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Marked Elevation of Brain Natriuretic Peptide Levels in Pericardial Fluid Is Closely Associated With Left Ventricular Dysfunction

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Objectives. **The purpose of this study was to investigate whether atrial and brain natriuretic peptides (ANP and BNP, respectively) represent autocrine/paracrine factors and are accumulated in pericardial fluid.**

Background. **ANP and BNP, systemic hormones produced by the heart, have elevated circulating levels in patients with heart failure. Recent evidence suggests that the heart itself is one of the target organs for these peptides.**

Methods. **With an immunoreactive radiometric assay, we measured the concentrations of these peptides in plasma and pericardial fluid simultaneously in 28 patients during coronary artery bypass graft surgery.**

Results. **The pericardial levels of BNP were markedly elevated in patients with impaired left ventricular function. We investigated the correlation of ANP and BNP levels in plasma or pericardial fluid with left ventricular hemodynamic variables. None of the hemodynamic variables correlated with ANP levels in plasma or pericardial fluid. Both plasma and pericardial fluid levels of BNP were significantly related to left ventricular end-diastolic and systolic volume indexes (LVEDVI and LVESVI, respectively). In addition, BNP pericardial fluid levels had closer relations with LVEDVI ($r = 0.679$, $p < 0.0001$) and LVESVI ($r = 0.686$, $p < 0.0001$) than did BNP plasma levels (LVEDVI: $r = 0.567$, $p = 0.0017$; LVESVI: $r = 0.607$, $p = 0.0010$). BNP levels in pericardial fluid but not in plasma correlated with left ventricular end-diastolic pressure ($r = 0.495$, $p = 0.0074$).**

Conclusions. **BNP levels in pericardial fluid served as more sensitive and accurate indicators of left ventricular dysfunction than did BNP levels in plasma. Thus, BNP may be secreted from the heart into the pericardial space in response to left ventricular dysfunction, and it may have a pathophysiologic role in heart failure as an autocrine/paracrine factor.**

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Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are members of a family of vasoactive substances that are produced by the heart (1–5). The synthesis of these peptides is augmented by the increased wall tension or stretch of cardiac chambers (6,7). Once these peptides are released from atrial or ventricular myocytes, most of them are believed to go into the bloodstream. Circulating peptides serve to unload the heart through their natriuretic, diuretic and vasodilative properties (1–3). Earlier studies (4,7–10) reported that plasma levels of ANP and BNP are elevated in patients with various heart diseases including cardiomyopathy and ischemic heart disease. These levels are roughly correlated with indicators of left ventricular systolic and diastolic dysfunction. However, the plasma levels of ANP and BNP change greatly with exercise, body position, anesthesia, surgical stress and minimal volume overload, among other factors. There is no direct evidence that their plasma levels provide accurate information on the left ventricular function of patients with heart failure.

Apart from acting as systemic hormones, ANP and BNP may have local actions at the site of their synthesis. Recent studies (11) demonstrated that genes for natriuretic peptide receptors are expressed in cardiac myocytes and fibroblasts and that ANP and BNP stimulate cyclic guanosine monophosphate (cGMP) accumulation in these cells. These findings support the hypothesis that the heart itself is one of the target organs for these peptides. In addition, natriuretic peptides are sufficient to inhibit deoxyribonucleic acid (DNA) synthesis in cardiac fibroblasts (12), suggesting their important paracrine role in the structural remodeling of the heart by regulating fibroblast growth. Thus, the local accumulation of these peptides adjacent to the heart might have important pathophysiologic significance. However, it is unknown whether 1) ANP and BNP are diffusible, more concentrated in pericardial fluid than in blood, and 2) their levels in pericardial fluid have prognostic and therapeutic implications. Therefore, the present study was designed to determine whether ANP and BNP are concentrated in pericardial fluid and whether these

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Abbreviations and Acronyms

ANP = atrial natriuretic peptide
BNP = brain natriuretic peptide
cGMP = cyclic guanosine monophosphate
DNA = deoxyribonucleic acid
EF = ejection fraction
LVEDP = left ventricular end-diastolic pressure
LVEDVI = left ventricular end-diastolic volume index
LVESVI = left ventricular end-systolic volume index

concentrations relate to the left ventricular dysfunction in patients with ischemic heart disease.

Methods

Patient profile. The 28 patients in this study underwent coronary artery bypass graft surgery because of double- (n = 12) or triple- (n = 16) vessel disease. The mean age of the patients (22 men and 6 women) was 66 ± 7 years. All patients were clinically evaluated before the operation by cardiac catheterization. None showed signs of decompensated congestive heart failure at the time of operation and cardiac catheterization. All underwent biplane left ventriculography and selective coronary angiography according to standard techniques. The left ventricular end-diastolic and end-systolic volume indexes (LVEDVI and LVESVI, respectively) and the ejection fraction (EF) were calculated from left ventricular cineangiograms performed in the right anterior oblique projection by the method of Kennedy et al. (13). The patients were classified into two groups: group I, the 5 patients with normal left ventricular function (i.e., EF ≥50% and left ventricular end-diastolic pressure [LVEDP] ≤18 mm Hg), and group II, the 23 patients with impaired left ventricular function (i.e., abnormal values for EF or LVEDP).

All patients gave written informed consent. The study protocol was approved by the ethical committee on human research of Takeda Hospital.

Sampling of plasma and pericardial fluid. Immediately after the incision of the pericardium, undiluted samples of pericardial fluid were obtained before heparinization. At the same time, blood was withdrawn from the cannulated brachial artery. These samples were collected in sterile tubes, placed immediately on ice, clarified by centrifugation at 3,000 *g* for 10 min at 4°C and rapidly frozen at −80°C.

Measurement of plasma and pericardial fluid levels of ANP and BNP by radioimmunoassay. BNP levels in plasma and pericardial fluid were measured as previously described (4,7–9). Briefly, [Tyr^{82}]-human BNP [83-108] (1 μg) was radioiodinated by the chloramine T method. The specific activity of ^{125}I-labeled [Tyr^{82}]-human BNP [83-108] ranged from 500 to 900 μCi/μg. The monoclonal antibody against human BNP (4) (final dilution of ascites, 1:5 × 10^6) was incubated with either standard human BNP or samples in 0.2-ml assay buffer (50 mmol/liter phosphate buffer, pH 7.4, containing 0.1% gelatin [Merck, Darmstadt, Germany], 0.1% Triton X-100, 1 mmol/liter Na_2EDTA, 0.2 mmol/liter L-cystine and 0.1% NaN_3) for 24 h at 4°C. Bound and free ligands were separated by adding 1.0 ml of a suspension of dextran-coated charcoal, consisting of 250 mg of Norit SX Plus (Norit Vereenging NV, The Netherlands) and 25 mg of Dextran T-70 (Pharmacia, Uppsala, Sweden) in 100 ml of 50 mmol/liter phosphate buffer, pH 7.4, containing 0.01% merthiolate.

The measurement of ANP levels in plasma and pericardial fluid by radioimmunoassay was performed as reported previously (14).

Statistical analysis. Clinicopathologic data are expressed as the mean value ± SD. Statistical comparisons were performed by chi-square analysis, Student *t* test, or one-way analysis of variance with multiple comparisons, when appropriate. Significance was designated at the probability value of $p < 0.01$. Linear regression analysis was used to assess the relation between natriuretic peptide levels and hemodynamic variables.

Table 1. Clinical Characteristics of Patients with Ischemic Heart Disease, With Normal (group I) or Impaired (group II) Left Ventricular Function

	Group I (n = 5)	Group II (n = 23)	p Value
Male/female	4/1	19/4	NS
History of MI	0/5	10/23	NS
EF (%)	57 ± 4	43 ± 9	< 0.005
LVEDP (mm Hg)	15 ± 6	25 ± 8	< 0.05
LVEDVI (ml/m^2)	57 ± 19	97 ± 45	0.06
LVESVI (ml/m^2)	24 ± 10	53 ± 40	0.06
Mean AoP (mm Hg)	104 ± 16	103 ± 16	NS

Data are presented as number of patients or mean value ± SD. AoP = aortic pressure; EF = ejection fraction; LVEDP = left ventricular end-diastolic pressure; LVEDVI = left ventricular end-diastolic volume index; LVESVI = left ventricular end-systolic volume index; MI = myocardial infarction.

Results

Plasma and pericardial fluid levels of ANP and BNP. Table 1 shows the clinical characteristics of patients in group I and group II. No group I patient but 10 of the 23 group II patients had an old myocardial infarction. EF was lower and LVEDP, LVEDVI and LVESVI were higher in group II than in group I. However, there were no differences in gender or mean aortic pressure between the two groups. Figure 1 shows plasma and pericardial fluid levels of ANP and BNP in the two groups. In group I, plasma levels of BNP were lower than those of ANP. In group II, plasma ANP and BNP levels were both higher than plasma levels in group I, but BNP plasma levels were higher than ANP levels. Thus, the plasma level ratio in group II compared with group I was much higher in BNP (group II/I 22-fold) than in ANP (group II/I 3.9-fold). ANP and BNP levels were both higher in pericardial fluid than in plasma. In contrast to plasma levels, pericardial fluid levels of BNP were higher than those of ANP in both groups. Similar to

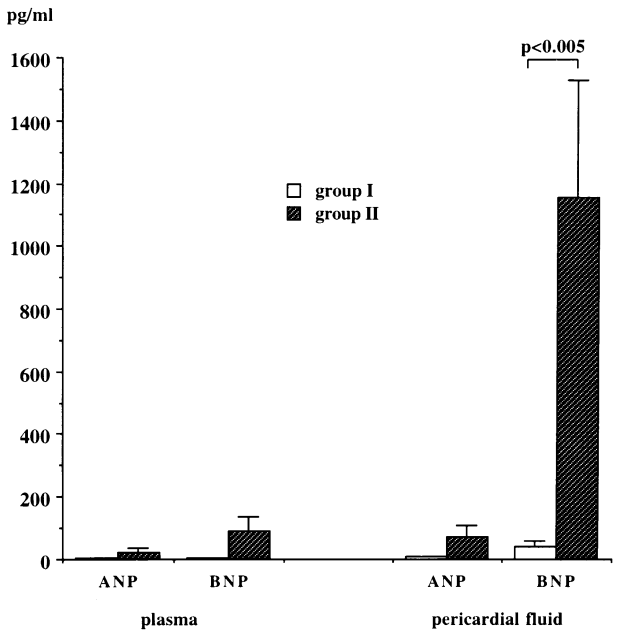

Figure 1. ANP and BNP levels in the plasma and pericardial fluid of patients with ischemic heart disease with normal (group I, n = 5) or impaired (group II, n = 23) left ventricular function.

plasma levels, the pericardial fluid level ratio in group II compared with group I was much higher for BNP (27-fold) than for ANP (8.9-fold). Further, the elevation of ANP and BNP levels in group II compared with group I was greater in pericardial fluid than in plasma. Thus, BNP levels in the pericardial fluid of group II patients were extremely high (Fig. 1).

Relations between natriuretic peptide levels in plasma or pericardial fluid and indicators for left ventricular function. Table 2 shows the correlations between hemodynamic variables and ANP or BNP levels in plasma or pericardial fluid. In plasma, ANP levels correlated with none of the hemodynamic variables, but BNP levels positively correlated with LVEDVI ($r = 0.567$, $p = 0.0017$) and LVESVI ($r = 0.607$, $p = 0.0010$). In pericardial fluid, ANP levels correlated with none of the hemodynamic variables. Notably (Fig. 2), BNP levels had a very close positive relation with LVESVI ($r = 0.686$, $p < 0.0001$) and LVEDVI ($r = 0.679$, $p < 0.0001$) and a significant relation with LVEDP ($r = 0.495$, $p = 0.0074$). None of these peptides levels in plasma or pericardial fluid correlated with age, heart rate or aortic pressure. In summary, 1) BNP had a much closer relation with left ventricular hemodynamic variables than did ANP in both plasma and pericardial fluid; and 2) BNP had closer relations with these variables in pericardial fluid than in plasma.

Effect of mitral regurgitation, hypertension and myocardial ischemia on natriuretic peptide levels in plasma or pericardial fluid. In group II, no patient had significant mitral regurgitation greater than Seller's grade II, as assessed by left ventriculography performed during cardiac catheterization. Only one patient had trivial mitral regurgitation (Seller's grade I). However, this patient had normal left atrial dimension. In addition, the hemodynamic variables and the ANP and BNP levels in plasma and pericardial fluid of this patient were within a range of the variation in group II. Therefore, the effect of mitral regurgitation on peptide levels in group II may be minimal, if any.

Group II contained nine patients with significant hypertension (>160/95 mm Hg) at the time of cardiac catheterization. However, these patients showed no evidence of left ventricular hypertrophy by echocardiography. Their ANP and BNP levels in plasma and pericardial fluid and hemodynamic variables including left ventricular EF, LVEDP, LVEDVI and LVESVI did not differ from those without hypertension. Therefore, the effect of this factor on peptide levels may be minimal, if any.

We further classified the patients into the following four ischemic groups: stable angina (n = 7), unstable angina (n = 7), recent myocardial infarction (n = 4, within 3 months from the onset of infarction) and old myocardial infarction (n = 10). No patient was status post acute myocardial infarction (within 3 days from the onset of infarction). Hemodynamic variables and the ANP and BNP levels of each patient group are shown in Table 3. There were no significant differences in hemodynamic variables and peptide levels between the groups with stable and unstable angina or between the groups with old and recent myocardial infarction.

Table 2. Regression Analyses of Association Between Hemodynamic Variables and Plasma or Pericardial Fluid Levels of Atrial and Brain Natriuretic Peptides

	ANP in Plasma		BNP in Plasma		ANP in Pericardial Fluid		BNP in Pericardial Fluid	
	Coefficient	p Value	Coefficient	p Value	Coefficient	p Value	Coefficient	p Value
Patient age (yr)	r = 0.319	0.0984	r = 0.113	0.5660	r = 0.299	0.1221	r = 0.101	0.6090
Heart rate (beats/min)	r = 0.152	0.4480	r = 0.323	0.1006	r = 0.252	0.2055	r = 0.310	0.1152
Mean AoP (mm Hg)	r = 0.120	0.9539	r = 0.092	0.6693	r = 0.163	0.4475	r = 0.122	0.5685
LVEDP (mm Hg)	r = 0.150	0.4453	r = 0.395	0.0375	r = 0.245	0.2085	r = 0.495	0.0074
LVEDVI (ml/m^2)	r = 0.236	0.2267	r = 0.567	0.0017	r = 0.367	0.0550	r = 0.679	< 0.0001
LVESVI (ml/m^2)	r = 0.310	0.1231	r = 0.607	0.0010	r = 0.417	0.0343	r = 0.686	< 0.0001
EF (%)	−r = 0.238	0.2220	−r = 0.372	0.0513	−r = 0.318	0.0988	−r = 0.411	0.0300

ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; other abbreviations as in Table 1.

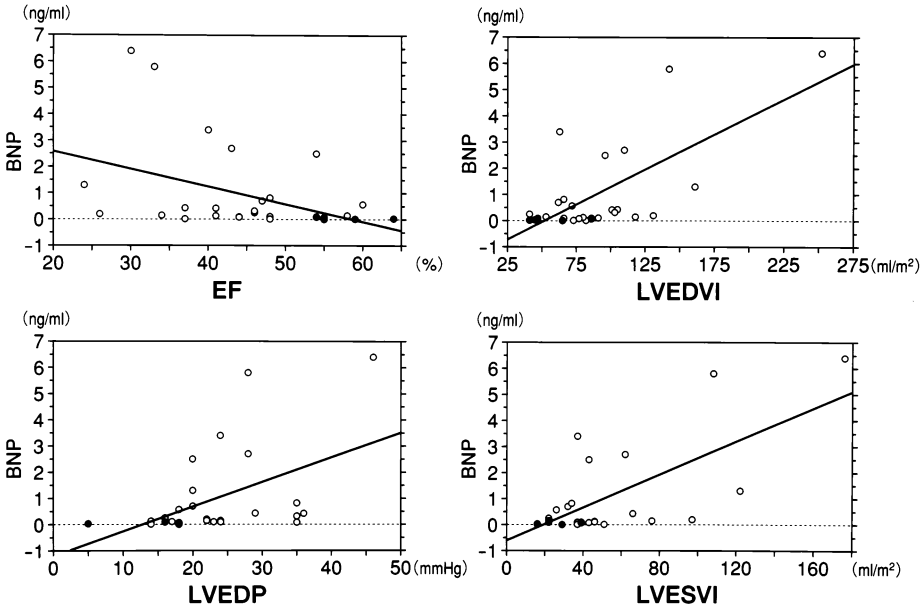

Figure 2. Correlations between pericardial fluid BNP levels and EF, LVEDP and LVEDVI or LVESVI in patients with normal (group I, **solid circles**) or impaired (group II, **open circles**) left ventricular function.

Discussion

Significance of ANP and BNP in left ventricular dysfunction. ANP and BNP are heart-derived peptides that are activated by the increased wall tension or stretch of cardiac chambers (1–7). Earlier studies (4,7–10) reported elevated plasma levels of ANP and BNP in association with left ventricular dysfunction. However, because the plasma levels of these peptides vary greatly from time to time in response to various minimal stresses, it is unknown whether they provide accurate information on the disease status and prognosis of patients with ischemic heart disease. In addition, as recent studies suggest that the heart itself is one of the target organs for natriuretic peptides, it is important to establish whether ANP and BNP are accumulated in the pericardial space. Our present results revealed that the levels of ANP and BNP were higher in pericardial fluid than in plasma, and that BNP levels in pericardial fluid had closer relations with indicators of left ventricular dysfunction than did BNP levels in plasma.

In healthy persons, the plasma concentration of BNP has been reported to be lower than that of ANP (4,7–9). Conversely, in chronic heart failure, the plasma BNP/ANP ratio is reversed. Furthermore, BNP levels increase more rapidly than ANP levels in the acute phase of myocardial infarction (8) and in obstructive form of hypertrophic cardiomyopathy (9). Compatible with these findings, the present study demonstrated that both plasma and pericardial fluid levels of BNP increased more markedly in association with left ventricular dysfunction than did those of ANP. BNP is secreted predominantly from the ventricle in response to ventricular wall stress and stretch (4,7–9). In contrast, the atria provide main source of plasma ANP (1,2), although increased amounts of ANP are secreted from the ventricle in patients with heart failure (4,7). Circu-

Table 3. Influence of Myocardial Ischemia on Atrial and Brain Natriuretic Peptide Levels in Plasma or Pericardial Fluid*

	Angina Pectoris		Myocardial Infarction	
	Stable (n = 7)	Unstable (n = 7)	Recent (n = 4)	Old (n = 10)
Plasma ANP (pg/ml)	9 ± 2	6 ± 1	89 ± 84	11 ± 4
Plasma BNP (pg/ml)	5 ± 0	18 ± 6	261 ± 236	94 ± 41
Pericardial fluid ANP (pg/ml)	15 ± 6	15 ± 4	177 ± 168	83 ± 41
Pericardial fluid BNP (pg/ml)	144 ± 73	308 ± 92	1,720 ± 1,370	1,682 ± 663
EF (%)	55 ± 2	48 ± 3	41 ± 4	38 ± 3
LVEDP (mm Hg)	18 ± 1	23 ± 4	26 ± 4	25 ± 3
LVEDVI (ml/m^2)	65 ± 7	75 ± 11	104 ± 16	112 ± 18
LVESVI (ml/m^2)	29 ± 4	33 ± 9	66 ± 17	71 ± 15

*There were no significant differences between patients with stable versus unstable angina or between patients with recent or old myocardial infarction for any of the variables listed. Data are expressed as mean value ± SD. Abbreviations as in Tables 1 and 2.

lating ANP and BNP are cleared through the clearance (C)-receptor (15–17) expressed in a wide variety of tissues. BNP has a lower affinity to the C-receptors than does ANP, and thus has a longer half-life than that of ANP (18). Thus, the sites of secretion and half-life may, at least in part, account for the differences in plasma and pericardial fluid levels between ANP and BNP.

Pericardial fluid BNP levels. The present results showed higher levels of ANP and BNP in pericardial fluid than in plasma. In the patients with normal left ventricular function, plasma levels of BNP were lower than those of ANP, but this relation was reversed in pericardial fluid. The higher levels of these peptides (especially BNP) in pericardial fluid than in plasma suggest that BNP is secreted more predominantly into the pericardial space rather than into the bloodstream, or that BNP has a longer half-life in the pericardial fluid than in plasma. Our study also showed that pericardial fluid BNP levels had closer relations with the indicators of left ventricular dysfunction than did plasma BNP levels. Because natriuretic peptides in plasma have relatively short half-lives, their plasma levels are rapidly and greatly changed by anesthesia or surgical stress. These findings indicate that pericardial fluid BNP levels provide more sensitive, stable and accurate information than do plasma BNP levels on left ventricular dysfunction due to ischemic heart disease.

Relations between ANP and BNP levels and left ventricular function. We defined group II patients as having impaired left ventricular function. However, it is possible that some of these patients have significant systolic dysfunction without diastolic dysfunction, some have both systolic and diastolic dysfunction and some have normal systolic but abnormal diastolic function. In addition, the secretion of ANP and BNP from the heart is accelerated by various stimuli, including catecholamines, endothelin and angiotensin II, among others. The levels of these endocrine factors as well as the mechanism causing congestive heart failure may vary in each patient. Thus, these factors may account for the variability seen in Figure 2. Further studies in a larger number of patients are needed to clarify the contribution of each factor to the levels of ANP and BNP in plasma and pericardial fluid.

Roles of pericardial fluid ANP and BNP. What are the pathophysiologic roles of ANP and BNP in pericardial fluid in patients with left ventricular dysfunction? Several lines of evidence support the idea that the heart itself is a site of action of ANP and BNP. 1) The physiologic function of isolated cardiac myocytes is altered by the administration of ANP (19,20). 2) The genes for all three natriuretic peptide receptor subtypes are expressed in the rat heart (11). 3) Both ANP and BNP stimulate cGMP generation in purified cardiac myocytes and in cardiac fibroblasts (11). 4) Both ANP and BNP inhibit DNA synthesis in cardiac fibroblasts (12). These results, taken together with the present findings indicating the accumulation of natriuretic peptides in the pericardial space, suggest that these peptides have a pathophysiologic role in heart failure as autocrine/paracrine factors. The upregulation of cGMP in cardiac myocytes and fibroblasts by ANP and BNP implies that these peptides inhibit the remodeling of the heart by regulating the growth of these cells. The reduction of cytosolic Ca^{2+} by ANP suggests that natriuretic peptides may have negative inotropic action. Further study is necessary to determine whether ANP and BNP have beneficial or harmful roles, as autocrine/paracrine factors, in patients with left ventricular dysfunction.

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