Effects of Altered Body Fluid Balance and High Blood Pressure on the Plasma Brain Natriuretic Peptide in Rats

The present study was aimed to investigate the regulatory mechanisms of BNP release. Effects of acute and chronic perturbations in body fluid balance, changes in BP, and regulatory roles of NO and endothelin systems on BNP release were examined in rats. Although acute extracellular volume expansion did not have significant effects on plasma BNP, prolonged high-salt intake increased plasma BNP levels. Plasma BNP levels were also higher in 2K1C rats compared with the control. Although infusion of L-NAME increased the plasma BNP in control, it did not further affect the plasma BNP in rats with high-salt intake. Although L-arginine (20 mg·kg^{-1} per min) per se did not have significant effects on plasma BNP, it blocked the stimulatory effect of L-NAME (200 μg·kg^{-1} per min). Plasma BNP was severalfold increased following a single injection of endothelin (0.3 μg/kg) in normal and high-salt intake groups, the magnitude of which was not significantly affected by the high-salt intake. Although indomethacin did not have significant effects on plasma BNP in normal rats, it blocked the stimulatory effect of 2K1C hypertension. It is concluded that BNP is regulated by chronic changes in body fluid balance and blood pressure. It is also suggested that endothelin and NO systems may directly regulate the secretion of BNP in vivo. An endogenous prostaglandin synthesis may be involved in the stimulated release of BNP in hypertension.

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Key Words : *Brain natriuretic peptide, Body fluid balance, Hypertension, Nitric oxide, Endothelin.*

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INTRODUCTION

Brain natriuretic peptide (BNP) has been, though originally isolated from porcine brain, demonstrated to be present in the highest concentration in the hearts of pigs (1), rats (2, 3), and humans (4). It may be cosecreted with atrial natriuretic peptide (ANP) from cardiac atrial granules (5), but the main source of BNP appears to be via a constitutive release from cardiac ventricular tissue (6). Its plasma levels are, along with ANP, elevated in disease states associated with fluid overload, such as heart failure (6) and renal failure (7), indicating that changes in total blood volume or central volume may influence its release. Furthermore, plasma BNP levels are higher in patients with essential hypertension than in normotensive controls, being in correlation with the level of the blood pressure (8~12). However, whether and to what extent the plasma BNP level is changed in response to an altered body fluid balance in hypertension are not clear.

Over the last decade, it has become clear that the endothelium-derived nitric oxide (NO) plays an important role in regulating the renal function (13, 14) and the release of other hormones affecting body fluid balance and blood pressure (15~17). No information, however, has been available about the interaction between NO and BNP in response to an altered fluid balance. Furthermore, the release of BNP may also be hypothesized to be mediated by a prostaglandin synthesis, as a stimulated-release of ANP is mediated by prostaglandin synthesis (18).

The present study was aimed to investigate the regulatory mechanisms of BNP release. Effects of acute and chronic perturbations in body fluid balance and changes in BP on the BNP release were examined. The interaction of BNP with other hormonal systems such as NO and endothelin were also investigated. Three different series of experiments were done, in which the effects of extracellular volume changes and high blood pressure, and regulatory role of other hormonal systems on the plasma BNP release were examined.

MATERIALS and METHODS

Effects of acute and chronic extracellular volume expansion on plasma BNP

Male Sprague-Dawley rats (230~270 g) were used. They were either supplied with normal or high-salt diet, in which the latter was achieved by supplying 0.9% saline as a drinking solution during one week.

On the experimental day, under thiopental anesthesia (50 mg/kg, i.p.), the left femoral artery was cannulated to measure arterial pressure and the vein to serve as an infusion route. A bladder catheter was implanted to collect urine samples.

Following the surgical preparation, a 30 to 60-min equilibration period was allowed to elapse before urine collection started. Urine was collected every 15 min by flushing the bladder with 1 mL of distilled water followed by 1 mL of air. Basal urinary data were obtained by averaging values of three consecutive periods before the volume expansion (VE) started.

Blood samples were taken from the femoral artery upon termination of the protocol. They were collected into EDTA-dipotassium salt tubes containing aprotinin [200 KIU/mL blood] and centrifuged at 4℃ (2,000 g for 15 min). The plasma was stored at -20℃ until analyzed for immunoreactive BNP (1-32) using a commercial radioimmunoassay kit (Peninsula, USA).

VE was achieved by intravenous infusion of saline (0.9 % NaCl) over 60 min, the total volume infused amounting up to 5% of the body weight.

Effects of hypertension on plasma BNP

Two-kidney, one clip (2K1C) hypertension was made in rats (150~200 g) by constricting the left renal artery with a silver clip (0.25 mm i, d.) under ketamine anesthesia; the contralateral kidney was left untouched. Sham-clipped rats served as control. They were used 4 weeks after clipping the artery.

For indomethacin-treatment, indomethacin (10 mg/kg, i.p.) was injected before the anesthetic was given.

Role of NO and endothelin systems

Effects of either precursor or inhibitor of NO synthesis, endothelin and an inhibitor of prostaglandin synthesis were examined.

L-Arginine, a precursor of NO synthesis, was infused for 90 min at the rate of 20 $mg \cdot kg^{-1}$ per min. N^G-nitro-L-arginine methyl ester (L-NAME), a competitive inhibitor of NO synthesis, was infused for 90 min at the rate of 200 $\mu g \cdot kg^{-1}$ per min (16 μL/min). Endothelin was injected intravenously as a single bolus (0.3 μg/kg), 10 min after which the blood sample for BNP assay was taken when the arterial pressure was normalized.

Statistical analysis

Differences between the groups were tested for their statistical significance using non-paired Student's *t*-test. Bonferroni adjustments were carried out for multiple comparisons, where applicable.

RESULTS

Effects of acute and chronic extracellular volume expansion and chronic salt-loading on plasma BNP

Fig. 1 shows the plasma levels of BNP before and during VE. The plasma level of BNP was not significantly altered by VE. However, the plasma BNP level was significantly higher in rats with high-salt intake compared with the control.

Effects of nitric oxide and endothelin systems on plasma BNP

Effects of L-NAME and L-arginine on the plasma BNP are shown in Fig. 2. L-NAME (200 $\mu g \cdot kg^{-1}$ per min) significantly increased the plasma BNP. Although L-arginine (20 $mg \cdot kg^{-1}$ per min) per se did not have significant effects on the plasma BNP, it blocked the stimulatory effect of L-NAME.

Fig. 3 shows plasma BNP levels following the infusion of L-NAME. The plasma BNP level was significantly

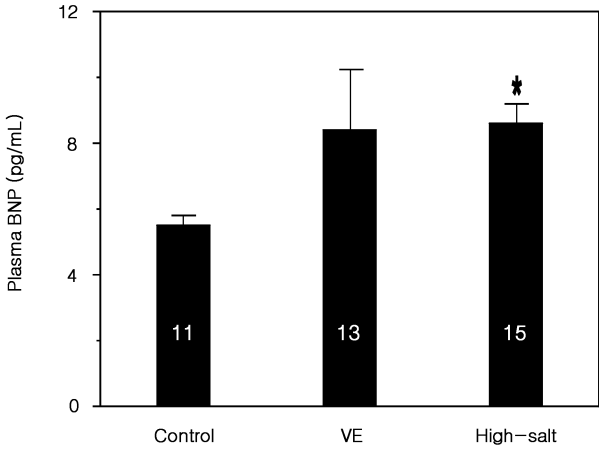

Fig. 1. Effects of acute extracellular volume-expansion (VE) and chronic salt-loading on plasma BNP levels. The numerals in bars represent numbers of rats. *p<0.05, compared with control.

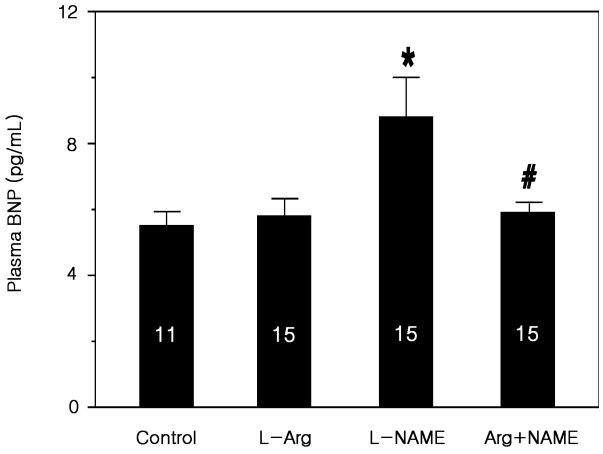

Fig. 2. Effects of L-arginine and L-NAME on plasma BNP levels. [Arg+NAME] group was infused with L-NAME and L-arginine. The numerals in bars represent numbers of rats. *p<0.01, compared with control. #p<0.05, compared with L-NAME group.

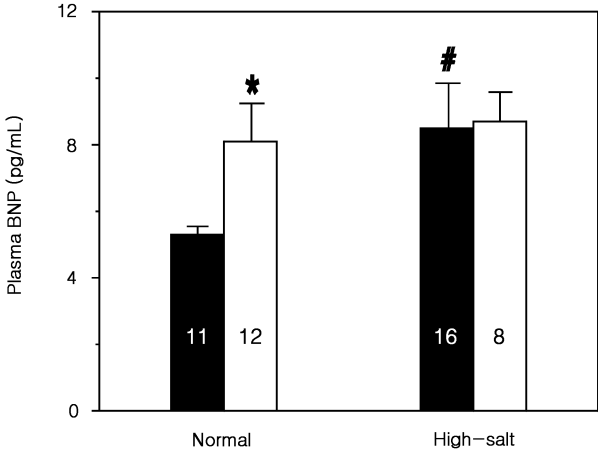

Fig. 3. Plasma BNP levels in control and high salt-fed rats. High-salt intake increased the plasma BNP levels, which was not further significantly affected by L-NAME. Symbols are: (■) control; (□) L-NAME. The numerals in bars represent numbers of rats. *p<0.01, compared with control. #p<0.01, compared with normal control.

higher in rats with high-salt intake than in control. L-NAME did not further affect the plasma BNP in rats with high-salt intake. The maximum pressor response to L-NAME was higher in rats with high-salt intake than in the control (△19.6±1.8 mmHg, n=8; vs. △11.0± 1.3 mmHg, n=12; p<0.01).

Fig. 4 depicts plasma BNP levels following the injection of endothelin in rats with normal and high-salt intake. The plasma BNP was severalfold increased by the endothelin (0.3 μg/kg), the magnitude of which did not significantly differ between the normal and high-salt groups.

Effects of hypertension on plasma BNP

Table 1 shows mean arterial pressure and plasma BNP levels in 2K1C and sham-clipped control rats. Mean arterial pressure in 2K1C rats was significantly higher than that in the control. Plasma BNP levels were also higher in 2K1C rats compared with those in the control. Although indomethacin did not have significant effects on plasma BNP in sham-clipped rats, it blocked the stimulatory effect of hypertension (Fig. 5).

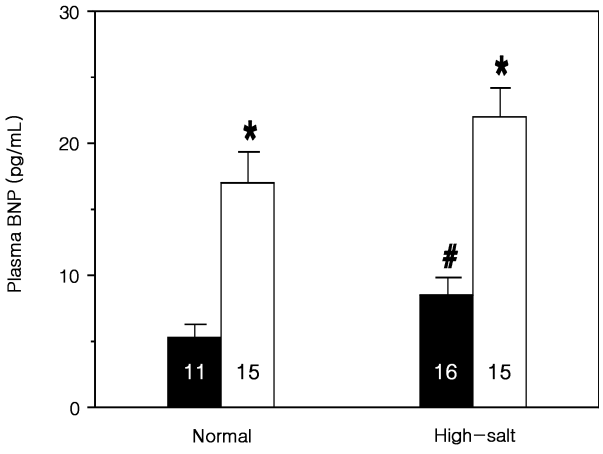

Fig. 4. Effects of endothelin on plasma BNP levels in rats with normal and high-salt intake. Symbols are: (■) control; (□) endothelin. The numerals in bars represent numbers of rats. *p<0.01, compared with control. #p<0.05, compared with normal control.

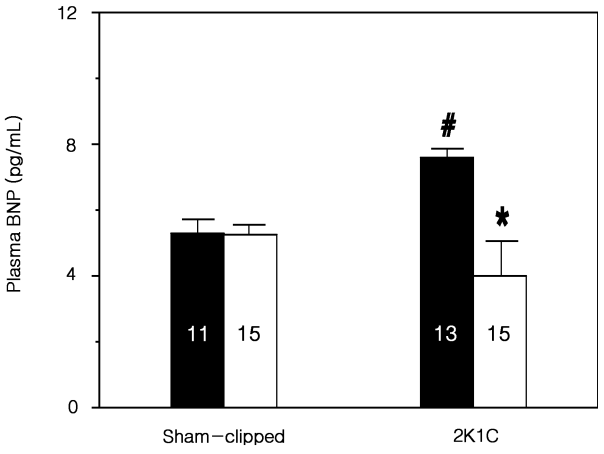

Fig. 5. Effects of indomethacin-treatment on plasma BNP levels in Sham-clipped and 2K1C rats. Symbols are: (■) control; (□) indomethacin. The numerals in bars represent numbers of rats. *p<0.01, compared with control. #p<0.01, compared with sham-clipped rats.

Table 1. Mean arterial pressure and plasma BNP levels in 2K1C and sham-clipped rats

	MAP (mmHg)	BNP (pg/mL)
Sham-clipped (n=11)	118 ± 4	5.29 ± 0.46
2K1C (n=13)	176 ± 11*	7.36 ± 0.45*

n=number of rats. *$p<0.01$, compared with sham-clipped.

DISCUSSION

The aim of the present study was to investigate the effects of acute and chronic changes in the body fluid balance on the plasma BNP. The plasma BNP was increased with increases of salt intake. Previous investigators also showed that chronic dietary salt loading increases plasma BNP as well as ANP (19, 20). Both BNP and ANP may be released into the circulation in response to a common stimulus during changes in sodium balance. In response to increased sodium and subsequent water retention on the high sodium intake, circulating volume may have been increased to stimulate their release.

However, an acute VE was ineffective in causing a change of plasma BNP, although a similar protocol caused an increase of ANP in our previous study (21). BNP may not always be cosecreted with ANP, and the effects on BNP may be of more complexity. Increases in plasma BNP levels have been reported during head-down postural changes (12), but not during saline-induced VE (19). Following acute intravenous saline loading in normotensive volunteers, despite the increase in plasma ANP, plasma BNP has been unchanged (22). These findings suggest differences in the release mechanisms of BNP and ANP in response to saline-induced VE. It is also possible that an acute change may not sensitively be transduced into an increased cosecretion of BNP. A chronic increase may only be effective in enhancing gene expression and protein synthesis for BNP.

On the other hand, BNP is synthesized predominantly by the ventricle (23), whereas ANP is mainly derived from the atrium. A ventricular source of BNP synthesis has been supported by the more BNP messenger RNA existing in the ventricle than in the atrium (6, 24). Expression of the BNP gene may also be regulated differently from that of the ANP gene (25, 26). In addition, BNP may be secreted in the ventricle via a constitutive pathway with a very small capacity for storage (22, 23). Therefore, the amount of stored BNP released into the circulation may be too small to detect a change in the expanding circulating volume, despite the volume-sensitive BNP release.

After an intravenous injection of endothelin, a severalfold increase of plasma BNP was shown. Endothelin may modulate the release of BNP in the same manner as it stimulates the ANP release (27, 28). Bruneau and deBold (29) also found that endothelin-1 caused increases in ANP and BNP release in isolated rat atria, up to two to three-fold. It has been further suggested that endothelin-1 stimulates the secretion of ANP and BNP by a direct mechanism, not through a hemodynamic change (30). In the present study, the maximal pressor response to endothelin was seen within 3 minutes, and the blood sample for BNP assay was taken after 10 minutes of endothelin injection when the arterial pressure was normalized. It is thus speculated that endothelin is a direct secretagogue for BNP. However, hypertension itself induced by endothelin may also affect the plasma BNP (see below) and the increase of the hormone may be manifested due to its longer half life compared with that for ANP (10, 31).

L-NAME infusion was associated with a significant increase of blood pressure. In addition, the pressor response to L-NAME was enhanced by high-salt intake, being in accord with previous findings (32, 33). These findings support the notion that L-NAME-induced hypertension is, at least in part, volume-dependent. On the other hand, BNP release was also stimulated by L-NAME. The specific effect of L-NAME on the NO system was supported by the blockade of the stimulated-release when L-NAME was infused along with L-arginine. It is likely that the BNP response reflects either an effect of the increased blood pressure or a direct tonic inhibitory role of endogenous NO in BNP secretion. On the other hand, it has been known that, L-arginine availability limits endothelium-dependent NO release only during prolonged increases in NO synthesis (34), and L-arginine is usually not a rate-limiting factor in the L-arginine-NO pathway in the healthy endothelium (35, 36). Therefore, no significant effect of L-arginine on the plasma BNP may not necessarily mean that NO is unrelated to the BNP system.

A marked increase of BNP was shown in pathophysiological conditions characterized by alterations of cardiac function and systemic hemodynamics, including hypertension (9, 11, 12). We also found in the present study that 2K1C rats showed significantly higher plasma BNP values than the control. In addition, the finding that indomethacin attenuated the stimulated release of BNP suggests that mechanisms leading to prostaglandin synthesis are involved in the BNP release. It is unlikely that basal BNP release is related with the cyclooxygenase system, however, since indomethacin has no significant effect on the basal level of plasma BNP in normotensive rats.

In summary, the present study demonstrates that BNP

is released in response to prolonged increases of dietary sodium intake and in hypertension. Prostaglandin synthesis may be involved in the stimulated-release of BNP in 2K1C hypertensive rats. It is also suggested that endothelin and NO systems have direct regulatory roles on the secretion of BNP.

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