Different Secretion Patterns of Atrial Natriuretic Peptide and Brain Natriuretic Peptide in Patients With Congestive Heart Failure

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Background. The plasma levels of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are increased in relation to the severity of congestive heart failure (CHF). This study was designed to examine whether the secretion patterns of ANP and BNP vary with underlying cardiac disorders of CHF with different degrees of overload in atria and ventricles.

Methods and Results. We measured plasma levels of ANP and BNP in the aorta in 20 patients with mitral stenosis (MS) in whom atria are mainly overloaded, 30 patients with dilated cardiomyopathy (DCM) in whom both atria and ventricles are overloaded, and 20 control subjects during cardiac catheterization. Pulmonary capillary wedge pressure (PCWP) was significantly higher in the MS and DCM groups (16.7±4.7 mm Hg and 15.1±7.7 mm Hg, respectively) than in the control group (7.2±1.1 mm Hg, p<0.01), whereas there was no significant difference between the MS and DCM groups. Left ventricular end-diastolic pressure (LVEDP) was significantly higher in the DCM group than in the MS group (16.4±7.8 mm Hg versus 7.6±2.0 mm Hg, p<0.01), and the level was comparable between the MS and control groups (7.6±2.0 mm Hg versus 6.8±1.2 mm Hg, p=NS). The plasma ANP level was significantly higher in the MS and DCM groups (356±169 pg/ml and 331±323 pg/ml, respectively) than in the control group (98±41 pg/ml, p<0.01), whereas there was no significant difference between the MS and DCM groups. The plasma BNP level was significantly higher in the DCM group than in the MS group (333±405 pg/ml versus 147±54 pg/ml, p<0.01), and the level was significantly higher in the MS group than in the control group (147±54 pg/ml versus <10 pg/ml, p<0.01). The plasma levels of ANP and BNP had a highly positive correlation with PCWP in the DCM group (p<0.01). On the other hand, in the MS group, the plasma ANP level had a highly significant correlation with PCWP (p<0.01) but the plasma BNP level did not.

Conclusions. We conclude that plasma levels of BNP mainly reflect the degree of ventricular overload and that the secretion patterns of ANP and BNP vary with underlying cardiac disorders of CHF with different degrees of overload in atria and ventricles. (Circulation 1993;87:464-469)

Key words: atrial natriuretic peptide • brain natriuretic peptide • cardiomyopathy, dilated • mitral stenosis

Atrial natriuretic peptide (ANP) has a wide range of potent biological effects including natriuresis, vasodilatation, and inhibition of renin and aldosterone secretion1-4 and plays an important role in the homeostasis of body fluids and blood pressure. The secretion of ANP is mainly regulated by stretch of atria, and its plasma levels are augmented in patients with congestive heart failure (CHF).5-10 We recently have shown that ANP is secreted from atria not only via the coronary sinus but also directly into the atrial cavity11 and that ANP is also secreted from ventricles of the patients with dilated cardiomyopathy.10,12,13

Brain natriuretic peptide (BNP) was first isolated from a porcine brain14 and subsequently from the hearts of pigs15 and rats.16-18 BNP forms a peptide family with ANP and may be involved in the regulation of blood pressure and fluid volume, as is ANP.19-21 We isolated human BNP from the human atrium and clarified its sequence of 32 amino acids.22 We established a specific radioimmunoassay for human BNP by developing a monoclonal antibody against it,23-25 and we demonstrated that BNP is a novel cardiac hormone secreted mainly from the ventricle in patients with CHF and hypertension23-27 and that plasma BNP levels are markedly increased in patients with CHF in proportion to its severity.23-25 The finding that the main source of the BNP secretion is the ventricle has raised the possibility that heart disorders with different degrees of overload in atria and ventricles may have different patterns of ANP and BNP secretion and different plasma levels of ANP and BNP. In the present study, we examined the
secretion patterns of ANP and BNP in patients with mitral stenosis (MS) in whom the atria are mainly overloaded and in those with dilated cardiomyopathy (DCM) in whom both the atria and ventricles are overloaded by measuring plasma levels of ANP and BNP using specific radioimmunoassays.

Methods

Patients
Study patients comprised three groups: the DCM group, the MS group, and the control group. The DCM group consisted of 30 patients (18 men and 12 women; ages ranging from 42 to 71 years, mean age, 58 years); the MS group consisted of 20 patients (12 men and eight women; ages ranging from 38 to 75 years, mean age, 51 years); and the control group consisted of 20 patients (11 men and nine women; ages ranging from 32 to 64 years, mean age, 47 years). According to New York Heart Association (NYHA) functional classification, 10 patients were in class I, nine patients were in class II, and 11 patients were in class III–IV in the DCM group; seven patients were in class I, six patients were in class II, and seven patients were in class III–IV in the MS group. The diagnosis of DCM was based on clinical history, physical examination, chest x-ray, ECG, echocardiogram, and cardiac catheterization including left ventriculography and coronary arteriography. Endomyocardial biopsy was also performed in each patient. The diagnosis of MS was based on clinical history, physical examination, chest x-ray, echocardiogram, and cardiac catheterization. Among 20 patients with MS, six patients had grade I mitral regurgitation (MR) according to the criteria by Nagle et al.,28 three patients had grade I aortic regurgitation (AR) according to the criteria by Sellers et al.,29 and six patients had both grade I MR and grade I AR. The patients received no diuretics or digitalis for 2 days before the study. Control subjects had been suspected of ischemic heart disease because of chest pain or ECG changes but subsequently were found to have no ischemic heart disease after catheterization. All of the control subjects had no other diseases and were not receiving medications.

Written informed consent was obtained from each patient and his or her family. This study protocol was in agreement with the guidelines of the ethical committee of our institution.

Cardiac Catheterization
Cardiac catheterization was performed in the morning with the patient in the fasting state. Using a Swan-Ganz catheter inserted into the femoral or subclavian vein, hemodynamic measurements including pulmonary arterial pressure, pulmonary capillary wedge pressure (PCWP), right atrial pressure, and cardiac output were performed. Cardiac output was determined by the thermodilution technique in triplicate. A Sones catheter then was placed through a brachial artery at the root of the aorta. Systemic pressure and left ventricular end-diastolic pressure (LVEDP) were measured, and blood samples for the measurement of the plasma levels of ANP and BNP in the aorta were obtained. After the examination of the pressure study and blood sampling, coronary angiography and left ventriculography were performed in each patient. Left ventricular ejection fraction (LVEF) was determined by left ventriculography. Endomyocardial biopsy was also performed in the patients with DCM.

Measurement of Plasma Levels of ANP and BNP
All blood samples were withdrawn into chilled plastic syringes and transferred to chilled siliconized disposable tubes that contained aprotinin (1,000 kallikrein inactivator units/ml) (Ohkura Pharmaceutical, Kyoto, Japan) and EDTA (1 mg/ml) and immediately placed on ice and centrifuged at 4°C. An aliquot of plasma was immediately frozen at ~80°C and thawed only once at the time of extraction. Plasma ANP concentration was measured with a specific radioimmunoassay for α-human ANP as previously reported.30,31 This radioimmunoassay recognizes a carboxyterminal sequence of ANP, and the minimal detectable quantity of α-human ANP is 1 pg per tube. The intra-assay and interassay coefficients of variation were 7.2% and 7.8%, respectively. The cross-reactivity with human BNP was <0.01% on a molar basis. The plasma BNP concentration was measured with a specific radioimmunoassay using a monoclonal antibody that recognizes the ring structure of human BNP, as previously reported.32–35 Cross-reaction for α-human ANP was <0.005% on a molar basis. The intra-assay and interassay coefficients of variation were 8.4% and 6.4%, respectively.

Statistical Analysis
Hemodynamic parameters and plasma levels of ANP and BNP were compared among more than three groups using one-way ANOVA followed by Scheffe’s test36 and between the MS and DCM groups in each stage of NYHA classification using the unpaired t test. The correlation of the plasma levels of ANP and BNP with hemodynamic parameters including PCWP, LVEDP, and LVEF was examined using a separate linear regression analysis in the MS and DCM groups. When there was not a significant linear correlation of ANP or BNP with a functional variable in the bivariate analyses, we hypothesized that the presence of MR or AR might be masking a relation. Thus, after first verifying that MR and AR were independent of each other and of the functional variable of interest (by Spearman’s method or a χ² test), we performed a multiple linear regression analysis33 with the plasma level of ANP or BNP as the dependent variable and MR, AR, and the functional variable as independent variables. When presence or absence of MR and AR was coded as a dummy variable (i.e., 0 = absence, 1 = presence), the general linear model to predict the plasma ANP or BNP level using hemodynamic parameter (PCWP, LVEDP, or LVEF), MR, and AR was as follows: Plasma level of ANP or BNP = C₁ + C₂(heemodynamic parameter) + C₃(MR) + C₄(AR) with a constant (C₁) and three linear coefficients (C₂, C₃, C₄).

All values are expressed as mean±SD. Statistical significance was defined as a probability value of less than 0.05.

Results

Hemodynamic Study and Plasma Levels of ANP and BNP
Table 1 shows the results of the hemodynamic study and the plasma levels of ANP and BNP.
PCWP increased significantly in proportion to the severity of NYHA classification in both the MS and the DCM groups. LVEDP increased significantly in proportion to the severity of NYHA classification in the DCM group and was significantly higher in the DCM group than in the MS group in each class. Cardiac index (CI) decreased significantly as the severity of NYHA classification increased in both the MS and the DCM groups, and there was no significant difference in the level between the two groups in any class. LVEF decreased significantly in proportion to the severity of NYHA classification in the DCM group but did not change in the MS group. LVEF was significantly lower in the DCM group than in the MS group in each class.

The plasma ANP level increased significantly in proportion to the severity of NYHA classification in both the MS and the DCM groups. There was, however, no significant difference in the plasma ANP level between the two groups in any class. The plasma BNP level was significantly higher in NYHA class I than in the control group and in NYHA class II than in class I, but there was no significant difference in the level between class II and class III–IV in the MS group. On the other hand, the plasma BNP level increased significantly in proportion to the severity of NYHA classification in the DCM group. The plasma BNP level was significantly higher in the DCM group than in the MS group in each class.

PCWP was significantly higher in the MS and DCM groups (16.7±4.7 mm Hg and 15.1±7.7 mm Hg, respectively) than in the control group (7.2±1.1 mm Hg, p<0.01), whereas there was no significant difference between the MS and DCM groups. LVEDP was significantly higher in the DCM group than in the MS group (16.4±7.8 mm Hg versus 7.6±2.0 mm Hg, p<0.01), and the level was comparable between the MS and control groups (7.6±2.0 mm Hg versus 6.8±1.2 mm Hg, p=NS). CI was significantly lower in the MS and DCM groups (2.6±0.6 l per min/m² and 2.3±0.7 l per min/m², respectively) than in the control group (3.2±0.3 l per min/m², p<0.01) whereas there was no significant difference between the MS and DCM groups. LVEF was significantly lower in the MS group than in the DCM group (31.7±13.1% versus 70.5±6.5%, p<0.01), and the level was comparable between the DCM and control groups (70.5±6.5% versus 75.9±4.3%, p=NS).

The plasma ANP level was significantly higher in the MS and DCM groups (356±169 pg/ml and 331±323 pg/ml, respectively) than in the control group (98±41 pg/ml, p<0.01), whereas there was no significant difference between the MS and DCM groups. The plasma BNP level was significantly higher in the DCM group than in the MS group (333±405 pg/ml versus 147±54 pg/ml, p<0.01), and the level was significantly higher in the MS group than in the control group (147±54 pg/ml versus <10 pg/ml, p<0.01).

**Univariate Analysis**

The correlations of the plasma ANP and BNP levels with hemodynamic parameters including PCWP, LVEDP, and LVEF in the MS and DCM groups are shown in Figures 1–3. When calculated on a separate linear regression analysis, there was a significant positive linear correlation between the plasma ANP level and PCWP in both the MS group (r=0.510, p<0.05) and the DCM group (r=0.710, p<0.01). However, a significant positive linear correlation was found between the plasma BNP level and PCWP only in the DCM group (r=0.611, p<0.01), and there was no significant corre-

<table>
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<th>TABLE 1. Hemodynamic Study and Plasma Levels of ANP and BNP</th>
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<td>Control</td>
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<td>PCWP (mm Hg)</td>
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<tr>
<td>MS</td>
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<tr>
<td>DCM</td>
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<tr>
<td>LVEDP (mm Hg)</td>
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<tr>
<td>MS</td>
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<td>DCM</td>
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<tr>
<td>CI (l per min/m²)</td>
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<td>LVEF (%)</td>
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<td>DCM</td>
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<td>ANP (pg/ml)</td>
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<td>DCM</td>
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<td>BNP (pg/ml)</td>
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<td>MS</td>
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<td>DCM</td>
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PCWP, pulmonary capillary wedge pressure; LVEDP, left ventricular end-diastolic pressure; CI, cardiac index; LVEF, left ventricular ejection fraction; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; NYHA, New York Heart Association; MS, mitral stenosis; DCM, dilated cardiomyopathy.

Values are expressed as mean±SD. *p<0.01 vs. control; †p<0.01 vs. NYHA class I; ‡p<0.01 vs. NYHA class II; $p<0.01 MS vs. DCM.
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ation between the plasma BNP level and PCWP in the MS group ($r=0.160, p=NS$) (Figure 1). There was a significant positive linear correlation between the plasma ANP level and LVEDP in the DCM group ($r=0.610, p<0.01$) but not in the MS group ($r=0.091, p=NS$). There was a significant positive linear correlation between the plasma BNP level and LVEDP in the DCM group ($r=0.610, p<0.01$) but not in the MS group ($r=0.143, p=NS$) (Figure 2). There was a significant negative linear correlation between the plasma ANP level and LVEF in the DCM group ($r=-0.457, p<0.05$) but not in the MS group ($r=0.089, p=NS$). There was a significant negative linear correlation between the plasma BNP level and LVEF in the DCM group ($r=-0.465, p<0.05$) but not in the MS group ($r=0.121, p=NS$) (Figure 3).

Multiple Linear Regression Analysis

Table 2 shows the results of the multiple linear regression analysis. There was a significant correlation between the plasma BNP level and PCWP in the MS group as shown in Table 2 (A) ($p<0.05$). There was, however, no significant correlation between other combinations (Table 2, B–D).

Discussion

BNP, the recently discovered natriuretic peptide, was isolated first from the brains of pigs and subsequently from the hearts of pigs and rats. BNP forms a natriuretic peptide family with ANP and may be involved in the regulation of blood pressure and blood volume, as is ANP. BNP has structural diversity among species, and we have determined human BNP as a 32-amino acid sequence. It has been subsequently elucidated that the biological actions of BNP are species specific, unlike those of ANP. We have also shown that BNP is secreted predominantly from the ventricles and that its plasma levels are markedly increased in patients with CHF in relation to its severity and surpass those of ANP in severe cases. We and others have shown that both ANP and BNP administered intravenously improve left ventricular function in patients with CHF by relieving both preload and afterload. These accumulating findings have raised the possibility that the secretion patterns of ANP and BNP in patients with CHF vary not only with the degree of CHF but also with underlying cardiac disorders with
In the stratification of patients by NYHA classification as shown in Table 1, both plasma levels of ANP and BNP increased in relation to the severity of CHF in the DCM group. However, in the MS group, only the plasma ANP level increased in relation to the severity of CHF and not the plasma BNP level. There was no significant difference in PCWP between the MS and DCM groups, indicating that the degree of overload in the left atrium was comparable between the two groups. The plasma ANP level was also comparable between the MS and DCM groups. On the other hand, the plasma BNP level was much higher in the DCM group than in the MS group.

As shown in Figures 1–3, the plasma levels of ANP and BNP had significant correlations with PCWP, LVEDP, and LVEF in the DCM group. However, in the MS group, a significant correlation was found only between the plasma ANP level and PCWP; the plasma BNP level did not correlate with PCWP, LVEDP, or LVEF in the MS group when calculated using a separate linear regression analysis.

All of these results clearly indicate that the plasma level of BNP predominantly reflects the degree of left ventricular overload and not necessarily that of atrial overload. This is in sharp contrast with that of ANP, which reflects mainly atrial overload.

The plasma BNP level was significantly higher in the MS group than in the control group. In the present study, the MS group included not only the patients with pure MS but also those with MS combined with grade I MR and/or AR. The left ventricular dysfunction induced by MR, AR, or MS may have increased left ventricular overload and augmented BNP secretion from the ventricle even in the patients with MS. A multiple linear regression analysis has shown that the plasma level of BNP is weakly but significantly correlated with PCWP even in the MS group. This finding is in agreement with our previous reports showing that BNP is secreted also from atria, although the amount of BNP secreted from atria is very small compared with that from ventricles in patients with CHF.

There was no significant correlation of the plasma ANP and BNP levels with LVEDP and LVEF in the MS group. This is probably because the left ventricular function was not impaired as much in the MS group.

The present study clearly demonstrates the different plasma levels and secretion patterns of BNP between the

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**TABLE 2. Multiple Linear Regression Analysis in the Mitral Stenosis Group**

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<tr>
<th>Variable</th>
<th>Coefficient and its significance</th>
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<tr>
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<td>(A)BNP (B)ANP (C)BNP (D)ANP (E)BNP</td>
</tr>
<tr>
<td>C, constant</td>
<td>12.84 -34.70 70.66 441.04 241.60</td>
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<tr>
<td>C, PCWP</td>
<td>3.70, p&lt;0.05 42.92, NS 0.95, NS</td>
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<tr>
<td>LVEDP</td>
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<tr>
<td>LVEF</td>
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<tr>
<td>C, MR</td>
<td>46.30, p&lt;0.05 28.14, NS 52.61, p&lt;0.05 58.81, NS 51.58, p&lt;0.01</td>
</tr>
<tr>
<td>C, AR</td>
<td>69.47, p&lt;0.01 -18.72, NS 59.47, p&lt;0.01 36.09, NS 53.07, p&lt;0.01</td>
</tr>
<tr>
<td>R²</td>
<td>0.68 0.28 0.59 0.057 0.654</td>
</tr>
</tbody>
</table>

BNP, brain natriuretic peptide; ANP, atrial natriuretic peptide; PCWP, pulmonary capillary wedge pressure; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; MR, mitral regurgitation; AR, aortic regurgitation.

Results are of a multiple linear regression analysis between the plasma BNP level and PCWP (A), the plasma ANP level and LVEDP (B), the plasma BNP level and LVEDP (C), the plasma ANP level and LVEF (D), and the plasma BNP level and LVEF (E) in the mitral stenosis group.
patients with MS and those with DCM, even though the MS group included not only the patients with pure MS but also those with MS combined grade I MR and AR.

Conclusions

The present study shows that underlying cardiac disorders of CHF with different degrees of overload in the atria and ventricles have different patterns of ANP and BNP secretion. The present results are also in agreement with our previous findings23–27 that BNP is a cardiac hormone secreted mainly from the ventricle.

References


M Yoshimura, H Yasue, K Okumura, H Ogawa, M Jougasaki, M Mukoyama, K Nakao and H Imura

Circulation. 1993;87:464-469
doi: 10.1161/01.CIR.87.2.464

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