Increases in Alpha- but Not Beta-Adrenoceptors in Hypertrophied Non-Infarcted Cardiac Muscles from Rats with Chronic Myocardial Infarction

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Abstract—The alteration in adrenoceptors in the hypertrophied right ventricle of the rats with myocardial infarction (MI) was examined. The density of alpha-1-adrenoceptors increased in four and twelve week old MI rats but not in one week old MI rats. The beta-adrenoceptors did not significantly change. It is probable that the increased density of alpha-adrenoceptors may be one of the causes for the increased responsiveness to the alpha-adrenergic agonist in the hypertrophied cardiac muscles of MI rats.

The changes in non-infarcted cardiac muscles after myocardial infarction (MI) in rats have been investigated by many morphologists and pathologists (1-4). The hypertrophy of non-infarcted muscles after coronary ligation in rats gradually progresses. However, the pharmacological alterations of non-infarcted muscles after MI, especially after chronic MI, have been examined by only a few investigators (5). We previously determined the responsiveness to alpha- and beta-adrenergic agonists of right ventricular muscles at one and four weeks after coronary ligation (5). The pD₂ value of the alpha-adrenergic agonist methoxamine increased at four weeks after the ligation, but those of the beta-adrenergic agonist dobutamine did not change. The alterations in adrenoceptors have been investigated in several models of cardiac hypertrophy (6, 7). In the previous study, the cardiac adrenoceptors in non-infarcted hypertrophied muscles were determined only at four weeks after the coronary ligation (5). Several authors have also examined the alteration of the cardiac beta-adrenoceptors in MI rats (8, 9). However, their results were inconsistent.

The reason for the discrepancy is unclear. These authors determined the adrenoceptors after different periods of MI, which may be one of the reasons. In the present study, we determined the alteration of the cardiac adrenoceptors in non-infarcted hypertrophied muscles at one, four and twelve weeks after the coronary ligation in rats.

Myocardial infarction was produced in male Wistar rats (200-220 g) by methods similar to those previously described (1, 10). Briefly, the rats were anesthetized with ether, followed by pentobarbitone (35 mg/kg), intubated and ventilated by a positive pressure respirator. The heart was exposed through an incision between the fourth to fifth, or fifth to sixth ribs on the left side. The left coronary artery was then ligated 1-2 mm from the origin with 5-0 nylon. The chest was closed, and the rat allowed to recover. There was a 20-30% mortality rate within the first 24 hr following this procedure. The sham-operated (SO) rats were treated quite similarly without the procedure of the coronary ligatation. Surviving rats were maintained under the same condition in a 12 hr light/dark cycle and given standard chow and water ad libitum.

At one, four and twelve weeks after the left coronary ligation or sham-operation, the body
weights were measured, and rats were killed by a blow on the head. The hearts were rapidly removed from the rats. After the atria were removed, the heart weights, i.e., the total ventricular weights, were measured. Then, the right ventricular free walls were excised and weighed for receptor binding assays. Because a large amount of protein was required for receptor binding assays, segments from two to five hearts were combined for the preparation of the membrane fraction. The right ventricular free walls were minced with a pair of scissors and homogenized in 10 to 20 times volume (W/V) of ice cold buffer (0.25 M sucrose, 5 mM Tris/HCl, 1 mM MgCl₂, pH=7.5) using a Brickman Polytron PT-10, setting of 6, for one 20-second period, followed by 10 strokes of a motor-driven teflon-glass homogenizer.

The homogenate was centrifuged at 1,000 x g for 10 min at 4°C. The supernatant was then centrifuged at 30,000 x g for 20 min at 4°C. The membrane pellet was washed with ice cold buffer (50 mM Tris/HCl, 10 mM MgCl₂, pH=7.5; buffer I) at a volume equal to 20 times the original wet weight and centrifuged at 30,000 x g for 20 min at 4°C. For the binding assay, the final pellet was resuspended in ice cold buffer I at a volume equal to 20 times the original wet weight.

Alpha-1- and beta-adrenoceptors were determined by a method similar to that previously described (11). For determination of alpha-1-adrenoceptors, membrane preparations (250 μl) were incubated with shaking for 20 min at 30°C with [3H]-prazosin (0.1-2.0 nM) in a total volume of 550 μl of buffer I containing 0.1 mg/ml bovine serum albumin. The incubations, which were performed in duplicate, were terminated by adding 4 ml of ice cold buffer I, and the radioactivities trapped on the filter disks were measured in an Aloka scintillation spectrometer (LSC-671) with a counting efficiency of 34%. Non-specific binding was defined as non-displaceable binding in the presence of 10 μM phentolamine, and the specific binding was defined as the difference between total and non-specific binding.

The binding assay of [3H]-dihydroalprenolol ([3H]-DHA) was carried out by the same procedure as that for [3H]-prazosin using [3H]-DHA (1.0-20 nM) instead of [3H]-prazosin, and non-specific binding of [3H]-DHA was defined as that in the presence of 10 μM of propranolol.

The protein concentration was measured by the method of Lowry et al. (12). The numerical results were expressed as the mean±S.E.M. Statistical analysis of these data was performed using Student's t-test, and a difference was considered significant when P<0.05.

Drugs used were [3H]-prazosin (80.9 Ci/mM), [3H]-dihydroalprenolol (54.8 Ci/mM) (New England Nuclear Corporation, Boston, MA), bovine serum albumin (Sigma), phentolamine hydrochloride (Ciba-Geigy) and propranolol hydrochloride (Sigma).

Table 1 shows the body weights and heart weights of MI and SO rats. The body weights of one, four and twelve week old MI and SO rats were not significantly different. The heart weights and right ventricular weights in one, four and twelve week old MI rats were significantly larger than those of SO rats. Thus, the ratios of heart weight/body weight in the three groups of the MI rats were significantly larger compared with those of SO rats. These ratios in MI rats increased time-dependently.

Table 1. Body, heart and ventricular weights in rats with myocardial infarction (MI) and sham-operated (SO) rats at one, four and twelve weeks after the operation.

<table>
<thead>
<tr>
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<th>1 week</th>
<th>4 weeks</th>
<th>12 weeks</th>
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<tbody>
<tr>
<td></td>
<td>SO</td>
<td>MI</td>
<td>SO</td>
</tr>
<tr>
<td>BW (g)</td>
<td>220±5</td>
<td>206±3</td>
<td>236±7</td>
</tr>
<tr>
<td>HW (mg)</td>
<td>608±25</td>
<td>783±30**</td>
<td>725±28</td>
</tr>
<tr>
<td>RVW (mg)</td>
<td>107±3</td>
<td>153±7**</td>
<td>125±6</td>
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<tr>
<td>HW/BW (%)</td>
<td>0.28±0.01</td>
<td>0.36±0.01**</td>
<td>0.30±0.02</td>
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Results are expressed as the mean±S.E.M. of eight animals. BW: body weight, HW: heart weight, RVW: right ventricular weight. **: P<0.01 vs. SO rats.
Table 2. Binding of [3H]-prazosin and [3H]-dihydroalprenolol to the right ventricle from sham-operated (SO) rats and rats with myocardial infarction (MI) at one, four or twelve weeks after the operation

<table>
<thead>
<tr>
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<th>1 week</th>
<th>4 weeks</th>
<th>12 weeks</th>
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<tbody>
<tr>
<td><strong>SO</strong> (fmol/mg protein)</td>
<td>B&lt;sub&gt;max&lt;/sub&gt;</td>
<td>K&lt;sub&gt;d&lt;/sub&gt;</td>
<td>B&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>62.9±3.0</td>
<td>0.28±0.02</td>
<td>33.2±3.2</td>
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<tr>
<td><strong>MI</strong></td>
<td>62.9±5.9</td>
<td>0.26±0.02</td>
<td>136.4±2.9</td>
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<th>12 weeks</th>
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<tr>
<td><strong>SO</strong> (fmol/mg protein)</td>
<td>B&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>68.5±1.7</td>
</tr>
<tr>
<td><strong>MI</strong></td>
<td>107.1±5.0**</td>
</tr>
</tbody>
</table>

Results are expressed as the mean±S.E.M. of six experiments. *, **: P<0.05 and 0.01 vs. SO rats, respectively.

Table 2 shows the results of the ligand binding at alpha-1 and beta-adrenoceptors in the right ventricle of MI and SO rats. The B<sub>max</sub> of [3H]-prazosin of the right ventricle in four and twelve week old MI rats significantly increased to a greater extent in the latter, but the B<sub>max</sub> in one week old MI rats was not significantly different from that of SO rats. The affinity for [3H]-prazosin expressed as a dissociation constant (K<sub>d</sub>) did not change in any of the MI rats. B<sub>max</sub> of [3H]-DHA showed a tendency to increase in MI rats, but these changes were not statistically significant. K<sub>d</sub> of [3H]-DHA also did not change in any of the MI rats.

In the present study, the right ventricular weights of MI rats gradually increased time-dependently. A similar change was also observed in the heart weight in MI rats. These results were consistent with our previous results (5). In the twelve week old MI rats, the increase in the heart weight (1.45 times of SO rats) was to a less extent than that in the right ventricular weight (2.52 times of SO rats), probably because the former contained the infarcted tissue which might not increase.

The B<sub>max</sub> of alpha-1-adrenoceptors increased in four and twelve week old MI rats to a larger extent in the latter rats, but in one week old MI rats, the B<sub>max</sub> did not increase. The result of four week old MI rats was consistent with the result in the previous study (5). In the previous study, the responsiveness to an alpha-agonist, methoxamine, of the papillary muscles of the right ventricle increased in four week old MI rats but not in one week old MI rats (5). Thus, it seems that the increased density of alpha-1-adrenoceptors at least partially contributes to the increased responsiveness to the alpha-agonist in the hypertrophied ventricular muscles in MI rats. The present results, however, do not deny the possibility that an alteration in some subcellular processes which are involved in the alpha-adrenoceptors-mediated inotropic effect may also contribute to the increased responsiveness to the alpha-agonist. The beta-adrenoceptors did not significantly change in one, four (5) and twelve week old MI rats, which is consistent with our previous result that the responsiveness to the beta-agonist dobutamine did not change in MI rats (5). Increases in the B<sub>max</sub> of alpha-adrenoceptors have been observed in several models of cardiac hypertrophy such as spontaneously hypertensive rats (6) and rats with aortic constriction (7). In hereditary cardiomyopathy of the Syrian hamster, the responsiveness to an alpha-, but not beta-agonist, increased (13). Thus, the increased density of alpha-adrenoceptors seems to be a common phenomenon in cardiac hypertrophy.

In one week old MI rats, the B<sub>max</sub> of alpha-1-adrenoceptors did not increase on the contrast to the significant hypertrophy of the
right ventricle. Although the stimulation of alpha-adrenoceptors is one of the causes of cardiac hypertrophy (14), other factors such as pressure- or volume-overload also cause the hypertrophy (1, 15). It seems that these factors in combination cause cardiac hypertrophy in MI rats.

In conclusion, the density of alpha-1-adrenoceptors increased in the hypertrophied non-infarcted cardiac muscles in the rats with myocardial infarction as observed in other models of cardiac hypertrophy. The increased density of alpha-1-adrenoceptors may partially contribute to the increased responsiveness to the alpha-agonist of the hypertrophied cardiac muscles of the MI rats.

References
1 Fishbein, M.C., MacLean, D. and Maroko, P.R.: Experimental myocardial infarction in the rat. Qualitative and quantitative changes during pathologic evolution. Am. J. Physiol. 90, 57–70 (1978)