Localization and Mechanism of Secretion of B-Type Natriuretic Peptide in Comparison With Those of A-Type Natriuretic Peptide in Normal Subjects and Patients With Heart Failure

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**Background** B-type or brain natriuretic peptide (BNP) is a novel natriuretic peptide secreted from the heart that forms a peptide family with A-type or atrial natriuretic peptide (ANP), and its plasma level has been shown to be increased in patients with congestive heart failure. This study was designed to examine the sources and mechanisms of the secretion of BNP in comparison with those of ANP in control subjects and in patients with heart failure.

**Methods and Results** We measured the plasma levels of BNP as well as ANP in 16 patients with dilated cardiomyopathy (11 men and 5 women; mean age, 59 years) and 18 control subjects (9 men and 9 women; mean age, 54 years) by sampling blood from the femoral vein, the aortic root, the anterior interventricular vein (AIV), and the coronary sinus using the newly developed immunoradiometric assay systems. In the control subjects, there was no significant difference in the plasma ANP level between the aortic root and the AIV (24.0±5.2 pg/mL versus 32.2±17.0 pg/mL), but there was a highly significant step-up of the level between the AIV and the coronary sinus (32.2±17.0 pg/mL versus 371.4±111.1 pg/mL, P<.001). In contrast, there was a significant step-up of the plasma BNP level between the aortic root and the AIV (8.6±6.4 pg/mL versus 19.0±11.5 pg/mL, P<.01) but not between the AIV and the coronary sinus (19.0±11.5 pg/mL versus 28.8±14.0 pg/mL). On the other hand, in patients with dilated cardiomyopathy, there was a significant step-up in the plasma ANP level between the aortic root and the AIV (280.6±183.7 pg/mL versus 612.3±431.6 pg/mL, P<.01) and between the AIV and the coronary sinus (612.3±431.6 pg/mL versus 1229.0±772.7 pg/mL, P<.01). There was a significant step-up in the plasma BNP level between the aortic root and the AIV (268.4±293.2 pg/mL versus 511.6±458.1 pg/mL, P<.01) but not between the AIV and the coronary sinus (511.6±458.1 pg/mL versus 520.7±455.3 pg/mL) in patients with dilated cardiomyopathy. The arteriovenous difference at the AIV of the plasma level of BNP had a significant positive correlation with left ventricular end-systolic volume index (r=0.859, P<.001) and a significant negative correlation with left ventricular ejection fraction (r=−.735, P<.001).

**Conclusions** We conclude that (1) BNP is secreted mainly from the left ventricle in normal adult humans as well as in patients with left ventricular dysfunction, whereas ANP is secreted from atria in normal adult humans and also from the left ventricle in patients with left ventricular dysfunction; (2) secretion of BNP as well as ANP from the left ventricle increases in proportion to the severity of the left ventricular dysfunction, suggesting that the secretions of ANP and BNP from the left ventricle are regulated mainly by wall tension of the left ventricle; and (3) the peripheral plasma levels of ANP and BNP reflect the secretion rate of these hormones from the left ventricle and may be used as a marker of the degree of left ventricular dysfunction in patients with left ventricular dysfunction. (Circulation. 1994;90:195-203.)

**Key Words** • heart failure, congestive • veins • ventricles

A-type or atrial natriuretic peptide (ANP) is a hormone with a wide range of potent biological effects, including natriuresis, diuresis, vasodilatation, and inhibition of the renin-angiotensin-aldosterone system and the sympathetic nervous system.1-6 ANP is mainly synthesized in and secreted from atria in adult mammals, and its plasma levels are increased in patients with congestive heart failure.1-5,7-10 There is a positive linear relation between plasma ANP level and atrial pressure, indicating that atrial pressure or stretch plays an important role in regulating secretion of ANP.10,11 We and others have shown that ANP is synthesized in and secreted also from ventricles in patients with congestive heart failure.12-15

B-type or brain natriuretic peptide (BNP), first isolated from porcine brain16 and subsequently from the hearts of humans as well as pigs and rats,17,18 forms a peptide family with ANP and may be involved in the regulation of blood pressure and fluid volume. We isolated human BNP from the human atrium and clarified its sequence of 32 amino acids.19 We have established a specific radioimmunoassay for human BNP by developing a monoclonal antibody against it and have shown that BNP is a novel cardiac hormone secreted mainly from the ventricles in patients with congestive heart failure and hypertension and that plasma levels of BNP are markedly increased in patients with congestive heart failure.20-22 Recently, Wei and his coworkers23

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also reported that plasma BNP levels are increased in patients with heart failure.

However, the mechanism of the secretion of BNP is not known in patients with heart failure. The localization and mechanism of secretion of BNP are also not known in normal subjects mainly because plasma BNP level in normal subjects is very low, and its measurement requires a fairly large amount of blood by the extraction method previously used. Very recently, we have developed a highly sensitive and specific immunoradiometric assay system that measures plasma levels of human BNP and ANP without extraction by using two monoclonal antibodies, one recognizing the ring structure and the other the C-terminal of the peptides, respectively.24-26

We have shown that ANP is released into the general circulation mainly by way of the coronary sinus.7,27 Because the coronary sinus receives blood from both the atria and ventricles, and because the anterior interventricular vein (AIV), which lies in the anterior interventricular groove and empties into the coronary sinus, drains the ventricles only (mainly the left ventricle and not the atria28-30), the difference of plasma levels of ANP and BNP between the AIV and the aortic root reflects the amount of the hormones released mainly from the left ventricle and not the atria.14

The present study was designed to examine the localization and mechanism of secretion of BNP in comparison with those of ANP, in normal subjects as compared with patients with heart failure, by sampling blood for ANP and BNP from the aortic root, the AIV, and the coronary sinus using this newly developed assay system.

Methods

Patients

Sixteen patients were the subjects of this study: 11 men and 5 women (ages ranging from 48 to 72 with a mean age of 59 years) with dilated cardiomyopathy (DCM) in whom diagnostic cardiac catheterization was performed and in whom insertion of a catheter into the AIV was possible. The diagnosis of DCM was based on history, physical examination, chest roentgenogram, ECG, echocardiogram, cardiac catheterization, and angiography including left ventriculography and coronary arteriography. Endomyocardial biopsy was also done in 8 of the patients. All patients were free of ischemic heart disease, hypertension, valvular heart disease, congenital malformation of heart and vessels, or intrinsic pulmonary or renal diseases. The mean serum creatinine level in the study patients was 0.80±0.20 mg/dL, with a range of 0.6 to 1.3 mg/dL. Eleven of the patients had been taking diuretics singly (n=2), in combination with digitalis (n=2), in combination with digitalis and nitrates (n=2), and in combination with angiotensin-converting enzyme inhibitors (n=5). Three patients had been on angiotensin-converting enzyme inhibitors alone, and 2 patients had been receiving no drugs. Angiotensin-converting enzyme inhibitors were withdrawn for 3 to 5 days before the study in all 8 patients who had been taking these drugs. All other drugs were discontinued at least 1 day before the study.

We selected as control subjects 18 patients without heart muscle diseases and heart failure (9 men and 9 women, ages ranging from 40 to 73, with a mean age of 54 years) in whom diagnostic cardiac catheterization, including coronary arteriography and left ventriculography, was performed and in whom insertion of a catheter into the AIV was possible. The group consisted of 15 patients with chest pain syndrome with normal coronary arteriograms and 3 patients with ECG abnormalities with normal coronary arteriograms. None of them had myocardial infarction, hypertension, cardiac hypertrophy, or other heart muscle diseases. None of them was receiving therapy at the time of the study. The mean serum creatinine level in the control subjects was 0.75±0.19 mg/mL, with a range of 0.5 to 1.1 mg/mL.

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 at our institution.

Cardiac Catheterization

Cardiac catheterization was performed in the morning with patients 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 made. Cardiac output was determined by the thermodilution technique in tripleate. After the right heart catheterization was done, a 6F Goodale-Lubin catheter was placed in the coronary sinus by way of a brachial vein. The catheter was then advanced to the AIV under fluoroscopy by using a guide wire. The position of the catheter tip in the AIV was confirmed by injection of contrast dye. This was the critical part of the study, and the position in the right atrium and the proximal half of the AIV was not visualized were excluded from the study. A Sones catheter was placed at the root of the aorta by way of a brachial artery. Then sampling of blood for ANP and BNP was done simultaneously at the root of the aorta, the AIV, the coronary sinus, and the femoral vein, with care taken to draw blood samples slowly from the AIV. Systemic arterial pressure and left ventricular end-diastolic pressure (LVEDP) were then measured, and coronary arteriography and left ventriculography were performed in each patient. Left ventricular ejection fraction (LVEF), end-systolic volume index (LVESVI), and end-diastolic volume index (LVEDVI) were determined with the left ventriculogram.

Measurement of Plasma Levels of ANP and BNP

An aliquot of plasma was immediately frozen at −80°C. All blood samples were withdrawn into chilled plastic syringes, transferred to chilled siliconized disposable tubes containing aprotonin (1,000 kallikrein inactivator units per milliliter; Ohkura Pharmaceutical) and EDTA (1 mg/mL), and immediately placed on ice and centrifuged at 4°C. Samples were thawed only once, at the time of analysis.

Plasma ANP concentration was measured with a specific immunoradiometric assay for α-human ANP (Shionoria ANP kit) as previously reported.31 This assay system uses two monoclonal antibodies against α-human ANP, one recognizing a carboxyterminal sequence and the other the ring structure of ANP, and measures α-human ANP by sandwiching it between the two antibodies without extraction of plasma. The minimal detectable quantity of α-human ANP is 5 pg/mL. The intra-assay and interassay coefficients of variation were 4.7% and 5.8%, respectively, and the recovery rate of 20 to 600 pg/mL of α-human ANP added to plasma was 99.2±3.0%. The correlation between the plasma level of α-human ANP measured by this method and that by the extraction method was highly significant, in the range of 20 to 1500 pg/mL (r=0.97, P<.001). The cross-reactivity with human BNP was <0.001% on a molar basis.

The plasma BNP concentration was measured with a new and specific immunoradiometric assay for human BNP as previously reported.24-26 This assay system uses two monoclonal antibodies against human BNP, one recognizing a carboxyterminal sequence and the other the ring structure of human BNP, and measures human BNP by sandwiching it between the two antibodies without extraction of plasma. The minimal detectable quantity of human BNP is 2 pg/mL. The intra-assay
and interassay coefficients of variation were 5.3% and 5.9%, respectively, and the recovery rate of 10 to 300 pg/mL of human BNP added to plasma was 105.7±5.4%. The correlation between the plasma level of human BNP measured by this method and that by the extraction method was highly significant, in the range of 0 to 1500 pg/mL (r=.98, P<.001). Cross-reactivity for α-human ANP was <0.001% on a molar basis. The plasma samples frozen at −80°C were stable for the assays for more than 20 days after sampling, and all samples were measured within 2 weeks after sampling.

Statistical Analysis

Hemodynamic parameters and plasma levels of ANP and BNP were compared between the DCM group and the control group using the unpaired t test. Plasma levels of ANP and BNP among the various sampling sites were compared using one-way ANOVA followed by Dunnett’s test. The correlation of the plasma levels of ANP and BNP with hemodynamic parameters was examined using linear regression analysis.

All values were expressed as mean±SD. Statistical significance was defined as a probability value of less than .05.

Results

Cardiac Catheterization Data

The table shows the cardiac catheterization data for the patients with DCM and the control subjects. PCWP and LVESVI were significantly increased and cardiac index and LVEF were significantly decreased in patients with DCM as compared with control subjects.

Plasma ANP and BNP Levels

The plasma ANP level was significantly increased at all sampling sites, including the femoral vein, the aortic root, the AIV, and the coronary sinus in patients with DCM as compared with control subjects (207.1±156.2 pg/mL versus 9.8±4.1 pg/mL, P<.001; 280.6±183.7 pg/mL versus 24.0±5.2 pg/mL, P<.001; 612.3±431.6 pg/mL versus 32.2±17.0 pg/mL, P<.001; and 1229.0±772.7 pg/mL versus 371.4±111.1 pg/mL, P<.001; respectively), as shown in the upper panel of Fig 1. Plasma BNP level was also significantly increased at all sampling sites, including the femoral vein, the aortic root, the AIV, and the coronary sinus in patients with DCM as compared with control subjects (235.9±239.4 pg/mL versus 6.9±5.9 pg/mL, P<.001; 268.4±293.2 pg/mL versus 8.6±6.4 pg/mL, P<.001; 511.6±458.1 pg/mL versus 19.0±11.5 pg/mL, P<.001; and 529.7±455.3 pg/mL versus 28.8±14.0 pg/mL, P<.001; respectively), as shown in the lower panel of Fig 1.

In the control subjects, there was no significant difference in the plasma ANP level between the aortic root and the AIV (24.0±5.2 pg/mL versus 32.2±17.0 pg/mL), but there was a highly significant step-up of the level between the AIV and the coronary sinus (32.2±17.0 pg/mL versus 371.4±111.1 pg/mL, P<.001), as shown in the left panel of Fig 2. In contrast, there was a significant step-up of the plasma BNP level between the aortic root and the AIV (8.6±6.4 pg/mL versus 19.0±11.5 pg/mL, P<.001) but not between the AIV and the coronary sinus (19.0±11.5 pg/mL versus 28.8±14.0 pg/mL), as shown in the left panel of Fig 2. On the other hand, in patients with DCM, there was a significant
step-up in the plasma ANP level between the aortic root and the AIV (280.6±183.7 pg/mL versus 612.3±431.6 pg/mL, \( P<.01 \)) as well as between the AIV and the coronary sinus (612.3±431.6 pg/mL versus 1229.0±772.7 pg/mL, \( P<.01 \)), as shown in the right panel of Fig 2. There was a significant step-up in the plasma BNP level between the aortic root and the AIV (268.4±293.2 pg/mL versus 511.6±458.1 pg/mL, \( P<.01 \)) but not between the AIV and the coronary sinus (511.6±458.1 pg/mL versus 529.7±455.3 pg/mL) in patients with DCM, as shown in the right panel of Fig 2.

Correlation of Plasma Levels of ANP and BNP With Cardiac Catheterization Data

The arteriovenous difference at the AIV of plasma level of ANP had a significant positive correlation with PCWP (\( r=.656, P<.001 \)) and LVEDP (\( r=.644, P<.001 \)) and a significant negative correlation with cardiac index (\( r=-.617, P<.001 \)), as shown in the upper panel of Fig 3. The arteriovenous difference at the AIV of plasma level of BNP also had a significant positive correlation with PCWP (\( r=.663, P<.001 \)) and LVEDP (\( r=.694, P<.001 \)) and a significant negative correlation with cardiac index (\( r=-.675, P<.001 \)).

Fig 3. Graphs showing the correlation of arteriovenous difference of plasma levels of A-type natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) at the anterior interventricular vein (Plasma ANP [AIV-Ao] [top] and Plasma BNP [AIV-Ao] [bottom]) with pulmonary capillary wedge pressure (PCWP), left ventricular end diastolic pressure (LVEDP), and cardiac index (CI) in control subjects and in patients with dilated cardiomyopathy (DCM). Ao indicates aortic root.
The arteriovenous difference of plasma level of ANP at the AIV had a significant negative correlation with LVESVI (\( r = -.676, P < .001 \)) and a significant positive correlation with LVEDVI (\( r = .757, P < .001 \)), as shown in the upper panel of Fig 4. The arteriovenous difference of plasma BNP level at the AIV had a significant negative correlation with LVESVI (\( r = -.735, P < .001 \)) and a significant positive correlation with LVEDVI (\( r = .817, P < .001 \)), as shown in the lower panel of Fig 4. The plasma ANP level at the femoral vein had a significant positive correlation with PCWP (\( r = .844, P < .001 \)) and LVEDP (\( r = .819, P < .001 \)) and a significant negative correlation with cardiac index (\( r = -.764, P < .001 \)), as shown in the upper panel of Fig 5. The plasma BNP level at the femoral vein had a significant positive correlation with PCWP (\( r = .713, P < .001 \)) and LVEDP (\( r = .700, P < .001 \)) and a significant negative correlation with cardiac index (\( r = -.553, P < .001 \)), as shown in the lower panel of Fig 5.

The plasma level of ANP at the femoral vein had a significant negative correlation with LVESVI (\( r = -.827, P < .001 \)) and LVEDVI (\( r = .781, P < .001 \)), as shown in the upper panel of Fig 6. The plasma BNP level at the femoral vein had a significant negative correlation with LVESVI (\( r = -.828, P < .001 \)) and LVEDVI (\( r = -.798, P < .001 \)), as shown in the lower panel of Fig 6. The ratio of the arteriovenous difference at the AIV to that at the coronary sinus of plasma ANP level had a significant positive correlation with PCWP (\( r = .715, P < .001 \)) and LVEDP (\( r = .706, P < .001 \)) and a significant negative correlation with LVESVI (\( r = -.729, P < .001 \)), as shown in the upper panel of Fig 7. In contrast, this ratio for BNP had no correlation with the hemodynamic parameters and LVEF, as shown in the lower panel of Fig 7.

**Discussion**

BNP is a novel cardiac hormone mainly synthesized in and secreted from the ventricles and forms a natriuretic peptide family with ANP.\(^\text{16-21}\) We and others have shown that plasma BNP and ANP levels are increased in patients with chronic heart failure.\(^\text{20-23}\) However, the precise sources and mechanisms for secretion of BNP are not known in normal subjects as compared with patients with chronic heart failure and in comparison with ANP. In the present study, blood sampling for ANP and BNP was done in the femoral vein, the aortic root, the AIV, and the coronary sinus simultaneously. The plasma level of BNP was much lower than that of ANP in the control subjects. The plasma levels of both ANP and BNP were significantly increased at all sampling sites, including the femoral vein, the aortic root, the AIV, and the coronary sinus in the patients with DCM as compared with the control subjects. These findings are in agreement with the results of our previous studies.\(^\text{14,20-22}\) The increased plasma levels of ANP and
BNP were not due to the decrease in glomerular filtration rate because serum creatinine levels were within normal range in most of the patients.

In the control subjects, there was no significant difference in the plasma ANP level between the aortic root and the AIV, but there was a highly significant
step-up in the level between the AIV and the coronary sinus. Because the AIV drains the ventricles only, mainly the left ventricle and not the atria, and the coronary sinus drains most of the heart including the atria and ventricles,\textsuperscript{14} this implies that ANP is not secreted significantly from the ventricles but is released into the coronary sinus system from atria, as blood flows downstream from the AIV to the coronary sinus in normal subjects.\textsuperscript{14} However, the possibility that some synthesis and/or release of ANP occur in the normal ventricle cannot be conclusively excluded because secretion from the ventricle of ANP and BNP are qualitatively similar in patients with heart failure, as will be discussed later. In contrast, there was a significant step-up of the plasma BNP level between the aortic root and the AIV, but there was no significant difference in the level between the AIV and the coronary sinus, indicating that the BNP is secreted mainly from the left ventricle and not from the atria in the control subjects.

In the patients with DCM, on the other hand, there was a highly significant step-up in the plasma ANP level between the aortic root and the AIV and between the AIV and the coronary sinus. These findings are in agreement with the results of our previous studies\textsuperscript{14,15} and indicate that increased amounts of ANP are released from the left ventricle, as well as the heart as a whole, into the circulation in the patients with DCM. There was a significant step-up in the plasma level of BNP between the aortic root and the AIV, but there was no significant difference in the level between the AIV and the coronary sinus. BNP is thus secreted mainly from the left ventricle and not from the atria in patients with DCM and control subjects. There is a possibility that ANP and BNP may also be secreted from the right ventricle in the patients with DCM because the AIV may also drain the right ventricle. However, the patients studied had left-sided heart failure only, as suggested by the normal right atrial pressures.

The amounts of BNP secreted from the left ventricle as well as those of ANP, as reflected by the arteriovenous difference of the levels at the AIV, had a significant positive correlation with PCWP, LVEDP, LVESVI, and LVEDVI and a significant negative correlation with cardiac index and LVEF, respectively. This indicates that BNP as well as ANP is released increasingly from the left ventricle as left ventricular function deteriorates. Thus, the present study suggests that increased wall tension or stretch as a consequence of volume overload may stimulate the secretion of BNP as well as ANP from the left ventricle. The demonstration in this study that the amounts of BNP secreted from the left ventricle, as well as those of ANP, had the highest significant positive correlation with the LVESVI and LVEDVI supports this interpretation.

The plasma level of BNP and of ANP at the peripheral (femoral) vein had a significant positive correlation with PCWP, LVEDP, LVESVI, and LVEDVI and a significant negative correlation with cardiac index and LVEF, respectively. This indicates that plasma level of BNP, as well as that of ANP, at the peripheral vein reflects the amounts of the hormones secreted from the
left ventricle and suggests that peripheral plasma level of these hormones may be used as a marker of left ventricular dysfunction.\textsuperscript{33,34}

The ratio of ANP released from the left ventricle to that released from the heart as a whole, as reflected by the ratio of the arteriovenous difference of the level at the AIV to that at the coronary sinus, had a significant positive correlation with PCWP and LVEDP and a significant negative correlation with LVEF, indicating that the amounts of ANP released from the left ventricle increase in proportion to the severity of left ventricular dysfunction. In contrast, the ratio of BNP released from the left ventricle to that from the heart as a whole had no correlation with the degree of left ventricular dysfunction. This again indicates that BNP is secreted mainly from the left ventricle regardless of the degree of left ventricular dysfunction.

Limitations

In the present study, we inserted a catheter into the AIV for sampling blood from the AIV, which drains ventricles only, mainly the left ventricle. Because there is individual variation in the anatomy and development of the AIV, it was not always possible to insert the catheter into the AIV in all patients. Moreover, the possibility of back flow from the great cardiac vein, which is proximal to the AIV, could not be completely excluded when blood samples were drawn forcibly. Thus, we excluded the patients in whom at least the proximal half of the AIV could not be visualized with the injection of contrast dye, and blood samples were drawn slowly in the present study.

Conclusions

We conclude that (1) BNP is secreted mainly from the left ventricle in normal adult humans as well as in patients with left ventricular dysfunction, whereas ANP is secreted from atria in normal adult humans and also from the left ventricle in patients with left ventricular dysfunction; (2) the ratio of BNP released from the left ventricle to that from the heart as a whole had no correlation with the degree of left ventricular dysfunction, indicating that BNP is secreted mainly from the left ventricle regardless of the degree of left ventricular dysfunction, whereas the ratio of ANP released from the left ventricle to that from the heart as a whole increases with the severity of left ventricular dysfunction; (3) secretion of BNP as well as ANP from the left ventricle increases in proportion to the severity of the left ventricular dysfunction, suggesting that the secretions of ANP and BNP from the left ventricle are regulated mainly by wall tension of the left ventricle; and (4) the peripheral plasma level of ANP and BNP reflects the secretion rate of these hormones from the left ventricle and may be used as a marker of the degree of left ventricular dysfunction in patients with left ventricular dysfunction.

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