Augmented Expression of Atrial Natriuretic Polypeptide Gene in Ventricles of Spontaneously Hypertensive Rats (SHR) and SHR-Stroke Prone

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Tissue levels of atrial natriuretic polypeptide (ANP) and ANP messenger RNA (mRNA) in the atrium and ventricle were measured simultaneously in spontaneously hypertensive rats (SHR) and its substrain, SHR-stroke prone (SHRSP), and these levels were compared with those in control Wistar-Kyoto rats (WKY). At 27 weeks of age with established hypertension and ventricular hypertrophy, ANPmRNA levels in ventricles from SHR and SHRSP were markedly increased, and total contents of ventricular ANPmRNA in SHR and SHRSP were approximately 50% and 250%, respectively, of the corresponding atrial contents. However, levels and total contents of atrial ANPmRNA in SHR and SHRSP were similar to those of WKY, and the total content of ventricular ANPmRNA in WKY was only 6% of the content of atrial ANPmRNA. ANP concentrations in ventricles of SHR and SHRSP were increased in association with the augmentation of ANPmRNA levels. During the prehypertensive stage at 6 weeks of age, slight increases in levels and total contents of ANPmRNA and ANP in the ventricle were observed only in SHRSP. These results demonstrate that the expression of the ANP gene is markedly augmented in ventricles of SHR and SHRSP, especially of SHRSP, at the stage of established hypertension and ventricular hypertrophy, and they also suggest that these genetically hypertensive rats are one of the best animal models to investigate the biosynthesis, storage, and secretion of ventricular ANP. (Circulation Research 1988;62:926–930)

Atrial natriuretic polypeptide (ANP) is a cardiac hormone with diuretic, natriuretic, and vasorelaxant activities, and it is implicated in the control of blood pressure, body fluid, and cardiovascular homeostasis. De Bold et al. first reported that atrial extracts possess natriuretic, diuretic, and hypotensive activities, whereas ventricular extracts do not possess activities. Similarly, histological studies showed that mammalian atrial cardiocytes contain numerous specific atrial granules, whereas these granules are rarely found in ventricular cardiocytes. In addition, previous investigations using radioimmunoassay (RIA) and blot hybridization revealed the presence of large quantities of ANP or ANP messenger RNA (ANPmRNA) in the atrium but not in the ventricle.

Recently, however, evidence has accumulated indicating that ANP is synthesized in various extra-atrial tissues, including the ventricle. Subsequently, the gene expression of ANP in the ventricle has been reported to be increased in neonatal rats and decreased during the 1st week of life. We have also demonstrated that human fetal ventricles contain considerable amounts of ANP and that its level in the ventricle decreases in parallel with fetal development.

The spontaneously hypertensive rat (SHR) and its rat substrain, SHR-stroke prone (SHRSP), are genetically hypertensive rat strains that have been studied widely and intensively in hypertension research. SHR develops hypertension by the 7th week of age. SHRSP exhibits more severe hypertension, with adult males having blood pressures over 230 mm Hg. In addition, in SHR and SHRSP, a gradual increase in cardiac mass occurs as a function of age-related increase in blood pressure and results in natural progression of hypertrophy. Several laboratories, including ours, have demonstrated that plasma ANP concentrations are significantly elevated in SHR and SHRSP and have proposed that the increased ANP secretion in these rats is a possible compensatory response to hypertension. We and others have also reported that plasma ANP concentrations are elevated in patients with essential hypertension. Therefore, the intensive research on the biosynthesis and secretion of ANP in these hypertensive models could lead us to a better understanding of the implication of ANP in hypertension and such complications as ventricular hypertrophy.

In the present study, we measured ANPmRNA and ANP levels in atria and ventricles of SHR and SHRSP.
and of their control strain, Wistar-Kyoto rats (WKY), using the blot hybridization technique and specific RIA for ANP.

Materials and Methods

Animals

Male SHR and SHRSP at ages of 6 and 27 weeks, and age-matched male WKY were used in the present study. Rats were maintained in Shionogi Research Laboratories (Shionogi, Osaka, Japan). Animals were housed in a temperature-, humidity-, and light-controlled room and had free access to water and standard rat chow CA-1 (Japan CLEA, Tokyo) containing 0.50% sodium and 0.84% potassium. Systolic blood pressure was measured by the indirect tail-cuff method. Brief profiles of rats used in the present study are summarized in Table 1.

Hearts

Hearts were removed from decapitated rats; they were immediately dissected into four parts, bilateral atria and ventricles, and were placed on ice. To avoid contamination of the atrial tissue, the apical half of the ventricle was used for measurements of ANPmRNA and ANP in the ventricle. Tissues were weighed, frozen in liquid nitrogen, and stored at -70°C until use.

RNA Extraction and Northern Blotting Analysis

RNA extraction was performed for three groups of pooled samples (n = 5 x 3), and the ANPmRNA level was measured by the Northern blotting analysis according to methods described previously. Labeled 368-bp restriction fragment of the rat ANP complementary DNA (ANPcDNA) was used as a probe. Specific activity was approximately 1 x 10^9 cpmp/µg.

Peptide Extraction and Radioimmunoassay

ANP was extracted from individual samples (n = 5), and the ANP concentration was measured by RIA as previously reported. This RIA recognizes a carboxy-terminal fragment of α-ANP, α-ANP[17-28], and is equally specific for both rat α-ANP and human α-ANP. The cross-reactivity with rat γ-ANP in the RIA is 100% on a molar basis. The minimal detectable quantity of rat α-ANP is 1 pg/tube.

Statistical Analysis

The values were compared by Duncan’s multiple range test after one-way analysis of variance.

Results

ANPmRNA and ANP in SHR, SHRSP, and WKY at 27 Weeks of Age

As shown in Table 1, hypertension and ventricular hypertrophy developed in SHR and SHRSP at the 27th week. Ventricular hypertrophy of SHRSP was more conspicuous than that of SHR as judged from the ratio of ventricular weight to body weight. Figure 1 shows the results of Northern blot analysis of RNA from WKY, SHR, and SHRSP hearts. Total RNA extracted from atri and ventricles of SHR and SHRSP contained a hybridizing RNA band of the same size (approximately 0.95 kilo base pair) as atrial ANPmRNA from WKY. The density or the radioactivity in the hybridized band showed a linear relationship with the amount of RNA applied to the electrophoresis gel.

Table 2 shows levels and total contents of both ANPmRNA and ANP in WKY, SHR, and SHRSP. Atrial levels and total contents of both ANPmRNA of SHR and SHRSP at the 27th week were similar to those of age-matched WKY. However, ANPmRNA levels in ventricles of both SHR and SHRSP were markedly increased and were approximately seven and 30 times higher than that of WKY, respectively. In SHRSP, the ANPmRNA level in the ventricle reached up to 20% of that in the atrium. Total contents of ANPmRNA in the ventricles of SHR and SHRSP were about 10 and 40 times higher than that of WKY, respectively. Consequently, the total ventricular content of ANPmRNA reached up to 50% of the atrial content in SHR, and even to 250% in SHRSP, although it was only 6% in WKY.

ANP concentrations and contents in atria of SHR and SHRSP at the 27th week were similar to those of age-matched WKY. ANP concentrations in ventricles of SHR and SHRSP were increased as were the ANPmRNA levels and were approximately three and eight times higher than that of WKY, respectively. Total contents of ANP in ventricles of SHR and SHRSP were about four and 10 times higher than that of WKY, respectively. Consequently, total ANP contents in

### Table 1. Profiles of Wistar-Kyoto, Spontaneously Hypertensive (SHR), and SHR-Stroke Prone Rat Strains

<table>
<thead>
<tr>
<th>Variable</th>
<th>6 Weeks</th>
<th>27 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>96.3 ± 2.7</td>
<td>97.6 ± 3.4</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>109.7 ± 2.8</td>
<td>125.1 ± 6.5</td>
</tr>
<tr>
<td>Atrial weight (mg)</td>
<td>54.7 ± 2.4</td>
<td>53.0 ± 3.2</td>
</tr>
<tr>
<td>Ventricular weight (mg)</td>
<td>220 ± 32</td>
<td>295 ± 45</td>
</tr>
<tr>
<td>Atrial w/body wt (%)</td>
<td>0.057 ± 0.003</td>
<td>0.054 ± 0.004</td>
</tr>
<tr>
<td>Ventricular w/body wt (%)</td>
<td>0.229 ± 0.033</td>
<td>0.303 ± 0.049*</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rat strain; SHR, spontaneously hypertensive rat strain; SHRSP, SHR-stroke prone rat strain.

*p<0.05; †p<0.01 vs. age-matched WKY; ‡p<0.01, §p<0.05 vs. age-matched SHR.
FIGURE 1. Northern blot analysis of total RNA from atria and ventricles of WKY, SHR, and SHRSP. One microgram and 10 μg of total RNA from atria and ventricles, respectively, was fractionated on 1.4% agarose gel, transferred to the nylon membrane, and hybridized to a [³²P]ANP complementary DNA probe. kbp, kilo base pair.

ventricles of SHR and SHRSP were about 3% and 7%, respectively, of those in corresponding atria, while total content was only 0.6% in WKY.

**ANPmRNA and ANP in SHR, SHRSP, and WKY at 6 Weeks of Age**

SHR and SHRSP at the 6th week and their age-matched control WKY were studied before the development of hypertension. At 6 weeks of age, blood pressure of SHRSP was slightly, but significantly, higher than that of WKY, but it did not reach the range of hypertension as shown in Table 1. Ventricular hypertrophy was observed in both SHR and SHRSP at this age. The variable of ventricular wt/body wt was larger in SHR and SHRSP than in WKY. This variable of 6-week SHRSP was larger than that of 27-week WKY.

As shown in Table 2, atrial levels and contents of ANPmRNA of SHR and SHRSP at the 6th week were similar to those of age-matched WKY. ANPmRNA levels and contents (arbitrary units) are expressed as relative levels to those in the atrium of WKY at 27 weeks of age (ANPmRNA level in 1 μg of total RNA from 27-week-old WKY atria is 1.0 unit).

**TABLE 2. Levels and Contents of ANPmRNA and ANP in Hearts From Wistar-Kyoto, Spontaneously Hypertensive (SHR), and SHR-Stroke Prone Rat Strains**

<table>
<thead>
<tr>
<th>ANP</th>
<th>WKY 6 Weeks</th>
<th>SHR 6 Weeks</th>
<th>SHRSP 6 Weeks</th>
<th>WKY 27 Weeks</th>
<th>SHR 27 Weeks</th>
<th>SHRSP 27 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue ANPmRNA level (μg/unit)</td>
<td>540±43</td>
<td>460±43</td>
<td>440±41</td>
<td>450±42</td>
<td>480±45</td>
<td>410±38</td>
</tr>
<tr>
<td>Ventricle</td>
<td>3.5±0.32</td>
<td>3.2±0.29</td>
<td>14±1.3††</td>
<td>2.6±0.23</td>
<td>17±1.6†</td>
<td>81±7.6††</td>
</tr>
<tr>
<td>Total content of ANPmRNA (μg/unit)</td>
<td>26±3.4</td>
<td>26±2.4</td>
<td>20±2.4</td>
<td>55±9.4</td>
<td>68±9.4</td>
<td>46±4.7</td>
</tr>
<tr>
<td>Atrium</td>
<td>0.81±0.10</td>
<td>1.0±0.11</td>
<td>3.4±0.30*§</td>
<td>3.1±0.19</td>
<td>35±3.9†</td>
<td>110±10††</td>
</tr>
<tr>
<td>Ventricle</td>
<td>43.6±8.2</td>
<td>58.7±8.8</td>
<td>52.4±8.0</td>
<td>69.5±4.2</td>
<td>59.1±9.5</td>
<td>61.9±7.8</td>
</tr>
<tr>
<td>Tissue ANP concentration (μg/unit)</td>
<td>0.026±0.008</td>
<td>0.050±0.006</td>
<td>0.126±0.016††</td>
<td>0.040±0.002</td>
<td>0.124±0.020*</td>
<td>0.329±0.045§</td>
</tr>
<tr>
<td>Atrium</td>
<td>2.40±0.32</td>
<td>3.05±0.32</td>
<td>2.21±0.33</td>
<td>8.26±0.45</td>
<td>8.18±1.00</td>
<td>6.81±0.75</td>
</tr>
<tr>
<td>Ventricle</td>
<td>0.006±0.002</td>
<td>0.016±0.004</td>
<td>0.032±0.006*</td>
<td>0.049±0.006</td>
<td>0.210±0.042*</td>
<td>0.467±0.059† §</td>
</tr>
<tr>
<td>Total content of ANP (μg/unit)</td>
<td>119</td>
<td>154</td>
<td>123</td>
<td>151</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>Atrium</td>
<td>7.4</td>
<td>15.6</td>
<td>9.3</td>
<td>15.4</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>Ventricle</td>
<td>288</td>
<td>288</td>
<td>288</td>
<td>288</td>
<td>288</td>
<td></td>
</tr>
</tbody>
</table>

ANPmRNA, atrial natriuretic peptide messenger RNA; WKY, Wistar-Kyoto rat strain; SHR, spontaneously hypertensive rat strain; SHRSP, SHR-stroke prone rat strain.

The experiment concerning ANPmRNA was repeated three times. Each experiment was performed with pooled sample (n = 5). The experiment concerning ANP was performed with individual samples (n = 5), as described in "Materials and Methods."
levels in ventricles of WKY and SHR were approximately 1% of those in corresponding atria. The ANPmRNA level in the ventricle of SHRSP was 3% of that in the corresponding atrium. The total content of ventricular ANPmRNA in SHR was similar to that in WKY, but SHRSP showed a three-times higher increase of the ANPmRNA content in the ventricle. Consequently, the total content of ventricular ANPmRNA was about 20% of atrial ANPmRNA content in SHRSP.

ANP concentrations in atria of SHR and SHRSP tended to be higher than that of WKY, but the differences were not significant. ANP contents in atria of SHR and SHRSP were similar to that of WKY at this age. Ventricular ANP concentrations in SHR and SHRSP were approximately two and five times higher than that of WKY, respectively. Ventricular ANP contents of SHR and SHRSP were about three and five times higher than that of WKY, respectively. The ANP concentration and content in the ventricle were, however, only 0.2% and 1.4%, respectively, of those in the atrium, even in SHRSP.

**Comparison of ANPmRNA and ANP Levels Between 6-Week-Old and 27-Week-Old Rats**

Atrial ANPmRNA and ANP levels of these three strains at the 6th week were similar to those at the 27th week. However, ventricular ANPmRNA and ANP levels contrast with atrial ANPmRNA and ANP levels. Although ANPmRNA levels in ventricles of WKY at the 6th and 27th week were similar, ANPmRNA levels in ventricles of SHR and SHRSP at the 27th week were about five and six times higher than those at the 6th week, respectively. Ventricular ANP levels in SHR and SHRSP at the 27th week were approximately 2.5 and three times higher than those at the 6th week, respectively, and the ventricular ANP level in WKY at the 27th week was 1.5 times higher than that at the 6th week. Ventricular levels of ANPmRNA and ANP in 6-week SHRSP were higher than those in 27-week WKY.

**Comparison of ANPmRNA and ANP Levels Between Atria and Ventricles of WKY, SHR, and SHRSP**

The ratios of ANP to ANPmRNA levels in atria were approximately one order of magnitude higher than those in ventricles in all three strains as shown in Table 2.

**Discussion**

The present study demonstrates that the gene expression of ANP is substantially augmented in ventricles of SHR and SHRSP, especially in the latter, at the stage of established hypertension and cardiac hypertrophy. In the present study, levels and contents of ANPmRNA and ANP in the atrium and ventricle in control WKY were consistent with those recently reported, indicating the validity of our assay systems.

It is of great interest that ventricular ANPmRNA levels in WKY, SHR and SHRSP at the 27th week were approximately 0.6%, 3.5%, and 20% of corresponding atrial ANPmRNA levels, respectively, in spite of similar levels of atrial ANPmRNA in these strains. Consequently, the total content of ventricular ANPmRNA was about half of that of atrial ANPmRNA in SHR and reached 250% of the total atrial content in SHRSP. This finding means that considerable amounts of ANP are synthesized in SHR and SHRSP ventricles. It is likely, therefore, that the ventricle of SHR and SHRSP, especially of SHRSP, is potentially an additional source of plasma ANP.

In the present study, the ratios of ANP to ANPmRNA levels in ventricles were about one order of magnitude lower than those in atria in all three strains. This finding is in agreement with the recent observation of ANPmRNA and ANP levels in ventricles of normal adult rats and is also consistent with the results of the pulse-chase experiment performed by Bloch et al that neonatal ventricular cardiocytes in culture release ANP more rapidly after biosynthesis than atrial cardiocytes. Therefore, our results along with those of Bloch et al raise the possibility that ANP is secreted from the ventricle more rapidly than from the atrium and that ventricular ANP contributes to the elevation of the plasma ANP level in SHR and SHRSP, especially in SHRSP. However, further studies are needed to clarify whether ANP secretion from the ventricle is constitutive or regulated under physiological and pathophysiological conditions and to clarify the efficiency of the translation and the turnover of ANPmRNA in the ventricle. In such cases, SHRSP would be one of the best experimental models.

The gene expression of ANP in the ventricle has been reported to be increased in neonatal rats and decreased during the 1st week of life. We have also demonstrated that human fetal ventricles contain considerable amounts of ANP that decrease in parallel with fetal development. In addition, we have recently observed the increased ANP gene expression in the ventricle of dilated cardiomyopathy. It is conceivable, therefore, that the augmented expression of the ANP gene in SHR and SHRSP ventricles shown in the present study is the increased expression of the ANP gene in the diseased ventricle. Changes in myosin heavy chain or light chain isoenzymic pattern and switching of the corresponding mRNAs have been demonstrated in animals with a hypertrophic ventricle and in SHR. The increased amount of fetal myosin light chain 1-like protein has also been reported in the ventricle of DCM.

The implication of the augmented expression of the ANP gene in the diseased ventricle remains unclear at present, but the hypertrophic process of ventricular cardiocytes and/or other factors including hemodynamic changes may contribute to the increased expression of the ANP gene in the diseased ventricle. In the present study, ventricular ANPmRNA and ANP levels of SHR and SHRSP increased along with the progression of ventricular hypertrophy. This observation is consistent with the recent results by Day et al that the experimentally hypertrophied rat ventricle induced by
aortic constriction shows the high ANP mRNA level. These results suggest that the augmentation of the expression of the ANP gene in the diseased ventricle is ascribed to ventricular hypertrophy and that the augmented expression of the ANP gene is one of the self-compensatory mechanisms of the heart.

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References

KEY WORDS • ANP • ANP messenger RNA • gene expression • ventricle • spontaneously hypertensive rats
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