Expression and Distribution of Brain Natriuretic Peptide in Human Right Atria

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Objectives. We investigated expression of brain natriuretic peptide (BNP) as well as atrial natriuretic peptide (ANP) and their genes in human right atria. Their relations with atrial pressure were also examined.

Background. The BNP plays a role in electrolyte-fluid homeostasis such as ANP. The tissue level is reported to be elevated in the failing ventricles. However, expression and transmural distribution of BNP in the atria remain unclear.

Methods. Expression of ANP and BNP was immunohistochemically investigated in the right atrial (RA) specimens from 21 patients who had undergone cardiac surgery. The mRNA of specimens were quantitatively measured by Northern blot analysis and also evaluated by in situ hybridization. In addition, plasma levels of ANP and BNP were measured in the patients.

Results. The BNP immunoreactivity was diffusely seen in RA tissue of patients with mean RA pressure (mRAP) of 5 mm Hg or more, but it was noted only in the subendocardial half of the atria of those with mRAP less than 5 mm Hg. There was a significant correlation between the incidence of BNP-positive myocytes and mRAP (r = 0.850, p < 0.0001). Conversely, ANP-positive myocytes were found diffusely in all cases. In Northern blot analysis, the mRNAs levels of ANP and BNP in the atrial tissue were positively correlated with the mRAP (ANP, p = 0.775, p < 0.005 and BNP, p = 0.771, p < 0.005). In situ hybridization confirmed these findings. The mRNA levels were significantly correlated to each other (r = 0.845, p < 0.0002). Plasma ANP and BNP levels were elevated in the patients compared with that in controls; however, none were significantly correlated with the mRAP.

Conclusions. Expression of BNP and BNP mRNA is augmented in the atria with increased pressure, and distributed predominantly in the subendocardial side. The level of BNP mRNA was well correlated with that of ANP mRNA. Thus, these two genes might be commonly regulated in response to atrial pressure.

(J Am Coll Cardiol 1998;32:1832–8)

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Brain natriuretic peptide (BNP) shares biological and structural similarities with atrial natriuretic peptide (ANP) (1–3). Both appear to play a role in fluid-electrolyte homeostasis (3–5) and are produced in hearts of various animals and humans (2,3). The major source of circulating BNP is thought to be ventricular myocytes (6) and that of circulating ANP is atrial myocytes (6,7). Plasma ANP level and atrial ANP expression are known to be augmented in patients with atrial hemodynamic overload (6,7). In ventricle, both ANP and BNP are overexpressed, being correlated with severity of congestive heart failure (CHF) or ventricular hemodynamic overload (6,9–12). Contrary to these findings, expression of BNP in human atria has not been well elucidated. Furthermore, the relationship between expression of BNP in the atria and atrial hemodynamics has not been investigated in humans.

In the present study, we investigated the expression and tissue localization of both peptides (ANP and BNP) in human right atrial (RA) appendages obtained at surgery, using immunohistochemistry, Northern blot analysis, and in situ hybridization. We also measured plasma levels of both peptides with radioimmunoassay. The relation between expression of these peptide and hemodynamic parameters, especially mean right atrial pressure (mRAP), was clarified.

Methods

Patients’ profiles. Specimens were obtained from 21 patients during cardiac surgery (11 males and 10 females, mean age 59 ± 11 years) (Table 1): 3 patients with atrial septal defect, 2 with mitral stenosis, 1 with mitral regurgitation, 1 with aortic regurgitation, 1 with aortic stenosis, 10 with angina...
pectoris (n = 6) and/or old myocardial infarction (n = 4), 2 with aortic aneurysm and 1 with left atrial myxoma. Two patients were in New York Heart Association (NYHA) functional class IV, 4 in class III, 12 in class II, and 2 in class I. The NYHA class was evaluated on the day of catheterization. Patients were hemodynamically stable during 15 ± 7 days between catheterization and operation, and there was no change in therapeutic regimens during that period. All patients had restriction of dietary sodium intake less than 10 g/day, and all medications were stopped on the days of catheterization and operation.

Informed consent was obtained from each patient. This study was approved by the ethical committee on human research of Kyoto University.

**Tissue preparation.** Specimens were obtained from the cannulation site in the RA appendage before starting extracorporeal circulation. Sampling site was the same because of surgical maneuver. For immunohistochemistry and in situ hybridization, specimens were fixed in paraformaldehyde and embedded in OCT-compound (Miles, Elkhart, Indiana) and stored at −70°C. Specimens for RNA analysis were obtained from 12 of 21 patients (three with atrial septal defect, two with mitral stenosis, one with mitral regurgitation, five with angina pectoris, one with left atrial myxoma), frozen and stored at −70°C until use.

**Immunohistochemistry.** Sections were stained by the indirect immunoperoxidase method using antibodies against ANP and BNP as reported previously (9,12–15). Evaluation of immunohistological findings were carried out by two individuals independently and who did not know patient information such as diagnosis and hemodynamic parameters. To grade the immunostaining of BNP, we used a semiquantitative scoring system as follows: BNP-positive and -negative myocytes were counted in 20 random high-power fields (HPF, ×200) in a subendocardial half and epicardial half in each specimen, and percent ratio of BNP-positive myocytes to total count of myocytes was calculated (BNP score).

**Northern blot analysis.** Total cellular RNA was prepared using single-step RNA extraction (16). Human ANP cDNA (17) and human BNP cDNA (18) probes were labeled with [alpha-32P]-dCTP using random-priming method. The radioactivity of hybridized band was measured using a BAS analyzing system (Fuji Film, Japan). The mean value of RA-specific

### Abbreviations and Acronyms

- ANP = atrial natriuretic peptide
- BNP = brain natriuretic peptide
- CHF = congestive heart failure
- mRAP = mean right atrial pressure
- NYHA = New York Heart Association
- RA = right atrial

### Table 1. Patient Data

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Pt, patient; Dx, diagnosis; NYHA, New York Heart Association functional class; mRAP, mean right atrial pressure; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; a.u., arbitrary unit; MS, mitral stenosis; Myx, myxoma; MR, mitral regurgitation; ASD, atrial septal defect; OMI, old myocardial infarction; AP, angina pectoris; AR, aortic regurgitation; AS, aortic stenosis; AA, aortic aneurysm; diff, diffuse; subend, subendocardial; –, not measured.
mRNA level normalized by beta-2-microglobulin cDNA (19) was arbitrarily set as 1.0 for each mRNA species.

**Digoxigenin-labeled riboprobes and in situ hybridization.** Human ANP and BNP cDNA probes used in the Northern blot were subcloned into pBluescript SK⁻ (Stratagene, La Jolla, California) and pCR ™II (Invitrogen, Carlsbad, California), respectively. Riboprobes were transcribed using digoxigenin-labeled dUTP (Boehringer Mannheim, Germany) with an appropriate RNA polymerase (19).

In situ hybridization for ANP mRNA and BNP mRNA was performed on cryosections according to the method previously reported (20).

**Measurement of plasma levels of ANP and BNP.** Plasma was sampled in 18 of the study patients and 5 normal control subjects. Blood was sampled at 6:00 AM while the subjects were fasting in a recumbent position. Measurement was performed with radioimmunoassay as previously reported (6,7).

**Statistical analysis.** We set the cutoff point of the mRAP at 5 mm Hg. This was based on the mRAP of 2 to 4 mm Hg (3 ± 1 mm Hg), which was calculated from 31 normal control subjects (17 men and 14 women). They underwent cardiac catheterization because of chest pain, minimal ECG changes or arrhythmias, but examinations revealed no specific abnormalities.

Data were expressed as the mean ± SD. Statistical comparisons were performed using unpaired t test and chi-square test appropriately. Selected variables were compared using linear regression analysis. Multiple regression analysis was used to determine correlations between the results. Statistical significance was designated as a probability of less than 0.05.

**Results**

**Immunohistochemical expression of ANP and BNP.** In all the sections examined, ANP immunoreactivity was evident in the cytoplasm of all myocytes, and definite difference in its distribution and intensity was not found (Fig. 1, A, B, and G). Conversely, BNP immunoreactivity was relatively weaker than ANP immunoreactivity (Fig. 1, D). The BNP score is listed in Table 1. This value of immunoreactive BNP was positively correlated with mRAP (r = 0.850, p < 0.0001) (Fig. 2).

**Northern blot analysis.** The radioactivity of the hybridized band of beta-2-microglobulin was similar in each case and was not correlated with the mRAP (Fig. 3). Atrial mRNA levels of BNP and ANP normalized by beta-2-microglobulin were correlated positively with the mRAP (ANP mRNA: r = 0.775, p < 0.005 and BNP mRNA: r = 0.771, p < 0.005) (Fig. 4, A and B).
and they were correlated with each other ($r = 0.845$, $p < 0.0002$) (Fig. 4, C).

**In situ hybridization of BNP and ANP.** Signals of in situ hybridization were dark precipitates in the cytoplasm (Fig. 5, A to E). Hybridization signals of ANP and BNP mRNA were distributed like immunoreactive BNP. The mRNA signals of ANP and BNP were found only in the subendocardial half of the atria in patients with mRAP less than 5 mm Hg (Fig. 5, A and B). In those with mRAP of 5 mm Hg or more, signals showed diffuse and transmural distribution (Fig. 5, C to E).

**Plasma levels of ANP and BNP.** The plasma levels of ANP and BNP in the study patients ($n = 18$) were 40.8 ± 30.0 and 62.8 ± 68.1 pg/ml, respectively, which were significantly higher
than those in normal control subjects (n = 5); the plasma levels of ANP and BNP were 19.2 ± 5.9 and 6.6 ± 2.7 pg/ml, respectively. We performed multiple regression analysis to assess the degree to which one of the hemodynamic parameters (mRAP, right ventricular end-diastolic pressure, left ventricular end-diastolic pressure, and ejection fraction) was associated with the plasma levels of ANP and BNP. Among them, the left ventricular end-diastolic pressure showed significant relations with the plasma ANP and BNP levels (Table 2). However, no statistically significant relation was identified between the mRAP and plasma levels of ANP and BNP.

### Discussion

**Expression of BNP in human right atria.** In the present study, we have shown expression of BNP by immunohistochemistry and that of BNP mRNA by Northern blot analysis and in situ hybridization in human RA tissue. We examined them in the right atrial appendage. As reported by Rodeheffer et al. (21), atrial appendage is representative of the whole atria on ANP expression in normal and failing hearts. However, investigations in other parts of the atria as well as in the appendage might be desirable. We did not evaluate the expression of ANP and BNP in the left atria, either, which is also to be further examined.

The BNP-positive myocytes were predominantly localized in the atrial subendocardial region. Semiquantitative measurement by BNP score confirmed this. According to in situ hybridization, the mRNA signals of BNP showed a distribution pattern similar to the immunostaining of BNP. We previously showed a transmural gradient of BNP expression in the porcine normal atria (13). In the present study, we have evidenced a transmural gradient of BNP at both peptide and gene levels in human right atrial wall.

**Regulatory mechanisms for BNP expression in the atria.** Different from the predominant expression of BNP in the subendocardial side of RA in patients with mRAP less than 5 mm Hg, BNP-positive myocytes were found diffusely in the atrial wall in patients with mRAP of 5 mm Hg or more. The BNP mRNA was also increased in these patients. There was a positive correlation between BNP score and mRAP and between the level of BNP mRNA and mRAP. We also found a positive correlation between BNP mRNA and ANP mRNA. These suggest that synthesis of BNP and ANP in human right atria may be commonly associated with atrial pressure.

Increased intracavitary pressure generally accompanies augmentation of wall stress (Laplace’s law). The expression of ANP and BNP is augmented in the left ventricles of the failing hearts (6,7,9–12,18,22) or in the ventricles of hypertrophic cardiomyopathy without CHF (14,23). In these reports, increased wall stress and local stress are supposed to evoke the expression of the ANP and BNP genes in the left ventricles. In fact, wall stress decreases from the endocardial side to the epicardial side (24,25). Therefore, this regulation mechanism of natriuretic peptides in the ventricle would also work in the atria.

Wall stress generally induces wall stretch at physiological states. Atrial stretch causes secretion of ANP from atria of

### Table 2. Correlation Coefficients Relating Hemodynamic Parameters to Plasma Levels of ANP and BNP

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<th>Parameters</th>
<th>Plasma ANP (pg/ml)</th>
<th>Plasma BNP (pg/ml)</th>
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<tr>
<td></td>
<td>r-Value</td>
<td>p-Value</td>
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<td>mRAP (mm Hg)</td>
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<td>RVEDP (mm Hg)</td>
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<td>EF (%)</td>
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*Statistically significant. See Table 1 for abbreviations.
BNP showed the transmural gradient in the atria. Second, the findings. First, immunoreactive BNP and mRNA signals of BNP is incomplete. Such an incomplete compensation may add to the systemic natriuretic peptide levels. Circulating ANP and BNP levels and atrial mRNA expression of these peptide were increased in patients with atrial septal defect and mitral stenosis in the present study. In these patients, right ventricular overload was seen, in addition to atrial overload. It is well-known that right ventricular overload increases ANP and BNP expression in the right ventricles (6,34). We performed multiple regression analysis to assess the degree to which of the hemodynamic parameters, mRAP in particular, was associated with the plasma levels of ANP and BNP. We found significant correlations only in the left ventricular end-diastolic pressure, but not in mRAP. This suggests that the right atrial secretion of ANP and BNP does not contribute significantly to the systemic levels in the study patients. However, it is possible that left atria may have been a far larger source of the peptides in some patients studied. Thus, the present analysis may have underestimated the overall atrial contribution to circulating levels of ANP and BNP because of a lack of information regarding left atria. In addition, an analysis is necessary to confirm the present result, using a larger patient population accompanied by higher mRAP. Augmentation of BNP expression in the right atria with increased mRAP may be one of the compensatory mechanisms of hearts against volume overload when considering the pharmacological effects of BNP such as vasodilation and natriuresis (1–5), but the lack of relationship between plasma level of BNP and mRAP indirectly suggests that the compensation by atrial BNP is incomplete. Such an incomplete compensation may justify the clinical use of exogenous natriuretic peptides.

Conclusions. The present study revealed the following findings. First, immunoreactive BNP and mRNA signals of BNP showed the transmural gradient in the atria. Second, the atrial expression of BNP was augmented in response to the increased atrial pressure and was well correlated with the mRAP. Third, atrial expression of BNP mRNA was well correlated with that of ANP mRNA. Thus, these two genes may share some common regulatory mechanisms, and atrial pressure was suggested to be one of them.

We thank M. Hatsuoka, Y. Hataguchi and R. Nonaka for their technical assistance with immunohistochemistry; K. Hayashi and F. Ota for their secretarial assistance; the staff members of Cardio Disease Center, Rakukai Otowa Hospital for their help in preparing hemodynamics data; and the staff members of our laboratory, especially Dr. Y. Nishiyama (Second Department of Pathology, Kyoto University School of Medicine), for their encouragement and advice.

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