Atrial Natriuretic Peptide in Chronic Heart Failure in the Rat: A Correlation with Ventricular Dysfunction

KAZUO TSUNODA, G. PETER HODSMAN, ERIC SUMITHRAN, AND COLIN I. JOHNSTON

To assess the relation between atrial natriuretic peptide and ventricular dysfunction, we simultaneously measured both atrial and plasma immunoreactive atrial natriuretic peptide concentrations in rats 4 weeks after myocardial infarction induced by left coronary artery ligation. When compared to controls (n = 39), rats with infarction (n = 16) had markedly elevated plasma immunoreactive atrial natriuretic peptide concentrations (1205.8 ± 180.9 vs. 126.7 ± 8.9 pg/ml, p<0.001) and reduced immunoreactive atrial natriuretic peptide concentrations in right and left atria (31.4 ± 4.6 vs. 61.2 ± 3.2 ng/mg, p<0.001; 14.9 ± 2.2 vs. 32.7 ± 4.5 ng/mg, p<0.001, respectively). Right ventricular weight increased in proportion to infarct size, and both were correlated with plasma immunoreactive atrial natriuretic peptide levels (r = 0.825, p<0.001 and r = 0.816, p<0.001, respectively). Right atrial immunoreactive atrial natriuretic peptide content was significantly higher than left in both controls and rats with infarction. Both right and left atrial immunoreactive atrial natriuretic peptide concentrations were negatively correlated with both right ventricular weight as well as plasma immunoreactive atrial natriuretic peptide concentrations (right atrium: r = -0.816, p<0.001, r = -0.708, p<0.01; left atrium: r = -0.687, p<0.01, r = -0.644, p<0.01, respectively). These results suggest that chronic stimulation of atrial natriuretic peptide release from both atria is associated with increased turnover and depleted stores of atrial natriuretic peptide in atria in proportion to the severity of heart failure. It also suggests that plasma atrial natriuretic peptide levels may be used as a reliable index of cardiac decompensation in chronic heart failure. (Circulation Research 1986;59:256-261)

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ing ligation, a time by which necrotic myocardium has been completely replaced by scar tissue, the rats were sacrificed by decapitation. The heart, lungs, and liver were immediately removed and weighed. The right and left atria were individually dissected, weighed, frozen in liquid nitrogen, and stored at -70°C until extraction. The right and left ventricles were dissected, separated, and weighed. The left ventricles (including intraventricular septum) were fixed in 10% buffered formalin for histological study. The weights of ventricles, atria, livers, and lungs were corrected for body weight. Blood was obtained by decapitation for the measurement of plasma IR-ANP by radioimmunoassay. The infarct was quantitated histologically by projection and planimetry using the method of Pfeffer et al. 17

Measurement of Atrial and Plasma IR-ANP

ANP was extracted from the atria by a method reported previously. 19 The atria were boiled for 15 minutes in 10 volumes of 0.1 M acetic acid to inactivate proteases and then homogenized with a polytron homogenizer. The homogenate was centrifuged at 5,000 rpm for 30 minutes at 4°C, and the supernatant was stored at -70°C until assay. Atrial ANP is expressed as both concentration per milligram atrial weight and as content per gram body weight.

Blood samples were taken into plastic tubes containing the following inhibitors: disodium methylenebisaminetetraacetic acid (EDTA-2Na, AJAX Chemicals Ltd., Sydney, 1 mg/ml), aprotinin (Trasylol, Bayer, 500 kallikrein inhibitor units/ml), and soybean trypsin inhibitor (SBTI, Sigma, 50 BAEE units/ml). Plasma IR-ANP was extracted using Vycor glass beads. To 1 ml of plasma, 30 mg of Vycor glass (140 mesh, Society of the Association of Trade with America, Geneva) was added and agitated for 30 minutes at 4°C. After 2 minutes centrifugation at 3500 rpm, the supernatant was discarded and the glass powder was washed with 2 ml of distilled water. Adsorbed IR-ANP was eluted from the glass powder with 1 ml of purified acetone–water mixture (60:40) containing 0.1% trifluoroacetic acid (TFA, BDH Ltd., Dorset, U.K.) during 30 minutes agitation at 4°C. The acetone was evaporated, the aqueous solution lyophilized and reconstituted in buffer for assay. Recovery of added synthetic α-rat ANP (15–2000 pg, 28 amino acid, Peptide Institute Inc., Osaka, Japan) from rat plasma was 94.0 ± 2.5% (mean ± SEM, n = 8). Serial dilution of the atrial and plasma extracts showed parallelism to the synthetic ANP standards. Results were not corrected for recovery.

A 0.1 M Tris-acetate buffer, pH 7.4, containing 0.1% bovine serum albumin (Fraction V, Sigma), aprotinin (500 kallikrein inhibitor units/ml), SBTI (50 BAEE units/ml), 0.1% iso-octylphenoxypolyethoxyethanol (Triton X-100, Packard Ins., Ill.), EDTA-2Na (1 mg/ml), and 0.02% sodium azide, was used to dissolve all reagents and to perform the radioimmunoassay. Fifty microliters of synthetic α-rat ANP or diluted samples were incubated for 48 hours at 4°C with 450 μl of buffer, 100 μl of antiserum (1:5000, rabbit anti-α-human ANP) and 100 μl of iodinated 125I-ANP (~10,000 cpm). The separation of antibody-bound and free peptide was performed using goat anti-rabbit gamma globulin in the presence of normal rabbit serum. The sensitivity of the assay is 11 pg α-rat ANP per tube. Intraassay and interassay variances were 5.3% (n = 10) and 16.3% (n = 10), respectively. The anti-human ANP antibody cross-reacted 65% with α-rat ANP and was directed toward the ring of section of ANP. Deletions at the N- or C-terminus had no effect on the immunoreactivity, but disruption of the disulphide bond completely abolished immunoreactivity.

Statistical Analysis

The data were analyzed with a one-way analysis of variance. Duncan’s New Multiple-Range test, which permits multiple testing among treatment means, was then employed. Regression lines were fitted by the method of least squares. P < 0.05 was considered significant. Data are presented as mean ± SEM.

Results

Myocardial Infarct Size and Cardiac Weights

Healed myocardial infarcts were present in 16 of 55 surviving rats. There was marked thinning of the infarcted area, where muscle was replaced with fibrous connective tissue. Right ventricular weight in infarcted rats was progressively elevated in proportion to infarct size (r = 0.864, p < 0.001, Figure 1). Rats with infarcts were, therefore, divided arbitrarily into three groups according to the ratio of right ventricular to body weight (mg/g), namely, Group I, <1.0; Group II, over 1.0 and under 1.4; Group III, >1.4.

Table 1 shows the body and the organ weights of control rats and rats with infaracts. There was no significant difference in body weight between controls and rats with infarction. Both right and left atrial weights were progressively increased in the infarcted rats; however, the values in Group I did not reach statistical significance. Left atrial hypertrophy was more marked than right. Increments in atrial weight were significantly correlated with increasing right ventricular weight (right atria: r = 0.594, p < 0.05; left atria: r = 0.832, p < 0.001). Lung weight was progressively increased in the infarcted groups and correlated significantly with right ventricular and left atrial weight.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Right ventricular weight (open bars) and infarct size (closed bars) in controls (C) and rats with infarction. I, II, and III indicate the subgroups described in “Results.”
Table 1. Body and Organ Weights (mg/g) in Controls and Rats with Infarction

<table>
<thead>
<tr>
<th>Numbers</th>
<th>Control</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt. (g)</td>
<td>39</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Organ wt. (mg/g b. wt.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricle</td>
<td>271 ± 4</td>
<td>255 ± 15</td>
<td>269 ± 10</td>
<td>251 ± 9</td>
<td>258 ± 6</td>
</tr>
<tr>
<td>Left atrium</td>
<td>2.40 ± 0.04</td>
<td>2.76 ± 0.08*</td>
<td>2.74 ± 0.13*</td>
<td>2.70 ± 0.06*</td>
<td>2.73 ± 0.08*</td>
</tr>
<tr>
<td>Right atrium</td>
<td>0.15 ± 0.01</td>
<td>0.19 ± 0.02</td>
<td>0.56 ± 0.04†</td>
<td>0.56 ± 0.04†</td>
<td>0.44 ± 0.05*</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.15 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.24 ± 0.03‡</td>
<td>0.27 ± 0.03*</td>
<td>0.23 ± 0.02‡</td>
</tr>
<tr>
<td>Liver</td>
<td>5.3 ± 0.1</td>
<td>5.5 ± 0.3</td>
<td>8.5 ± 0.8‡</td>
<td>14.8 ± 1.5*</td>
<td>9.9 ± 1.2*</td>
</tr>
<tr>
<td>Liver</td>
<td>32.1 ± 0.5</td>
<td>32.6 ± 0.9</td>
<td>32.8 ± 1.1</td>
<td>36.6 ± 1.4</td>
<td>34.3 ± 0.8</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM.
*p < 0.01, †p < 0.001, ‡p < 0.05, control vs. infarction group.

weights ($r = 0.873, p < 0.001$; $r = 0.661, p < 0.01$; respectively). Left ventricular weight was also slightly but significantly increased despite marked thinning of the ventricular free wall. There was no difference in liver weight between controls and animals with infarction.

**IR-ANP in Plasma and Atria**

Figure 2 shows the mean values of plasma IR-ANP in each group. Mean plasma ANP concentration in the control sham-operated group was 126.7 ± 8.9 pg/ml, similar to that of 10 nonoperated intact Wistar rats (114.2 ± 14.7 pg/ml). Mean plasma IR-ANP concentration in infarcted rats was tenfold higher (1205.8 ± 180.9 pg/ml, $p < 0.01$) than that of the control group. Plasma IR-ANP concentrations were significantly higher in Group I, II, and III (146.0 ± 80.7, $p < 0.05$; 1295.9 ± 118.4, $p < 0.001$ and 1752.2 ± 307.0 pg/ml, $p < 0.01$, respectively) than in the control group. The values in Group II and Group III were significantly higher ($p < 0.001$ and $p < 0.01$, respectively) than those in Group I, but the difference between Groups II and III was not significant. Plasma concentrations of IR-ANP were strongly correlated with right ventricular weight in infarcted rats (Figure 3: $r = 0.825, p < 0.001$) and with infarct size ($r = 0.816, p < 0.001$).

Table 2 shows the mean values of right and left atrial IR-ANP concentration and content in each group. The IR-ANP content of right and left atria in rats with myocardial infarcts was significantly decreased when compared with controls (11.55 ± 0.6 ng/g, $p < 0.05$). IR-ANP content in Group III was significantly less than in controls (9.97 ± 0.69 ng/g, $p < 0.01$); however, there were no significant differences among controls, Group I, and Group II. The IR-ANP content of both atrial in infarcted rats was significantly and negatively correlated with right ventricular weight and also with plasma ANP concentrations ($r = -0.596; p < 0.05$, $r = -0.649, p < 0.001$, respectively). Right atrial IR-ANP content was not decreased in Group I infarcted rats, but IR-ANP content in Groups II and III was significantly lower than in the control group and Group I. No significant difference was found between Group II and Group III. Right atrial IR-ANP content was significantly correlated with right ventricular weight and also with plasma IR-ANP concentration ($r = -0.744, p < 0.001$; $r = -0.633, p < 0.01$, respectively). On the other hand, there were no significant differences in left atrial ANP content between control and infarcted groups. The mean values of right atrial IR-ANP concentrations in Groups II and III were less than those of controls or Group I. Left atrial IR-ANP concentrations in Group II and Group III were also decreased signifi-
TABLE 2. Right (RA) and Left (LA) Atrial IR-ANP Concentration (ng IR-ANP/mg atrial weight) and Content (ng IR-ANP/g b. wt.)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Myocardial Infarction Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Numbers</td>
<td>I</td>
</tr>
<tr>
<td>RA IR-ANP concentration (ng/mg)</td>
<td>39</td>
<td>5</td>
</tr>
<tr>
<td>LA IR-ANP concentration (ng/mg)</td>
<td>61.1±3.2</td>
<td>54.6±5.3</td>
</tr>
<tr>
<td>RA IR-ANP content (ng/g)</td>
<td>3.7±0.44</td>
<td>25.5±3.7</td>
</tr>
<tr>
<td>LA IR-ANP content (ng/g)</td>
<td>4.67±0.34</td>
<td>4.75±0.80</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.
* p < 0.001, †p < 0.01, control vs. infarction group.

significantly compared with those of controls or Group I. No significant difference was observed between controls and Group I. Figure 4 shows the negative relations between right and left atrial IR-ANP concentrations and right ventricular weight (r = -0.816, p < 0.001; r = -0.687, p < 0.01, respectively). In addition, right and left atrial IR-ANP concentrations were strongly correlated inversely with plasma IR-ANP concentrations (r = -0.708, p < 0.01; r = -0.644, p < 0.01, respectively, Figure 5).

Discussion

The rat coronary artery ligation model of heart failure is particularly suitable for a study of this nature. This model of myocardial infarction has been shown to produce a wide range of left ventricular dysfunction in proportion to infarct size. In previous studies, the degree of right ventricular hypertrophy was directly correlated with infarct size, and hemodynamic changes including left ventricular end-diastolic and right ventricular systolic pressures. Right ventricular end-diastolic pressure and right atrial pressure were also increased, explaining the right ventricular hypertrophy observed in rats with large infarcts. For this reason, the degree of right ventricular hypertrophy was employed as an index of severity of heart failure in our study. Additional support for this approach comes from the lung and atrial weights, which, with infarct size, increased in proportion to the degree of right ventricular hypertrophy in rats with myocardial infarction.

Recently high levels of plasma IR-ANP have been reported in patients with heart failure, and increasing severity of heart failure was associated with progressively higher plasma levels of IR-ANP. A significant correlation between plasma IR-ANP level and pulmonary capillary wedge pressure has also been reported in humans, suggesting that increased atrial pressure is a stimulus for ANP release. Although we did not perform hemodynamic studies in the present experiment, they have been extensively measured and correlated with right ventricular hypertrophy in previous studies. Our present data show that plasma levels of IR-ANP are significantly increased in rats with heart failure compared with those in control animals and closely correlated with the degree of right ventricular hypertrophy and infarct size. These findings suggest that plasma ANP concentration increases in proportion to the degree of heart failure.

Two previous studies have reported both reduced content and increased content of atrial ANP in heart failure although both laboratories measured atrial ANP content in BIO 14.6 cardiomyopathic hamsters by bioassay. In our study, right atrial IR-ANP content was greatly reduced in rats with heart failure and this reduction was negatively correlated with right ventricular concentration.

FIGURE 4. Relations between right (*) and left (o) atrial IR-ANP concentrations and right ventricular weight \(r = -0.816, p < 0.001; r = -0.687, p < 0.01, \text{ respectively} \).

FIGURE 5. Relations between right (*) and left (o) atrial IR-ANP concentrations and plasma IR-ANP concentration \(r = -0.708, p < 0.01; r = -0.644, p < 0.01, \text{ respectively} \).
weight. Left atrial IR-ANP content was not reduced although the reason for these disparate results is not clear. The reduction in right atrial IR-ANP content was progressive with increasing severity heart failure. Interestingly, there was a very good correlation between reduced atrial ANP content and increased plasma ANP in these animals. This suggests that chronic stimulation leads to increased release of ANP, with elevated plasma ANP levels and reduced atrial peptide content. This is in keeping with the known behavior of other peptide hormones. IR-ANP concentration was much less in both the right and left atria of rats with heart failure compared with controls, which may also indicate depletion of atrial ANP content in chronic heart failure. This result also suggests a significant role for both atria in the release of ANP in at least this model of heart failure.

Although the metabolism of circulating ANP in plasma has not yet been clarified, it is likely that chronic stimulation of ANP release from both atria is responsible for the high levels of plasma IR-ANP found in heart failure. Atrial distension caused by a variety of stimuli, including volume loading, atrial tachycardia, and mitral obstruction, has been shown to stimulate ANP release. Other mechanisms, including neurohumoral factors such as catecholamines, vasopressin, and angiotensin-II may be involved directly in ANP release since preliminary studies have revealed that incubated atria secrete ANP under the influence of adrenaline and vasopressin. Although the direct effect of angiotensin-II on ANP release has not been confirmed, the possibility remains since angiotensin-II, adrenaline, and vasopressin share a common intracellular pathway. These observations could partially explain the mechanism of ANP release from the failing heart since atrial pressure, circulating catecholamine concentrations, vasopressin concentrations, and activity of the renin-angiotensin system are often increased in proportion to the severity of heart failure. Little is yet known of the pathophysiological significance of the high levels of plasma IR-ANP concentrations in heart failure and their relation to the sodium and water retention associated with the failing heart. It is somewhat paradoxical that plasma ANP levels are elevated in heart failure, as this is a condition associated with salt and water retention. However, it is possible that increased ANP levels represent a homeostatic response to increased volume and that this lessens the salt and water retention in heart failure. Possible mechanisms include not only its diuretic, natriuretic, and vasodilating action, but also reduction of aldosterone synthesis and suppression of vasopressin release. There is also a possibility of tachyphylaxis, and alteration of the effects of ANP on its target organs under pathophysiological conditions. Despite this apparent blunting of effect, it has been reported recently that synthetic ANP stimulates natriuresis without altering renal hemodynamics and causes an increase in coronary blood flow with a fall in coronary artery resistance in dogs with acute left ventricular failure. This indicates a possible therapeutic role for synthetic ANP or ANP analogues in the future treatment of human heart failure.

In conclusion: In rats with chronic heart failure secondary to healed myocardial infarction, chronic stimulation of ANP release is associated with depleted atrial ANP stores in proportion to the severity of heart failure. The results also suggest that plasma ANP levels may be used as a reliable index of cardiac decompensation in human heart failure, although the effects of treatment are as yet unknown. Further study is needed to elucidate the pathophysiological importance of ANP in heart failure.

Acknowledgments

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