Changes in Plasma Level of Alpha-Atrial Natriuretic Polypeptide (Alpha-ANP) and Responsiveness of the Aorta to Exogenous Alpha-ANP Subsequent to Myocardial Infarction in Rats

Yi-Ting WANG, Toshihiko UEMATSU, Ryuichi SATO
Yujiro HAYASHI and Mitsuyoshi NAKASHIMA
Department of Pharmacology, Hamamatsu University School of Medicine, Hamamatsu 431-31, Japan
Division of Clinical Pharmacology, Research Institute, Suntory Co., Ltd., Gunma 370-05, Japan

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Abstract—The changes in plasma level of alpha-atrial natriuretic polypeptide (alpha-ANP) and the relaxing responses to exogenous alpha-ANP of strips of rat aorta pretreated with methoxamine were examined at one, four and eight weeks after myocardial infarction induced by left coronary ligation. Responses to a beta-adrenergic stimulant, isoprenaline, and sodium nitroprusside of the vessel pretreated with high potassium were also evaluated up to twelve weeks. Plasma concentrations of immunoreactive alpha-ANP, which were measured at one, four and twelve weeks, were significantly elevated in rats with myocardial infarction (MI-rats) as compared with sham-operated rats (sham-rats). The relaxation responses of the aorta to exogenous alpha-ANP in MI-rats were significantly reduced at one and four weeks as compared with sham-rats. The difference was, however, less obvious at eight weeks. The responses to isoprenaline tended to be reduced from the 1st week to the 12th week, and the difference was significant at eight weeks, whereas those to methoxamine and sodium nitroprusside were unchanged. It is concluded that the MI-rats are partially resistant to the vasodilating effects of alpha-ANP and isoprenaline without any change in responses to the alpha-stimulant and sodium nitroprusside, although these changes are transient.

A number of peptides with potent natriuretic, diuretic and vasorelaxant properties have been recently isolated from human and animal hearts. Immunoreactive materials have also been found in the circulating blood of both humans and animals (1–4). Increased atrial pressure is considered to be a major stimulus for the release of atrial natriuretic peptide (ANP). Alpha-ANP, the most potent ANP, inhibits the contraction of isolated smooth muscle preparations (5, 6). The hypotensive effect of alpha-ANP was initially attributed to a reduction in peripheral resistance. In contrast, the recent studies in conscious animals have suggested that the fall in arterial pressure is primarily due to a reduction in venous return and consequently, reduction in cardiac output (7, 8). Therefore, the use of alpha-ANP is expected to improve the heart failure. There is also some evidence that the initial degree of vascular tone may determine whether ANP elicits a vasodilatory or even vasoconstrictor effect (9). Heart failure is a complex syndrome characterized by an inadequate tissue blood flow and some compensatory activations of various biological systems designated to maintain the tissue perfusion pressure (10). Plasma ANP is reported to be increased in patients (11–14) and animal models with heart failure (15–17). It has been shown that haemodynamic responses in rats with heart failure, which was experimentally induced, were partially resistant to exogenous alpha-ANP (18). However, the role of increased alpha-ANP is still unclear.
In this study, the relations between the elevated alpha-ANP levels in plasma and the responses of the aorta to exogenous alpha-ANP in rats with heart failure subsequent to left coronary ligation were examined.

Materials and Methods

Production of myocardial infarct in rats: Male Wistar rats, 8 weeks old and weighing 175–190 g, were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and myocardial infarction was produced by the method of Selye et al. (19) with a slight modification (20). In brief, the rats were intubated and ventilated by a positive-pressure respirator. The electrocardiogram (ECG) of lead II was continuously monitored on an ink-writing oscillograph. A left thoracotomy was performed at the fourth intercostal space to ligate the left coronary artery between the outflow tract of the pulmonary artery and the left auricle (MI-rats). Thereafter, the thorax was immediately closed. When the left coronary artery was successfully ligated, the ST-T complex of ECG was elevated, and ventricular arrhythmias were successively observed. It has been confirmed that the rat, which showed both of these ECG changes, exhibited a myocardial infarction of 27.3±0.92% (means±S.E.M., n=10) of the total ventricles at 24 hr after ligation in a separate series of experiments using a histochemical staining technique with nitrobluetetrazolium (Sigma). The sham-operated rats underwent the same surgical procedures without the coronary ligation. There was a mortality rate of 20–30% during the first week after the production of the myocardial infarct. Surviving rats were maintained under identical conditions in a 12 hr light/dark cycle and fed on standard rat chow and water ad libitum.

Plasma alpha-ANP: In a separate series of experiments, 1 ml of blood was drawn from the external jugular vein of the MI- and sham-operated rats under pentobarbital anesthesia at one and twelve weeks after the operations. The blood was transferred immediately into an ice-cold tube containing 0.5% EDTA and aprotinin (5000 U/ml). The separated plasma was stocked at −80°C until analyzed. Alpha-ANP was measured with the radio-immunoassay method described by Miyata et al. (21).

At four weeks after the operation, blood (2 ml) was drawn by puncturing the inferior vena cava, and plasma alpha-ANP concentration was measured by a different radio-immunoassay method at another institution (Special Reference Laboratory Co.).

Tissue preparation: MI-rats and sham-operated rats were stunned by a blow on the head and killed by bleeding at one, four, eight and twelve weeks after the operation. The thoracic aorta was rapidly excised and trimmed of excess fat and connective tissue. Then, the aorta was helically cut into 2×10 mm strips. The strips were suspended in a 10-ml organ bath containing Krebs solution of the following composition: 119 mM NaCl, 1.8 mM KCl, 1.17 mM KH2PO4, 2.5 mM MgSO4·7H2O, 1.6 mM CaCl2·6H2O, 25.0 mM NaHCO3 and 5.5 mM glucose. The solution was continually gassed with a mixture of 95% O2 and 5% CO2 (pH 7.4). The resting tension of the strip was adjusted to 1 g. The developed tension was recorded on a polygraph (6000 series, Nihon Kohden) with a force displacement transducer (TB–651T, Nihon Kohden). The bathing solution was changed every 15 min, and the strip was allowed to equilibrate for 1.5 hr. Following equilibration, the contraction was elicited by KCl (40 mM), two times. After the contraction reached its maximum, the bathing solution was changed three times in quick succession, and the bathing fluid was changed every 15 min for the following 60 min. The dose–response curve for methoxamine was obtained in a cumulative manner. The relaxant effect of alpha-ANP or sodium nitroprusside was studied in a strip pre-contracted with methoxamine (10−5 M). To study the relaxation induced by isoprenaline or sodium nitroprusside, the strip was pretreated with KCl (40 mM) in the presence of prazosin (10−7 M). All relaxing substances were added into the bathing solution cumulatively after the pre-contraction reached a plateau. Relaxation values were expressed as a percentage of the relaxation observed with a supramaximally effective concentration of papaverine (10−4 M), which was added at the end of the experiment.

Drugs used in the present study were: alpha-human ANP (Peptide Institute),
isoprenaline hydrochloride (Tokyo Kasei), methoxamine hydrochloride (Nippon Shin-yaku), papaverine hydrochloride (Wako Pure Chemical Industry), sodium nitroprusside (Sigma) and sodium pentobarbital (Tokyo Kasei).

Statistics: The numerical results were expressed as means±S.E.M. Statistical analyses were performed using Student’s t-test, and differences were considered significant when P<0.05. When the values were expressed as a percent of the maximal response, the values were transformed into arcsine, and then the t-test was performed.

**Results**

Body and heart weights: All MI-rats showed a marked thinning of the infarcted area where the muscle was replaced with fibrous connective tissue and signs of cardiac failure such as pulmonary congestion and thoracic effusion at the time of sacrifice. The changes in body weight following the operations are shown in Table 1. The mean body weights of MI-rats tended to be smaller than those of sham-operated rats at any occasion, though there was no significant difference between the groups. On the other hand, the mean heart weights of MI-rats were significantly larger than those of sham-operated rats at 8 weeks (Table 1). As a result, the ratios of heart weight to body weight of MI-rats showed significantly larger values than those of sham-operated rats at all observation times.

Plasma alpha-AN P concentration was clearly elevated in the MI-rats at all time-points examined when compared with the sham-operated rats. The mean concentrations in the sham-operated and MI-rats were as follows: 260.5±19.2 pg/ml vs. 517.7±86.0 pg/ml at 1 week (P<0.05), 350±209 vs. 2270±491 at 4 weeks (P<0.05), and 358.0±38.7 vs. 660.3±110.7 at 12 weeks (P<0.05).

Response to alpha-AN P: Alpha-AN P induced a concentration-dependent relaxation in the methoxamine-pretreated aorta of both sham-operated and MI-rats. However, the relaxant responses to alpha-AN P were attenuated in the MI-rats at 1 (Fig. 1, 1W) and 4 weeks (Fig. 1, 4W) after operation as compared with the sham-operated rats; significant differences were observed at the concentrations of 10⁻⁸ to 10⁻⁷ g/ml (1 week) and at the concentrations of 10⁻⁸ to 3x10⁻⁷ g/ml (4 weeks). The difference was not obvious at 8 weeks (Fig. 1, 8W).

Responses to isoprenaline: There were no significant differences in relaxant response of the aorta to isoprenaline at any concentrations from 1 to 4 weeks (Fig. 2, 1W, 4W). At 8 weeks after operation the responses of MI-rats were significantly attenuated at the concentrations of 10⁻⁸ to 10⁻⁵ M (Fig. 2, 8W). The significant difference was absent again at 12 weeks (Fig. 2, 12W).

Responses to methoxamine and sodium nitroprusside: Methoxamine and sodium nitroprusside exhibited concentration-dependent contraction and relaxation of the aorta, respectively. At any time after the

<table>
<thead>
<tr>
<th>Group</th>
<th>B.W. (g)</th>
<th>H.W. (g)</th>
<th>H/B (×10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1W Sham</td>
<td>210.0±9.3</td>
<td>0.55±0.02</td>
<td>2.63±0.09</td>
</tr>
<tr>
<td>MI</td>
<td>189.6±7.8</td>
<td>0.63±0.03</td>
<td>3.33±0.16**</td>
</tr>
<tr>
<td>4W Sham</td>
<td>277.0±9.2</td>
<td>0.77±0.05</td>
<td>2.74±0.07</td>
</tr>
<tr>
<td>MI</td>
<td>251.7±19.8</td>
<td>0.88±0.06</td>
<td>3.56±0.28*</td>
</tr>
<tr>
<td>8W Sham</td>
<td>315.0±6.2</td>
<td>0.64±0.03</td>
<td>2.68±0.01</td>
</tr>
<tr>
<td>MI</td>
<td>304.7±8.4</td>
<td>1.10±0.10*</td>
<td>3.62±0.36*</td>
</tr>
</tbody>
</table>

B.W.: Body weight, H.W.: Heart weight, H/B: Ratio of heart weight to body weight, Sham: Sham-operated rats, MI: Myocardial infarcted rats, 1W: One week, 4W: Four weeks, 8W: Eight weeks. Results represent means±S.E.M. of 6 experiments. *: P<0.05, **: P<0.01, as compared with the sham-operated rats.
Fig. 1. Cumulative concentration-response curves to alpha-ANP in sham-operated rats (○) and rats with myocardial infarct (●) at 1 week (1W), 4 weeks (4W) and 8 weeks (8W) after left coronary artery ligation. Results represent means±S.E.M. of 6 experiments. *P<0.05, **P<0.01, ***P<0.001, as compared with the sham-operated rats.

Fig. 2. Cumulative concentration-response curves to isoprenaline in sham-operated rats (○) and rats with myocardial infarct (●) at 1 week (1W), 4 weeks (4W), 8 weeks (8W) and 12 weeks (12W) after left coronary ligation. Results represent means±S.E.M. of 6 experiments. **P<0.01, ***P<0.001, as compared with the sham-operated rats.

operation, there were no significant differences in the concentration-response relations of the aorta for both methoxamine and sodium nitroprusside between the sham-operated and MI-rats when the aorta was pre-contracted with KCl (Table 2). There is also no significant difference in relaxant response of the aorta to sodium nitroprusside when the aorta was pre-contracted with methoxamine at any concentrations at 4 weeks (data not shown).

Discussion
The present study showed that plasma concentrations of alpha-ANP are clearly elevated in rats with cardiac failure subsequent to myocardial infarction and that the aorta excised from these rats is resistant to the relaxing effects of exogenous alpha-ANP and isoprenaline transiently in the time sequence after the occurrence of myocardial infarct. These results suggest that the sensitivity of aorta exposed to elevated plasma concentrations of alpha-ANP subsequent to myocardial infarct may be partly attenuated. Elevated plasma alpha-ANP was reported in rats with cardiac failure at 6 weeks after the ligation of coronary artery (18). In our study, the
Table 2. Maximum contraction, EC50 to methoxamine and maximum relaxation to nitroprusside in aorta of myocardial infarcted rats and sham-operated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Max. Cont. (g) to methoxamine</th>
<th>EC50 (×10^-6 M) to methoxamine</th>
<th>Max. Relax. (%) to nitroprusside</th>
</tr>
</thead>
<tbody>
<tr>
<td>1W Sham</td>
<td>0.75±0.06</td>
<td>1.3±0.9</td>
<td>94.0±1.9</td>
</tr>
<tr>
<td>MI</td>
<td>0.60±0.06</td>
<td>1.0±0.8</td>
<td>91.3±2.1</td>
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<tr>
<td>4W Sham</td>
<td>0.86±0.05</td>
<td>1.0±0.8</td>
<td>91.9±1.1</td>
</tr>
<tr>
<td>MI</td>
<td>0.82±0.07</td>
<td>0.7±0.9</td>
<td>90.2±2.3</td>
</tr>
<tr>
<td>8W Sham</td>
<td>0.90±0.01</td>
<td>1.5±0.8</td>
<td>92.7±1.3</td>
</tr>
<tr>
<td>MI</td>
<td>1.00±0.04</td>
<td>1.1±0.8</td>
<td>90.4±2.3</td>
</tr>
</tbody>
</table>

Max. Cont.: Maximum contraction, Max. Relax.: Maximum relaxation, Sham: Sham-operated rats, MI: Myocardial infarcted rats. 1W: One week, 4W: Four weeks, 8W: Eight weeks. Results represent means±S.E.M. of 6 experiments.

circulating plasma immunoreactive alpha-ANP was markedly elevated in the MI-rats at 1 and 4 weeks and marginally significant elevation was observed at 12 weeks after operation as compared with the sham-operated rats.

As reported (22), the aorta pretreated with high KCl was resistant to the relaxing effect of alpha-ANP in our preliminary experiment, and therefore we used methoxamine as the pretreatment drug. Exogenous alpha-ANP relaxed the aorta pre-contracted with methoxamine in a concentration-dependent manner. The relaxation was attenuated in the MI-rats at 1 and 4 weeks, as shown in Fig. 1. It has been reported that rats with experimentally induced myocardial infarction were partially resistant to the hypotensive effect of exogenous ANP (18) and an extensive myocardial infarct was associated with a significant decrease in receptor density for ANP in the renal medulla of rats at 4 weeks after the ligation of the coronary artery (23). These data suggest that the circulating ANP levels may be involved in the regulation mechanisms for the ANP binding sites in target tissues. The present study shows that the attenuation in the relaxation of aorta to exogenous alpha-ANP was almost negatively correlated with the degree of elevation in plasma alpha-ANP; i.e., the attenuation was more evident at 1 and 4 weeks, when the elevation of plasma alpha-ANP showed higher significance. We did not measure plasma ANP at 8 weeks, when the attenuation in relaxing response was not significant, but at 12 weeks, the elevation in plasma alpha-ANP was marginally significant. Recent in vitro observations by Hirata et al. (24) demonstrated that ANP receptors in cultured vascular smooth muscle cells are regulated downward by a prolonged exposure of the cells to ANP. It is widely accepted that circulating peptide hormones may directly regulate the numbers of receptors at target cells, and changes in the ambient concentrations of peptide hormones are associated with reciprocal changes in the density of their specific binding sites. These ideas are in good agreement with our results and may provide an insight into the mechanism of possibly attenuated responses to ANP in the state of heart failure.

The effect of ANP on vascular smooth muscles could be mimicked by sodium nitroprusside (6). Although the mechanisms through which ANP and sodium nitroprusside exert their vascular relaxations are not fully clarified, both agents increase the intracellular second messenger guanosine 3',5'-cyclic monophosphate (cGMP), which may lead to vascular relaxation. The degrees of relaxation induced by ANP and sodium nitroprusside were reported to correlate well with those in the elevation of intracellular cGMP through the activation of guanylate cyclase (6). The present results showing the decreased sensitivity of the aorta not to sodium nitroprusside but to alpha-ANP in the MI-rats also suggest that the mechanism involved in its effect may not be due to some alternation after the formation of cGMP.
Plasma concentrations of catecholamines exhibit continuous progressive increases as patients demonstrate continuous deterioration of left ventricular function (25). Enhanced activity of the sympathetic nervous system, which leads to elevated concentrations of circulating catecholamines and their increased urinary excretion in the post-infarct period (26–29), may reflect an important reserve mechanism for maintaining cardiac performance. However, elevated concentrations of circulating catecholamines do not necessarily prevent the development of heart failure (30, 31) and result even in a subsequent down-regulation for beta-adrenergic receptors, leading to their decreased density and loss of sensitivity to beta-adrenergic stimulation (32). In our study, the responsiveness of the aorta from MI-rats to isoprenaline was reduced as compared with the sham-operated rats at 8 weeks after coronary ligation, while that to methoxamine showed no significant change throughout the study. In heart failure, the decreased cardiac performance may be compensated for by an increase in vascular tone to maintain the arterial blood pressure and venous return. The attenuation of the response that occurs in the case of the beta-adrenergic, but not the alpha-adrenergic one, may serve this purpose, although the underlying mechanism needs to be clarified further.

In conclusion, our results support the idea that there should be a reduced sensitivity to high endogenous and exogenous alpha-ANP in heart failure (18). This may be partly due to the decreased vascular sensitivity to ANP, which is consistent with the recent report that there is a decreased density of alpha-ANP specific binding sites in the renal medulla of rats with chronic heart failure (23).

References


