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Short communication

# Effects of C-type natriuretic peptide on renal vasoconstriction in dogs

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**Abstract**

Intrarenal arterial infusion of C-type natriuretic peptide (CNP, 50 ng/kg per min) increased urine flow rate without affecting glomerular filtration rate. Intrarenal arterial bolus injection of angiotensin II (25, 50 and 100 ng) or of norepinephrine (0.25, 0.5 and 1.0 μg) reduced renal blood flow. The blood flow response induced by angiotensin II was slightly attenuated but the response induced by norepinephrine was unaffected during CNP infusion. These results suggest that exogenous CNP, even at the pharmacological dose that can induce diuresis, has little effect on the canine renal vasculature. © 1997 Elsevier Science B.V.

*Keywords:* Kidney; CNP (C-type natriuretic peptide); Angiotensin II; Norepinephrine; Vasoconstriction; (Dog)

## 1. Introduction

Atrial natriuretic peptide (ANP) plays an important role in the control of extracellular fluid volume and peripheral vascular resistance (Goetz, 1988). We had reported that ANP suppressed the renal vasoconstriction induced by angiotensin II (Hisa et al., 1992) and by endogenous and exogenous norepinephrine (Tomura et al., 1991; Hisa et al., 1992) and the hypofiltration induced by angiotensin II (Tomura et al., 1995) in anesthetized dogs. ANP thus may be able to modulate the humoral and neural control of renal hemodynamics.

C-type natriuretic peptide (CNP), which has a sequence highly homologous to that of ANP (Sudoh et al., 1990), is also supposed to participate in cardiovascular homeostasis. CNP immunoreactivity and mRNA coding for CNP have been confirmed in the kidney (Nir et al., 1994; Terada et al., 1994). On the other hand, effects of CNP on renal functions are unclear. CNP reduces blood pressure and cardiac output but does not induce natriuresis or renal vasodilation in anesthetized dogs (Stingo et al., 1992; Clavell et al., 1993). However, CNP can dilate afferent arterioles preconstricted with norepinephrine in the perfused kidney of rats (Amin et al., 1996). It is therefore possible that CNP counteracts vasoconstrictor stimuli in the kidney despite its lack of natriuretic and direct vasodilator actions.

In the present study, we examined whether CNP affects the angiotensin II- and norepinephrine-induced vasoconstriction in the kidney in vivo. Decreases in renal blood flow induced by intrarenal arterial injection of angiotensin II and norepinephrine were compared before and during infusion of CNP in anesthetized dogs.

## 2. Materials and methods

### 2.1. Animal preparation

Mongrel dogs of either sex, weighing 9–14 kg, were anesthetized with $Na^+$ pentobarbital (30 mg/kg i.v.), then intubated and ventilated artificially with room air. Decamethonium bromide (0.25 mg/kg i.v.) was given to prevent spontaneous active respiratory movements. Anesthesia was maintained by a continuous i.v. infusion of $Na^+$ pentobarbital at a rate of 5 mg/kg per h throughout the experiments. The right brachial artery was cannulated to measure systemic blood pressure with a pressure transducer (Nihon Kohden, Tokyo, Japan, TP-200T). The left kidney was exposed by a retroperitoneal flank incision. All visible renal nerves were dissected away from the renal vessels and cut after ligation. An electromagnetic flow probe (2.5–3.5 mm in diameter, Nihon Kohden) was attached at the renal artery to measure renal blood flow with

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a square-wave flowmeter (Nihon Kohden, MF-27). A curved 25 gauge needle connected to a polyethylene tube was inserted into the renal artery for continuous CNP infusion. In experiments with agonist injection, a second 25 gauge needle was placed in the renal artery for bolus intrarenal arterial injection. Mean arterial pressure, heart rate and renal blood flow were recorded with a polygraph system (Nihon Kohden). After completion of surgery, 60–90 min was allowed for stabilization. The animals were divided into three groups.

### 2.2. Experimental protocols

#### 2.2.1. Group 1 (n = 6)

Inulin, dissolved in 0.45% NaCl and 0.25% glucose, was given intravenously at a priming dose of 50 mg/kg and at a maintenance dose of 1 mg/kg per min (0.1 ml/kg per min). More than 60 min was allowed for stabilization with monitoring of renal hemodynamics and urine flow rate. Urine was collected over 10 min and arterial blood was withdrawn at the midpoint of the urine collection to obtain basal values. CNP (human CNP-22, Osaka Protein Research Foundation, Osaka, Japan) was then infused into the renal artery, using a motor-driven infusion pump (Harvard Apparatus, Millis, MA, USA, Model 975), at increasing doses of 10 and 50 ng/kg per min for 30 min each. Urine and arterial blood samples were collected twice, beginning 10 min after the start of CNP infusion at each dose. Samples for recovery values were collected about 60 min after stopping the CNP infusion.

#### 2.2.2. Group 2 (n = 6) and group 3 (n = 6)

An intrarenal arterial bolus injection of angiotensin II (Osaka Protein Research Foundation) 25, 50 and 100 ng (group 2) or of norepinephrine (Sigma, St. Louis, MO, USA) 0.25, 0.5 and 1.0 μg (group 3) was given at 7 to 10

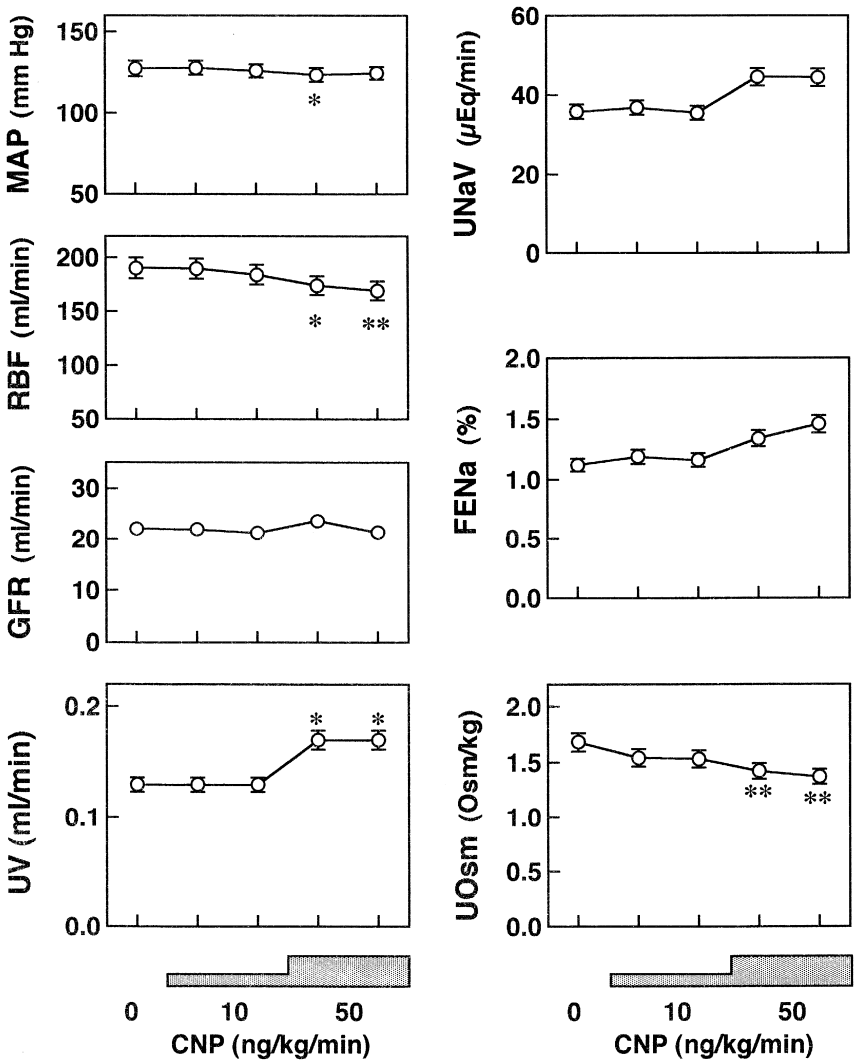


Fig. 1. Effects of C-type natriuretic peptide (CNP) on urine formation (group 1, $n = 6$). Values are means ± S.E. MAP, mean arterial pressure; RBF, renal blood flow; GFR, glomerular filtration rate; UV, urine flow rate; UNaV, urinary $Na^+$ excretion; FENa, fractional excretion of $Na^+$; UOsm, urine osmolarity. CNP was infused into the renal artery at increasing rates, 10 and 50 ng/kg per min. * $P < 0.05$ and * * $P < 0.01$ compared with basal (dose zero) values.

min intervals. After the control responses had been obtained, CNP was infused into the renal artery at increasing rates, 10 and 50 ng/kg per min. Ten minutes later, the drug injection was repeated during CNP infusion at each dose.

### 2.3. Data analysis

All values are expressed as means ± S.E. The data were analyzed by analysis of variance for multifactor repeated measures. Dunnett's test was used for multiple comparisons. Differences with a $P$ value less than 0.05 were considered to be statistically significant.

## 3. Results

Intrarenal arterial infusion of CNP at 10 ng/kg per min did not affect hemodynamics or urine formation (group 1, Fig. 1). CNP infusion at 50 ng/kg per min increased urine flow rate. CNP at the high dose also tended to increase urinary $Na^+$ excretion and fractional excretion of $Na^+$, but the changes were not statistically significant (Fig. 1). The increased values of these parameters returned to their basal levels after the CNP infusion was stopped (data are not shown). Systemic blood pressure, renal blood flow and urine osmolarity decreased during the high-dose CNP infusion, whereas glomerular filtration rate remained unaffected throughout the experiments (Fig. 1).

Bolus injection of angiotensin II (group 2) or of norepinephrine (group 3) into the renal artery reduced renal blood flow in a dose-dependent manner. Fig. 2 shows the agonist-induced blood flow responses as percentage changes from basal levels. Basal renal blood flow (obtained just before the first agonist injection) in the control period and CNP infusion periods (10 and 50 ng/kg per min) were: 155 ± 13, 145 ± 14 and 140 ± 13 ml/min in group 2, and 141 ± 19, 134 ± 20 and 127 ± 19 ml/min in group 3, respectively. Intrarenal arterial infusion of CNP did not affect the angiotensin II- or norepinephrine-induced reductions in renal blood flow except that the blood flow response induced by 50 ng of angiotensin II was slightly attenuated (Fig. 2).

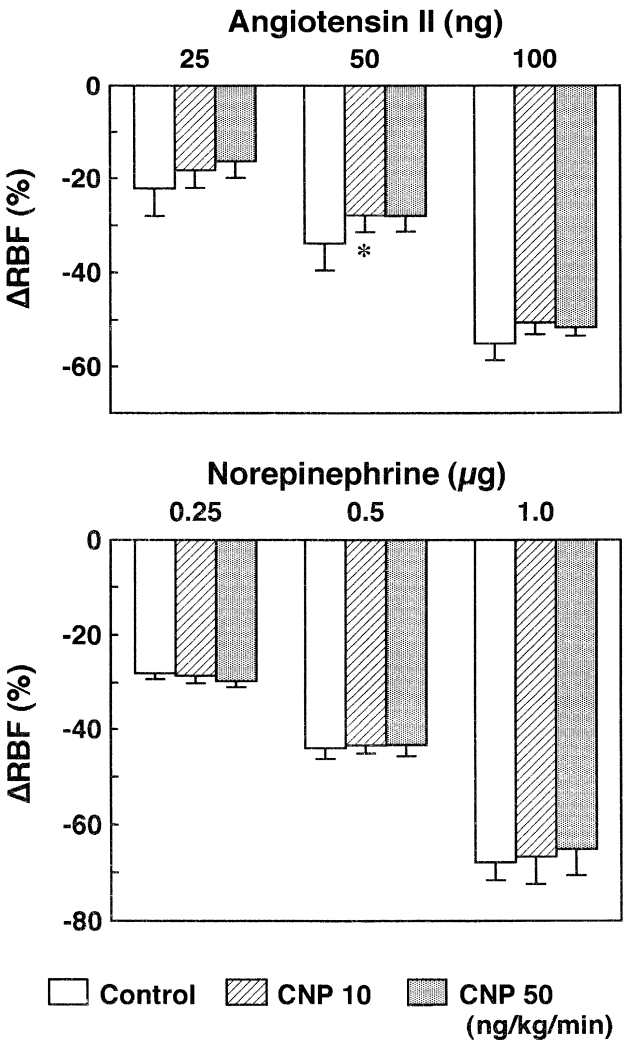


Fig. 2. Effects of C-type natriuretic peptide (CNP) on renal vasoconstriction induced by intrarenal arterial injection of angiotensin II (A, group 1, $n = 6$) and of norepinephrine (B, group 2, $n = 6$). Values are means ± S.E. RBF, renal blood flow. CNP was infused into the renal artery at increasing rates, 10 and 50 ng/kg per min. * $P < 0.05$ compared with corresponding control values.

## 4. Discussion

Although systemic administration of CNP induces natriuresis and diuresis in anesthetized rats (Sudoh et al., 1990) and in conscious sheep (Charles et al., 1995), Clavell et al. (1993) reported that intrarenal arterial infusion of CNP at 1 and 5 ng/kg per min did not affect urinary parameters in anesthetized dogs. In the present study, the higher dose of CNP (50 ng/kg per min) increased urine flow rate by about 40%. CNP also tended to increase urinary $Na^+$ excretion (by about 20%). These results demonstrate that CNP at the pharmacologically high dose can induce diuresis and moderate natriuresis in dogs.

mRNA for natriuretic peptide B-type receptors to which CNP binds with high selectivity is widely distributed in both rat renal vasculature and tubular segments (Terada et al., 1994). Since there was no increase in renal blood flow or glomerular filtration rate during the CNP infusion, the CNP-induced diuresis observed in the present study could be related to its action on renal tubular reabsorption. It is also possible that CNP dilates vasa recta, an effect which may not be detected as changes in total renal blood flow, and thereby washes out medullary solutes thus reducing the interstitial osmotic gradient.

However, the potency of CNP as a natriuretic peptide is less than that of ANP. Whereas intrarenal arterial infusion of ANP at 10 ng/kg per min (3.3 pmol/kg per min) increased urine flow rate and absolute and fractional excretion of $Na^+$ more than 2 fold in anesthetized dogs (Takagi et al., 1993), CNP infusion at 10 ng/kg per min (4.5

pmol/kg per min) did not affect the renal parameters in the present study.

In our previous study, intrarenal arterial infusion of ANP (10 and 50 ng/kg per min) dose dependently attenuated the decreases in renal blood flow induced by angiotensin II and by norepinephrine in anesthetized dogs (Hisa et al., 1992). Amin et al. (1996) reported that CNP and ANP dilated juxtamedullary afferent arterioles preconstricted with norepinephrine to the same extent in rats. The vascular action of CNP is suggested to depend on prostaglandins and nitric oxide (Amin et al., 1996). In the present study, however, CNP infusion exerted almost no inhibitory effect on the angiotensin II- or norepinephrine-induced decrease in renal blood flow. Although CNP at 10 ng/kg per min slightly attenuated the blood flow response induced by angiotensin II, CNP at 50 ng/kg per min did not further suppress the blood flow response. CNP might be able to counteract constriction of renal medullary vessels, but it is unlikely that CNP plays a significant role in the control of total renal hemodynamics in dogs.

CNP immunoreactivity has been found in the systemic circulation (Clavell et al., 1993) and renal epithelial cells produce CNP (Nir et al., 1994) in dogs. Therefore it could be postulated that endogenous CNP mostly exerts its effect on the kidney, thus blunting renal actions of exogenous CNP. However, we observed that HS-142-1, an antagonist for natriuretic peptide A- and B-type receptors (Sano et al., 1992), failed to affect the urinary $Na^+$ and water excretion or the angiotensin II-induced renal blood flow response in dogs (unpublished data). Thus endogenous CNP does not seem to play a substantial role in the kidney of normal anesthetized dogs, although enhanced renal CNP production was demonstrated in endothelin-infused dogs (Nir et al., 1994).

In summary, the present results suggested that CNP can induce diuresis but does not interfere with the angiotensin II- or norepinephrine-induced vasoconstriction in the dog kidney in vivo. Higher doses of CNP than ANP are required to affect urine formation.

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