Urol Res (2002) 30: 97–101
DOI 10.1007/s00240-002-0242-9

ORIGINAL PAPER

Soo Wan Kim · Yingshun Li · Sunmi Kim
Yoon Wha Oh · Jong Un Lee

Local renal and vascular natriuretic peptide system in obstructive uropathic rats

Received: 6 August 2001 / Accepted: 1 February 2002 / Published online: 12 March 2002

Abstract The present study was aimed at investigating whether the regulation of the local natriuretic peptide system is altered in the kidney and the vasculature in obstructive uropathy. Male Sprague-Dawley rats were bilaterally obstructed by ligation of the proximal ureters for 48 h. Control rats were treated in the same way, except that no ligature was made. The mRNA expression of the various isoforms of atrial, brain, and C-type natriuretic peptide (ANP, BNP, CNP) and different subtypes of natriuretic peptide receptor-A, -B, and -C (NPR-A, NPR-B, NPR-C) was determined in the kidney and the thoracic aorta by reverse transcription-polymerase chain reaction. The basal and stimulated activities of particulate guanylyl cyclase were also examined. Following the bilateral ureteral obstruction, the expression of ANP, BNP, and CNP was increased in the aorta as well as in the kidney. Contrary to this, the expression of NPR-A, NPR-B, and NPR-C was decreased both in the kidney and the aorta. Accordingly, the guanylyl cyclase activity was significantly decreased in response to natriuretic peptides. ANP relaxed phenylephrine-precontracted aortic rings in a dose-dependent manner, the degree of which was significantly diminished. Our results suggest that the local synthesis of natriuretic peptides is increased in the kidney and in the vasculature in obstructive uropathy.

S.W. Kim (✉)
Department of Internal Medicine,
Chonnam National University Medical School,
5 Hak-dong, Gwangju 501–746, Korea
E-mail: skimw@chonnam.ac.kr
Tel.: +82-62-2204266; Fax: +82-62-225-8578

Y. Li · S. Kim · Y.W. Oh · J.U. Lee
Department of Physiology,
Chonnam National University Medical School,
5 Hak-dong, Gwangju 501–746, Korea

J.U. Lee
Chonnam National University Research
Institute of Medical Sciences, 5 Hak-dong,
Gwangju 501–746, Korea

Keywords Natriuretic peptide · Natriuretic peptide receptor · Bilateral ureteral obstruction

Introduction

Natriuretic peptides (NP) are known to play a role in cardiovascular homeostasis. Among the subtypes of NP receptor (NPR) thus far known, NPR-A has high affinities both for atrial NP (ANP) and brain NP (BNP), whereas NPR-B is selectively stimulated by C-type NP (CNP). They are linked to particulate guanylyl cyclase and their activation results in a secondary formation of cGMP [2]. Contrary to this, NPR-C binds to all the known NP ligands less tightly [1, 3], acting in their metabolic clearance [2]. The biological activity of the NP system may be affected by the tissue expression of NPR as well as of NP.

The local NP system may exert an important role in its own right. Indeed, we previously demonstrated that postobstructive natriuresis is in part associated with an enhanced activity of the local ANP system [8, 9]. In the vasculature, the local NP system plays a role in vasorelaxation [4, 6, 12]. An altered regulation of the vascular NP system may exist in association with ureteral obstruction, since the blood pressure may be increased following the obstruction of the urinary tract [7, 13].

The present study was aimed at investigating whether the local NP system is altered in the vasculature and the kidney in association with an obstructed kidney. The expression of the various isoforms of NP and of NPR was quantified in the kidney and the aorta by reverse transcription-polymerase chain reaction (RT-PCR). The activity of particulate guanylyl cyclase was also determined in the glomeruli, papilla, and aorta.

Materials and methods

Animals

Male Sprague-Dawley rats weighing 200–250 g were used. They were kept in accordance with the Chonnam National University

Guidelines of Experimental Animal Care and Use. The abdominal cavity was opened and a 2-0 silk ligature was proximally placed on both ureters while the rats were under anesthesia with ketamine (50 mg/kg, i.p.). After closure of the abdomen, the animals were kept for 48 h during which time they were given food and water ad libitum. Control rats were treated in the same way, except that no ureteral ligature was made.

On the day of experimentation, the systolic blood pressure was measured by the tail-cuff method. The rats were then killed by decapitation in a conscious state. In six experimental and control rats, the kidneys and the aortae were quickly removed and stored at –70°C until used for RT-PCR. The kidneys and aortas were used for the measurement of guanylyl cyclase activity in another eight rats in each group, and the thoracic aorta was isolated and examined of its isometric tension in response to ANP on the same day.

RNA extraction and RT-PCR

Total RNA was isolated according to the protocol of the Ultraspec RNA isolation system (Biotecx Laboratories, Houston, Tex., USA). RNA concentrations were determined by measuring the absorbance at 260 nm. The mRNA expression was determined by RT-PCR. For RT, 5 μg total RNA was incubated with reverse transcriptase (Gibco BRL, Grand Island, N.Y., USA; 200 U), RNasin (10 U), dNTP mix (10 mM), DTT (0.1 M), $MgCl_2$ (25 mM), oligo(dT) (0.5 μg/μl), and reaction buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl] in a final volume of 20 μl at 42°C for 50 min. After the inactivation of the reverse transcriptase at 72°C for 15 min, 2 μl cDNA was subjected to PCR amplification.

PCR was conducted in a final volume of 20 μl containing 10 pmole of each primer, dNTP mix (250 μmol), $MgCl_2$ (1.5 mmol), and Taq polymerase (0.3 U, Super Taq, Super-Bio, Suwon, Korea) using a thermal cycler (M.J. Research, Watertown, Mass., USA). The PCR profiles for NPR-A, NPR-B, and NPR-C were 35 cycles and consisted of 95°C for 1 min, 58°C for 1 min, and 72°C for 1 min. For ANP, 32 cycles of 95°C for 1 min, 58°C for 1 min, and 72°C for 1 min were used. For BNP, 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min were used. For CNP, 60 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 30 s andfor β-actin, 35 cycles of 94°C for 45 sec, 56°C for 45 sec, and 72°C for 90 s were used. The final extension was ended with 5 min of elongation at 72°C. The primers were adopted as described by previous investigators [10]. The PCR products were size fractionated by 1.2% agarose gel electrophoresis, and visualized under UV light with ethidium bromide staining. The PCR products were quantified by IMAGER and 1D MAIN (Bioneer, Cheongwon, Korea).

Membrane preparation and guanylyl cyclase activity

The glomeruli was isolated from the cortex by the graded sieve method. In brief, the kidney was decapsulated and the cortex was filtered through standard sieves (250, 150, 125, and 75 μm, consecutively). The glomeruli on the 75 μm sieve were collected by centrifugation (1,000 *g* for 15 min at 4°C). The glomeruli, papilla, or thoracic aorta were homogenized in an ice-cold buffer (50 mM Tris-HCl, pH 8.0, containing EDTA (1 mM), phenylmethylsulfonyl fluoride (0.2 mM), and sucrose (250 mM). The homogenate was centrifuged at 1,000 *g* for 10 min at 4°C, and the supernatant was again centrifuged at 100,000 *g* for 60 min at 4°C. The membrane pellet was washed three times with Tris HCl (50 mM, pH 7.4) and resuspended. Protein concentrations were determined using the bicinchonic acid assay kit (BioRad, Hercules, Calif., USA).

The activity of particulate guanylyl cyclase was determined in the membrane aliquots by the method of Winquist et al. [14] with a slight modification. They were incubated for 15 min at 37°C in Tris-HCl (50 mM, pH 7.6) containing 3-isobutyl-1-methylxanthine (1 mM), guanosine triphosphate (1 mM), adenosine triphosphate (1 mM), phosphocreatinine (15 mM), creatine phosphokinase (80 μg/ml), magnesium chloride (4 mM), plus 0–10 μM ANP, BNP, or CNP. Incubations were stopped by adding ice-cold sodium acetate (50 mM, pH 5.0) and boiling for 5 min. Samples were then centrifuged at 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 sodium acetate buffer (50 mM, pH 4.8), and then 100 μl of diluted cGMP antiserum (Calbiochem-Novabiochem, San Diego, Calif., USA) and iodinated cGMP (10,000 cpm/100 μl, specific activity = 2,200 Ci/mmol, DuPont-New England Nuclear, Wilmington, Del., USA) were added and incubated for 24 h at 4°C. The bound form was separated from the free form by charcoal suspension. The results were expressed as picomoles of cGMP generated per mg protein per min.

Recording isometric tension

The thoracic aorta was isolated and cut into rings of 5-mm length. Each ring was suspended in a tissue bath containing physiological salt solution (PSS) at 37°C, while being continuously bubbled with 95% O_2–5% CO_2 (pH 7.4). The baseline load placed on the ring was 2.0 g, and the changes in isometric tension were recorded using a force-displacement transducer (Grass FT03; Quincy, Mass., USA). The composition of PSS used was NaCl 112 mM, KCl mM5, $NaHCO_3$ mM25, KH_2PO_4 mM1.0, $MgSO_4$ 1.2 mM, $CaCl_2$ 2.5 mM, and glucose 11.5 mM. The aortic ring was precontracted with EC_{80} phenylephrine (3.5×10^{-6} M), and the response to ANP was calculated as a percentage over the phenylephrine-induced maximum contraction.

Statistical analysis

The results are expressed as means ± SEM. The statistical significance of differences between the groups was determined using the unpaired Student's *t*-test. *P*-values less than 0.05 were considered to be significant.

Results

Expression of NP and NPR

Systolic blood pressure was significantly increased following bilateral ureteral obstruction (BUO) (169 ± 10 mmHg in the experimental vs 110 ± 9 mmHg in the control; $n = 6$ each, $P < 0.01$). Accordingly, in the kidney the mRNA expression of ANP, BNP, and CNP was increased (Fig. 1), whereas that of NPR-A, NPR-B, and NPR-C was decreased (Fig. 2). Similarly, in the aorta, the expression of ANP, BNP, and CNP was increased (Fig. 3), whereas that of NPR-A, NPR-B, and NPR-C was decreased (Fig. 4).

Guanylyl cyclase activity and isometric tension

Figure 5 shows the activity of particulate guanylyl cyclase evoked by ANP, BNP, and CNP in the glomerulus, papilla, and aorta. ANP increased the guanylyl cyclase activity in the glomerulus and papilla, however, the degree was significantly reduced in BUO. BNP and CNP also activated guanylyl cyclase, the magnitude of which was also reduced in BUO. The guanylyl cyclase activities evoked by ANP, BNP, and CNP were also decreased in the aorta in BUO. The ANP-induced relaxation of

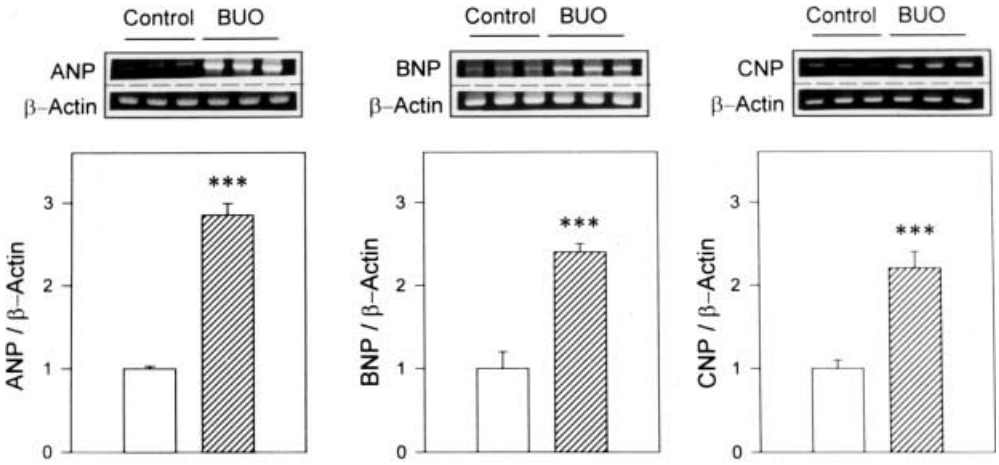

Fig. 1. Expression of ANP, BNP and CNP mRNA in the kidney. *Fluorographs* show ethidium bromide-stained agarose gels containing RT-PCR products, and *columns* show densitometric data representing control and BUO groups (mean ± SEM, $n = 6$). ***$P < 0.001$ vs control

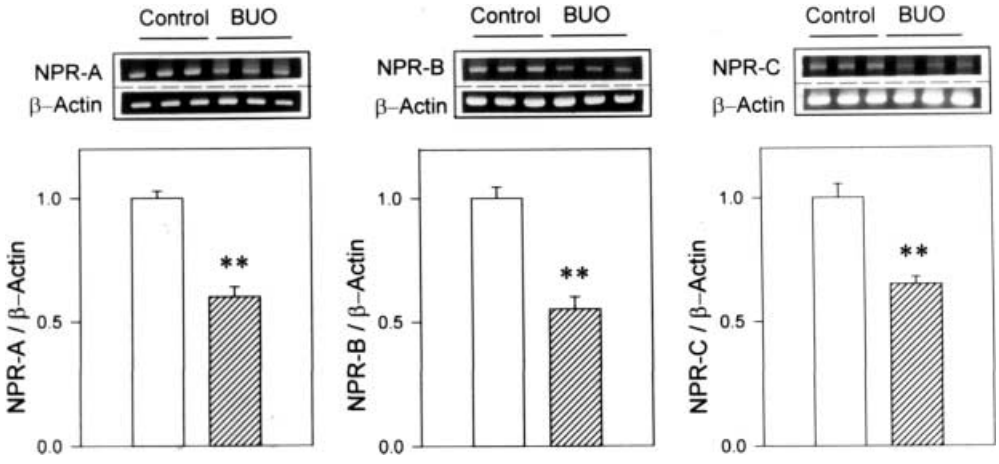

Fig. 2. Expression of NPR-A, NPR-B and NPR-C mRNA in the kidney. Legends as in Fig. 1. **$P < 0.01$ vs control

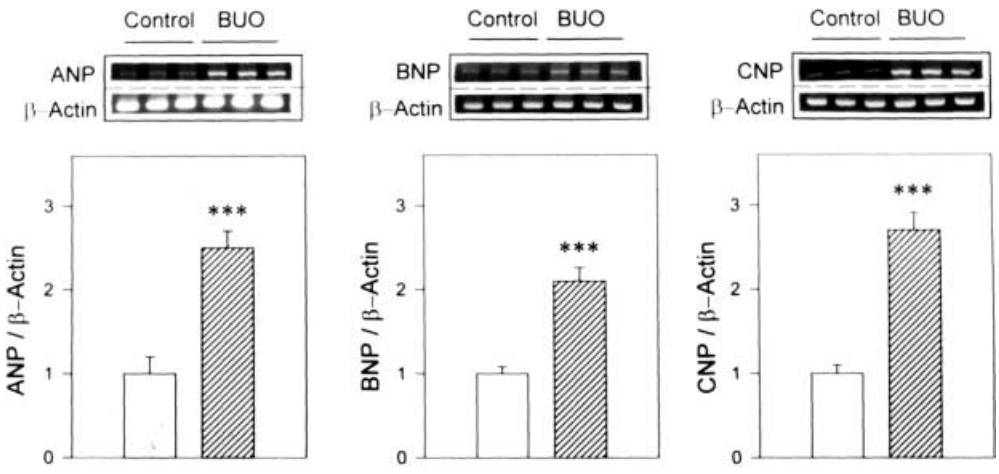

Fig. 3. Expression of ANP, BNP and CNP mRNA in the thoracic aorta. Legends as in Fig. 1. ***$P < 0.001$ vs control

phenylephrine-precontracted aortic rings was significantly attenuated in BUO (Fig. 6).

Discussion

The present study demonstrated that the mRNA expression of ANP, BNP, and CNP was increased in the obstructed kidney and the thoracic aorta following ureteral obstruction. This occurred along with an increase in systemic blood pressure. A substantial vasoconstriction of the renal vascular bed has usually been observed after ureteral obstruction, thereby reducing the glomerular filtration rate and the effective renal plasma flow [11]. The increased synthesis of NP may permit prolonged diuresis in the previously obstructed kidney,

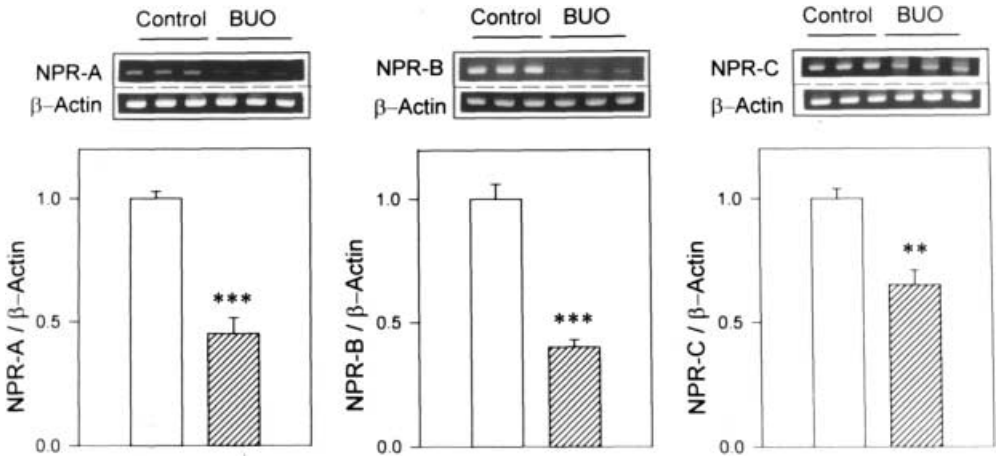

Fig. 4. Expression of NPR-A, NPR-B and NPR-C mRNA in the thoracic aorta. Legends as in Fig. 1. $^{**}P < 0.01$, $^{***}P < 0.001$ vs control

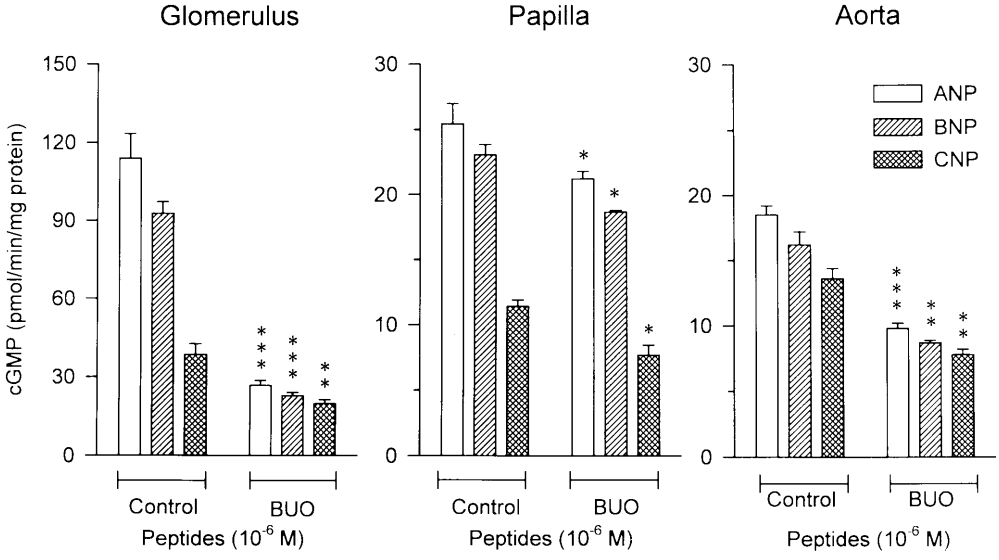

Fig. 5. cGMP production in response to ANP, BNP and CNP in the glomeruli, papilla and aorta. Each *column* represents the mean ± SEM of eight experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs control

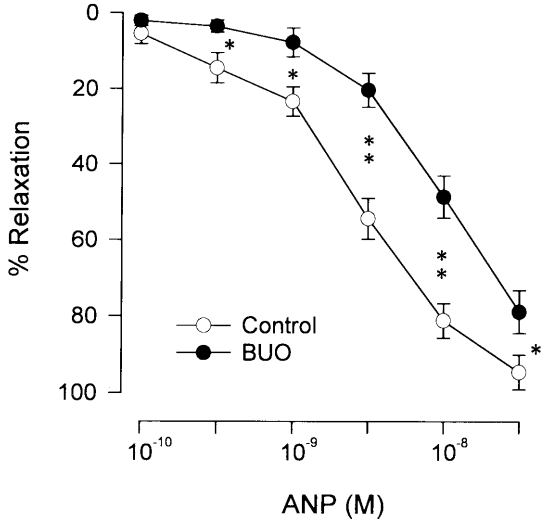

Fig. 6. Isometric tension responses to cumulative doses of ANP in the isolated aortic rings. Each point represents mean ± SEM of eight experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control

this being a compensatory mechanism to preserve renal function. Locally synthesized NP in the vasculature may then compensate for the hypertension induced by fluid volume retention.

Contrary to this, the expression of the different subtypes of NPR was decreased in the kidney and the aorta in BUO. An elevated tubular pressure, hypertension and uremic milieu may be responsible for the reduced expression of functional receptors. However, it has been shown that exposure to ANP results in a reduction of NPR in cultured vascular smooth muscle cells [5]. In this context, the down-regulation of NPR may be attributed to an enhanced local synthesis of NP.

The altered expression of NPR-A and NPR-B may be manifested by an altered biological effect of the NP system. The effects of NP may be dissipated when the expression of its active receptors, NPR-A and NPR-B, is decreased. Indeed, the decreased NPR-A and NPR-B expression was associated with a decrease in guanylyl cyclase activity in BUO. However, it has been shown that the decreased guanylyl cyclase activity is rapidly

reversible upon releasing the ureteral obstruction [9]. Taken together, the enhanced local synthesis of NP along with a rapid recovery of guanylyl cyclase activity may allow an increased natriuresis in a previously obstructed kidney.

In the aorta, the expression of the different subtypes of NPR was decreased in BUO. The NP-mediated vasodilation, as well as the particulate guanylyl cyclase activity, was significantly attenuated in BUO. Although these findings may be related with the increased blood pressure, their pathophysiological significance remains to be further explained.

In summary, the expression of ANP, BNP and CNP was increased in the kidney and the aorta in BUO. The expression of NPR-A, NPR-B, and NPR-C was decreased, along with decreased guanylyl cyclase activity. These may be causally related with the postobstructive diuresis and the altered hemodynamic regulation and blood pressure in BUO.

Acknowledgments This work was supported by a research grant from KOSEF through HRC (2001G0301) and Chonnam University Hospital Research Institute of Clinical Medicine (2002).

References

1. Chang MS, Lowe DG, Lewis M, Hellmiss R, Chen E, Goeddel DV (1989) Differential activation by atrial and brain natriuretic peptides of two different receptor guanylate cyclases. Nature 341: 68
2. Drewett JG, Garbers DL (1994) The family of guanylyl cyclase receptors and their ligands. Endocr Rev 15: 135
3. Fuller F, Porter JG, Arfsten AE, Miller J, Schilling JW, Scarborough RM, Lewicki JA, Schenk DB (1988) Atrial natriuretic peptide clearance receptor. Complete sequence and functional expression of cDNA clones. J Biol Chem 263: 9395
4. Gardner DG, Deschepper CF, Baxter JD (1987) The gene for the atrial natriuretic factor is expressed in the aortic arch. Hypertension 19: 103
5. Hirata Y, Hirose S, Takata S, Takagi Y, Matsubara H (1987) Down-regulation of atrial natriuretic peptide receptor and cyclic GMP response in cultured rat vascular smooth muscle cells. Eur J Pharmacol 135: 439
6. Itoh H, Pratt RE, Dzau VJ (1990) Atrial natriuretic peptide inhibits hypertrophy of vascular smooth muscle cells. J Clin Invest 86: 1690
7. Jones DA, George NJ, O'Reilly PH, Barnard RJ (1987) Reversible hypertension associated with unrecognised high pressure chronic retention of urine. Lancet 1: 1052
8. Kim SW, Lee JU, Choi KC (2001) Increased expression of atrial natriuretic peptide in ureteral obstructed kidneys in rats. Scand J Urol Nephrol 35: 163
9. Kim SW, Lee JU, Park JW, Hong JH, Kook H, Choi C, Choi KC (2001) Increased expression of atrial natriuretic peptide in the kidney of rats with bilateral ureteral obstruction. Kidney Int 59: 1274
10. Mistry SK, Chatterjee PK, Weerackody RP, Hawksworth GM, Knott RM, McLay JS (1998) Evidence for atrial natriuretic factor induced natriuretic peptide receptor subtype switching in rat proximal tubular cells during culture. Exp Nephrol 6: 104
11. Purkerson ML, Klahr S (1989) Prior inhibition of vasoconstrictors normalizes GFR in postobstructed kidneys. Kidney Int 35: 1305
12. Suga S, Nakao K, Itoh H, Komatsu Y, Ogawa Y, Hama N, Imura H (1992) Endothelial production of C-type natriuretic peptide and its marked augmentation by transforming growth factor-β: Possible existence of "vascular natriuretic peptide system". J Clin Invest 95: 2442
13. Weidmann P, Beretta-Piccoli C, Hirsch D, Reubi FC, Massry SG (1977) Curable hypertension with unilateral hydronephrosis. Studies on the role of circulating renin. Ann Intern Med 87: 437
14. Winquist RJ, Faison EP, Waldman SA, Schwartz K, Murad F, Rapoport RM (1984) Atrial natriuretic factor elicits an endothelium-independent relaxation and activates particulate guanylate cyclase in vascular smooth muscle. Proc Natl Acad Sci USA 81: 7661