Atrial Natriuretic Peptide Distribution in Fetal and Failed Adult Human Hearts

Hidetsugu Tsuchimochi, MD, Fumihiko Kurimoto, PhD, Keiko Ieki, MD, Hidekazu Koyama, DVM, Fumimaro Takaku, MD, Masatoshi Kawana, MD, Shin-ichi Kimata, MD, and Yoshio Yazaki, MD

The distributions of atrial natriuretic peptide (ANP) in human hearts during the developmental stage and in adult pathological states was examined with an antibody specific to human α-ANP. With immunoblotting and immunofluorescence methods, we found that a 17-kDa protein, which is a pro ANP, was expressed in human fetal ventricles, in which the numbers of myofibers containing ANP granules were more abundant in the subendocardial region than the subepicardial region. As determined by radioimmunoassay, the content of immunoreactive ANP (per milligram protein) in the developing heart was greatest in the left atrium and occurred decreasingly in the right atrium, right ventricle, and left ventricle, respectively. Because ANP content in the left ventricle declined during the progress of gestation in developing hearts and because it was very low, if ever detectable, in normal adult hearts, ventricular ANP expression appears to be developmentally regulated from the early gestational stage. However, it was reexpressed in the ventricles of patients who had suffered from severe congestive heart failure. In this situation, we found that the ventricular ANP expression was more marked in patients with dilated cardiomyopathy than in patients with severe valvular disease. Interestingly, in the ventricles of patients with dilated cardiomyopathy, ANP contents were higher in the left ventricular free wall than in the right ventricular free wall, although the left ventricular subendocardium contained more ANP than the subepicardium, showing a transmural gradient similar to that expressed in fetal ventricles. Thus, the expression of ANP in human ventricles is developmentally regulated from the early gestational stage, and even adult ventricular myofibers can synthesize ANP during severe congestive heart failure. Considering the ventricular site that is subjected to an overload in fetal and adult pathological states and considering that myofibres in the subendocardial region normally suffer from a greater wall stress than do those in the epicardial region, it is reasonable to hypothesize that focal factors, such as wall stress, contribute to the expression of ventricular ANP. (Circulation 1988;78:920–927)

It is generally accepted that mammalian cardiac atria synthesize and secrete atrial natriuretic peptides (ANPs) with natriuretic, diuretic, and smooth muscle relaxant properties.1 Several investigators have reported that plasma ANP levels are elevated in patients with congestive heart failure.2–5 Because congestive heart failure is characterized by sodium retention and increased vascular resistance, the high levels of circulating plasma ANP may be a compensatory physiological response to this condition. Until recently, ANP was believed to be produced only by atrial and not by ventricular myocytes. However, recent reports have demonstrated that ventricular myocytes, in vivo and in vitro, can synthesize and secrete ANP during the very early neonatal or fetal stages.6,7 and other reports have shown that in rats, ANP messenger ribonucleic acid increases in ventricles subjected to volume overload or pressure overload, and it increases in those treated with dexamethasone.8,9 More recently, we have detected ANP granules in the ventricular myofibers of patients who had suffered from severe congestive heart failure due to dilated cardiomyopathy.10 These results suggest that the expression of

From The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku and from the Institute of Cardiovascular Research (M.K., S.K.), Tokyo Women’s Medical College, Kawada-cho 10, Ichigaya, Shinjuku-ku, Tokyo, 113 Japan.

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Address for correspondence: H. Tsuchimochi, MD, The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo, 113 Japan.

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ANP in ventricles may be developmentally regulated and that a ventricular ANP coding gene that is dormant under normal conditions could be expressed in certain pathological states. However, the expression and the distribution of ANP in developing human hearts and in pathological adult hearts has not yet been characterized. Therefore, we examined human fetal, normal, and pathological hearts, obtained at autopsy, to characterize the ANP expression in human hearts.

Subjects and Methods

Tissue Sources

Fetal hearts were obtained from fetuses of 9, 14, 16, 18, 21, 22, and 23 weeks of gestation. All fetuses were aborted in legal accordance with the Eugenic Protection Act in Japan (which prohibits artificial abortion at any stage after the 24th week of gestation), and they showed no cardiac malformation or other disease when examined macroscopically. Thirty hearts obtained at autopsy from patients with or without cardiac disease were also examined. Of these, 17 patients (nine men and eight women; 23–81 years old; mean age, 59.3 years) had shown no evidence of cardiovascular disease and had not suffered from congestive heart failure before their death. Seven patients (patients 1–7, Table 1) had dilated cardiomyopathy according to echocardiography and cardiac catheterization, and all had suffered from severe, chronic congestive heart failure (New York Heart Association functional Class IV). Six other patients (patients 8–13, Table 1) had also suffered from severe congestive heart failure due to various forms of valvular disease. The clinical data of cardiac disease before death and heart weights at autopsy are presented in Table 1. All autopsied samples were obtained within 16 hours of death.

Tissue specimens were excised from autopsied heart samples and stored at −80 °C until use. In some patients, these specimens were also embedded in a compound (Tissue Teck II, Miles Laboratories, Naperville, Illinois), chilled in liquid nitrogen, and then stored at −80 °C until use. Although only portions of heart tissue (e.g., parts of the right and left atrial appendages and left ventricular free walls), rather than whole hearts, were available for analysis, in five patients with dilated cardiomyopathy and in seven patients without cardiac disease, we obtained ventricular cross sections including the left and right ventricular free walls and the interventricular septum.

Production of Anti-Human α-ANP Antibody

Synthetic human α-ANP (ha-ANP) and 125I-labeled ha-ANP were purchased from Peptide Institute (Osaka, Japan) and Amersham (Little Chalfont, UK), respectively. At first, 1 mg ha-ANP was conjugated with 10 mg porcine thyroglobulin with 1-ethyl-3-dimethylaminopropyl carbodiimide hydrochloride (Peptide Institute),11 and the combination was then dialyzed against 0.9% saline. Conjugated ha-ANP was emulsified with an equal volume of complete Freund’s adjuvant (Difco Laboratories, Detroit, Michigan) and was injected into rabbits five times at intervals of 2 or 3 weeks. The anti–ha-ANP activity of the serum was assessed by its capability to immunoprecipitate 125I-labeled ha-ANP.

Immunoblotting

Samples devoid of connective tissue were weighed and cut into small pieces with scissors and then were homogenized in 3 volumes (vol/wt) of extraction buffer (10 mM phosphate buffer, 140 mM NaCl: phosphate-buffered solution, pH 7.2, 0.5 mM phenylmethyl

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of Patients With Cardiac Disease</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Clinical diagnosis</td>
<td>Age</td>
<td>Sex</td>
<td>LVds (mm)</td>
</tr>
<tr>
<td>1</td>
<td>Dilated cardiomyopathy</td>
<td>26</td>
<td>F</td>
<td>81/73</td>
</tr>
<tr>
<td>2</td>
<td>Dilated cardiomyopathy</td>
<td>52</td>
<td>M</td>
<td>63/58</td>
</tr>
<tr>
<td>3</td>
<td>Dilated cardiomyopathy</td>
<td>73</td>
<td>F</td>
<td>68/58</td>
</tr>
<tr>
<td>4</td>
<td>Dilated cardiomyopathy</td>
<td>70</td>
<td>F</td>
<td>75/70</td>
</tr>
<tr>
<td>5</td>
<td>Dilated cardiomyopathy</td>
<td>68</td>
<td>F</td>
<td>57/52</td>
</tr>
<tr>
<td>6</td>
<td>Dilated cardiomyopathy</td>
<td>59</td>
<td>M</td>
<td>63/56</td>
</tr>
<tr>
<td>7</td>
<td>Dilated cardiomyopathy</td>
<td>71</td>
<td>M</td>
<td>75/68</td>
</tr>
<tr>
<td>8</td>
<td>Mitral stenosis</td>
<td>61</td>
<td>F</td>
<td>47/34</td>
</tr>
<tr>
<td>9</td>
<td>Aortic regurgitation</td>
<td>35</td>
<td>F</td>
<td>58/31</td>
</tr>
<tr>
<td>10</td>
<td>Mitral regurgitation, aortic regurgitation, tricuspid regurgitation</td>
<td>45</td>
<td>F</td>
<td>74/60</td>
</tr>
<tr>
<td>11</td>
<td>Aortic regurgitation</td>
<td>48</td>
<td>F</td>
<td>80/75</td>
</tr>
<tr>
<td>12</td>
<td>Aortic regurgitation, aortitis</td>
<td>38</td>
<td>F</td>
<td>61/43</td>
</tr>
<tr>
<td>13</td>
<td>Aortic stenosis and regurgitation, mitral regurgitation</td>
<td>73</td>
<td>M</td>
<td>66/54</td>
</tr>
</tbody>
</table>

ANP contents were determined in the left subendocardium of each heart. LVds, left ventricular diastolic/systolic dimension determined by echocardiography; CTR, cardiothoracic ratio; ANP, atrial natriuretic peptide; ND, not determined; F, female; M, male.
sulfonyl fluoride, 500 units/ml aprotinin) by a micro glass homogenizer. After centrifugation for 10 minutes at 10,000g, the supernatant was brought to 62.5 mM tris(hydroxymethyl)aminomethane (Tris) (pH 7.2), 2% sodium dodecyl sulphate (SDS), 0.01% bromophenol blue (BPB), and 10% glycerol by adding 0.33 volumes concentrated Tris-SDS-BPB-glycerol solution, and it was then subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE, %T = 18%) by the method of Laemmli. Reducing agents were avoided to prevent proteins from loss of immunoreactivity. ha-ANP was also subjected to SDS-PAGE. Proteins were then transferred to polyvinylidene difluoride membranes (Immobilon transfer membrane, Millipore, Bedford, Massachusetts) by the method of Towbin et al, and one was stained with amido black, and the other protein was stained with the anti-ANP antibody. Bound antibody was detected with an Immune Blot Kit (Bio Rad Labs, Richmond, California).

Radioimmunoassay

Samples were homogenized in 10 volumes extraction buffer and diluted to appropriate concentrations in radioimmunoassay (RIA) buffer (100 mM phosphate-buffered solution at pH 7.2, 0.1% bovine serum albumin, 0.05% Tween 20, 0.1% NaCl, and 500 units/ml aprotinin). Then, 100 µl diluted extract or various amounts of unlabeled ha-ANP were placed in tubes, together with 100 µl RIA buffer, and 50 µl diluted anti-ANP antibody, and the mixtures were incubated at 4°C for 24 hours, after which 125I-labeled ha-ANP (about 2,500 cpm) in 50 µl RIA buffer was added to each sample. After incubation at 4°C overnight, free and bound ANP fractions were separated by precipitation with a sufficient amount of anti-rabbit immunoglobulin G in 12.5% polyethylene glycol 6000. After centrifugation, the supernatant was decanted, and the radioactivity in the precipitates was counted in an auto-gamma spectrometer. The amount of unlabeled ANP required to displace 50% of bound 125I-labeled ha-ANP was 23 pg, and the sensitivity of the procedure was 3 pg/tube. The interassay and intra-assay variations were 9.2% and 5.3%, respectively.

The RIA method revealed that the antibody cross-reacted with an efficiency of 44% to synthesized hβ-ANP. Cross-reactivity with synthetic ha-ANP was 100% but was 0% with synthetic ha-ANP and ha-ANP. When methionine at position 12 of ha-ANP was oxidized, the cross-reactivity was reduced to 40%.

Immunofluorescent Study

Cryostat sections, cut by a cryostat (International Equipment, Needham, Massachusetts) into 4 µm, were fixed in 4% paraformaldehyde for 1 minute and then in 95% methanol for 4 minutes. After being treated with anti-ANP serum for 40 minutes, sections were rinsed with 10 mM phosphate-buffered solution, and then incubated with fluorescein isothio-

cyanate (FITC)–labeled goat anti-rabbit immunoglobulin G (EY Laboratories, San Mateo, California) for 40 minutes. Sections were examined by an epifluorescent microscope equipped with a filter for fluorescein isothiocyanate.

Other Methods

Protein concentrations were determined by the method of Lowry et al. Statistical analyses were performed by two-way analyses of variance, the Student’s t test, and a correlation study. Data are expressed as mean ± SEM.

Results

Ventricles of Fetuses and Patients With Dilated Cardiomyopathy Expressed a 17-kDa ANP

With the immunoblotting method, the anti-ANP antibody was found to react strongly with the 3-kDa ha-ANP and weakly with its 6-, 9-, 12-, and 15-kDa polymerized molecules (Figures 1A and 1B). Some atrially extracted proteins reacted with the antibody, but proteins from normal adult ventricular extracts did not. The relative molecular weights of the major atrial proteins showing reaction with the antibody were 6, 17, and 21 kDa, of which the
ANP Contents and the Distribution of Myofibers Expressing ANP in the Developing Hearts

To quantitatively evaluate the expression of ANP in the fetal heart, we measured the amount of ANP in the ventricles of each fetal stage by the RIA method. In the left ventricle of the examined hearts, ANP amounts were higher in the early fetal stages, which showed a good correlation with time (weeks) of gestation ($r = -0.84, p < 0.05$) (Figure 2). This supported the idea that the ventricular expression of ANP is developmentally regulated from a very early fetal stage. The contents of ANP (per mg protein) in each ventricular chamber and the interventricular septum were also determined (Figure 3) in fetal hearts of midgestational stage (18–23 weeks). In the atria, we found consistently higher ANP contents in the left atrium than in the right atrium (Figure 3A). On the other hand, in the ventricles, higher ANP levels were observed in the right ventricle than in the left ventricle or interventricular septum (Figure 3B). Ventricular ANP contents in this midgestational stage were approximately one fourth to one third of those expressed in atria. Because hearts at the early developmental stage are very small, we could not determine the concentration of ANP in each atrium by the RIA method, but by immunohistochemical studies, we found that the difference in ANP expression between the right atrium and left atrium existed from the early fetal stage. Figure 4 shows atria from hearts of 9 weeks of gestation stained with anti-ANP antibody. Although almost all atrial myofibers in the left atrium were stained, most myofibers in the right atrium gave a negative reaction in this stage. Thus, the difference in expression of ANP between the right atrium and the left atrium can be observed from the very early developmental stage.

In the ventricles of the early developing hearts, immunohistochemical studies revealed that the ANP producing myocytes were clustered more in the subendocardial than in the subepicardial region of both free walls (Figure 5). This tendency toward a transmural gradient in the expression of ANP was also observed in the midfetal stage. Therefore, it was suggested that ventricular myofibers expressing ANP are confined to the subendocardial region in the fetal stage.

Contents and Distributions of ANP in Ventricles of Patients With Congestive Heart Failure

As demonstrated in Figure 1, most of the ventricles from patients with dilated cardiomyopathy expressed significant amounts of ANP. To determine whether the ventricles of patients with congestive heart failure due to causes other than dilated cardiomyopathy were expressing ANP, we examined the ventricles of patients who had died from severe congestive heart failure due to valvular heart disease. Figure 6 shows ANP amounts in the left ventricular subendocardium of patients without cardiac disease (normal group, $n = 17$), with congestive heart failure due to valvular heart disease (the valvular heart disease group, $n = 6$) and with congestive heart failure due to dilated cardiomyopathy (the dilated cardiomyopathy group, $n = 7$). Even in

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**Figure 2.** Plot of correlation between amount of left ventricular atrial natriuretic peptide (ANP) and time (weeks) of gestation. ANP amounts were higher in the early fetal stage and lower in the midgestational stage, which show good correlation with weeks of gestation. ir, immunoreactive.

**Figure 3.** Plots of atrial natriuretic peptide (ANP) contents in the right atrium (RA), left atrium (LA), left ventricle (LV), interventricular septum (IVS), and right ventricle (RV) in midgestational fetal stages (18, 21, 22, and 23 fetal weeks [WF]). Panel A: In these stages, ANP contents were higher in the LA than in the RA. Panel B: In ventricles, ANP contents were consistently higher in the RV than in the LV or IVS. Bars indicate the mean±SEM.
the ventricles of the normal group, ANP was detectable by the RIA method but only in very small quantities (1.8±0.26 ng/mg protein). In the ventricles of the valvular heart disease group, although clearly visible bands were not observed by the immunoblotting method (data not shown), ANP content measured by the RIA method was found to be significantly greater (4.70±0.98 ng/mg protein) than in the normal group (p<0.05). The highest ANP contents were detected in the ventricles of the dilated cardiomyopathy group (10.99±1.68 ng/mg protein). Although patients in the dilated cardiomyopathy and the valvular heart disease groups had suffered similarly from congestive heart failure before death, the ANP amounts in ventricles from the dilated cardiomyopathy group were significantly higher than those in the valvular heart disease group (p<0.01) and, needless to say, higher than those in the normal group (p<0.01).

In the ventricular cross sections, including the left and right ventricles and interventricular septum obtained from the dilated cardiomyopathy group (n=5), ANP contents in almost all regions were significantly higher than in the corresponding regions of the normal group (n=7) (Figure 7). It is noteworthy that even papillary muscle expressed considerable quantities of ANP. The higher ANP contents were observed in the subendocardium and in papillary muscles of the left ventricle compared with the corresponding regions of the right ventricle, and thus, it was suggested that in the ventricles of patients with dilated cardiomyopathy, ANP contents were higher in the left ventricle than in the right ventricle. We found also that ANP contents were consistently higher in the left subendocardial region than in the subepicardial region, which shows a transmural gradient in the dilated cardiomyopathy group. This transmural gradient in the expression of ANP was similar to that in fetal hearts by immunohistochemical studies. We also examined the ANP contents (per milligram protein) in the right and left atrial appendages of the normal and dilated cardiomyopathy groups (Figure 8). In the normal group, although seven of 10 patients showed higher ANP contents in the right atrial appendage than in the left atrial appendage, there was no significant difference when all patients were examined. Similarly, in the dilated cardiomyopathy group, we found no differ-
ence in the contents of ANP between right and left atrial appendages. In addition, we found no significant difference in both atrial ANP contents between the normal and dilated cardiomyopathy groups. Thus, it was suggested that both atria of the dilated cardiomyopathy group used in this study contained essentially the same quantity of ANP (per milligram protein) as was observed in the normal group atria. To confirm that ANP detected by immunoblotting and RIA methods was actually expressed by the ventricular myocytes, we performed immunohistochemical studies with cryostat sections (Figure 9). Although a granular staining pattern was observed in the perinuclear region and in the cytoplasm of the adult atria obtained from the normal and dilated cardiomyopathy groups (Figures 9A and 9C), such clear granular staining was negligible or completely absent in the normal group ventricles (Figure 9B). However, in the ventricles of dilated cardiomyopathy patients, as we have previously reported,\(^\text{10}\) considerable numbers of stained granules were clearly observed in the cytoplasm of ventricular myofibers (Figure 9D). In the ventricles of the valvular heart disease group, small quantities of granules were also detected in some patients (data not shown). Thus, the ventricular myofibers themselves were found to express ANP in the patients suffering from congestive heart failure.

Discussion

In this study, we have demonstrated that ANP expression in the ventricular myofibers is regulated developmentally; that is, these proteins are abundantly expressed in fetal ventricles but are hardly detected in normal adult ventricles. However, in the ventricular myofibers of patients with congestive heart failure, these proteins are again significantly present. Our results suggest that the ANP encoding gene, transiently expressed in the fetal stage but almost dormant in the adult stage, can be reinduced when ventricular myofibers are subjected to special circumstances such as those observed in congestive heart failure. Another interesting finding was that, in the fetal stage, ANP content (per milligram protein) was higher in the right ventricle than in the left ventricle, but in pathological adult conditions such as observed in dilated cardiomyopathy, the left ventricle expressed more ANP than did the right ventricle. In addition, we found the existence of an ANP-containing myofiber transmural gradient in both fetal and dilated cardiomyopathic ventricles, which is a finding also reported for neonatal rats.\(^\text{6}\) Considering the ventricular sites that are subjected to an overload in both states and considering that myofibers in the subendocardial region normally suffer from a greater wall stress than do those in the epicardial region, our results...
suggest that local factors, such as wall stress, may contribute to the ventricular expression of ANP. The major protein detected by the antibody had a relative molecular weight of 17 kDa as determined by SDS-PAGE. As far as we know, ANPs of about 17 kDa are prepro ANP or pro ANP. Although the calculated molecular weights of prepro ANP and pro ANP from complementary deoxyribonucleic acid–encoding ANP in humans are 16.4 kDa and 13 kDa, respectively,15–17 elegant studies by Bloch et al.16–18 showed that a 17-kDa protein determined by SDS-PAGE has been identified as a pro ANP in rats. Because there is remarkable homology between human and rat pro ANP in amino acid sequences15 and because pro ANP is also known to be a major ANP in mammalian atria,15,19 the 17-kDa protein detected in this study is likely to be a pro ANP. The other proteins, larger (21 kDa) and smaller (6 kDa) than the 17-kDa molecule, which reacted with the antibody are unknown but may correspond to a prepro ANP and β-ANP, respectively. Because we have no Northern blotting data, one may speculate that ventricular ANP might be extracted from circulating blood by the ventricular myofibers. However, this possibility can be eliminated because the main ANP contained in the ventricular tissue should, as discussed above, be a pro ANP, which is believed to be a precursor form of the ANP and cardiodiastatic circulating in the blood.15–17 Moreover, the granular staining pattern in the ventricles suggests that ANPs are stored in the secreting granules. Thus, it is reasonable to assume that the ventricular myofibers themselves synthesize ANPs and possibly secrete them.

Our findings are compatible with the earlier studies that demonstrated high-circulatory ANP levels in patients with congestive heart failure.2–5 Although

the amounts of ANP appear to be less in the ventricular than in the atrial myofibers, the total amount of these proteins secreted by the ventricle should not be ignored because ventricular mass is significantly larger than atrial mass. Therefore, it is possible that the ANP secreted from the ventricle may significantly contribute to circulatory ANP levels in patients suffering from severe congestive heart failure. Although ventricular ANP expression is not specific to dilated cardiomyopathy, the amounts of ANP expressed in the ventricles of dilated cardiomyopathy patients are considerably greater than amounts in other cardiac disease, and thus, dilated cardiomyopathy should be particularly identified as a cardiac disease that induces this ventricular ANP expression.

Because we could not obtain whole hearts, the determination of the whole ANP content in each chamber was not performed. However, when ANP content was expressed per milligram of protein, we found characteristic ANP distributions in fetal and adult hearts, which led us to hypothesize that focal factors such as wall stress contribute to the expression of ANP. The pattern of the ANP distribution in the right ventricle and left ventricle during development differed from that of pathological adult ventricles. In fetal ventricles, ANP contents (per milligram protein) were higher in the right ventricle than in the left ventricle, but in adult patients with dilated cardiomyopathy, ANP tissue levels were higher in the left ventricular subendocardium than in the right ventricular subendocardium. Because the right ventricle pumps against a higher resistance than does the left ventricle in the fetal stage,20 it is

Figure 8. Plots of atrial natriuretic peptide (ANP) contents in the right atrium (RA) and left atrium (LA) of normal (NORMAL) adult hearts and dilated cardiomyopathy (DCM) patient hearts. No statistical differences in ANP contents were observed between the RA and LA in normal and pathological states.

Figure 9. Photomicrographs of immunohistochemical detection of atrial natriuretic peptide (ANP) in adult normal and diseased hearts (magnification, ×320). Although a granular staining pattern was observed in the perinuclear region, in the cytoplasm of the adult atria from patients without dilated cardiomyopathy (DCM) (Panel A) and with DCM (Panel C), such a clear granular staining was negligible or completely absent in ventricles from the normal group (Panel B). However, considerable numbers of stained granules were clearly observed in the cytoplasm of the DCM group (Panel D) ventricular myofibers.
assumed that the right ventricle is subjected to an overload during this stage. On the other hand, because patients with dilated cardiomyopathy suffer primarily from left ventricular failure and have an elevated systemic vascular resistance, it is assumed that the left ventricle is subjected to more severe overload in this situation than is the right ventricle. Therefore, the difference in expression of ANP in the right and left ventricles, between the fetal and pathological states might depend, in part, on the site of ventricular overload. This hypothesis was further supported by the existence of an ANP-containing myofiber transmural gradient in ventricles from patients with dilated cardiomyopathy and fetuses. Because myofibers in the subendocardial region normally suffer from a greater wall stress than do those in the epicardial region, subendocardial myofibers may be able to sense an overload and, thus, synthesize ANP in these situations. The causes of the difference in the amount of ANP (per milligram protein) between the right and left atria during the fetal stage remain to be determined. However, one possible explanation may be related to the existence of a large right-to-left shunt through the foramen ovale. The fetal left atrium must receive a large volume of blood and eject it to the left ventricle, whereas the right atrium may function as a conductor of this large shunt. Thus, the left atrium may be subjected to an overload compared with the right atrium. In any case, these characteristic distributions of ANP suggest that local factors such as wall stress probably contribute to the expression of ANP.

Lastly, the reason why a 6-kDa ANP was also detected in the ventricles of patients with dilated cardiomyopathy, but not in fetal ventricles, was not elucidated by this study. Speculatively, a pro ANP may be actively processing to β-ANP in ventricles of patients with dilated cardiomyopathy, or perhaps, the fetal ventricles examined in this study were not sufficiently developed to express the enzyme required to convert a pro ANP to its derivatives. However, further investigation is needed to elucidate this matter.

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References


KEY WORDS: atrial natriuretic peptide • congestive heart failure • dilated cardiomyopathy • valvular heart disease
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