Ventricular Expression of Atrial Natriuretic Polypeptide and Its Relations With Hemodynamics and Histology in Dilated Human Hearts

Immunohistochemical Study of the Endomyocardial Biopsy Specimens

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To investigate the mechanism of expression of atrial natriuretic polypeptide (ANP) in human ventricles, we conducted an immunohistochemical study of ANP in biventricular endomyocardial biopsy specimens obtained from a total of 49 patients with cardiac dilatation due to dilated cardiomyopathy (21 patients), postmyocarditis (18 patients), or volume overload (five patients) and subjects with no dilatation as controls (five patients). Four-micron thick sections were stained by an indirect immunoperoxidase method using monoclonal antibody to α-human ANP as the primary antibody. The frequency of ANP-present myocytes was calculated in each specimen and compared with clinical, echocardiographic, hemodynamic, angiographic, and histologic parameters. ANP-present myocytes were noted in all of the 21 patients with dilated cardiomyopathy, in 11 of the 18 patients with postmyocarditis, in four of the five patients with volume overload, and in zero of the five controls. The mean percentage of ANP-present myocytes was significantly greater in the left-side specimens (35±37%) than in the right-side ones (2±4%). The percentage of ANP-present myocytes in the left-side specimens significantly correlated with peak systolic or end-diastolic wall stress (r=0.67 and 0.58), left ventricular end-systolic or end-diastolic volume index (r=0.75 and 0.69), or left ventricular end-diastolic pressure (r=0.42) and inversely correlated with ejection fraction (r=-0.73), systolic left ventricular wall thickness (r=-0.58), or cardiac index (r=-0.30). Expression of ANP was rarely seen in the cases with normal wall stresses, normal ejection fraction, normal volume, or normal myocyte size. However, it was seen frequently even in hearts with normal levels of left ventricular end-diastolic pressure and cardiac index (compensated hearts). The percent of ANP-present myocytes in both sides significantly correlated with size of myocytes (r=0.48 at right and r=0.57 at left side) or degree of fibrosis (r=0.45 at right and r=0.48 at left side). These results suggest that ANP expression is augmented in the dilated ventricles regardless of the causes of dilatation and that the augmentation is a compensatory mechanism as prevention against decompensation responding to reduced contractility, excess of wall stresses, or both, concomitantly occurring with cardiac dilatation and myocardial hypertrophy. (Circulation 1989;80:1137–1147)
atrial natriuretic polypeptide (ANP) is a circulating hormone released from atrial myocytes that regulates the fluid, electrolytes, and vascular homeostasis. Its plasma concentration increases in patients with congestive heart failure, probably due to compensatory mechanisms. In fact, the relation between plasma ANP and hemodynamics has been studied precisely. ANP is expressed in fetal ventricles, but markedly decreases in normal adult ventricles. However, in the failing human heart, the hormone is reproduced and resecreted from ventricular myocytes, likewise in animal preparations under various conditions. Recently, we reported an increase in tissue ANP and ANP gene in the ventricular walls of hearts from patients with dilated cardiomyopathy and ischemic damage from old myocardial infarction. However, it is still not clear which factors, either clinical or pathologic, are contributing or relating to the reexpression of ANP in human ventricles. Therefore, in the present study, using ventricular endomyocardial specimens obtained from human hearts with primary or secondary dilation, we studied the immunohistochemical expression of ventricular ANP and analyzed its relations to clinical (including hemodynamics and cardiac imagings) and histopathologic parameters.

Methods

Patient Profile

Both right- and left-side endomyocardial biopsy specimens from each of 49 patients were studied. There were 36 men and 13 women (age range, 16–70 years). All patients were evaluated clinically by both noninvasive and invasive methods. Based on clinical, echocardiographic, hemodynamic, angiographic, and histologic findings, patients were assigned to one of the following groups: ventricular dilation due to dilated cardiomyopathy (21 patients), ventricular dilation due to postmyocarditis (18 patients), ventricular dilation due to volume overload (five patients), and control subjects without any demonstrative organic cardiac disease (five subjects). The diagnosis of dilated cardiomyopathy was made according to the definition and classification proposed by the World Health Organization–International Society and Federation of Cardiology task force. The postmyocarditis group included patients who had clinical evidence of acute myocarditis more than 1 month earlier, were given the histologic diagnosis of acute myocarditis at the acute stage by the right ventricular endomyocardial biopsy, or both. The right- and left-side endomyocardial biopsies at the late stage, from which the biopsied specimens were used for the present study, revealed persistent, healing, or healed myocarditis. The histologic definition of myocarditis was based on the “Dallas classification system.” The group with volume overload consisted of patients with pure aortic regurgitation. The control group included patients who had been clinically suspected of some cardiac disease because of chest pain, minimal electrocardiographic change, or arrhythmia but for whom invasive examinations of coronary angiography and biopsy findings were not diagnostic. Thirty patients (16 with dilated cardiomyopathy, 10 with postmyocarditis, and four with volume overload) were given drugs with various combinations of digitalis, diuretics (furosemide, spironolactone, trichlormethazide), vasodilators, and antiarrhythmics. However, no drugs were given on the day of biopsy examination.

Echocardiographic, Hemodynamic, and Angiographic Evaluation

In all patients, the two-dimensional echocardiographic study was performed no more than 3 days before invasive examinations. The ventricular septal thickness at diastolic (VSTd) and systolic phase (VSTs) and left ventricular free wall thickness at the respective phases (LVTd and LVTs) were recorded.

After premedication with 5 mg diazepam, all patients underwent both right- and left-heart catheterization, biplane left ventriculography, and selective coronary angiography with standard techniques. The heart rate and pressures from the right and left heart were recorded, and the cardiac index (CI) was estimated by the thermodilution method. In cases with volume overload due to aortic regurgitation, the regurgitant fraction was measured by the thermodilution method. Left ventricular end-diastolic and end-systolic volume indexes (EDVI and ESVI) and the ejection fraction (EF) were calculated from the left ventricular cineangiogram performed in the right anterior oblique projection by Kennedy’s method (see Kennedy et al). Furthermore, left ventricular high-fidelity pressure measurements were obtained in 20 patients (eight with dilated cardiomyopathy, seven with postmyocarditis, two with volume overload, and three control subjects) with a Millar 7F micromanometer angiocatheter during the left ventriculography. In these patients, peak systolic and end-diastolic circumferential wall stress (Sps and Ssd) were calculated according to the method previously proposed.

Endomyocardial Biopsy Procedure and Histologic Evaluation

Biopsy specimens were obtained during the cardiac catheterization from both the right ventricular side of the ventricular septum and the left ventricular free wall by a Konno-Sakakibara or Kawai biopsy catheter in all patients. Two or three biopsy specimens each from the right and left side were obtained from every patient. These specimens were immediately fixed with a 10% buffered-formalin solution, dehydrated, embedded in paraffin, and cut into 4-μm thick sections. From light microscopic observations of the sections stained with hematoxylin and eosin or Masson’s trichrome, histologic parameters such as myocyte size (mean diameter,
TABLE 1. Echocardiographic, Hemodynamic, and Angiographic Patient Data

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (yr)</th>
<th>Gender (M/F)</th>
<th>Duration of illness (mo)</th>
<th>EDVI/ESVI (ml/m²)</th>
<th>VSTa (mm)</th>
<th>LVTa (mm)</th>
<th>LVSP/ LVEDP (mm Hg)</th>
<th>RVSP/ RVEDP (mm Hg)</th>
<th>EDVI/ESVI (ml/m²)</th>
<th>EF (%)</th>
<th>CI (ml/m²)</th>
<th>Heart rate (dynes/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>47±14</td>
<td>3/2</td>
<td></td>
<td>70±15</td>
<td>9±0</td>
<td>9±1</td>
<td>118±23</td>
<td>24±5</td>
<td>3.1±0.5</td>
<td>68±6</td>
<td>404±49</td>
<td>86±3</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>21</td>
<td>42±16</td>
<td>17/4</td>
<td>46±2</td>
<td>132±33C</td>
<td>9±1</td>
<td>9±1</td>
<td>114±18</td>
<td>29±12</td>
<td>31±12C,A</td>
<td>2.6±0.7</td>
<td>78±20</td>
<td>605±130C</td>
</tr>
<tr>
<td>Postmyocarditis</td>
<td>18</td>
<td>42±15</td>
<td>13/5</td>
<td>34±6</td>
<td>97±45D</td>
<td>9±1</td>
<td>10±1</td>
<td>122±28</td>
<td>25±5</td>
<td>58±17D</td>
<td>3.2±1.0</td>
<td>69±16</td>
<td>456±148</td>
</tr>
<tr>
<td>Volume overload</td>
<td>5</td>
<td>39±14</td>
<td>3/2</td>
<td>74±103</td>
<td>134±30C</td>
<td>12±5</td>
<td>10±4</td>
<td>132±35</td>
<td>33±17</td>
<td>49±11C,D</td>
<td>3.5±0.5</td>
<td>64±8</td>
<td>459±45</td>
</tr>
</tbody>
</table>

EDVI/ESVI, left ventricular end-diastolic and end-systolic volume index; VSTa/LVTa, diastolic and systolic ventricular septal thickness; LVTa/LVTs, diastolic and systolic left ventricular free wall thickness; LVSP/LVEDP, left ventricular peak systolic and end-diastolic pressure; RVSP/RVEDP, right ventricular peak systolic and end-diastolic pressure; EF, ejection fraction; CI, cardiac index; Sos, peak circumferential wall stress; Ssd, end-diastolic circumferential wall stress.

*p<0.05 different from controls (C), dilated cardiomyopathy (D), postmyocarditis (M), or volume overload (A) (one-way analysis with multiple comparisons).

30–50 myocytes per specimen) and degree of fibrosis (from 0 to 3) were evaluated. The extent of fibrosis was graded as 0 (no fibrosis), 1 (mild, when an isolated small scar or mild interstitial fibrous tissue formation, or both, was identified), 2 (moderate, intermediate between mild and severe), or 3 (severe, characterized by extreme replacement scarring occupying substantial portions of the section). In addition, the mean number of inflammatory cells (mean number of total polymorphonuclear leukocytes, lymphocytes, and plasma cells per high power field) was calculated.

Immunohistochemical Procedure and Evaluation

A monoclonal antibody to α-human ANP was prepared as previously described. The recognized epitope of the monoclonal antibody is located in the N-terminal half of the ring structure of α-human ANP including Met residue. This radioimmunoassay can recognize not only α-human ANP but also β-human ANP with a cross-reactivity of 80% on a molar basis and γ-human ANP on an equimolar basis. Immunohistochemical reactions according to an indirect immunoperoxidase method were performed with some technical modifications. In the first step, intrinsic peroxidase activity was inhibited by the addition of 0.3% hydrogen peroxide in 0.01 mol/l phosphate-buffered saline (PBS), and nonspecific binding was blocked with normal goat serum. To inhibit the nonspecific staining of lipofuscin granules that may often have been confusing for the observers, it took as long as 20 minutes for the addition of the hydrogen peroxidase solution. As the primary antibody, the monoclonal antibody was added (dilution of the ascite, 1:1-1,000) to the sections for 48 hours at 4°C. In the second step, peroxidase-conjugated F(ab')2 fragment of the secondary antibody (goat anti-mouse IgG[H+L], Jackson ImmunoResearch Laboratories) was added for 45 minutes at room temperature. Then, the sections were stained with 45 mg 3,3’-diaminobenzidine tetrahydrochloride (Sigma Chemical, St. Louis, Missouri) and 0.05% hydrogen peroxide in 100 ml 0.05 mol/l Tris buffer solution for 3 minutes at room temperature. Between each step, the sections were washed four times (10 minutes each) with 0.01 mol/l PBS. Finally, counterstaining with hematoxylin or periodic acid shift (PAS) was carried out. PAS staining was used to confirm the absence of the pulse-conducting system. The specificity of the immunologic reactions was controlled by replacing the primary antibody with normal mouse serum or PBS. A tissue preparation of the right atrial appendage obtained from a patient during aortocoronary bypass surgery was used as a positive control section.

The presence of immunoreactive ANP was assessed by light microscopic examination. Using the largest of the two or three specimens obtained from each of the right and left ventricular biopsies, ANP in the myocyte was graded as present or absent, and the percentage of ANP-present myocytes to a total of 30–50 arbitrarily selected myocytes with clearly cut nuclei in each specimen was calculated. Two blinded trained observers reviewed the sections. The variability between the two observers was 1±1%.

Statistical Analysis

Clinicopathologic data were expressed as mean±SD. Statistical comparisons were performed using χ² analysis, Student’s t test, and one-way analysis of variance with multiple comparisons when appropriate. Values were considered significant at p less than 0.05.

Results

Table 1 summarizes echocardiographic, hemodynamic, and angiographic patient data. The three groups with diseased hearts had dilated left ventricular cavity and, therefore, a significant difference in EDVI from the control group. VSTa and LVTa showed no difference among the groups, but VSTa and LVTa were significantly thinner in dilated cardiomyopathy than in that of the control group. Of ventricular pressures, left
ventricular end-diastolic pressure (LVEDP) was significantly elevated in dilated cardiomyopathy compared with the controls. EF was significantly reduced in the hearts with dilated cardiomyopathy and volume overload compared with controls, whereas no difference in CI was seen between the groups. $S_{ed}$ and $S_{ps}$ of dilated cardiomyopathy and $S_{ed}$ of postmyocarditis were significantly greater than those of controls. There was no significant difference in age, sex distribution, duration of illness, and heart rate between any of the groups. In the volume-overload group, the mean regurgitant fraction was 27±6% (20–34%).

Table 2 summarizes the histopathologic data on the biopsy specimens. Left-side biopsy specimens of all of the diseased groups showed larger myocytes and a greater degree of fibrosis than in the control group. In all groups, the size was greater in the left-side than in the right-side specimens.

**Immunohistochemical Expression of ANP**

ANP was observed as a fine, granular, dark-brown immunoreactive substance in the cytoplasm of ventricular myocytes in dilated hearts (Figure 1). It tended to concentrate around the nuclei, although it was also distributed in the peripheral cytoplasm. Lipofuscin granules were much bigger and not dark brown and, therefore, could be easily distinguished from ANP granules. No immunopositive substances were seen in the endocardium or any of the connective tissues. None of the specimens contained the pulse-conducting system. The sections without primary antibody did not show any immunoreactivity (Figure 1). As a positive control section, the tissue preparation from the right atrial appendage showed very strong immunoreactivity in the cytoplasm of the myocytes (Figure 1).

The incidence of patients with ANP-positive ventricles was zero of the five control patients, 21 of the 21 patients with dilated cardiomyopathy, 11 of the 18 patients with postmyocarditis, and four of the five patients with aortic regurgitation. The mean percentage of ANP-present myocytes in the right- and left-side specimens were 2±4% and 53±33% in dilated cardiomyopathy, 2±4% and 21±35% in postmyocarditis, and 3±5% and 42±43% in aortic regurgitation (Table 2). In the left-side specimens, it was greater in dilated cardiomyopathy than in postmyocarditis.

In all groups with diseased hearts, the incidence of the specimens with ANP and the mean percentage of ANP-present myocytes were higher in the left than in the right-side specimens (Tables 2 and 3). A correlation was noted between the mean percentage of ANP-present myocytes in the left and the right sides (Figure 2).

**Relations of Ventricular ANP to Clinicopathologic Parameters**

Table 4 shows the coefficients of correlation between the percentage of ANP-present myocytes in the right- and left-side biopsy specimens and clinicopathologic parameters in the patients examined. Duration of illness did not correlate with the percentage of ANP-present myocytes in each side. The percentage of ANP-present myocytes in the right- and left-side specimens were correlated with EDVI, ESVI, LVEDP, and RVSP and were inversely correlated with VST and EF (Figure 3). The percent of ANP-present myocytes in the left-side specimens was positively correlated with both $S_{ed}$ and $S_{ps}$ and was inversely correlated with LVT and CI (Figures 3 and 4). However, it showed no correlation with LVT, VST, LVSP, RVEDP, or heart rate. A significant correlation was seen between LVEDP and RVSP ($r=0.70, p<0.001$).

The percentage of ANP-present myocytes in the left- and the right-side biopsy specimens correlated with the mean size of myocytes and the grade of fibrosis (Figure 5). However, it did not correlate with the number of inflammatory cells in either of the two sides.

In the left-side specimens, ANP-present myocytes were seen in only a few cases of the hearts with normal range of EF, $S_{ps}$, $S_{ed}$, EDVI, ESVI, or myocyte size, whereas they were noted in many of the hearts with normal CI or LVEDP range (Figures 3–5).

**Discussion**

The monoclonal antibody to $\alpha$-human ANP that we used had cross-reactivities of 80% with $\beta$-human ANP and 100% with $\gamma$-human ANP. Thus, we
FIGURE 1. Microphotograph of immunohistochemical preparation of tissue obtained during ventricular endomyocardial biopsy or surgery. Panel A: The left ventricular endomyocardial biopsy specimen from a case of dilated cardiomyopathy. In this specimen, 98% (49 of 50 myocytes counted) of the myocytes were ANP present. Original magnification, ×200. Panel B: Section obtained from the same patient as in Panel A treated with nonimmune mouse serum. No evidence of immunoperoxidase activity. Original magnification, ×200. Panel C: The right ventricular endomyocardial specimen from a case of postmyocarditis with histologic diagnosis of healing myocarditis. ANP granules were noted in the myocytes surrounded by some residual inflammatory cells and fibrous tissues. Original magnification, ×200. Panel D: High-power view of the section from Panel C. Immunoreactive ANP granules present mainly in a perinuclear region of a myocyte. Original magnification, ×400. Panel E: The right ventricular endomyocardial biopsy specimen from a normal case, the control group. No myocytes show immunoreactive ANP granules. Original magnification, ×200. Panel F: Positive control section of the right atrial appendage obtained during cardiac surgery. ANP granules with very strong immunoreactivity were seen mainly in the perinuclear regions. Original magnification, ×200.

observed immunohistochemical expressions of α-, β-, and γ-human ANP.

The present study revealed that ventricular ANP was augmented in various forms of cardiac dilatation: dilatation due to unknown origin (dilated cardiomyopathy), dilatation due to inflammatory damage (postmyocarditis), and dilatation due to volume overload (aortic regurgitation). Our previous report
TABLE 3. Incidence of Patients With ANP-Present Myocytes in Endomyocardial Biopsy Specimens

<table>
<thead>
<tr>
<th>Patients with ANP-present myocytes</th>
<th>Both sides</th>
<th>RVB</th>
<th>LVB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>21</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Postmyocarditis</td>
<td>18</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Volume overload</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

RVB, LVB, right- and left-side endomyocardial biopsy specimens.

*p<0.05 (χ² analysis).

showed that it was also augmented in the dilated heart due to ischemic damage (old myocardial infarction). Thus, ventricular ANP is likely to be augmented in dilated hearts regardless of the cause of dilatation. However, significant differences were seen between the groups of patients with dilated cardiomyopathy and postmyocarditis in both the incidence of the patients with ANP-present myocytes and the mean percentage of ANP-present myocytes in the left-side specimens; those of patients with dilated cardiomyopathy were greater than those with postmyocarditis. Compared with postmyocarditis group, the dilated cardiomyopathy group's EDVI, ESVI, SED, and myocyte size were significantly greater and VST%, LVT%, and EF were significantly less. Therefore, the difference of degree of ventricular ANP expression may be explained by the differences of hemodynamics or histologic variables rather than by disease specificity.

![FIGURE 2. Plots of comparison and correlation of the percentage of ANP-present myocytes between the right- and left-side ventricular endomyocardial biopsy specimens in the 49 patients examined. The comparison was treated by paired t test. Correlation coefficient and regression line were obtained from the 49 patients examined. RVB, LVB, right- and left-side endomyocardial biopsy specimens; •, dilated cardiomyopathy; ○, postmyocarditis; ▲, volume overload; △, control.](http://circ.ahajournals.org/)
The present results showed that left ventricular ANP expression correlated positively with EDVI, ESVI, and LVEDP and inversely with VSTd and LVTs. These parameters are directly associated with Sps and Sed. Thus, both peak systolic and end-diastolic left ventricular wall stresses, indicators of afterload and preload, respectively, seem to be decisively responsible for the augmentation of left ventricular ANP. The mean percentage of ANP-present myocytes was lower in the right-side biopsy specimens obtained from the right ventricular side of the ventricular septum than in the left-side specimens obtained from the endocardial side of the left ventricular free wall. Wall stress is lower in the epicardial than in the endocardial side, and the right ventricular side of the ventricular septum can be regarded as the epicardial side of the left ventricle. In the present study, positive correlation was evident between the right-side ventricular ANP and Sed of the left ventricle. In addition, the mean percentage of ANP-present myocytes correlated with RVSP but not with RVEDP. Generally, RVSP is one of the indicators of left ventricular dysfunction. These suggest that ANP in the right ventricular side of the ventricular septum is also influenced by left ventricular wall stress transmitted through the ventricular septum rather than by right ventricular wall stress. However, in the present immunohistochemical study, ANP-present myocytes and ANP-absent ones coexisted in a small specimen despite presumably being under the same hemodynamic conditions. It is difficult to explain this phenomenon from hemodynamic stress. The coexistence may be related to the coexistence of myocytes with various sizes in the small ventricular biopsy sample, although we do not know the precise pathogenesis of them.

It is established that ANP is expressed in fetal ventricles but markedly decreases in normal adult ventricles. At present, the precise mechanism of reexpression of ventricular ANP after the increase of wall stress is unknown. We found a positive correlation between the percentage of ANP-present myocytes and myocyte size. Fetal types of actin or myosin have been reported to be detected in some forms of diseased ventricles with myocyte hypertrophy. Therefore, ventricular ANP expression, as well as myocyte hypertrophy and reinduction of the other fetoproteins, would be one of the phenomena of cell reversion.

In the left ventricular diseases, the contractility reduces, the hemodynamic burdens exceed, or both occur. The various compensatory mechanisms (e.g., increase of EDVI [Frank-Starling's law], hypertrophy of myocytes) appear against them. When CI and LVEDP are normalized by the compensatory mechanisms, it is called compensatory phase. When CI decreases and LVEDP increases despite the compensatory mechanisms, it is called decompensatory phase. In the present data, the percentage of ANP-present myocytes in the left ventricular biopsy specimens correlated inversely with EF as an indicator of contractility and positively with Sps and Sed as indicators of hemodynamic burdens. The augmentation of left ventricular ANP correlated with increase of EDVI, hypertrophy of myocytes, decrease of CI, and increase of LVEDP. It has been reported that ventricular ANP increases in hearts with decompensatory phase (failing hearts). However, the expression of the ANP was frequently evident even in the left ventricular free wall of many hearts with compensatory phase (normal CI and normal LVEDP), whereas it was evident in only a few hearts with normal EF, normal Sps and Sed, normal EDVI, or normal myocyte size. These indicate that expression of ventricular ANP is a self-compensatory mechanism of “prevention against decompensation” in response to reduced contractility, ventricular wall stress, or both that concomitantly occurs with volume expansion and hypertrophy of myocytes.
**Figure 1:**

- **EDVI (ml/m²)** vs. ANP-present myocytes (%)
  - RVB: $r = -0.45$, $n = 49$, $P < 0.01$
  - LVB: $r = 0.68$, $n = 49$, $P < 0.001$

- **ESVI (ml/m²)** vs. ANP-present myocytes (%)
  - RVB: $r = -0.30$, $n = 49$, $P < 0.05$
  - LVB: $r = 0.75$, $n = 49$, $P < 0.001$

- **VSTs (mm)** vs. ANP-present myocytes (%)
  - RVB: $r = 0.49$, $n = 49$, $P < 0.001$
  - LVB: $r = 0.69$, $n = 49$, $P < 0.001$

- **LVTS (mm)** vs. ANP-present myocytes (%)
  - RVB: $r = 0.42$, $n = 49$, $P < 0.01$
  - LVB: $r = 0.50$, $n = 49$, $P < 0.001$

- **RVSP (mmHg)** vs. ANP-present myocytes (%)
  - RVB: $r = -0.58$, $n = 49$, $P < 0.001$
  - LVB: $r = -0.73$, $n = 49$, $P < 0.001$

- **LVEDP (mmHg)** vs. ANP-present myocytes (%)
  - RVB: $r = 0.49$, $n = 49$, $P < 0.001$
  - LVB: $r = 0.36$, $n = 49$, $P < 0.05$

- **EF (%)** vs. ANP-present myocytes (%)
  - RVB: $r = -0.40$, $n = 49$, $P < 0.01$
  - LVB: $r = -0.73$, $n = 49$, $P < 0.001$

- **CI (ml/min/m²)** vs. ANP-present myocytes (%)
  - RVB: $r = -0.30$, $n = 49$, $P < 0.05$
  - LVB: $r = -0.36$, $n = 49$, $P < 0.05$
To check on sampling errors, we examined the variability of the percentage of ANP-present myocytes between different sections of the same specimen and among different specimens from the same patient. The variability was minimal: 1±1% in the different sections of the same specimen and 3±2% in the different specimens from the same patient. However, some variant specimens were also seen in this study, although the correlations were indeed significant between ventricular ANP and various clinical or morphologic parameters. These may be attributed to the limitation of a biopsy study, which is too small to represent the whole ventricle.

Although all medications were stopped on the day of invasive examination, we believe their influences cannot be completely negligible. However, we did not analyze the influence of the drugs on ventricular ANP expression. The strict analysis was impossible because varying amounts and combinations of many drugs had been given to the patients according to their clinical states. To date, there have been no strict data on the intervention of drugs on ANP in humans, and, therefore, further analyses are expected in this field.

Last, the benefits of the semiquantitative immunohistochemical method for ANP used in this study were that a direct comparison with histopathologic findings in the same tissue was possible and that formalin-fixed, paraffin-embedded tissue blocks were available.

**Conclusion**

Ventricular expression of ANP was immunohistochemically demonstrated in endomyocardial biopsy specimens obtained from various forms of cardiac dilatation, and its augmentation is likely to be one of various compensatory mechanisms in response to reduced contractility, excess of ventricular wall stresses, or both.

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