Interaction on Metabolic Clearance Between A-Type and B-Type Natriuretic Peptides in Patients With Heart Failure

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A-type and B-type natriuretic peptides (ANP and BNP) are secreted into the systemic circulation via the coronary sinus. Plasma levels of ANP and BNP at the coronary sinus should directly determine the systemic circulating levels. However, the metabolic clearance of these hormones are dependent on similar systems, natriuretic peptide clearance receptor (NPR-C) and neutral endopeptidase 24.11 (NEP), suggesting a possible interaction between ANP and BNP on metabolic clearance. In this study, we examined the interaction on metabolic clearance in patients with heart failure. We obtained blood samples from the coronary sinus and aortic root in 100 patients with heart failure and 28 control subjects. The difference in ANP and BNP levels between the coronary sinus and the aortic root is reflected partly by the metabolic clearance in the pulmonary circulation. In this study, we examined the possible interaction on metabolic clearance between ANP and BNP using a statistical procedure. The ratio of the level of BNP to ANP (BNP/ANP) was significantly higher in the aortic root than in the coronary sinus at any stage of heart failure. We performed multiple regression analysis using ANP and BNP levels at the coronary sinus as independent variables (X_1 and X_2, respectively) and the ANP level at the aortic root as a dependent variable (Y). The analysis showed that both X_1 and X_2 were significant variables in the equation. On the other hand, we performed the same analysis using the BNP level at the aortic root as a dependent variable (Y). The analysis showed that only X_2 was a significant variable in the equation. This study suggests that (1) the metabolic clearance in the pulmonary circulation is higher for ANP versus BNP and (2) the amount of ANP cleared in the pulmonary circulation depends on the amount of both ANP and BNP secreted from the heart, whereas the amount of BNP cleared in the pulmonary circulation is dependent solely on the amount of BNP secreted from the heart.

THE CARDIAC HORMONES A-type or atrial natriuretic peptide (ANP) and B-type or brain natriuretic peptide (BNP) have a wide range of potent biological effects, including vasodilating action, natriuretic action, and inhibition of the renin-angiotensin-aldosterone and sympathetic nervous systems.[1-6] ANP and BNP are secreted from the heart into the systemic circulation via the coronary sinus, and plasma levels of ANP and BNP are markedly increased in the peripheral veins in patients with heart failure.[7-13] Furthermore, BNP is rapidly secreted from the ventricles by acute ventricular overload and from the infarcted ventricles.[14,15]

We have examined the secretion patterns of ANP and BNP from failing hearts using blood samples obtained from these patients during cardiac catheterization.[9,11,12] We have shown that ANP is secreted mainly from the atria in normal subjects and mild heart failure patients, and that ANP secretion is increased from the ventricles according to the severity of heart failure. On the other hand, BNP is secreted mainly from the ventricles regardless of the severity of heart failure.

To evaluate the roles of the natriuretic peptide family in the pathophysiology of heart failure, we have to consider not only the synthesis/secretion of these peptides but also the metabolic clearance. Until now, there have been reports about the metabolic clearance of ANP and BNP by the infusion of pharmacological doses of hormone and by the tracer method in patients with heart failure.[11,16-25] The analyses showed that the half-life of ANP is shorter than that of BNP[11,16,22] and the metabolic clearance ratio of ANP changes according to the clinical severity of heart failure.[18] It has also been reported that ANP and BNP are cleared from the circulation via the natriuretic peptide clearance receptor (NPR-C) and neutral endopeptidase 24.11 ([NEP] EN 3.4.24.11) in the lung, kidney, liver, and peripheral vascular beds.[24-34] However, there are no reports about the interaction on metabolic clearance between endogenous ANP and BNP in patients with heart failure.

In this context, we hypothesized that the interaction of ANP and BNP in binding to NPR-C and NEP may exert an influence on each peptide's metabolic clearance. We then examined the metabolic clearance of endogenous ANP and BNP using blood obtained from patients with heart failure during cardiac catheterization.

SUBJECTS AND METHODS

Patients

This study involved a total of 128 subjects, 100 patients with heart failure (57 men and 43 women; mean age, 63.3 ± 0.5 years) and 28 control subjects (12 men and 16 women; mean age, 61.8 ± 1.0 years).

The degree of heart failure was New York Heart Association (NYHA) class I in 34 patients, class II in 27, class III in 27, and class IV in 12. The underlying cardiac disorder was dilated cardiomyopathy in 52 patients and old myocardial infarction in 48 patients. The diagnosis was based on the medical history, physical examination, chest roentgenogram, electrocardiogram, echocardiogram, and cardiac catheterization including left ventriculography and coronary arteriography. In all patients, diagnostic catheterization was performed and insertion of a catheter into the coronary sinus was possible. The mean serum creatinine level in the patients was 1.0 ± 0.3 mg/mL, with a range of 0.5 to 1.9. Medication was stopped at least 24 hours before the examination

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in all patients, except 3 patients who had nitrates 12 hours before the study.

The control subjects also underwent diagnostic cardiac catheterization, including coronary arteriography and left ventriculography. The group consisted of 20 patients with chest pain syndrome with normal coronary arteriograms and 8 patients with electrocardiographic abnormalities but normal coronary arteriograms. None of them had ischemic heart disease, hypertension, or cardiac hypertrophy. The mean serum creatinine level in the control subjects was 0.8 ± 0.2 mg/mL, with a range of 0.5 to 1.2.

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

Cardiac Catheterization

Cardiac catheterization was performed in the morning with the patients in a fasting state. Using a Swan-Ganz catheter inserted into the femoral or subclavian vein, hemodynamic measurements, including pulmonary capillary wedge pressure and cardiac output, were obtained. Cardiac output was determined using the thermodilution technique in triplicate. After the right heart catheterization, a 6F Goodale-lubin catheter was placed in the coronary sinus by way of a brachial vein. The position of the catheter tip was conformed by injection of contrast dye. A Judkins catheter was placed at the root of the aorta by way of a femoral artery. Then, blood sampling for ANP and BNP was performed simultaneously at the aortic root and the coronary sinus. The systemic arterial pressure, heart rate, and left ventricular end-diastolic pressure were measured, and coronary arteriography and left ventriculography were performed. The left ventricular ejection fraction was determined by left ventriculograms.

Measurement of ANP and BNP

An aliquot of plasma was immediately frozen at −80°C. All blood samples were obtained with chilled plastic syringes, transferred to chilled siliconized disposable tubes containing aprotinin (1,000 kallikrein inactivator U/mL; Ohkura Pharmaceutical, Tokyo, Japan) and EDTA (1 mg/mL), and immediately placed on ice and centrifuged at 4°C. Samples were thawed at once at the time of analysis. The plasma concentration of ANP and BNP was measured with a specific immunoradiometric assay for human α-ANP (ANP kit; Shionoria, Osaka, Japan) and BNP (Shionoria BNP kit).[35,36] These assay systems were used without extraction of the plasma.

Statistical Analysis

A paired or unpaired T test was performed to compare 2 variables. In the analysis of the change in the BNP/ANP ratio with the severity of heart failure, we used 1-way ANOVA.

When the correlation coefficient for the linear regression line is low, we should postulate that the presence of other factor(s) might be masking the relation. Therefore, it is recommended that multivariate analysis is performed in addition. In the present study, we performed multiple linear regression analysis followed by stepwise selection as follows: $Y = C_0 + C_1X_1 + C_2X_2$. This is a general linear model to predict Y (plasma ANP or BNP at the aortic root) using X_1 (plasma ANP at the coronary sinus) and X_2 (plasma BNP at the coronary sinus) as independent variables with a constant (C_0) and 2 linear coefficients (C_1 and C_2).

All values are expressed as the mean ± SE. Statistical significance was defined as a *P* value less than .05.

RESULTS

Cardiac Catheterization

Table 1 shows cardiac catheterization data for the patients with heart failure and the control subjects. The heart rate, mean blood pressure, pulmonary capillary wedge pressure, and left ventricular end-diastolic pressure were significantly increased and the cardiac index and left ventricular ejection fraction were significantly decreased in patients with heart failure as compared with the control subjects.

Table 1. Cardiac Catheterization Data (N = 128)

Group	HR (bpm)	mBP (mm Hg)	PCWP (mm Hg)	LVEDP (mm Hg)	CI ($L \cdot min^{-1} \cdot m^{-2}$)	LVEF (%)
Control (n = 28)	66 ± 3	93 ± 5	5 ± 1	5 ± 1	2.8 ± 0.2	77 ± 1
Heart failure (n = 100)	77 ± 5	98 ± 6	13 ± 1	16 ± 1	2.3 ± 0.7	50 ± 4
P	<.01	<.01	<.001	<.001	<.01	<.001

Abbreviations: HR, heart rate; mBP, mean blood pressure; PCWP, pulmonary capillary wedge pressure; LVEDP, left ventricular end-diastolic pressure; CI, cardiac index; LVEF, left ventricular ejection fraction.

Plasma ANP and BNP at the Coronary Sinus and Aortic Root

Plasma levels of ANP and BNP were increased in proportion to the severity of heart failure at both the coronary sinus and the aortic root (Fig 1). The ratio of the plasma level of BNP to ANP (BNP/ANP) increased stepwise as heart failure advanced at each sampling site, suggesting that the degree of increase was more marked for plasma BNP versus ANP. The BNP/ANP ratio was significantly higher in the aortic root versus the coronary sinus in any stage of heart failure ($P < .01$, respectively) (Fig 2).

Relationship of Plasma ANP and BNP Between the Coronary Sinus and Aortic Root

Figure 3 shows the relationship of plasma levels of ANP and BNP between the coronary sinus and the aortic root. There was a highly significant linear correlation between the levels at the coronary sinus and those at the aortic root ($r = .939$), suggesting that BNP was cleared from the circulation at a fixed ratio regardless of the plasma level at the coronary sinus. In other words, the plasma level of BNP at the coronary sinus directly determined the level at the aortic root. A significant linear correlation was also recognized between plasma levels of ANP at the coronary sinus and those at the aortic root. However, the coefficient of correlation for this linear regression was relatively low ($r = .712$) compared with that for BNP ($r = .939$).

Statistical Analysis by Multiple Linear Regression Analysis

Because the correlation coefficient of the linear regression line for ANP was relatively low ($r = .712$), we hypothesized that the interaction of ANP and BNP in the clearance of ANP might mask the relation: the plasma level of BNP as well as ANP at the coronary sinus would exert an influence on the plasma level of ANP at the aortic root. To prove this hypothesis, we performed a multiple linear regression analysis (Table 2).

When we used the plasma levels of ANP and BNP at the coronary sinus independent variables (X_1 and X_2, respectively) and the ANP level at the aortic root a dependent variable (Y, ANP plasma level at the aortic root), both X_1 and X_2 were

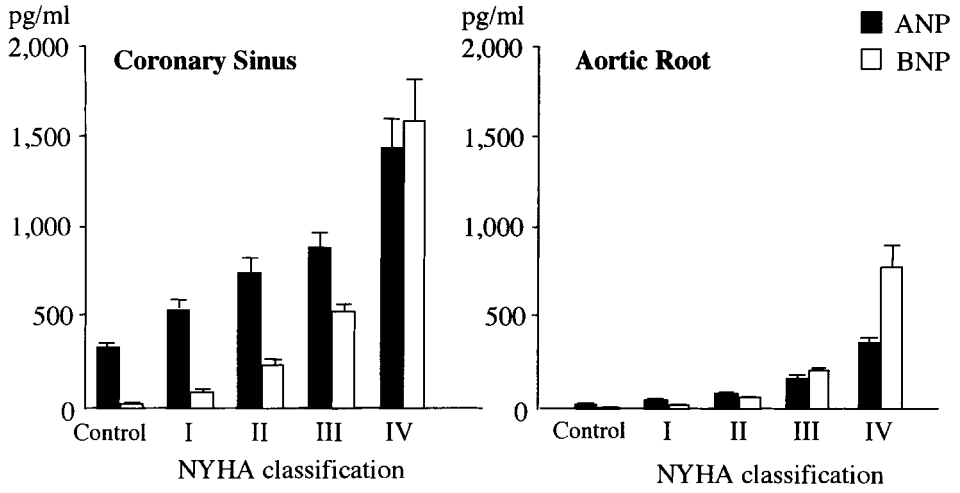

Fig 1. Plasma levels of ANP and BNP in the coronary sinus and aortic root in patients with heart failure and control subjects.

significant in the equation ($P < .001$). On the other hand, when we used the plasma levels of ANP and BNP at the coronary sinus independent variables (X_1 and X_2, respectively) and the plasma level of BNP at the aortic root a dependent variable (Y, BNP plasma level at the aortic root), X_2 was a significant variable ($P < .001$), but not X_1, in the equation.

DISCUSSION

In research on the pathophysiological roles of the natriuretic peptide family in heart failure, the study of the metabolic system of ANP and BNP is as indispensable as the synthesis/secretion system. In this study, we examined the clearance of endogenous ANP and BNP by simultaneously measuring plasma levels in the coronary sinus and the aortic root in patients with heart failure.

The degree of increase in the plasma level was more marked for BNP versus ANP at both the coronary sinus and the aortic root (Figs 1 and 2). These results are attributed to the marked production of BNP over ANP in heart failure. Also, the ratio of the plasma level of BNP and ANP (BNP/ANP) was significantly greater in the aortic root versus the coronary sinus at each stage of heart failure. This result can be explained by the fact that the half-life is shorter for ANP compared with BNP.[11,16,22] The degradation in plasma ANP and BNP between the coronary sinus and the aortic root is thought to be mainly due to the clearance in the pulmonary circulation. Thus, the metabolic clearance in the pulmonary circulation would be higher for ANP compared with BNP.

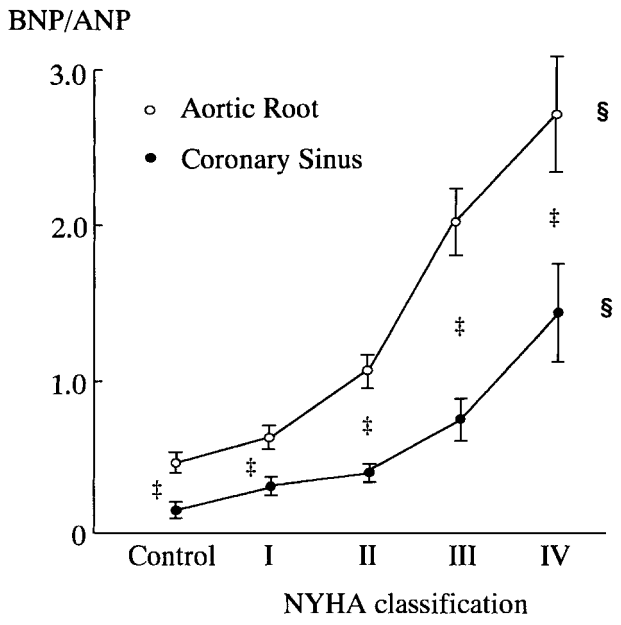

Fig 2. BNP/ANP ratio according to the severity of heart failure. ‡$P < .01$, coronary sinus *v* aortic root, §$P < .01$ by 1-way ANOVA.

It is quite natural to speculate that the plasma levels of ANP and BNP at the coronary sinus should determine those at the aortic root. Thus, we examined the relationship of the plasma level of ANP and BNP between the coronary sinus and the aortic root, respectively (Fig 3). The slope of the linear regression line was steeper for BNP compared with ANP. This result is also explained by the shorter half-life of ANP versus BNP. However, the correlation coefficient was relatively low for ANP compared with BNP. This suggests that the plasma level of ANP at the coronary sinus did not directly determine that at the aortic root, whereas the plasma level of BNP at the coronary sinus directly determined that at the aortic root. Some factors may exert an influence on ANP clearance in the pulmonary circulation.

We then hypothesized that the plasma level of BNP as well as ANP at the coronary sinus would exert an influence on the plasma level of ANP at the aortic root. This idea was derived from the evidence that both ANP and BNP are metabolized by the same clearance system of the NPR-C and NEP and the 2 peptides should bind competitively to them.[24-34] To probe the interaction of ANP and BNP in the pulmonary clearance, we used multiple linear regression analysis followed by a stepwise selection (Table 2). Initially, we examined whether the plasma level of ANP at the aortic root depends on plasma ANP and BNP levels at the coronary sinus. This result suggests that the plasma level of ANP at the aortic root depends on plasma level of both ANP and BNP at the coronary sinus and that plasma ANP and BNP at the coronary sinus almost equally contributed (C_1, 0.119; C_2, 0.092). Secondarily, we examined whether the plasma level of BNP at the aortic root depends on plasma ANP and BNP levels at the coronary sinus by the same statistical

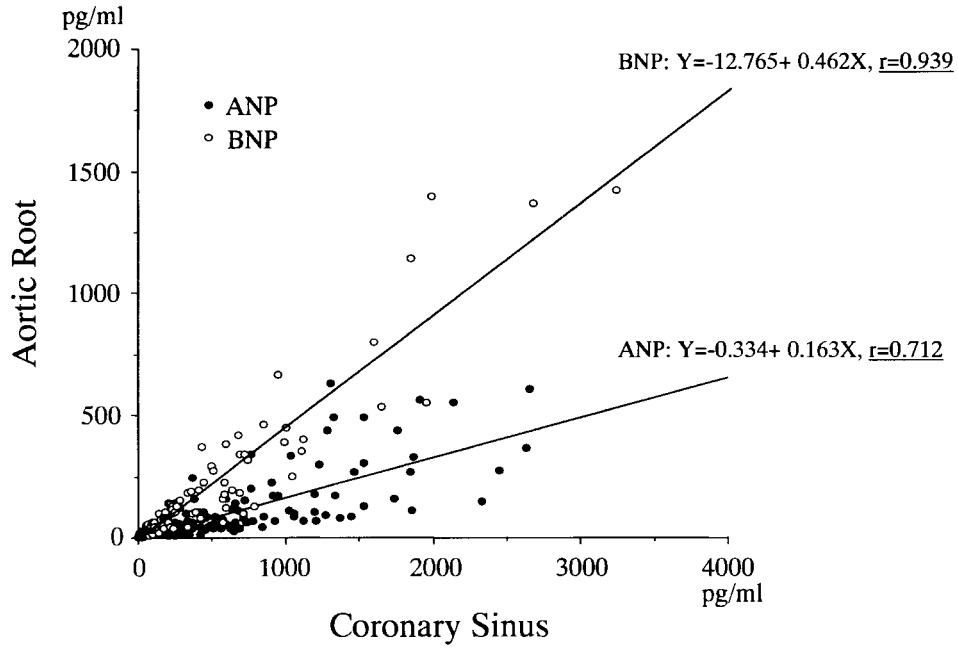

Fig 3. Correlations for plasma ANP and BNP between the coronary sinus and aortic root.

method. As a result, the variable, plasma BNP at the coronary sinus, was significant in the equation, but not plasma ANP (Table 2). These results suggest that the plasma level of BNP at the aortic root depends on plasma BNP but not plasma ANP at the coronary sinus.

It is interesting that the plasma level of BNP at the coronary sinus influenced ANP clearance in the pulmonary circulation. We have not yet clearly determined the reason for this. However, we can explain it by the difference in the affinity of ANP and BNP for NPR-C and NEP in the pulmonary circulation. The affinity of ANP for NPR-C and NEP was much stronger than that of BNP.[16,29,30] Thus, there may be a smaller amount of BNP that combines with NPR-C and NEP compared with ANP. If the amount of ANP secreted from the heart is increased, it is difficult for the influence to reach to the clearance of BNP by NPR-C and NEP—the size of the change in the amount of BNP cleared by them should be small. On the other hand, if the amount of BNP secreted from the heart is increased, the influence easily reaches to the clearance of ANP by NPR-C and NEP, and the size of the change in the amount of ANP cleared by them should be large.

We previously reported that the endogenous plasma level of ANP was increased after an infusion of synthetic BNP.[37] It is probable that a large amount of infused BNP competes with endogenous ANP for binding to NPR-C and NEP, resulting in an increased amount of ANP that is not cleared. This study also suggests that BNP competes with the metabolic clearance of ANP. In the future, BNP will be used in the treatment of heart failure. This effect may be more beneficial in treatment with BNP versus ANP.

Table 2. Multiple Regression Analysis: Final Significant Variables in the Equation Using Forward Stepwise Selection

Equation	Definition
ANP	
$Y = -0.835 + 0.119X_1 + 0.092X_2$	Y: ANP at the aortic root
$r = .779$, $P < .0001$	X_1: ANP at the coronary sinus
X_1: $P < .001$	X_2: BNP at the coronary sinus
X_2: $P < .001$	
BNP	
$Y = -12.765 + 0.462X_2$	Y: BNP at the aortic root
$r = .939$, $P < .0001$	X_1: ANP at the coronary sinus
X_1: not in the equation	X_2: BNP at the coronary sinus
X_2: $P < .001$	

It has been shown that NPR-C and NEP are activated and have a role in natriuretic peptide metabolism in heart failure.[32,33] Although the clearance system of ANP and BNP is thought to be activated also in the pulmonary circulation, the present results show the competitive binding of ANP and BNP to NPR-C and NEP, suggesting that NPR-C and NEP are saturated with the ligands ANP and BNP in heart failure.

It has been clearly shown that NEP activity is concentrated mostly within the brush border of the proximal tubule of the kidney.[38,39] However, in this study, we examined the clearance system of ANP and BNP only in the pulmonary circulation. The renal clearance of ANP and BNP and the possible interaction between them should be discussed in the future using another methodology.

BNP has structural diversity among species, and we have determined human BNP as a 32–amino acid sequence.[40] It has been subsequently elucidated that the biological actions of BNPs are species-specific, unlike those of ANP.[41] Thus, the pathophysiological analysis of BNP obtained with animal models is hardly applicable to humans. Because this was a study using patients with heart failure, the present results will offer important information for the future clinical application of ANP and BNP in the treatment of heart failure. Nonetheless, it may be too simplistic to draw a conclusion regarding the metabolic clearance of ANP and BNP based on regional plasma levels of ANP and BNP. A microbolus injection of radioactive ANP and BNP would be the ideal method to assess the clearance.

In summary, the present study shows that the clearance of ANP in the pulmonary circulation depends on the amount of ANP and BNP secreted from the heart. On the other hand, the clearance of BNP in the pulmonary circulation was influenced solely by the amount of BNP secreted from the heart. This result may be due to the similar clearance systems of ANP and BNP but their different affinities for NPR-C and NEP in the pulmonary circulation.

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