Diminished Responsiveness to Cardiac $\beta_1$-Adrenoceptor Agonists in Rats with Chronic Heart Failure Following Myocardial Infarction

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The present study was undertaken to determine whether cardiac response to $\beta_1$-adrenergic agonists is altered in rats with chronic heart failure (CHF), and whether this alteration is related to $\beta$-adrenergic receptor down-regulation in the viable tissue of the left ventricle of these rats. For this purpose, the cardiac response to denopamine, a selective $\beta_1$-adrenergic agonist, and the change in cardiac $\beta$-adrenoceptor density were examined in rats with CHF. A non-selective $\beta$-adrenergic agonist, isoprenaline, was also examined as a comparison. Cardiac output and stroke volume indices were reduced 12 weeks after left coronary artery ligation, suggesting that CHF had developed at this time. Denopamine (2, 4, and 8 $\mu$g/kg i.v.), and isoprenaline (0.01 $\mu$g/kg i.v.) increased the cardiac output and stroke volume indices in sham-operated rats, whereas such increases were attenuated in the CHF rat. The cardiac $\beta$-adrenergic receptor density, measured by [H]CGP-12177 binding assay, was reduced in homogenates and microsomal membranes in the viable tissue of the left ventricle of the CHF rat (homogenates: 29% reduction, microsomal membrane: 23% reduction). These results suggest that the cardiac responsiveness to denopamine is diminished in the CHF rat and this alteration is accounted for, in part, by a decrease in cardiac $\beta$-adrenoceptor density.

Key words $\beta$-adrenergic receptor; cardiac output; chronic heart failure; denopamine; stroke volume

Heart failure is characterized by inadequate cardiac output during exercise or rest, which is accompanied by activation of compensatory mechanisms. One of these mechanisms is activation of the sympathetic nervous system.1-3 This activation results in an increase in circulating norepinephrines,4-9 which is considered to be a symptomatic and prognostic feature of chronic heart failure (CHF).7-9 Chronic exposure of cardiac muscles to high levels of circulating cateholamines, however, may blunt their responsiveness to exogenous $\beta$-adrenoceptor agonists and compromise the ability of endogenous cateholamines to support cardiac function.10-12 Such a decrease in cardiac $\beta$-adrenergic responsiveness may be, in part, due to change in the cardiac $\beta$-adrenergic receptor system.

In rat models of CHF, Cherg et al.13 have shown that inotropic responses of the uninfarcted myocardium to cateholamines are impaired despite the absence of $\beta$-receptor down-regulation. Yamamoto et al.14 also showed that there was no change in $\beta$-adrenergic receptor density and dissocitation constant in the ventricular septum. In contrast, Warner et al.15 have shown that the response of papillary muscle to isoprenaline was impaired and cardiac $\beta$-adrenergic receptor density was reduced. Thus, the loss of cardiac $\beta$-adrenergic receptors in rat CHF models remains controversial. The present study was undertaken to determine whether the cardiac response to a $\beta_1$-selective adrenoceptor agonist is reduced in CHF rats, and whether this alteration is related to $\beta$-adrenoceptor down-regulation in the viable tissue of the left ventricle of these rats. For this purpose, the effects of denopamine, a selective $\beta_1$-adrenoceptor agonist, on the cardiac output and $\beta$-adrenoceptor density of the myocardium were examined in sham-operated and CHF rats. For this purpose of comparison, the effects of isoprenaline, a non-selective adrenoceptor agonist, were also examined.

MATERIALS AND METHODS

Male Wistar rats (SLC, Shizuoka, Japan), weighing 200—260 g, were used in the present study. Before and during the experiments, they were fed standard rat chow and tap water ad libitum, housed in polyethylene cages, and maintained under a 12-h light/dark cycle according to the Guidelines of Experimental Animal Care issued by the Prime Minister's Office of Japan.

Preparation of Rats with CHF Myocardial infarction was induced in 22 rats by occlusion of the left coronary artery as described previously.16 Animals were anesthetized with ether and the skin was incised along the left sternal border and the fourth rib was cut proximal to the sternum. The pericardial sac was perforated and the heart was exteriorized through the intracostal space. The left coronary artery was ligated approximately 2 mm from its origin with a 5-0 silk suture. The heart was repositioned in the chest. During the operation, the rats were maintained under a positive-pressure ventilation. Approximately 34% of the rats died within 1 week of surgery. Fifteen sham-operated animals were treated similarly except for the coronary artery ligation. Hemodynamic and biochemical assessments were performed 12 weeks after the operation. In a previous study, we showed that rats with coronary artery ligation exhibited decreased cardiac output and stroke volume indices 8 and 12 weeks after the operation, but not at 2 and 4 weeks.10 These results indicate that CHF with low cardiac output is established 12 weeks after coronary artery ligation.

Measurements of Hemodynamic Parameters Hemodynamic parameters in the CHF and sham-operated animals were determined as described previously.16 Twelve weeks after the operation, the animal was anesthetized with a mixture of nitrous oxide—oxygen (3:1) containing 0.5—2.5% halothane. The animals were warmed by means of an electronic panel heater to maintain their body temp-

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perature at 36.5±0.5°C during the experiment. Under positive ventilation, right thoracotomy was performed and an electromagnetic flow meter with a diameter of 2 to 2.5 mm (model MFV-3100, Nihonkohden, Tokyo) was placed around the ascending aorta to measure aortic blood flow as described previously.16) Mean arterial pressure (MAP) was measured via a cannula inserted into the left femoral artery by means of a pressure transducer (model DX-360, Nihonkohden, Tokyo). Heart rate measurement was triggered by changes in systemic blood pressure (model AT-601G, Nihonkohden, Tokyo). After a 10 min equilibration period, the parameters were recorded on a thermal-pen recorder for 10 min (model RTA-1200, Nihonkohden, Tokyo). Cardiac output and stroke volume indices were calculated by dividing the aortic flow by the body weight and the cardiac output index by the heart rate, respectively. Systemic vascular resistance (SVR) was calculated by dividing the mean arterial pressure by the cardiac output index.17)

The Effects of the β-Adrenergic Receptor Agonists, Denopamine and Isoprenaline, on Hemodynamic Parameters After measurement of the baseline values of the hemodynamic parameters, the effects of the β-adrenoceptor agonists (denopamine and isoprenaline) were examined. Denopamine, at doses of 2, 4 and 8 μg/kg, and isoprenaline, at a dose of 0.01 μg/kg were administered intravenously through the cannula inserted into the right femoral vein. The drugs were dissolved in 0.9% NaCl to yield a concentration of 1 mg/ml and diluted to the desired concentrations with 0.9% NaCl. All drug solutions were freshly prepared. The injection volume of each drug was less than 0.2 ml per rat. The effects of these agents on aortic flow, mean arterial pressure and heart rate were recorded as described above. The peak values of these parameters were then determined. In a preliminary study, we performed blood gas analysis on the experimental animals using a blood gas analyzer (model 288 Blood Gas System, Ciba-Corning, Medfield, MA). The pH, pO2, and pCO2 of the animals were 7.41±0.02, 90.0±11.5 mmHg and 36.5±1.2 mmHg; respectively 5 min after starting the experiment and 7.36±0.03, 100.8±3.2 mmHg and 30.5±3.5 mmHg (n=6), respectively after 45 min. The results of the gas analyses indicate that the present experiment was carried out under stable conditions.

Measurements of Tissue Weight and Infarct Size After measurement of the aortic flow, the animals were sacrificed by intravenous administration of 1 ml 30 mM KCl, and divided into two groups. In one group, the heart and lungs were isolated, rinsed with ice-cold saline, blotted and weighed. In the other group, the heart was isolated and cut into 8 slices with a width of 1 mm from the base. The slices were stained with 0.0125% nitro blue tetrazolium chloride (NBT). The infarct and remaining left ventricular areas were determined by the method of Pfeffer et al.18)

Preparation of Samples for Receptor Binding Assay The experiment was designed to determine the specific binding capacity of [3H]CGP-12177 to cardiac β-adrenergic receptors of CHF and sham-operated animals according to the method of Gopalarishnan et al.19) with a minor modification. Several reports have shown that there is a significant reduction in total binding site densities (ca. 70—80%) when radioligand binding assays were performed using microsomal membranes prepared by the differential centrifugation method.20,21) Therefore, to minimize this and the potential artifact that may result from the preparation of membranes by the differential centrifugation method, we carried out the binding study using both homogenate and microsomal membrane preparations. Animals were sacrificed by decapitation, and the hearts quickly isolated and rinsed in ice-cold 50 mM tris(hydroxymethyl)aminomethane (Tris)–HCl buffer (pH 7.2). After the atria and connective tissue were removed, the heart was separated into 3 sections; scar tissue, the remaining left ventricle including the interventricular septum and the right ventricle. Their wet weights were determined. After weighing, the remaining left ventricle was homogenized in 10 vol/g wet weight of Tris–HCl buffer with two 15 s periods at the maximal speed with a Polytron homogenizer (PT-10, Kinematica, Switzerland). The homogenates were filtered through four layers of gauze and the filtrate was used for the radioligand receptor binding assay. In another experiment, assays were also performed using microsomal membrane fractions prepared by centrifuging the homogenate at 1100 x g for 20 min, followed by centrifugation of the supernatant at 4500 x g for 45 min. The final pellet was resuspended and used for the binding assay.

To obtain the total binding capacity, the homogenates (250—350 μg protein) or microsomal membranes (200—300 μg protein) were incubated in 0.5-ml buffer with 5 x 10^{-11} to 1 x 10^{-9} M [3H]CGP-12177 at 25°C for 60 min. The reaction mixture was filtered through a glass-fiber filter (GC-50, Advantec., Ltd., Japan) and washed twice with 2-ml volumes ice-cold buffer. After drying the filter paper, radioactivity was determined by liquid scintillation spectrometry (LSC-1000, Aloka, Japan) with an efficiency of 55—65%. The nonspecific binding capacity was determined in the presence of 10^{-6} M propranolol. The specific binding activity of CGP-12177 to cardiac homogenates was estimated by subtracting the nonspecific binding activity from the total binding activity. The binding parameters were determined by linear least-squares fitting using the method of Scatchard.22)

Protein and DNA Measurements Protein was determined by the method of Lowry et al.,23) using bovine serum albumin as a standard. DNA concentrations were measured by fluorescence spectroscopy using Hoechst dye 33258 with calf thymus DNA (sodium salt) type I as a standard.24)

Drugs [3H]CGP-12177 (specific activity, 1.96 TBq/mmoll) was obtained from Amersham Japan Co., Ltd. (Tokyo, Japan). 1-Isoprenaline hydrochloride was purchased from Sigma Chemical Company (St. Louis, U.S.A.) and denopamine was a gift from Tanabe Seiyaku Co., Ltd (Osaka, Japan).

Statistical Analysis Data are expressed as mean±S.E.M. Statistical significance was estimated by an unpaired Student’s t-test for the comparison of variables between the sham-operated and CHF groups.
Table 1. Tissue Weights in Rats with Chronic Heart Failure

<table>
<thead>
<tr>
<th>Conditions</th>
<th>BW (g)</th>
<th>LV (mg)</th>
<th>LV/BW (mg/g)</th>
<th>RV (mg)</th>
<th>RV/BW (mg/g)</th>
<th>Lung (mg)</th>
<th>Lung/BW (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>338 ± 4</td>
<td>720 ± 50</td>
<td>2.12 ± 0.14</td>
<td>148 ± 13</td>
<td>0.44 ± 0.04</td>
<td>1503 ± 95</td>
<td>4.46 ± 0.34</td>
</tr>
<tr>
<td>CHF</td>
<td>324 ± 9</td>
<td>707 ± 16</td>
<td>2.19 ± 0.09</td>
<td>349 ± 45*</td>
<td>1.07 ± 0.13*</td>
<td>3202 ± 188*</td>
<td>9.86 ± 0.43*</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 5 to 6 experiments. *Significantly different from the sham-operated group (p < 0.05). Abbreviations: BW, body weight; LV, left ventricular weight; RV, right ventricular weight; Lung, lung weight; CHF, chronic heart failure.

Table 2. Hemodynamic Parameters and Infarct Size, in Rats with Chronic Heart Failure

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Cardiac output index (ml/min/kg)</th>
<th>Stroke volume index (ml/beat/kg)</th>
<th>MAP (mmHg)</th>
<th>Heart rate (beats/min)</th>
<th>SVR (mmHg/ml/min/kg)</th>
<th>Infarct size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>188 ± 7</td>
<td>0.49 ± 0.02</td>
<td>105 ± 4</td>
<td>383 ± 13</td>
<td>0.56 ± 0.02</td>
<td>N.D.</td>
</tr>
<tr>
<td>CHF</td>
<td>126 ± 6*</td>
<td>0.36 ± 0.02*</td>
<td>90 ± 3*</td>
<td>351 ± 18</td>
<td>0.72 ± 0.02*</td>
<td>40 ± 3</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 5 experiments. *Significantly different from the sham-operated group (p < 0.05). Abbreviations: MAP, mean arterial pressure; SVR, systemic vascular resistance; CHF, chronic heart failure.

RESULTS

**Tissue Weight and Infarct Size** Changes in tissue weight of the CHF rats are shown in Table 1. The right ventricular weight, right ventricular weight/body weight ratio, lung weight and lung weight/body weight ratio of the CHF rats were higher than those of the sham-operated rats.

**Hemodynamic Parameters and Infarct Size in Rats with CHF** The hemodynamic parameters are shown in Table 2. The cardiac output and stroke volume indices and MAP were lower and the SVR was higher in rats with CHF than in sham-operated rats. There were no significant differences in heart rate between sham-operated and CHF rats. The infarct size of the CHF rats was about 40% of the left ventricle.

**Effects of β-Adrenoceptor Agonist on Hemodynamic Parameters** The effects of β-adrenoceptor agonists on hemodynamic parameters are shown in Figs. 1—3. Denopamine increased the cardiac output and stroke volume indices in a dose-dependent manner in sham-operated rats and an increase in those indices was also observed following isoprenaline. These responses were attenuated in CHF rats. Increases in MAP and heart rate and a decrease in SVR were observed following the administration of β-adrenoceptor agonists to sham-operated rats. These changes were relatively small in the CHF rats.

**Radioligand Binding Study** The β-adrenergic receptor density in the left ventricular homogenates and microsomal membranes of CHF rats are shown in Table 3. The specific [3H]CGP-12177 binding capacity to the ventricular homogenates ranged from 50 to 70% of the total binding in the homogenates, and from 70 to 80% of the total binding in the microsomal membranes of the left ventricle, and was independent of the degree of cardiac failure. The maximum [3H]CGP-12177 binding site densities in the left ventricular homogenates and microsomal membranes of CHF rats were reduced without any significant change in the equilibrium dissociation constant (Kd), when expressed per mg protein. The maximum specific binding site density of CHF rats remained low regardless of whether the density is calculated in terms of protein content, wet weight or DNA content.

**DISCUSSION**

The present study has shown that about a 40% degree of infarction of the left ventricle was induced 12 weeks after coronary artery ligation. Decreases in cardiac output and stroke volume indices, and an increase in SVR were observed 12 weeks after the operation. These findings indicate that CHF with low cardiac output was established 12 weeks after the operation.

In the present study, we measured cardiac output in the anesthetized rat to examine the effects of cardiotoxic agents.
Fig. 2. Effects of the β-Adrenergic Receptor Agonist, Denopamine, on Mean Arterial Pressure (MAP) (Upper Panel), Systemic Vascular Resistance (SVR) (Middle Panel) and Heart Rate (Lower Panel) in Sham-Operated Rats (Sham, □), and Rats with Chronic Heart Failure (CHF, ■) 12 Weeks after the Operation

The agents were administered intravenously through a cannula inserted into a femoral vein. Values represent the mean ± S.E.M. of 5 experiments.

on cardiac function. Because only approximately 40% of the left ventricle was infarcted in the CHF rats, cardiotonic agents could exert effects on the remaining viable myocardium. The decreased cardiac responsiveness might be due to a loss of normal myocardial mass in CHF rats. However, the left ventricular weight and left ventricular weight/body weight ratio in CHF rats was not reduced 12 weeks after the operation, despite the loss of myocardial mass. In the previous study, we observed that the left ventricular weight/body weight and the water content of the left ventricle, a marker of edema, were unchanged from the 1st to 12th week after operation.16 We also observed thinning of the left ventricular infarct-free wall. These finding appear to indicate that the remaining left ventricle are hypertrophied after acute myocardial infarction. Furthermore, we observed in the preliminary study that the response of cardiac output and stroke volume to β-adrenoceptor agonists in CHF rats 1 week after myocardial infarction was greater than 12 weeks after the operation, despite a similar sized infarct of the left ventricle (data not shown). Therefore, the loss of β-adrenergic responsiveness appears to be due to desensitization of the remaining myocardium.

We noted that the β-adrenergic receptor density, measured by [3H]CGP-12177 binding assay, was reduced in homogenates and microsomal membranes of the failing heart, which is consistent with the reports of other investigators.25,26 The exact mechanism underlying the decrease in the number of β-adrenergic receptors was not addressed in the present study. One possible explanation is that chronic exposure of the cardiac membrane to high levels of circulating catecholamines results in a β-adrenergic receptor down-regulation, as postulated by

Table 3. β-Adrenergic Receptor Density in Homogenates and Microsomal Membranes of the Remaining Left Ventricle in Rats with Chronic Heart Failure

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Homogenates</th>
<th>Sham</th>
<th>CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.15 ± 0.01</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>$K_d$ (nm)</td>
<td></td>
<td>19.07 ± 0.70</td>
<td>13.59 ± 1.12*</td>
</tr>
<tr>
<td>$B_{max}$ (pmol/mg protein)</td>
<td></td>
<td>1.91 ± 0.09</td>
<td>1.19 ± 0.14*</td>
</tr>
<tr>
<td>$B_{max}$ (fmol/µg DNA)</td>
<td></td>
<td>1.44 ± 0.15</td>
<td>0.85 ± 0.11*</td>
</tr>
<tr>
<td>Microsomal membranes</td>
<td></td>
<td>0.22 ± 0.05</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>$K_d$ (nm)</td>
<td></td>
<td>42.13 ± 2.24</td>
<td>32.62 ± 0.16*</td>
</tr>
<tr>
<td>$B_{max}$ (fmol/mg protein)</td>
<td></td>
<td>0.20 ± 0.03</td>
<td>0.25 ± 0.05</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 4 to 5 (homogenates) and 3 to 4 (microsomal membranes) experiments. * Significantly different from the sham-operated group (p < 0.05). The $B_{max}$ value is expressed as either units per mg protein, g wet tissue or µg DNA. Abbreviations: CHF, chronic heart failure; $B_{max}$, maximal binding capacity; $K_d$, dissociation constant.
several investigators.\cite{1-7} Such a decrease in cardiac β-adrenergic receptor density may blunt its responsiveness to exogenous β-adrenergic agonists and compromise the ability of endogenous catecholamines to support cardiac function.\cite{7,2,3} Consequently, cardiac β-adrenergic receptor down-regulation may account for the decreased positive inotropic response to β-adrenergic agonists.

It is likely that the reduction in cardiac β-adrenergic receptor density results in a decreased response to β-adrenergic agonists. However, as described in the introduction, Cherg\textit{ et al.}\cite{13} have shown that an impaired inotropic response of the uninfarcted myocardium to catecholamines is present in rats, despite the absence of β-receptor down-regulation 3 weeks after left coronary artery ligation. Yamamoto \textit{et al.}\cite{14} also showed no change in β-adrenergic receptor density and dissipation constant in the ventricular septum of rats 2 weeks after operation. In contrast, Warner \textit{et al.}\cite{15} have shown that the response of papillary muscle to isoprenaline was impaired and cardiac β-adrenergic receptor density was decreased in rats 9 weeks after coronary artery ligation. The differences between these observations may be attributed to the examination period of post-myocardial infarction. In the present study, the reduction in β-adrenergic receptor density of the remaining left ventricle was observed in rats 12 weeks after the operation, and cardiac output and stroke volume indices were reduced. We have shown in a previous study\cite{16} that no decrease in cardiac output was observed 4 weeks after the operation. Thus, the down-regulation of β-adrenergic receptors in the left ventricle may occur at a later stage in the development of CHF. We did not observe the decrease in cardiac β-adrenergic receptor density in the CHF rats 1 week after coronary artery ligation in the preliminary study (data not shown). This finding supports our above hypothesis.

The decrease in cardiac β-adrenergic receptor density was relatively small compared with the decrease in cardiac response to β-adrenergic agonist. Thus, other mechanisms may also be involved in the impairment of the β-adrenergic receptor pathway in the failing heart. Ungerer \textit{et al.}\cite{28} reported that, in the failing heart, the function of the remaining receptors was impaired due to phosphorylation by β-adrenergic receptor kinase. In addition to biochemical changes at the receptor site, Gi proteins have been reported to be increased in the failing human heart.\cite{8,29,30} Increased Gi-mediated inhibition may compromise the ability of the failing heart to generate sufficient amounts of cAMP. Such changes may play a role in the decreased responsiveness to catecholamines in the failing heart.

In the present study, we observed that the right ventricular hypertrophy in CHF rats occurred in association with left ventricular hypertrophy. In the previous study, we have shown that the increase in left ventricular end-diastolic pressure in CHF rats 12 weeks after the operation.\cite{16} The increase in left ventricular end-diastolic pressure may lead to pulmonary hypertension in this animals. Such pulmonary hypertension may result in the development of right ventricular hypertrophy.

In conclusion, the cardiac response to the β\textsubscript{1}-selective adrenergic receptor stimulated dopamine was reduced in the CHF animals. This finding suggests that the β-adrenergic system is severely impaired in the failing heart. This change is, at least in part, due to cardiac β-adrenergic down-regulation in the viable myocardium.

**REFERENCES**