Ventricular Expression of Brain Natriuretic Peptide in Hypertrophic Cardiomyopathy

Koji Hasegawa, MD; Hisayoshi Fujiwara, MD; Kiyoshi Doyama, MD; Masami Miyamae, MD; Takako Fujiwara, MD; Shinichi Suga, MD; Masashi Mukoyama, MD; Kazuwa Nakao, MD; Hiroo Imura, MD; and Shigetake Sasayama, MD

Background. Brain natriuretic peptide (BNP), as a cardiac hormone, is expressed together with atrial natriuretic peptide (ANP) in the ventricles in congestive heart failure. However, the ventricular expression of BNP in hypertrophic cardiomyopathy (HCM) with normal systolic function is still unclear.

Methods and Results: The study population consisted of 39 HCM patients with asymmetric septal hypertrophy and 10 control subjects without any specific cardiac disease. Eleven cases of HCM were obstructive (HOCM), and the other 28 cases were nonobstructive (HNCM). All of these patients had a normal ejection fraction. Immunohistochemical analysis of endomyocardial biopsy specimens with specific monoclonal antibodies showed BNP immunoreactivity in the HOCM group (5/10, 50%) but not in the HNCM group (0/22) or in control subjects (0/5). In HOCM, left ventricular end-diastolic pressure was significantly higher in the BNP-positive patients than the BNP-negative patients. Histological changes such as myocardial fiber disarray, hypertrophy of myocytes, and fibrosis were greater in BNP-positive patients than BNP-negative patients in HCM. However, the expression had no significant relation with other clinical parameters. The elevation of the BNP plasma level versus control subjects was marked in both HOCM (85-fold) and HNCM (23-fold). By contrast, the elevation of the ANP plasma level versus control subjects was mild in HOCM (5.7-fold) and HNCM (4.2-fold). The ratio of BNP level to ANP level was higher in HOCM (4.16) than in HNCM (1.46) and control subjects (0.28), and it was higher than the ratio previously reported for severe congestive heart failure (1.72).

Conclusions. These findings suggest that BNP is expressed in the ventricular myocytes of HCM with normal systolic function. In HOCM, ventricular expression of BNP may be augmented in response to both obstruction and diastolic dysfunction. (Circulation 1993;88:372-380)

Key Words • antibodies • natriuretic peptides

Brain natriuretic peptide (BNP) was first isolated from the porcine brain, and it has a striking similarity to atrial natriuretic peptide (ANP) with regard to both its amino acid sequence and pharmacological spectrum.1 BNP is also synthesized in and secreted from the porcine heart.2,3 Unlike ANP, BNP shows species variations in both its structure and tissue distribution.4-6 It has been shown that patients with congestive heart failure had extremely high plasma levels of BNP and that its secretion from the ventricles is much accelerated in this condition.7-9 These findings suggest that BNP has pathophysiological importance in heart failure as a cardiac hormone secreted predominantly from the ventricles.

Although ANP is expressed in fetal ventricles, its expression is markedly reduced in normal adult ventricles.10,11 However, ventricular ANP is reexpressed in various pathological heart conditions.12-17 Expression of both ANP and BNP is prominently augmented in the ventricles in severe congestive heart failure.7,8 However, the ventricular expression of BNP in relation to ANP in hypertrophic cardiomyopathy (HCM) with normal systolic function is still not clear. To elucidate this expression, we carried out this study, in which the immunohistochemical expression of BNP and ANP in endomyocardial biopsy specimens was related to routine hemodynamic parameters obtained from cardiac catheterization in patients with HCM. Since the secretory turnover in ANP and BNP differs,7,9 we also measured their plasma levels in HCM.

Methods

Patient Profile

All of the 39 patients with HCM in this study were evaluated clinically by both noninvasive and invasive methods. The mean age of these patients (28 men and 11 women) was 49±16 years. All patients had normal sinus rhythm and normal ejection fraction and were categorized as New York Heart Association (NYHA) functional class I. No patient had apparent history or clinical findings of congestive heart failure. The diagnosis of HCM was made according to the definition and classification proposed by the World Health Organiza-
Hemodynamic and Angiographic Evaluation

None of the patients received any drugs on the day of the invasive examination. All patients underwent both right and left heart catheterization, biplane left ventriculography, and selective coronary angiography according to standard techniques. The heart rate and the right and left heart pressures were recorded, and the cardiac index was estimated by the thermodilution method. The left ventricular end-diastolic and end-systolic volume indexes and the ejection fraction were calculated from left ventricular cineangiograms performed in the right anterior oblique projection by the method of Kennedy et al.19

Endomyocardial Biopsy Procedure and Histological Evaluation

Ventricular specimens were obtained by endomyocardial biopsy during cardiac catheterization in 32 patients with HCM (10 with HOCM, 22 with HNCM) and 5 normal control subjects. In all of these patients, biopsy specimens were obtained at cardiac catheterization from both the right ventricular side of the ventricular septum (RVB) and the left ventricular free wall (LVB) with a Konno-Sakakibara20 or Kawai-Kitaura21 biopsy catheter. Two or three biopsy specimens from the RVB and LVB were obtained from every patient. These specimens were immediately fixed with 4% paraformaldehyde in 0.01 mol/L phosphate-buffered saline, dehydrated, embedded in paraffin, and cut into 4-μm-thick sections. From light microscopic observations of the sections stained with hematoxylin-eosin or Masson's trichrome, histological parameters such as myocyte size (mean diameter, 30 to 50 myocytes per specimen), degree of fibrosis (from 0 to 3), and degree of myofiber disarray (from 0 to 3) were evaluated. The extent of fibrosis was graded as 0 (no fibrosis), 1 (mild, when an isolated scar or mild interstitial fibrous tissue formation or both were identified), 2 (moderate, intermediate between mild and severe), or 3 (severe, characterized by extreme replacement scarring occupying substantial portions of the section). For myocardial fiber disarray, the abnormal disarray defined in our previous reports was graded according to the severity and the extent of the findings in the 4-μm-thick sections as follows: 0 (no myofiber disarray), 1 (mild, only minimal and focal myofiber disarray), 2 (moderate, intermediate between mild and severe), and 3 (severe disorientation of myofibers covering more than three quarters of a section). Two blinded observers reviewed the sections, and the variability between the observers was 0.1±0.4 regarding the grade of fibrosis and 0.1±0.3 for the grade of disarray.

Monoclonal Antibodies

Monoclonal antibodies against alpha human ANP22 and human BNP7 were prepared as described previously. The epitope recognized by the monoclonal antibody against alpha human ANP is located in the N-terminal half of the ring structure that includes the Met12 residue. Radioimmunoassay with this antibody allows the recognition of not only alpha human ANP but also beta human ANP, with a cross-reactivity of 80% on a molar basis. Gamma human ANP is recognized on an equimolar basis; the cross-reactivity of this antibody with human BNP-32 and gamma human BNP is <0.01%. The monoclonal antibody against human BNP belonged to the IgG1 subclass. Analysis by Scatchard plot revealed a high affinity for human BNP, with an association constant of 1.0×10^11 per mole. The radioimmunoassay revealed that the antibody recognized the ring structure of human BNP. The cross-reactivity of the anti-BNP antibody with alpha human ANP, beta human ANP, gamma human ANP, and rat BNP was <0.005%, and porcine BNP-32 showed a cross-reactivity of 0.1% on a molar basis by radioimmunoassay. The intra-assay and interassay coefficients of variation were 8.4% (n=8) and 6.4% (n=8), respectively.

Light Microscopic Immunohistochemistry

Serial 4-μm-thick sections were alternately immunostained with antibodies to ANP and BNP to compare their distribution. Immunohistochemical reactions using an indirect immunoperoxidase method were obtained as described previously.3,8,13-16,23 Briefly, the first step involved the inhibition of intrinsic peroxidase activity by the addition of 0.3% hydrogen peroxide in phosphate-buffered saline. Nonspecific binding was blocked with normal goat serum. As a primary antibody, the monoclonal antibody described above (dilution of ascites to 1:1000) was incubated with the sections for 48 hours at 4°C. In the second step, the peroxidase-conjugated F(ab')2 fragment of the secondary antibody (goat antihuman IgG[H+L], Jackson Immunoresearch Laboratories) was added to the sections for 45 minutes at room temperature. Sections were then washed with 45 mg of 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co, St Louis, Mo) and 0.05% hydrogen peroxide in 100 mL of 0.05 mol/L Tris buffer solution (pH 7.6) for 3 minutes at room temperature. Sections were then washed four times for 10 minutes each time.
with phosphate-buffered saline between each step. Finally, counterstaining with hematoxylin was performed.

Immunohistochemical staining was performed on the two largest and different specimens taken from the ventricular side of the same heart and on sections from three different depths of the same specimen. The presence of immunoreactive ANP or BNP was assessed by light microscopy. Two blinded trained observers reviewed the sections and graded ANP and BNP as present or absent in the myocytes. ANP- and BNP-containing specimens (for RVB and LVB) were defined as those that contained at least one section in which ANP or BNP was present. Unanimity on the grade of ANP or BNP was reached by the two observers for all sections.

Immunohistochemical Control Sections

Although both monoclonal antibodies showed a high degree of specificity when the radioimmunoassay was performed,7,21 control experiments were carried out to verify the specificity of the reaction by light microscopic immunocytochemistry. These experiments included preabsorption of the primary antibody with the corresponding peptide antigen (1 nmol of peptide per mL of diluted antisera) and substitution of the primary antibody with nonimmune mouse serum. In addition, to exclude the possibility of cross-reaction between ANP and BNP, the primary antibody was preabsorbed with a heterologous antigen (1 nmol/mL).

As a positive control section, a tissue preparation of the right atrial appendage was obtained from a patient during aortocoronary bypass surgery.

Plasma Sampling

Plasma was sampled in 14 HCM patients (5 with HOCM and 9 with HNCM) and in 5 normal control subjects. After the drugs were stopped overnight, blood was withdrawn from the antecubital vein at 9:00 AM while the subjects were in a recumbent position. The samples were immediately transferred to chilled siliconized glass tubes containing Na3EDTA (1 mg/mL) and aprotonin (1 million IU/mL, Ohkura Pharmaceutical, Kyoto, Japan) and centrifuged at 4°C. Plasma was frozen immediately and stored at -70°C until assay.

Measurement of Plasma Levels by Radioimmunoassay

Synthetic human BNP (human BNP [77-108]) and [Tyr82]-human BNP [83-108] were donated by Professor H. Matsuo at the National Cardiovascular Center, Suita, Japan. Alpha human ANP (human ANP [99-126]) was purchased from the Peptide Institute, Inc, Minoh, Japan.

[Tyr82]-Human BNP [83-108] (1 µg) was radioiodinated by the chloramine T method as described previously.24 The specific activity of 125I-labeled [Tyr82]-human BNP [83-108] ranged from 500 to 900 µCi/µg. The monoclonal antibody against human BNP (final dilution of ascites, 1:5×10⁶) was incubated with either standard human BNP or samples in 0.2 mL assay buffer (50 mmol/L phosphate buffer, pH 7.4, containing 0.1% gelatin [Merck], 0.1% Triton X-100, 1 mmol/L Na3EDTA, 0.2 mmol/L L-cystine, and 0.1% NaCl) for 24 hours 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 Vereening NV, The Netherlands) and 25 mg of Dextran T-70 (Pharmacia, Uppsala, Sweden) in 100 mL of 50 mmol/L phosphate buffer, pH 7.4, containing 0.01% merthiolate, as described previously.24

Measurement of the concentrations of plasma BNP-like immunoreactivity was performed with or without extraction. In the assay performed without extraction, 25 µL of plasma was added to the incubation mixture. Hormone-free plasma, prepared by passing normal plasma through a Sep-Pak C18 cartridge (Waters Associates, Milford, Mass),25 was used for constructing the standard curve and diluting plasma samples. For the radioimmunoassay performed with extraction, peptides were extracted from 5 to 10 mL plasma with a Sep-Pak C18 cartridge, as described previously.25 The mean recovery of 3 to 15 fmol/mL human BNP added to plasma was 70%. The minimal detectable concentrations of BNP-like immunoreactivity in plasma with and without extraction were 0.4 and 10 fmol/mL, respectively.

Measurement of the ANP plasma level by radioimmunoassay was performed as reported previously,24,25

Statistical Analysis

Clinicopathological data were expressed as the mean±SD. Statistical comparisons were performed by χ² analysis, Student's t test, or one-way ANOVA with multiple comparisons, when appropriate. Statistical significance was designated at a probability value of P<.05.

Results

Immunohistochemical Expression of BNP

Negative control sections incubated with the primary antibody preabsorbed with the corresponding peptide antigen showed no positive signals (Fig 1, a and b). The positive control sections of the right atrial appendage showed dark brown immunoreactive ANP (Fig 1, c) or BNP (Fig 1, d) products in the cytoplasm of atrial myocytes. No immunopositive products were seen in the endocardium or in any of the connective tissues. ANP immunoreactivity was strong, whereas BNP immunoreactivity was relatively weak, being localized in the subendocardial atrial myocytes. The positive signals were not changed by preabsorption with the heterologous antigen (Fig 1, e and f).

In endomyocardial biopsy specimens, none of the control group showed ANP (Fig 2, a) or BNP (Fig 2, b) immunoreactivity (0/5). However, BNP was found in 50% (5/10; 2 with both LVB and RVB, 1 with LVB, and 2 with RVB) of the HOCM patients (Fig 2, d through f) but not in the HNCM patients (0/22). In contrast, ANP was present in 60% (6/10; 2 with both LVB and RVB, 2 with LVB only, and 2 with RVB only) of the HOCM patients (Fig 2, c) and 37% (8/22; 1 with both LVB and RVB, 3 with LVB only, and 4 with RVB only) of the HNCM patients. All of the BNP-positive patients had positive staining for ANP. ANP immunoreactivity showed a fine granular pattern in the ventricular myocytes of HNCM and HOCM (Fig 2, c). BNP immunoreactivity usually showed a fine granular pattern in ventricular myocytes of HOCM resembling ANP but somewhat more diffusely spread throughout the cytoplasm than ANP (Fig 2, d through f). None of the
endomyocardial biopsy specimens contained a pulse conduction system.

Table 1 shows a comparison of the clinical values for the control subjects, HNCM patients, and HOCM patients with and without ANP or BNP expression. In HNCM and HOCM, all of the BNP-positive patients were positive for ANP. Thus, patients were divided into the following three groups: group 1, ANP-positive and BNP-positive; group 2, ANP-positive and BNP-negative; and group 3, ANP-negative and BNP-negative. In HOCM, since group 2 consisted of only one patient, clinical values were compared between BNP-positive (group 1) and BNP-negative (groups 2 and 3) patients. Left ventricular end-diastolic pressure (LVEDP) did not differ between the HOCM (15±4 mm Hg) and the HNCM (14±5 mm Hg) patients. However, LVEDP was significantly higher in the BNP-positive than in the BNP-negative HOCM patients. The other parameters, including ejection fraction, left ventricular volume indexes, cardiac index, right-side heart pressures, and left ventricular wall thickness, showed no significant difference between BNP-positive and BNP-negative patients in HOCM. The basal intraventricular pressure gradient did not differ between the BNP-positive (55±43 mm Hg) and BNP-negative (42±15 mm Hg) HOCM patients. In HOCM, BNP-positivity did not differ among patients receiving calcium antagonists alone (33%, 1/3), those with β-blockers alone (50%, 2/4), and those with both calcium antagonists and β-blockers (67%, 2/3). In HNCM, since there were no patients in group 1, clinical values were compared between ANP-positive (group 2) and ANP-negative (group 3) patients. However, none of the clinical parameters showed a significant difference between ANP-positive and ANP-negative patients in HNCM.

The immunohistochemical expression of BNP in HCM was related to histological findings of RVB and LVB separately, as shown in Table 2. In LVB of HCM, the myocyte size was greater in the BNP-present specimens than in the BNP-absent specimens. The BNP-present RVB of HCM showed more severe grades of fibrosis and myofiber disarray than did the BNP-absent specimens. The immunohistochemical expression of ANP in HCM also had a significant relation with myocyte hypertrophy in LVB and disarray or fibrosis in RVB.
FIG 2. Photomicrographs showing immunohistochemistry of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) in endomyocardial biopsy specimens (panels a through f). None of the control subjects showed immunoreactivity for ANP (panel a) or BNP (panel b). However, in the ventricular myocytes of hypertrophic nonobstructive cardiomyopathy, fine granular brown immunoreactive products of ANP (panel c) or BNP (panels d through f) were observed. Note that BNP was somewhat diffusely spread throughout the cytoplasm in ventricular myocytes (panels d through f). Original magnification ×200; bar=50 μm.

Plasma BNP Level

Fig 3 shows plasma levels of ANP and BNP in patients with HCM and in normal control subjects. The plasma BNP level in control subjects was 1.5±1.1 fmol/mL, whereas the ANP level in the same plasma samples was 5.5±2.0 fmol/mL. Plasma BNP levels were significantly increased in patients with HNCM (12.1 to 55.1 fmol/mL; mean, 35.3±20.7 fmol/mL) and HOCM (24.6 to 212.9 fmol/mL; mean, 128.0±76.2 fmol/mL) compared with control values. The elevation of plasma BNP levels versus control values was marked both in HOCM (85-fold) and in HNCM (23-fold). The BNP plasma level was significantly higher in the HCM patients with LVEDP ≥20 mm Hg (n=3, 188.7±18.6 fmol/mL) than those with LVEDP <20 mm Hg (n=11, 36.9±12.2 fmol/mL). Plasma ANP levels were significantly elevated in patients with HNCM (9.7 to 52.4 fmol/mL; mean, 23.3±11.5 fmol/mL) and HOCM (11.5 to 59.3 fmol/mL; mean, 31.2±16.8 fmol/mL) compared with control values. In contrast to the elevation of BNP, the elevation of ANP levels versus control values was mild in HOCM (5.7-fold) and HNCM (4.2-fold).
TABLE 1. Comparison of Clinical Values Between Patients With and Without Ventricular Atrial or Brain Natriuretic Peptide Expression

<table>
<thead>
<tr>
<th>Group</th>
<th>LVEDVI, LVEDVI (mL/m²)</th>
<th>AOSP, LVEDP (mm Hg)</th>
<th>PCWP (mm Hg)</th>
<th>RVSP, RVEDP (mm Hg)</th>
<th>CI (L/min⁻¹·m⁻²)</th>
<th>EF (%)</th>
<th>VST, LVT (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP(+), BNP(+)</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ANP(+), BNP(−)</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ANP(−), BNP(−)</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ANP(−), BNP(−)</td>
<td>5</td>
<td>70±15</td>
<td>118±23</td>
<td>7±2</td>
<td>24±5</td>
<td>3.1±0.5</td>
<td>68±8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25±10</td>
<td>7±2</td>
<td>4±3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOCM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP(+), BNP(+)</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ANP(+), BNP(−)</td>
<td>8</td>
<td>62±10</td>
<td>119±21</td>
<td>9±3</td>
<td>26±8</td>
<td>2.7±0.4</td>
<td>65±2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22±5</td>
<td>17±3</td>
<td>4±1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP(−), BNP(+)</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ANP(−), BNP(−)</td>
<td>14</td>
<td>71±11</td>
<td>124±18</td>
<td>7±3</td>
<td>24±5</td>
<td>2.8±0.4</td>
<td>67±7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23±7</td>
<td>14±5</td>
<td>5±3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HNCM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP(+), BNP(+)</td>
<td>5</td>
<td>67±16</td>
<td>117±21</td>
<td>7±3</td>
<td>24±2</td>
<td>3.4±0.8</td>
<td>64±8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25±12</td>
<td>20±2</td>
<td>5±1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP(+), BNP(−)</td>
<td>1</td>
<td>73</td>
<td>124</td>
<td>6</td>
<td>24</td>
<td>3.3</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
<td>15</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP(−), BNP(+)</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ANP(−), BNP(−)</td>
<td>4</td>
<td>60±14</td>
<td>113±11</td>
<td>7±2</td>
<td>29±7</td>
<td>2.8±0.4</td>
<td>72±4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17±6</td>
<td>13±4</td>
<td>5±1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LVEDVI and LVEDVI, left ventricular end-diastolic and end-systolic volume indexes; AOSP, aortic systolic pressure; LVEDP, left ventricular end-diastolic pressure; PCWP, pulmonary capillary wedge pressure; RVSP and RVEDP, right ventricular peak systolic and end-diastolic pressure; CI, cardiac index; EF, ejection fraction; VST and LVT, ventricular septal and left ventricular posterior wall thickness by two dimensional echocardiography; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; HNCM, hypertrophic nonobstructive cardiomyopathy; HOCM, hypertrophic obstructive cardiomyopathy; +, positive; −, negative.

* P<.05 different from BNP-negative HOCM patients.

The molar ratio of BNP to ANP in plasma varied markedly. In normal control subjects, the plasma BNP level was four times lower than the ANP level, whereas the mean plasma BNP level in patients with HNCM was slightly higher than the ANP level determined simultaneously. However, in HOCM patients, the plasma BNP level was much higher than the ANP plasma level. Thus, the mean plasma BNP/ANP ratio was significantly higher in HOCM (4.16±2.41) than in HNCM (1.46±0.34) or in control subjects (0.28±0.10). A previous study showed that in patients with severe congestive heart failure (NYHA class IV), both ANP and BNP plasma levels were markedly elevated (164.4±20.3 fmol/mL in ANP and 267.3±79.9 fmol/mL in BNP), with a mean plasma BNP/ANP ratio of 1.72. Thus, the plasma BNP/ANP ratio in our study was also higher in patients with HOCM than in those with severe congestive heart failure in the study referred to above.7

Relation Between Immunohistochemical Expression and Plasma Levels

Eight HCM patients (three with HOCM and five with HNCM) were evaluated by both immunohistochemistry and radiomimunoassay. BNP plasma levels were significantly higher in patients who were BNP-positive by immunohistochemistry (n=2, 176.7±9.2 fmol/mL) than in the BNP-negative patients (n=6, 32.3±13.7 fmol/mL). However, ANP plasma levels in ANP-positive (n=4, 23.3±10.2 fmol/mL) and ANP-negative patients (n=4, 22.2±5.1 fmol/mL) did not differ.

Discussion

BNP, as a cardiac hormone, is expressed predominantly in the ventricles of failing hearts together with ANP.8,9 We previously reported that the ventricular expression of BNP in dilated cardiomyopathy had a close relation with the impairment of systolic function.8 In this study, we demonstrated that BNP was expressed in the ventricular myocytes of HCM with normal systolic function. We detected ventricular BNP immunoreactivity in HOCM but not in HNCM. These findings suggest that the presence of the obstruction in HCM significantly contributes to the accelerated synthesis and secretion of ventricular BNP. In HOCM, the immunohistochemical expression of ventricular BNP and the BNP plasma level had significant relation with the elevation of LVEDP. Since all the HCM patients in this study had normal systolic function, the elevation of LVEDP may represent diminished compliance or diastolic dysfunction of ventricles. These findings suggest that BNP is expressed in the ventricular myocytes of HOCM in response to both obstruction and diastolic dysfunction.

It is well known that HCM patients with diastolic dysfunction may progress to congestive heart failure, although they have normal systolic function. However,
none of the patients studied here had an apparent history or clinical findings of congestive heart failure. Pulmonary capillary wedge pressure as an indicator of pulmonary congestion was within normal limits in all of the HCM patients and did not differ between immunohistochemically BNP-positive and BNP-negative HCM patients. These findings suggest that congestive heart failure is not indispensable for the ventricular expression of BNP. The immunohistochemical expression of ventricular BNP had a significant relation with myocardial fiber disarray, hypertrophy of myocytes, and fibrosis. Generally, these histological changes are one of the main causes of the diastolic dysfunction in HCM.26,27 Therefore, BNP expression may occur as a response to these histological changes.

In HCM, plasma BNP levels were significantly higher in patients who were BNP-positive by immunohistochemistry than in BNP-negative patients. These findings suggest that the ventricular expression of BNP contributes to the elevation of BNP plasma level, consistent with the idea that BNP is a cardiac hormone secreted from ventricles.7,9 In contrast, there was no significant relation between immunohistochemical expression and plasma levels of ANP in HCM. ANP is secreted predominantly from atria in normal states.7,9 In HCM, although ANP is expressed in the ventricles,14 atria as well as ventricles may provide a considerable source of circulating ANP.

The elevation of the BNP plasma level versus control levels was marked in both HNCM and HOCM. Since BNP is a cardiac hormone secreted from the ventricles,7,9 the elevation of BNP plasma level in HCM may represent accelerated ventricular secretion. However, ventricular BNP was immunohistochemically negative in all the HNCM patients and in half of the HOCM patients. This discrepancy may be attributed, in part, to the limitations of a biopsy study, in which specimens are too small to represent the whole ventricle. Moreover, a large portion of BNP is secreted into the bloodstream immediately after biosynthesis (constitutive pathway), and thus the retention time of BNP in ventricular myocytes may be very short.7,9 Therefore, in these BNP-negative patients, BNP may have been secreted from the ventricles too rapidly to be identified by immunohistochemistry.

In experimental settings, ventricular ANP gene induction is closely associated with myocardial cell hypertrophy.28,29 Although significant hypertrophy is evident by echocardiography and histology in all of the HCM patients studied here, ANP was immunohistochemically positive in only 37% of the HNCM patients and in only 60% of the HOCM patients. One possible reason for the lack of ANP staining in these patients is the sensitivity of immunohistochemistry. Since antigenicity may be partly damaged during the fixation and embedding process, the ANP staining may be immunohistochemically negative even if a small amount of ANP exists in the ventricular myocytes. The second possible reason is the site of biopsy. RVB specimens are obtained from the right ventricular side of the interventricular septum and LVB from the endocardial side of the left ventricular free wall. However, in the autopsied human heart with HCM, histological changes such as fibrosis, myocardial disarray, and myocardial cell hypertrophy are greatest in the middle portion of the ventricular septum.30-32 They are relatively small in the left

---

**TABLE 2. Comparison of Histological Parameters Between Specimens With and Without Atrial or Brain Natriuretic Peptide Expression**

<table>
<thead>
<tr>
<th>Group</th>
<th>Ventricular</th>
<th>n</th>
<th>Size of myocytes (μm)</th>
<th>Degree of fibrosis</th>
<th>Degree of disarray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>RVB</td>
<td>ANP(+)</td>
<td>0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ANP(-)</td>
<td>5</td>
<td>13±2</td>
<td>1±1</td>
</tr>
<tr>
<td></td>
<td>LVB</td>
<td>ANP(+)</td>
<td>0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ANP(-)</td>
<td>5</td>
<td>15±1</td>
<td>0±0</td>
</tr>
<tr>
<td>HCM</td>
<td>RVB</td>
<td>ANP(+)</td>
<td>9</td>
<td>19±5</td>
<td>2±1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ANP(-)</td>
<td>23</td>
<td>18±4</td>
<td>1±1</td>
</tr>
<tr>
<td></td>
<td>LVB</td>
<td>ANP(+)</td>
<td>8</td>
<td>22±1*</td>
<td>1±1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ANP(-)</td>
<td>24</td>
<td>19±3</td>
<td>1±1</td>
</tr>
<tr>
<td>Control</td>
<td>RVB</td>
<td>BNP(+)</td>
<td>0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BNP(-)</td>
<td>5</td>
<td>13±2</td>
<td>1±1</td>
</tr>
<tr>
<td></td>
<td>LVB</td>
<td>BNP(+)</td>
<td>0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BNP(-)</td>
<td>5</td>
<td>15±1</td>
<td>0±0</td>
</tr>
<tr>
<td>HCM</td>
<td>RVB</td>
<td>BNP(+)</td>
<td>4</td>
<td>19±6</td>
<td>2±1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BNP(-)</td>
<td>28</td>
<td>18±3</td>
<td>1±1</td>
</tr>
<tr>
<td></td>
<td>LVB</td>
<td>BNP(+)</td>
<td>3</td>
<td>23±0*</td>
<td>1±1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BNP(-)</td>
<td>29</td>
<td>19±3</td>
<td>1±1</td>
</tr>
</tbody>
</table>

ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; RVB, right ventricular side of ventricular septum; LVB, left ventricular free wall; HCM, hypertrophic cardiomyopathy.

*P<0.05 different from ANP- or BNP-negative specimens.
ventricular free wall and in the left ventricular or right ventricular endocardial side of the ventricular septum. Thus, endomyocardial biopsy specimens are not necessarily taken from the parts where myocardial hypertrophy is most evident.

The elevation of the plasma level in HCM versus control subjects was more prominent in BNP than ANP. However, the immunohistochemical positivity of BNP was slightly lower than that of ANP in HCM. This discrepancy may be explained by findings that BNP has a more rapid secretory turnover than ANP.7-9 Our immunohistochemical findings showed that, in ventricular myocytes of HOCM, BNP was more diffusely spread throughout the cytoplasm than ANP.33 These staining patterns might be related to differences in the secretory pathways of ANP and BNP. Even though BNP occurs to a lesser extent than ANP as a storage form in ventricular myocytes, the ventricular secretion of BNP via a constitutive pathway may be greatly accelerated. These findings suggest that in HCM with normal systolic function, the synthesis and secretion of BNP differ from those of ANP. However, the physiological activities of ANP and BNP are similar, including diuretic-natriuretic, hypotensive, and vasorelaxant effects. The binding affinity to ANP-A receptor or ANP-B receptor is similar between ANP and BNP, and the two peptides have a similar potency of cyclic GMP production. Therefore, further studies are needed to clarify the physiological differences between ANP and BNP.

In three of five HOCM patients, the BNP plasma level was markedly elevated compared with that in the HNCM patients. If the circulating BNP originates from ventricles, why is ventricular expression of BNP exaggerated in these patients? Recent studies have shown that BNP plasma level was much more elevated than ANP plasma level in the early phase of acute myocardial infarction.34 The BNP gene has an AT-nucleotide-rich sequence, implying messenger RNA instability in the 3'-untranslated region35,36; this sequence is lacking in the ANP gene.37 Rapid responses of the ventricular BNP synthesis and secretion to hemodynamic stress may be attributed in part to this unique structure of the BNP gene. In patients with severe congestive heart failure, in contrast, ANP and BNP were expressed together in the ventricles.7,8 Since the plasma levels of both ANP and BNP were elevated in these patients, the BNP/ANP-level ratio was only 1.72.7 These findings suggest that nonfailing ventricles under acute hemodynamic stress predominantly secrete BNP, whereas failing ventricles secrete both ANP and BNP. Thus, the more accelerated secretion of BNP than ANP in HOCM ventricles might indicate that the ventricles are under overload but are nonfailing. Further studies are continuing in our laboratory to clarify the difference in the mechanisms of ventricular synthesis and secretion between ANP and BNP.

Both ANP and BNP have been reported to have a beneficial effect on failing human hearts.38,39 In contrast, high levels of circulating BNP in HOCM may reduce preload and afterload, subsequently exacerbating obstruction. The accelerated secretion of BNP from HOCM ventricles would not compensate but rather would act to cause deterioration of hemodynamics. However, we have no data on the effect of anti-BNP antibody on hemodynamics in HOCM. Thus, the precise role of BNP in the pathophysiology of HCM should be investigated further.

Drug treatments differed between patients with obstructive and nonobstructive cardiomyopathy. Although all medications were stopped on the day of invasive examination, their influences cannot be completely excluded. Since the effects of drugs on BNP in humans have not been reported, further studies are awaited.

In conclusion, we demonstrated that BNP was expressed in the ventricular myocytes of HCM with normal systolic function. In HOCM, ventricular expression of BNP may be augmented in response to both obstructive and diastolic dysfunction.

Acknowledgments

We thank Drs. Masaru Tanaka, M. Katsuragawa, T. Hiy, K. Yamasaki, R. Yokota, T. Inada, and S. Ohtani in our laboratory for their encouragement and advice; J. Matsushita, R. Kashihara, A. Miyashita, and M. Kohno for their technical assistance; I. Hayashi for her secretarial work; Kazue Hasegawa for her assistance in preparing the manuscript; and D. Mrozek for reading it. This work was supported in part by research grants 02670414, 1990, and 0354253, 1991, from the Ministry of Education, Science, and Culture of Japan.

References


19. Kennedy JW, Trenholme SE, Kasser IS. Left ventricular volume and mass from single-plane cineangiogram: a comparison of
Ventricular expression of brain natriuretic peptide in hypertrophic cardiomyopathy.
K Hasegawa, H Fujiwara, K Doyama, M Miyamae, T Fujiwara, S Suga, M Mukoyama, K Nakao, H
Imura and S Sasayama

_Circulation_. 1993;88:372-380
doi: 10.1161/01.CIR.88.2.372

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/88/2/372

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
_Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office.
Once the online version of the published article for which permission is being requested is located, click Request
Permissions in the middle column of the Web page under Services. Further information about this process is
available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/