Chronic Effect of β-Adrenoceptor Blockade on Plasma Levels of Brain Natriuretic Peptide during Exercise in Essential Hypertension

Miho Tanaka, Yuku Ishizaka, Yuichiro Ishiyama, Johji Kato, and Osamu Kida, Kazuo Kitamura, Kenji Kangawa*, and Tanenao Eto

Many factors have been reported to stimulate the release of brain natriuretic peptide (BNP) as well as atrial natriuretic peptide (ANP). In hypertensive patients, however, little is known about whether these factors differ from those in normotensive subjects or if they are influenced by antihypertensive treatment. We measured the plasma concentrations of BNP and ANP in 12 hypertensive patients and examined the chronic effects of β-adrenoceptor blockade on BNP secretion during exercise with a bicycle ergometer. The exercise raised both plasma BNP and ANP with concomitant increases in systolic blood pressure, heart rate (HR) and plasma norepinephrine (NE) and epinephrine (Epi) before and after treatment. Before treatment, the changes in ANP and BNP correlated with that in HR (p<0.05). After treatment 4 wk of treatment, the change in ANP correlated with those in NE and Epi as well as HR. Multivariate regression analysis indicated that only NE was a significant stimulant for ANP secretion during the treatment period. As for BNP, HR was the only significant stimulant for its secretion both before and after treatment. In essential hypertension, β-adrenergic receptor blockade affected the factors stimulating exercise-induced ANP release but not those stimulating BNP release. BNP release, therefore, seems to be stimulated by similar but distinct factors from those that stimulate ANP release. (Hypertens Res 1996; 19: 239-245)

Key Words: atrial natriuretic peptide, brain natriuretic peptide, essential hypertension, exercise, β-adrenoceptor blockade

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are cardiac hormones synthesized mainly in the heart and secreted into the circulation. ANP has natriuretic and hypotensive activities and acts on its target organs to regulate blood pressure and water-electrolyte balance (1-13). Sudoh et al. originally discovered BNP in the porcine brain. Two distinct human BNPs (hBNPs), hBNP-32 and γ-BNP, were isolated from the human heart, which contains a higher concentration of BNP than the human brain (5, 7, 8). The amino acid sequence of hBNP differs markedly from that of human ANP (hANP) (9, 10). Since BNP has potent natriuretic and hypotensive activities when intravenously administered to rats (5, 11), hBNP is also thought to function together with hANP as a cardiac hormone involved in regulation of blood pressure and maintenance of body fluid homeostasis in humans.

The release of ANP is stimulated by several factors, including tachycardia and volume expansion associated with increased atrial pressure and stretch (4, 14, 15). Previous studies have demonstrated that sympathetic activity is an important factor for ANP release (16, 17). Cody et al. reported an exercise-induced increase in plasma ANP associated with increased plasma catecholamine levels in patients with essential hypertension (18). On the other hand, in patients with essential hypertension who were treated by α-adrenoceptor blockade, plasma ANP decreased, whereas short-term treatment by β-adrenoceptor blockade increased its plasma ANP levels (19).

Plasma BNP levels are elevated in patients with essential hypertension (20, 21), congestive heart failure (22, 23), and end stage renal disease (24, 25). Recently, we have reported that the exercise-induced release of BNP and ANP occurred in response to similar but slightly different sympathetic stimuli in normotensive subjects: ANP release was more sensitive to changes in NE, while BNP release was more sensitive to changes in Epi. On the other hand, in patients with hypertension the major stimulant for the release of both hormones is HR, indicating that the mediators for the release of BNP or ANP are switched by some factors involved in

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Received February 23, 1996; accepted in revised form September 2, 1996.
hypertension (26). However, little is known about factors that stimulate BNP release in humans. Furthermore, there is little information available on the long-term effects of α- and β-blockers on plasma BNP. In the present study, we examined the effects of chronic treatment with a β-blocker on the exercise-induced secretion of both ANP and BNP in patients with essential hypertension in order to elucidate stimuli for the release of these two natriuretic peptides.

Methods

Subjects and Protocol

Twelve patients with essential hypertension (11 men and 1 women, 45.0 ± 2.7 yr) took part in this study. We obtained informed consent from all patients. All patients who participated in the present study were in either the stage I or II of the World Health Organization Classification (WHO), and patients with secondary hypertension were excluded. All patients had been receiving antihypertensive drugs, such as diuretics, α-blockers, β-blockers, calcium antagonists, or ACE inhibitors. These drugs were discontinued at least 4 wk before the study. After an overnight fast, an exercise test was performed. The exercise test consisted of three step-up workloads in a sitting position with a bicycle ergometer (Cybex, Lumnex Inc., Bayshore, NY, USA). Before the exercise was started, the patients rested for 20 min, and then received an initial work-load of 25 W for 4 min. In the second stage, the work-load was 50 W for 4 min, and in the third, 75 W for 4 min. After these work loads, the patients rested again for 20 min. Blood pressure was measured with an automatic sphygmomanometer (Nippon Colin, Komaki, Aichi, Japan), and the electrocardiograph (Cardiosuper, San-ei Co. Ltd., Chiyoda, Tokyo, Japan) was monitored during the study. Blood samples were taken in the sitting position from an antecubital vein at three time points: pre-exercise, at maximum exercise, and during the recovery period. From the next morning after the exercise test, all subjects began to take a β1-selective adrenoceptor blocker (bisoprolol 5 mg/d). After 4 wk of treatment, they underwent a similar exercise test.

Preparation of Plasma Samples

Blood was collected into the ice-cooled tubes containing a 1/10 volume of 1.5% ethylenediamine tetraacetic acid (EDTA) and 500 kallikrein inactivation units (KIU) of aprotinin. Clear plasma was obtained by the centrifugation, repeated twice at 3,000 rpm (15 min, 4°C), and was diluted two-fold with 0.9% saline. The plasma was then loaded onto a Sep-Pak C18 cartridge (1 ml, Waters Associates, Milford, MA, USA) which had been equilibrated with 0.9% saline. The absorbed materials were eluted with 60% CH3CN containing 0.1% TFA, and the eluate was lyophilized. The dried materials were dissolved in 0.1 M sodium phosphate buffer (pH 7.4) containing 0.001% Triton X-100, and were loaded onto an anti-hBNP IgG immunoaffinity column (bed volume, 100 μl) prepared for the isolation of porcine BNP-32 as reported previously (10). In brief, the IgG fraction of antisera raised against hBNP-32 was prepared with Protein A-Sepharose CL-4B (Pharmacia Inc., Piscataway, NJ, USA), and was coupled with Affigel 10 (Bio-Rad Laboratories Ltd., Richmond, CA, USA). After washing the column with 0.1 M sodium phosphate buffer (pH 7.4), the absorbed materials were eluted with 1 M acetic acid containing 10% CH3CN. The samples for ANP were prepared with a similar procedure. Finally, the samples were desalted with a Sep-Pak C18 cartridge (0.3 ml, Waters Associates, Milford, MA, USA) and submitted to radioimmunoassay (RIA) for hANP and hBNP determination.

RIA for hANP and hBNP

The anti-hBNP antiserum raised in rabbits by immunization with hBNP-26-thyroglobulin conjugate recognized both hBNP-26 and hBNP-32 with the same affinity. The RIA for hBNP was performed as reported previously using hBNP-32 as a standard, and the free and bound tracer were separated with the double antibody method (7). When the antisem was used at a final dilution of 1: 210,000, the peptide was measurable in the range of 0.2 to 30 fmol/tube, and there was no cross-reactivity for hANP. The RIA for hANP was carried out as reported previously (27). This RIA system showed 0.004% cross-reactivity for hBNP-32.

Measurement of Other Hormones

Plasma catecholamine concentrations were measured by means of high-performance liquid chromatography combined with the trihydroxyindole fluorometric procedure.

Statistical Methods

All data are represented as the means ± standard error of the mean (SEM). Comparisons of the rest value with the maximum or recovery value were evaluated with ANOVA for the repeated measurements. Comparisons of the baseline values between pre- and post-treatment were performed with the unpaired Student’s t-test. A p value less than 0.05 was considered to indicate statistical significance. Relationships between the plasma natriuretic peptide concentrations and the other hormonal or hemodynamic values during exercise were assessed with simple regression analysis. For further elucidation of complex interrelationships among the changes in ANP, heart rate (HR), norepinephrine (NE), and epinephrine (Epi), multiple regression analysis was applied using the change in ANP as a dependent variable and the changes in other factors as independent variable.

Results

Table 1 shows the baseline characteristics in the hypertensive patients before and after treatment with bisoprolol. No abnormal value was found in body mass index (BMI) or blood chemistry including total protein, total cholesterol, fasting blood
glucose, and creatinine levels. Systolic blood pressure and diastolic blood pressure (SBP/DBP) before treatment (147 ± 3.2/98 ± 2.9 mmHg) were elevated, but decreased significantly (p < 0.05) to 131 ± 3.3/85 ± 3.1 mmHg after 4 wk of treatment with bisoprolol. The plasma ANP concentration after treatment (14.9 ± 2.3 pg/ml) was slightly higher than that before treatment (10.8 ± 2.3 pg/ml). The plasma BNP concentration after treatment (6.4 ± 1.7 pg/ml) was also slightly higher than before treatment (4.4 ± 1.2 pg/ml).

Figure 1 shows the changes in SBP, DBP, and HR during exercise. Both SBP and HR significantly (p < 0.05) increased during exercise before and after treatment. The DBP significantly (p < 0.05) decreased before treatment, whereas the change after treatment was not significant. SBP, DBP, and HR were significantly (p < 0.01) decreased after 4 wk of treatment with the β-blocker.

Changes in the plasma levels of NE and Epi during exercise are shown in Fig. 2. Both plasma NE and Epi levels significantly (p < 0.05) increased during exercise, and returned to the baseline during the recovery period both before and after treatment. The plasma NE level at maximum exercise after treatment was significantly (p < 0.05) higher than that before treatment.

Plasma concentrations of both ANP and BNP were measured with RIA at the same time points as the other variables. As shown in Fig. 3, plasma

### Table 1. Baseline Characteristics Before and After Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (M/F)</td>
<td>12(11/1)</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>45 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>167 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>68 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.0 ± 0.82</td>
<td></td>
</tr>
<tr>
<td>Serum total protein (mg/dl)</td>
<td>6.9 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dl)</td>
<td>173.3 ± 7.5</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>CTR (%)</td>
<td>46.6 ± 1.1</td>
<td>47.6 ± 1.0</td>
</tr>
<tr>
<td>LVH (SV1 + RVs, mV)</td>
<td>3.7 ± 0.2</td>
<td>3.8 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p < 0.05 vs. values before treatment.

Fig. 1. Changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) during exercise before (○—○) and after (●—●) treatment. Rest, at rest; Max, at maximum; Rec, at recovery. *p < 0.05, vs. values at rest. †p < 0.05, ††p < 0.01, vs. values before treatment.
ANP and BNP increased significantly \((p < 0.05)\) during exercise in both the control and the treatment periods, and returned to baseline during the recovery period. Although the response pattern of BNP to exercise after treatment was similar to that before treatment, ANP to exercise after treatment was greater than that before treatment. The plasma ANP level at maximum exercise after treatment, was significantly \((p < 0.05)\) higher than that before treatment. However, there was no significant difference in the maximal level of BNP in response to exercise between before \((8.1 \pm 2.3 \text{ pg/ml})\) and after \((10.9 \pm 3.7 \text{ pg/ml})\) treatment.

Table 2 shows the relations between the changes in the two natriuretic peptides and those in other variables during exercise. Univariate regression analysis revealed a common, significant correlation between the changes in the two natriuretic peptides and that in HR both before and after treatment. The change in ANP significantly \((p < 0.01)\) correlated with those in NE, Epi as well as HR after treatment. Repeatedly, the change in BNP correlated significantly \((p < 0.05)\) with only the change in HR both before and after treatment.

For stepwise multiple regression analysis, the change in ANP was used as a dependent variable and the changes in other variables such as HR, NE and Epi, as independent variables (Table 3). Only NE was significantly related to the change in ANP: standardized regression coefficients for HR, NE, and Epi were 0.001 (NS), 0.493 \((p < 0.05)\), and 0.408 (NS), respectively, and multiple R2 was 0.42.

**Discussion**

Both ANP and BNP are known to regulate body fluid homeostasis and blood pressure via hormonal and neural pathways (5). The plasma concentrations of these natriuretic peptides have been reported to increase in patients with congestive heart failure,
end stage renal disease, and essential hypertension (20-25) as compared with normal subjects. Several factors have been reported to stimulate ANP release in humans or experimental animals. These factors include an increase in systemic arterial pressure, atrial stretch, α- or β-adrenergic agonists, and vasopressin (4, 14-18, 28-30). These factors are also considered stimulants for BNP secretion; however, little is known with respect to differences in these factors between ANP and BNP. Thus, we previously studied factors that stimulate the secretion of these peptides not only during rest but also after exercise (26). The most remarkable findings were obtained after exercise, when ANP and BNP secretion was mediated mainly by NE or Epi in normal subjects and by HR in hypertensive patients. That study indicated that the mechanism responsible for the release of these peptides is altered by some factors involved in hypertension. The present study sought to determine if this mechanism is restored by antihypertensive treatment. Since catecholamines are the primary mediators of ANP and BNP secretion in normal subjects, β-adrenoceptor blockade was used for antihypertensive therapy.

There have been many studies of the short-term effects of β-adrenoceptor blockade on plasma ANP (19), but few have examined effects on BNP. Moreover, the chronic effect of β-adrenoceptor blockade on plasma BNP levels remains unknown. To our knowledge, this is the first report of the long-term effect of β-adrenoceptor blockade on BNP and ANP secretion in essential hypertension. In the present study, the baseline levels of plasma ANP and BNP after treatment tended to increase as compared with before treatment. Several factors may participate in the response of these peptides to β-adrenoceptor blockade. β-blockers usually cause bradycardia, decrease cardiac output (31) and in-
crease total peripheral resistance early after the initiation of treatment. These effects decrease with long-term treatment. The hemodynamic changes caused by β-adrenoceptor blockade may increase left ventricular filling pressure, resulting in atrial overload, which in turn stimulates the release of BNP and ANP. Another possible explanation for the β-blocker-induced increase in plasma BNP and ANP levels is exaggerated sympathetic activity associated with β-adrenoergic receptor blockade. When the α-adrenergic receptors are blocked, the plasma ANP level has been reported to fall (19, 35). Conversely, α-adrenergic stimulation increases the plasma ANP level and ANP secretion from the isolated rat heart (17, 34, 35). These findings suggest that α-adrenergic stimulation augmented by β-adrenergic receptor blockade triggers BNP release from the heart as well as ANP secretion. Furthermore, the influence of physical exercise on both BNP and ANP secretion was studied before and after long-term β-blocker administration. Plasma ANP and BNP levels significantly increased during exercise before and after treatment, returning to the baseline levels during the recovery period. Before treatment, the change in HR was the only significant factor for exercise-induced ANP release. These findings were consistent with those in our previous study in hypertensive patients (26). After β-blocker treatment, the exercise-induced ANP release significantly correlated with the increases in NE and Epi as well as the increase in HR. Despite the bradycardic effect of β-blocker treatment, the response of HR to exercise was dampened but still remained. However, in a previous study in normotensive subjects we have shown that changes in SBP, HR, and NE were significant stimuli for exercise-induced ANP release, among which NE was found to be the main factor on multiple regression analysis (26). These data raise the possibility that the mechanisms responsible for ANP release in essential hypertension were altered by some pathological factor, and further suggest that some of the mechanism can be restored, at least partially by the long-term β-blocker therapy. Although the exact mechanisms involved are unknown, as mentioned above, hemodynamic changes caused by β-adrenoceptor blockade, α-adrenergic receptor dominancy after β-blocker administration, or both may be

<table>
<thead>
<tr>
<th>Table 2. Correlation Coefficient between Changes in Natriuretic Peptides and Changes in Other Variables</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔANP vs. ΔSBP</td>
<td>0.073</td>
<td>0.337</td>
</tr>
<tr>
<td>ΔDBP</td>
<td>0.031</td>
<td>0.290</td>
</tr>
<tr>
<td>ΔHR</td>
<td>0.684*</td>
<td>0.663*</td>
</tr>
<tr>
<td>ΔNE</td>
<td>0.003</td>
<td>0.685*</td>
</tr>
<tr>
<td>ΔEpi</td>
<td>0.156</td>
<td>0.639*</td>
</tr>
<tr>
<td>ΔBNP vs. ΔSBP</td>
<td>0.166</td>
<td>0.322</td>
</tr>
<tr>
<td>ΔDBP</td>
<td>0.367</td>
<td>0.346</td>
</tr>
<tr>
<td>ΔHR</td>
<td>0.830*</td>
<td>0.634*</td>
</tr>
<tr>
<td>ΔNE</td>
<td>0.109</td>
<td>0.453</td>
</tr>
<tr>
<td>ΔEpi</td>
<td>0.249</td>
<td>0.481</td>
</tr>
<tr>
<td>ΔANP vs. ΔBNP</td>
<td>0.657*</td>
<td>0.773*</td>
</tr>
</tbody>
</table>

ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; NE, plasma norepinephrine; Epi, plasma epinephrine; *p < 0.05.

| Table 3. Relation between Change in Atrial Natriuretic Peptide (ΔANP) After Exercise and Changes in Correlating Variables by Stepwise Multiple Regression Analysis |
|-----------------------------------------------|----------------|----------------|----------------|----------------|
| | Dependent variable | Independent variable | β | t-value |
| ΔANP | ΔHR | 0.001 | 1.013 |
| ΔANP | ΔNE | 0.493 | 0.915* |
| ΔEpi | 0.408 | 1.997 |

HR, heart rate; NE, plasma norepinephrine; Epi, plasma epinephrine; β, standardized regression coefficient. *p < 0.05.
involved in the restoration of mechanisms for the stimulation of ANP secretion.

The present study in hypertensive patients demonstrated that the major factor responsible for the exercise-induced release of BNP was the change in HR, not only before but also after long-term β-blocker treatment. These findings indicate that BNP was released in response to rather different stimuli than those that stimulate ANP secretion. BNP is thought to be secreted mainly from the cardiac ventricles in response to many stimulating factors, even though it is also present in granules in the atrium, similar to ANP. Since the exercise-induced BNP release significantly correlated with HR and the change in HR also stimulates the release of these peptides from the atria, BNP may in fact originate primarily from the atria. Furthermore, the effect of β-blocker treatment on BNP secretion was also different from that on ANP secretion. Consequently, ANP release in response to sympathetic stimulation may be more sensitive that BNP release during β-blocker treatment.

In summary, the plasma levels of both ANP and BNP after 4 wk of treatment with bisoprolol were significantly higher than those before treatment. The exercise-induced release of both peptides was stimulated by the change in HR before treatment. After bisoprolol administration, however, the main stimulant for ANP release shifted from the change in HR to the change in NE, similar to the stimulation in normotensive subjects. These data suggest that β-blockers may restore mechanisms contributing to ANP secretion in essential hypertension by changing the dynamics of sympathetic activity. In contrast, the role of HR as the main stimulant for BNP release persisted even after long-term β-blocker treatment. These findings indicate that BNP was released in response to similar but distinctly different stimuli from those promoting the release of ANP, and also that BNP secretion was relatively resistant to β-blockade. Thus, it appears that BNP and ANP are respectively released by similar yet distinct regulators and function in harmony as cardiac hormones.

References


