRNA in BUO, BUR-4, and BUR-24. Each column represents mean ± SEM of six rats. ***$P < 0.001$ vs. control.

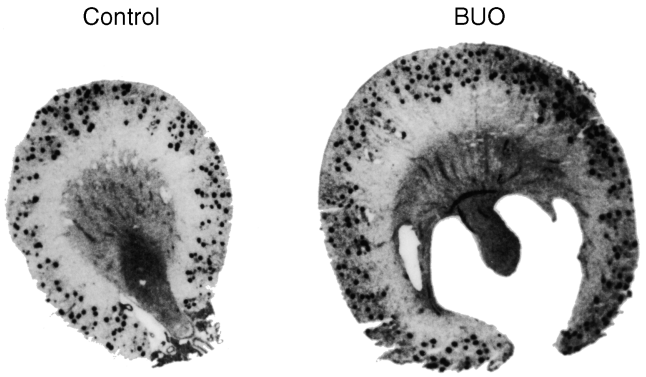

Fig. 6. Autoradiographic localization of ^{125}I-ANP binding sites in control and BUO kidneys. Frozen sections were incubated with ^{125}I-ANP (200 pmol/L).

DISCUSSION

It has been suggested that the endogenous ANP accounts for one fourth of the postobstructive diuresis [6]. The plasma ANP was indeed increased in BUO. An excessive retention of salt and water may occur during the period of obstruction, and hence, an expansion of extracellular fluid volume would account for the increased plasma ANP. When natriuretic and diuretic forces become manifest following the relief of obstruction in our study, however, the plasma ANP was decreased. Total volume excreted during the four-hour period of observation in BUR-4 was estimated at up to one half of the plasma volume. Therefore, the increase or the decrease of plasma ANP levels would merely reflect an increase or instant contraction of the extracellular fluid volume and may not be causally related to the postobstructive diuresis. This speculation may be in line with the previous notion that the degree of diuresis does not necessarily correlate with the plasma level of ANP [19, 20].

The locally synthesized ANP in the kidney has been suggested to play a regulatory role in the urinary sodium and water excretion. Furthermore, its role may be altered in various pathophysiological states such as in DOCA-salt treated [21], diabetic [22], and subtotal nephrectomized [23] rats. Postobstructive diuresis may also be attributed to an altered regulation of local ANP system in the kidney. Indeed, the present study showed that the postobstructive diuresis was associated with an enhanced renal expression of ANP mRNA and an increased urinary excretion of ANP. Furthermore, the urinary ANP excretion positively correlated with the urinary flow and sodium excretion. In contrast, the expression of ANP mRNA did not differ significantly from control in BUR-24, where the urinary parameters were also normalized. These findings support the hypothesis that an increased local synthesis of ANP is causally related to the postobstructive diuresis.

Although the enzymatic activity of endopeptidase contributes to the clearance of ANP, total ANP clearance is attributed mainly to its binding to NPR-C [24]. The present study showed a marked decrease of NPR-C mRNA in the kidney in BUO. The receptor-binding assay also showed a decreased maximal binding capacity for ANP in BUO, with the dissociation constant remaining unaffected. An elevated tubular pressure may be responsible for reductions of functional nephrons and receptors. However, a substantial reduction of ANP receptors without changing the affinity has been similarly shown after an exposure to ANP in cultured vascular smooth muscle cells [25]. Therefore, an enhanced local synthesis of ANP is more likely to be an event explaining the down-regulation of its receptors. It is suggested that not only an increased synthesis of ANP, but also a down-regulation of NPR-C contributes to the enhanced renal activity of ANP in BUO.

The expression of NPR-A that mediates the biological effect of ANP through generation of cGMP was also down-regulated, albeit to a small extent. The effect of local ANP may be dissipated when the expression of NPR-A is decreased. Along the decreased NPR-A expression, the ANP-evoked guanylyl cyclase activity was indeed decreased in BUO. However, the decreased activity was rapidly reversible upon releasing the ureteral obstruction. Local factors, possibly including the elevated tubular pressure, may only transiently affect the guanylyl cyclase activity in our experimental setup. The rapid recovery of guanylyl cyclase activity and the persistent increase of local ANP synthesis may then allow the postobstructive diuresis.

The urinary excretion of cGMP was not significantly altered. One may argue that the unaltered urinary excretion of cGMP may rule out a pathophysiological implica-

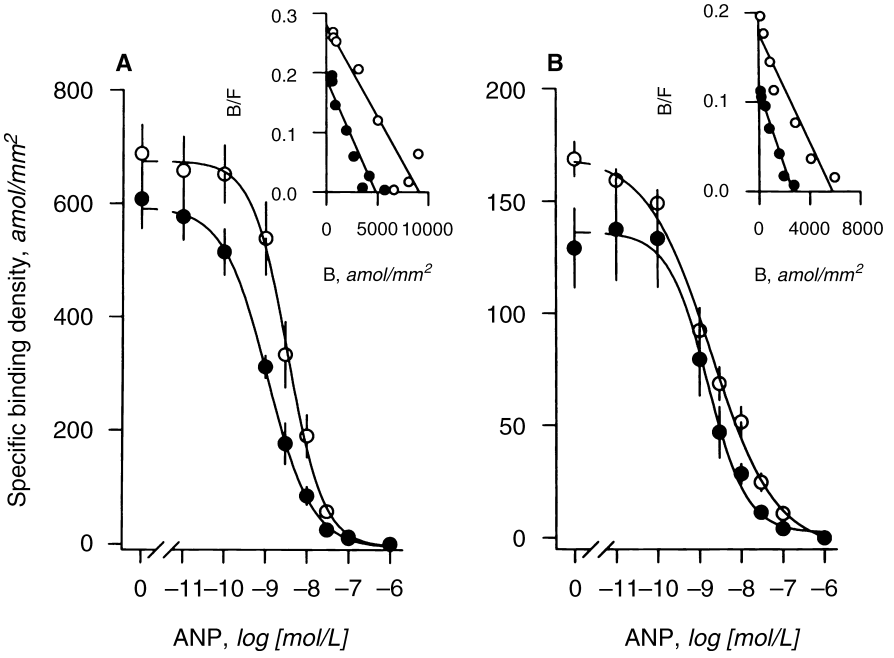

Fig. 7. Specific ^{125}I-ANP binding in the (*A*) glomerulus and (*B*) papilla. Symbols are: (○) control; (●) BUO. (*Inset*) Representative Scatchard plot. Abbreviations are: B/F, bound/free; B, bound. Each point represents mean ± SEM of eight experiments.

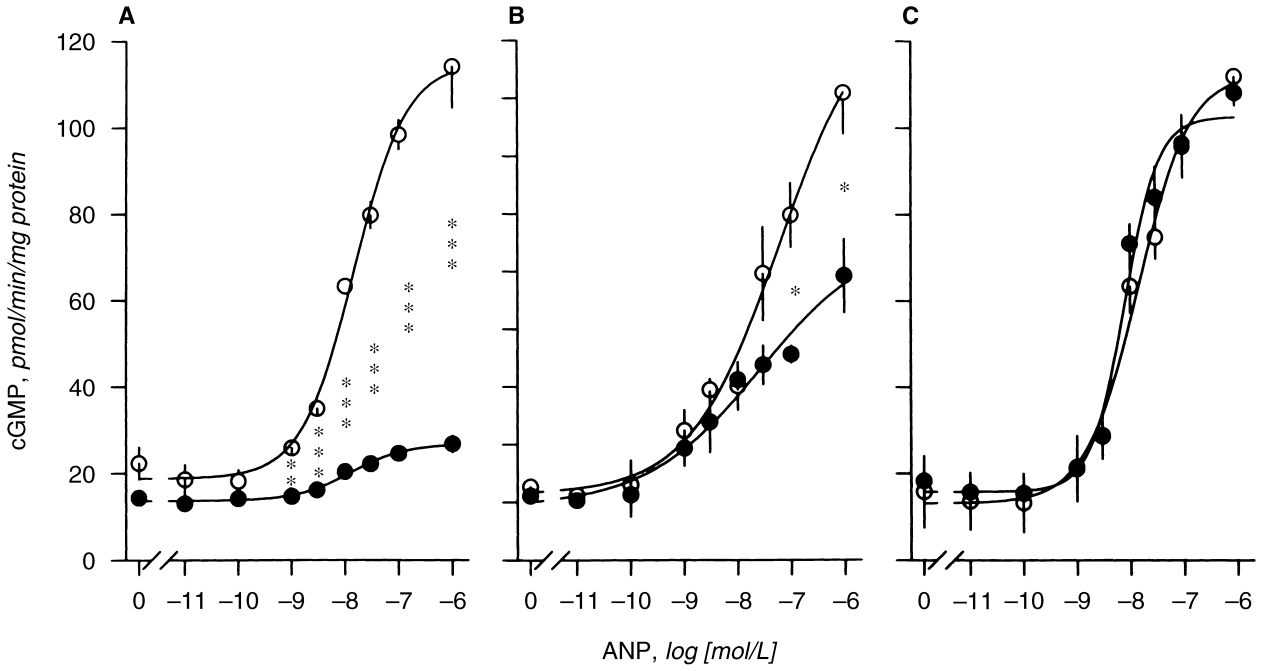

Fig. 8. cGMP production in response to ANP in the glomerulus in (*A*) BUO, (*B*) BUR-4, and (*C*) BUR-24 rats. Symbols are: (○) controls; (●) BUO or BUR. Each point represents mean ± SEM of eight experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs. control.

tion of ANP/cGMP pathway in the postobstructive diuresis. However, the urinary excretion of cGMP may not solely be a function of ANP, and other factors such as nitric oxide and heme oxygenase also exert their biological activity through a secondary formation of cGMP in the renal medulla [26, 27]. In fact, nitric oxide activity is decreased in rats with BUO as a consequence of diminished availability of L-arginine [28–30]. The decreased nitric oxide activity may result in a decrease of cGMP formation so that total urinary excretion of cGMP may also be affected. Therefore, measurements of urinary cGMP may not be a useful marker indicating the production and release of natriuretic peptides. Further studies are needed to delineate whether factors other than ANP are altered in association with the postobstructive diuresis.

In summary, it is suggested that the local synthesis of

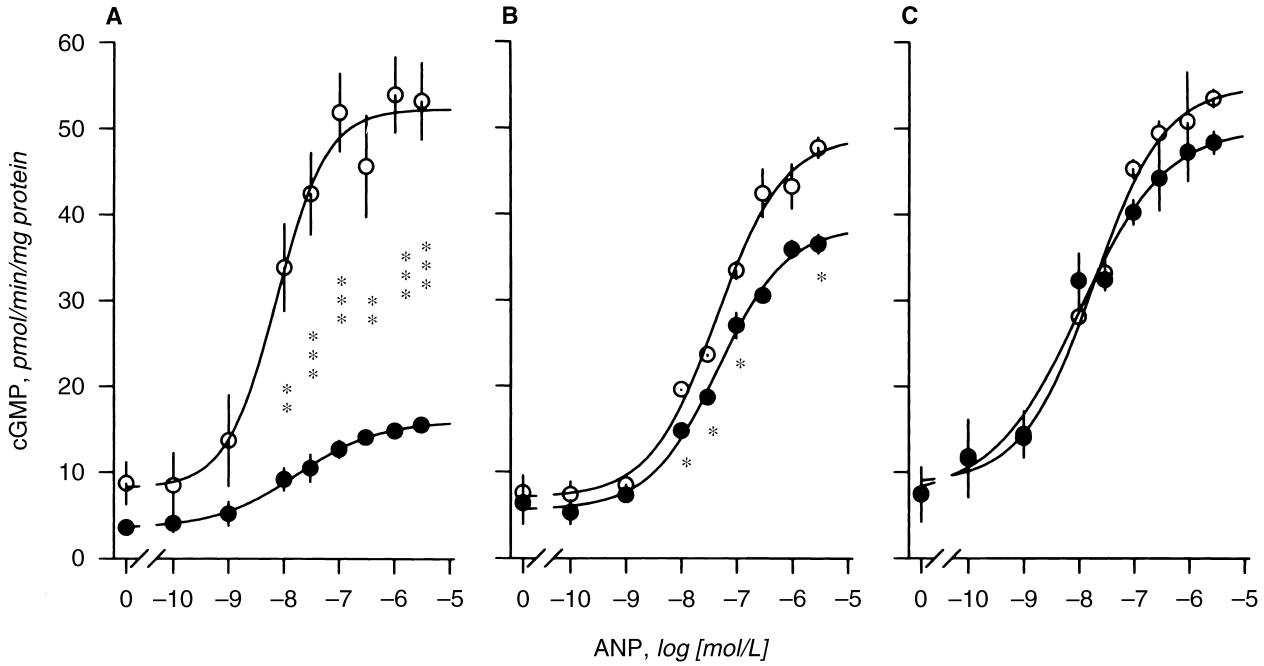

Fig. 9. cGMP production in response to ANP in the papilla in (*A*) BUO, (*B*) BUR-4, and (*C*) BUR-24. Symbols are: (○) controls; (●) BUO or BUR. Each point represents mean ± SEM of eight experiments. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ vs. control.

ANP is increased and the degradation is decreased by the ureteral obstruction. The enhanced ANP activity in the kidney may account in part for the postobstructive diuresis.

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