Abnormality in Plasma Catecholamines and Myocardial Adrenoceptors in Cardiomyopathic BIO 53.58 Syrian Hamsters and Improvement by Metoprolol Treatment

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ABSTRACT

The catecholaminergic neuronal activity and the densities of alpha-1 and beta adrenoceptors and angiotensin II receptors were simultaneously determined in BIO 53.58, a model of idiopathic dilated cardiomyopathy, and F1B control hamsters. Further, we examined the effect of repeated p.o. administration of metoprolol on these biochemical parameters. Compared with F1B control hamsters, there was a significant decrease in $B_{\text{max}}$ of specific binding of both (−)$[^{125}\text{I}]$iodocyanopindolol and $[^{3}\text{H}]$prazosin with a marked elevation of plasma catecholamine (mainly norepinephrine and epinephrine) concentrations, in BIO 53.58 hamsters at 11 and 18 weeks of age (severe cardiomyopathic stage), but not at 5 weeks of age. On the other hand, the $B_{\text{max}}$ value of myocardial $[^{125}\text{I}]$angiotensin II binding in BIO 53.58 hamsters was almost identical to that in F1B hamsters. These results suggest a development of down-regulation of myocardial beta and alpha-1 adrenoceptors because of an increased catecholaminergic neuronal activity with aging in BIO 53.58 hamsters. Repeated p.o. administration of a relatively low dose (1 mg/kg/day) of metoprolol for 7 weeks in 11-week-old BIO 53.58 hamsters caused a significant increase of myocardial (−)$[^{125}\text{I}]$iodocyanopindolol binding sites with a marked reduction in plasma catecholamine levels; this indicated a significant recovery to the F1B levels. The improvement of these biochemical parameters by metoprolol treatment was also accompanied by a significant decrease in the fibrosis in the heart in BIO 53.58 hamsters. These data suggest that catecholaminergic neurons and adrenoceptors play a part in the development of heart failure in idiopathic dilated cardiomyopathy. Consequently, the present study may provide a further pharmacological basis for the use of beta-1 adrenergic antagonists in patients with idiopathic dilated cardiomyopathy.

DCM is a disease of heart muscle characterized by left ventricular dilation and congestive heart failure. It has been previously shown that plasma norepinephrine levels in patients suffering from chronic heart failure that includes DCM are elevated and that this increase is apparently related to the severity of the disease (Chidsey et al., 1962; Thomas and Marks, 1978; Francis et al., 1982; Levine et al., 1982). Furthermore, in myocardial tissues obtained from patients with DCM undergoing heart transplantation, there is a marked loss of beta adrenoceptors in atrial and ventricular membranes (Bristow et al., 1982; Brodde et al., 1986, 1989; Böhm et al., 1988; Brodde, 1991; Steinfath et al., 1991). Several clinical studies have indicated beneficial effects of treatment with the beta-1 adrenoceptor antagonist metoprolol in DCM (Wangstein et al., 1983, 1989; Fowler and Bristow, 1985; Ishida et al., 1993; Yamada et al., 1996), although its mechanism of action has been not fully elucidated.

The BIO 14.6 strain of cardiomyopathic golden Syrian hamsters is a well-studied animal model of congestive heart failure that develops the following characteristic pathological changes: cardiac myolysis at 30 to 40 days of age, cardiac hypertrophy at approximately 150 days of age, cardiac dilation at approximately 250 days of age and frank congestive failure at approximately 1 year of age (Gertz, 1972). The biochemical changes in hearts of BIO 14.6 hamsters were previously examined by a number of investigators (Wagner et al., 1986; Kessler et al., 1989; Tawarahara et al., 1992; Watanabe et al., 1993). Unlike BIO 14.6 hamsters, BIO 53.58 hamsters do not develop myolysis or hypertrophy before dilation (Whitmer, 1987), and they have a significantly shorter life span and demonstrate reduced cardiac function at an earlier age than BIO 14.6 hamsters (Whitmer et al., 1988). Therefore, BIO 53.58 hamsters provide a model of cardiac dilation that contrasts with the hypertrophic model of BIO 14.6 strain.

ABBREVIATIONS: DCM, idiopathic dilated cardiomyopathy; CYP, cyanopindolol; AII, angiotensin II; $K_{d}$, apparent dissociation constant.
Some biochemical abnormalities occur in the myocardium of BIO 53.58 hamsters. Feldman et al. (1990) have reported that 100-day-old BIO 53.58 hamsters exerted a diminished contractile response to beta adrenoceptor stimulation with little change in myocardial beta adrenoceptor density. Consequently, it has been suggested that a substantial decrease in the guanine nucleotide-binding regulatory protein (Gα) bioactivity in hearts of BIO 53.58 hamsters contributes both to the diminished beta adrenoceptor-adenyl cyclase coupling and to the decreased hemodynamic responsiveness to beta adrenoceptor stimulation in hearts of these hamsters (Feldman et al., 1990; Tomita et al., 1994). Further, Kagawuchi et al. (1991, 1992) have shown that a prolonged high intracellular calcium level may lead to the death of myocytes in BIO 53.58 hamsters. However, as far as we know, little systematic information is available about the developmental change in catecholaminergic neuronal activity, including adrenoceptor density, in BIO 53.58 hamster hearts. Thus the purpose of this study was to determine whether the catecholaminergic neuronal activity and the densities of alpha-1, beta and AII receptors are altered with aging in hearts of BIO 53.58 hamsters compared with F1B control hamsters and further to examine the effect of repeated p.o. administration of metoprolol on these biochemical parameters in cardiomyopathic hamsters. Simultaneously, we also examined the pathological changes in hearts of these hamsters.

Materials and Methods

Animals. Male golden Syrian hamsters were obtained from Bio Breeders (Fitchburg, MA). Two genetically defined strains at 5, 11 and 18 weeks of age were studied: the cardiomyopathic BIO 53.58 hamsters and the normal control (F1B). The hamsters were allowed free access to food and water. After animals were anesthetized with pentobarbital (40 mg/kg i.p.), the blood was collected into tubes preloaded with EDTA from the descending aorta, and the hearts were perfused with 0.9% saline and excised. The hearts were processed for histopathological and biochemical studies. Plasma was separated by centrifugation. The plasma samples and a portion of the myocardial tissues were stored at −80°C. In the experiment with metoprolol treatment, 11-week-old BIO 53.58 hamsters received repeated p.o. administration of metoprolol at doses of 1, 10 and 50 mg/kg/day in the drinking water for 7 weeks.

Histopathological examination of myocardium. Hearts were processed for histopathological studies and quantitatively assessed as previously described (Wagner et al., 1989). Briefly, after fixation by immersion in a 10% phosphate-buffered formaldehyde solution for 24 hr, the hearts were embedded in paraffin. The tissues were cut into serial sections 5 μm thick with a sliding microtome (HM 400, Microm, Heiderberg). The serial sections were stained with hematoxylin and eosin, with Masson’s trichrome for connective tissue and by the Von Kossa method for calcium deposits. The areas of necrosis, calcium deposits, left ventricular cavity and fibrosis were quantitatively determined using a light microscope and an image analyzer (SP 500, Olympus, Tokyo).

Determination of plasma and myocardial catecholamines. The concentrations of norepinephrine, epinephrine and dopamine in plasma and myocardial tissues were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ECD), as previously described (Hansson et al., 1979; Hegestrand and Eichelman, 1981; Taylor et al., 1983). Briefly, the plasma protein was precipitated with trichloroacetic acid, and catecholamines were absorbed with alumina. To elute myocardial catecholamines, tissue protein was precipitated with perchloric acid, and catecholamines were absorbed on alumina. After elution with hydrochloric acid, concentrations of norepinephrine, epinephrine and dopamine in plasma and myocardial tissues were determined using HPLC with electrochemical detection. The HPLC system was constructed with a pump (655A-11, Hitachi) and an electrochemical detector (Model 5100A, ESA). The analysis was performed on the column: Nucleosil 7 C18(30 cm × 4.6 mm I.D.). The mobile phase for assay consisted of 0.1 M NaHPO 4 buffer (pH 4.0), 12.5% methanol, 0.01% EDTA-2Na and 1.25 mM SO 4 at a flow rate of 1.0 ml/min.

Measurements of alpha-1, beta and AII receptors. Ventricular myocardium was homogenized with a Polytron in 20 mM NaH 2 PO 4 buffer. After centrifugation of the homogenate at 40,000 × g for 20 min at 4°C, the resulting pellet was resuspended in 20 mM NaH 2 PO 4 buffer and recentrifuged. The final pellets were resuspended in assay buffer. The densities of alpha, beta and AII receptors in myocardial homogenates from F1B and BIO 53.58 hamsters were measured using [3H]prazosin, (−)[125I]CYP and [125I]AII, respectively, as previously described (Yamada et al., 1992, 1996; Nozawa et al., 1994). Briefly, myocardial homogenates (70–300 μg protein) were incubated with varying concentrations of [3H]prazosin (0.03–1 nM) for 30 min at 25°C in 50 mM Tris-HCl buffer (pH 7.5), of (−)[125I]CYP (3–140 pM) for 60 min at 37°C in 10 mM Tris-HCl buffer (containing 150 mM NaCl and 1 mM ascorbic acid, pH 7.2) and of [125I]AII (0.1–3.5 nM) for 60 min at 22°C in phosphate buffer (containing 50 mM NaH 2 PO 4, 100 mM NaCl, 10 mM MgCl 2, 1 mM EGTA and 0.2% BSA, pH 7.2). Incubation was terminated by rapid filtration over Whatman filters (GF/B or GF/C). Filters were washed with an additional 10 ml of ice-cold buffer, and the radioactivity of the filters in (−)[125I]CYP and [125I]AII assays was determined by a gamma-counter (Beckman Gamma 4000) at an efficiency of 70%. Tissue-bound radioactivity in the [3H]prazosin assay was extracted from the filters overnight in 5 ml of scintillation fluid (2 liters of toluene, 1 liter of Triton X-100, 15 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis-[2-(5-phenyloxazolyl)-benzene], and the radioactivity was determined by a liquid scintillation counter. Specific binding of each radioligand was defined experimentally as the difference between counts in the absence and presence of the following drugs: 10 μM phentolamine in the [3H]prazosin assay, 1 μM (−)-propranolol in the (−)[125I]CYP assay and 1 μM AII in the [125I]AII assay. All assays were conducted in duplicate. Every binding experiment was performed using fresh tissues. The protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Analysis of data. The analysis of binding data was performed as described previously (Yamada et al., 1980a). The K B and B max, values for [3H]prazosin, (−)[125I]CYP and [125I]AII were estimated by Rosenthal analysis of the saturation data (Rosenthal, 1967). Statistical analysis of data was performed with Welch’s t test and with one-way analysis of variance followed by Dunnett’s test for single and multiple comparisons, respectively.

Materials. [3H]Prazosin (2752.8 GBq/mmol), (−)[125I]CYP(81.4 TBq/mmol) and [125I]AII (Angiotensin II, Sar 1, [125I] Tyr 6, Ile 6, 8.4 TBq/mmol) were purchased from DuPont-NEN Co. Ltd (Boston, MA). Metoprolol tartrate was kindly donated by Fujisawa Pharmaceutical Co. (Ohsaka, Japan). Phentolamine hydrochloride, propranolol hydrochloride and AII were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were obtained from commercial sources.

Results

Pathological changes. The hearts from 18-week-old BIO 53.58 hamsters were substantially dilated when compared with the normal F1B controls of similar age. Representative sections from these hearts are shown in figure 1. The cavity area was significantly (P < .05) greater in left ventricles from BIO 53.58 hamsters (24.6 ± 3.5%, n = 6) than in the normal F1B controls (13.3 ± 1.7%, n = 5). The BIO 53.58 hamster
hearts also had largely focal lesions with calcification, necrosis and fibrosis, as demonstrated by significantly higher percentages of calcium deposits (2.36 ± 0.59% vs. 0.02 ± 0.01%, P < .05, n = 5–6), necrosis (6.30 ± 1.27% vs. 0.57 ± 0.25%, P < .01, n = 5–6) and fibrosis (16.2 ± 3.9% vs. 0.75 ± 0.20%, P < .05, n = 5–6) in the left ventricles of cardiomyopathic hamsters than in F1B controls.

The ratios of left ventricular weight to body weight in 18-week-old BIO 53.58 hamsters were not different from those of age-matched F1B controls (BIO 53.58 = 0.217 ± 0.004 mg/g, F1B = 0.231 ± 0.011 mg/g, n = 5).

Changes in catecholamine levels in plasma and myocardial tissues. There was a significantly higher level of norepinephrine and/or epinephrine in plasma of 11- and 18-week-old BIO 53.58 hamsters compared with age-matched F1B controls, but there was little difference between these hamsters in the plasma level of both catecholamines at 5 weeks of age (fig. 2A). The levels of norepinephrine and epinephrine in plasma of 18-week-old BIO 53.58 hamsters were twice those of age-matched F1B controls. The plasma concentration of dopamine was much lower than that of norepinephrine or epinephrine in these hamsters. There was a significantly lower level of dopamine in 11-week-old BIO 53.58 than in age-matched F1B controls.

As shown in figure 2B, the concentrations of norepinephrine, epinephrine and dopamine in myocardial tissues of 5- and 11-week-old BIO 53.58 hamsters were similar to those in age-matched F1B controls. In myocardial tissues of 18-week-old BIO 53.58 hamsters, the concentration of norepinephrine was significantly (44%) lower than that in age-matched F1B controls, with little significant change in the levels of epinephrine and dopamine.

Changes in myocardial alpha-1, beta and AII receptors. The Kd and Bmax values of specific (-)-[125I]CYP binding in myocardial tissues of 5-week-old BIO 53.58 hamsters were similar to those of age-matched F1B controls, and the Bmax values in 11- and 18-week-old cardiomyopathic hamsters were significantly decreased by 27% and 29%, respectively (table 1). Similarly, the Bmax values of myocardial [3H]prazosin binding in BIO 53.58 hamsters of both ages were significantly (26% and 30%, respectively) lower than those of age-matched F1B controls. On the other hand, the Kd values for both radioligands were not significantly different between these hamster strains at any age.

The Kd and Bmax values of myocardial [125I]AII binding in
were close to the values (2.1 (control) value, * P
a significant difference from the vehicle-treated BIO 53.58 hamsters
a percentage of the total area of the left ventricle. An asterisk indicates
Kossa. Each column represents the average
were stained with hematoxylin-eosin, Masson’s trichrome and Von
for 7 weeks. ** P < .01, *** P < .001, significantly different from the value for F1B hamsters.
Effects of repeated p.o. administration of metoprolol. The histopathological examination of hearts from 18-week-old BIO 53.58 hamsters treated p.o. with metoprolol (1, 10 and 50 mg/kg) for 7 weeks showed a tendency toward reduction of necrosis, calcium deposition and fibrosis, compared with those in hearts of control (vehicle-treated) age-matched BIO 53.58 hamsters.
The cavity area, calcium (Ca) deposition, necrosis and fibrosis were
18-week-old BIO 53.58 hamsters were 1.5 ± 0.1 nM and
23.9 ± 2.2 fmol/mg protein, respectively (n = 5), and they were close to the values (2.1 ± 0.2 nM and 26.8 ± 2.1 fmol/mg protein, respectively, n = 5) in age-matched F1B control hearts.

** TABLE 1 **
Kd and Bmax values for specific binding of (−)[125I]CYP and [3H]prazosin in myocardial homogenates of F1B and BIO 53.58 hamsters
Rosenthal analysis of specific binding of (−)[125I]CYP and [3H]prazosin in myocardial homogenates of hamsters was performed. Each value represents the mean ± S.E. of seven determinations.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Strain</th>
<th>Specific (−)[125I]CYP Binding</th>
<th></th>
<th>Specific [3H]prazosin Binding</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kd (pM)</td>
<td>Bmax (fmol/mg/protein)</td>
<td>Kd (pM)</td>
<td>Bmax (fmol/mg/protein)</td>
</tr>
<tr>
<td>5</td>
<td>F1B</td>
<td>25.9 ± 1.5</td>
<td>35.7 ± 2.5</td>
<td>62.8 ± 8.3</td>
<td>32.8 ± 3.6</td>
</tr>
<tr>
<td>11</td>
<td>BIO 53.58</td>
<td>30.3 ± 2.8</td>
<td>30.7 ± 4.7</td>
<td>55.6 ± 5.8</td>
<td>25.4 ± 2.6</td>
</tr>
<tr>
<td>18</td>
<td>F1B</td>
<td>12.8 ± 0.8</td>
<td>47.4 ± 3.3</td>
<td>25.7 ± 2.9</td>
<td>27.6 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>BIO 53.58</td>
<td>13.5 ± 1.1</td>
<td>34.6 ± 2.1**</td>
<td>28.2 ± 2.8</td>
<td>20.5 ± 1.1**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.0 ± 1.6</td>
<td>40.8 ± 1.5</td>
<td>38.2 ± 3.0</td>
<td>22.2 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.0 ± 1.3</td>
<td>28.8 ± 2.1***</td>
<td>38.9 ± 3.2</td>
<td>16.3 ± 0.9**</td>
</tr>
</tbody>
</table>

* P < .05, ** P < .01, *** P < .001, significantly different from the value for F1B hamsters.

18-week-old BIO 53.58 hamsters were 1.5 ± 0.1 nM and 23.9 ± 2.2 fmol/mg protein, respectively, and they were close to the values (2.1 ± 0.2 nM and 26.8 ± 2.1 fmol/mg protein, respectively, in age-matched F1B control hearts.

The histopathological examination of hearts from 18-week-old BIO 53.58 hamsters treated p.o. with metoprolol (1, 10 and 50 mg/kg) for 7 weeks showed a tendency toward reduction of necrosis, calcium deposition and fibrosis, compared with those in hearts of control (vehicle-treated) age-matched BIO 53.58 hamsters. The decrease in fibrosis by metoprolol at the dose of 1 mg/kg was statistically significant, but the reduction by higher doses of this drug (10 and 50 mg/kg) was not.

The plasma concentrations of norepinephrine, epinephrine and/or dopamine in BIO 53.58 hamsters were significantly decreased by the repeated p.o. administration of metoprolol at the dose of 1 or 10 mg/kg, although little further increase in the Bmax value in the treated cardiomyopathic hearts became close to that in the control F1B (table 3). The decrease in fibrosis by metoprolol at the dose of 1 mg/kg was statistically significant, but the reduction by higher doses of this drug (10 and 50 mg/kg) was not.

The plasma concentrations of norepinephrine, epinephrine and/or dopamine in BIO 53.58 hamsters were significantly decreased by the repeated p.o. administration of metoprolol at the dose of 1 or 10 mg/kg (table 2), and the catecholamine levels in the metoprolol-treated BIO 53.58 were similar to those in the age-matched (18-week-old) F1B controls (fig. 2A). Conversely, the concentrations of norepinephrine, epinephrine and/or dopamine in myocardial tissues of BIO 53.58 hamsters were significantly increased by the repeated p.o. administration of metoprolol at the dose of 1 or 10 mg/kg. Similarly, a higher dose (50 mg/kg) of metoprolol exerted, on the concentration of plasma dopamine and myocardial epinephrine, a significant effect that was similar to or less than the effects exerted by the lower doses of this drug. Thus, the repeated administration of a relatively low dose of metoprolol in cardiomyopathic hamsters effectively restored the plasma and myocardial catecholamines to the control F1B levels.

Repeated p.o. administration of metoprolol at the dose of 1 mg/kg in BIO 53.58 hamsters for 7 weeks increased significantly the Bmax for myocardial (−)[125I]CYP binding when compared with that of control cardiomyopathic hamsters (table 3); that is, the Bmax value in the treated cardiomyopathic hearts became close to that in the control F1B (table 1). The significant enhancement of myocardial (−)[125I]CYP binding sites was brought about by similar administration of metoprolol at the dose of 50 mg/kg, although little further increase in the Bmax value occurred. The Kd value for (−)[125I]CYP in myocardial tissues was little altered by the metoprolol treatment. In metoprolol-treated BIO 53.58 hamsters, there was little significant change in myocardial [3H]prazosin binding (Kd and Bmax values).

** Discussion **
BIO 53.58 hamsters at 18 weeks of age, compared with age-matched F1B control hamsters, exhibited an extreme dilation of the left ventricular cavity and thinning of the left ventricular wall, as previously reported (Feldman et al., 1990). Furthermore, these hearts had focal lesions with significantly higher percentages of calcification, necrosis and fibrosis. Previous studies have shown that plasma norepinephrine levels in patients suffering from chronic heart failure that includes DCM are elevated and that this increase is apparently related to the severity of the disease (Chidsey et al., 1962; Thomas and Marks, 1978; Francis et al., 1982; Levine et al., 1982). In addition, it has been suggested that chronic heart failure in patients with DCM is associated with a marked loss of myocardial beta adrenoreceptors and with subsensitivity to beta adrenergic stimulation in vitro and in vivo studies (Bristow et al., 1982; Brodde et al., 1986, 1989; Fowler et al., 1986; Böh m et al., 1988; Brodde, 1991; Stein fath et al., 1991). In accordance with these observations in the human, in 18-week-old BIO 53.58 hamsters at severe cardiomyopathic stage, there was a significant reduction in myocardial (−)[125I]CYP binding sites (Bmax) with a marked...
TABLE 2

Effect of repeated administration of metoprolol on concentration of norepinephrine, epinephrine and dopamine in plasma and myocardial tissues of BIO 53.58 hamsters

Metoprolol at doses of 1, 10 and 50 mg/kg/day was administered in the drinking water to BIO 53.58 hamsters for 7 weeks. The concentrations were measured by HPLC. Each value represents the mean ± S.E. of 6 to 7 determinations.

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>Plasma</th>
<th>Myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine (ng/ml or µg/g)</td>
<td>Epinephrine (ng/ml or µg/g)</td>
<td>Dopamine (ng/ml or µg/g)</td>
</tr>
<tr>
<td>Control</td>
<td>6.02 ± 1.03</td>
<td>9.57 ± 1.54</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>3.39 ± 0.65*</td>
<td>5.65 ± 1.39</td>
</tr>
<tr>
<td>10</td>
<td>2.14 ± 0.28**</td>
<td>4.03 ± 0.90*</td>
</tr>
<tr>
<td>50</td>
<td>4.11 ± 0.70</td>
<td>6.47 ± 0.93</td>
</tr>
</tbody>
</table>

* P < .05, ** P < .01, *** P < .001, significantly different from the value for the vehicle-treated BIO 53.58 hamsters (control).

TABLE 3

Effects of repeated administration of metoprolol on $K_d$ and $B_{max}$ values for specific (-)-[125I]CYP and [3H]prazosin binding in myocardial homogenates of BIO 53.58 hamsters

Metoprolol at doses of 1, 10 and 50 mg/kg/day was administered in the drinking water to BIO 53.58 hamsters for 7 weeks. Rosenthal analysis of specific (-)-[125I]CYP and [3H]prazosin binding in myocardial homogenates of hamsters was performed. Each value represents the mean ± S.E. of 6 to 7 determinations.

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>Specific (-)-[125I]CYP Binding</th>
<th>Specific [3H]prazosin Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_d$ (pM)</td>
<td>$B_{max}$ (fmol/mg protein)</td>
</tr>
<tr>
<td>Control</td>
<td>15.0 ± 1.3</td>
<td>28.8 ± 2.1</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>16.8 ± 1.5</td>
<td>40.0 ± 2.0**</td>
</tr>
<tr>
<td>10</td>
<td>15.5 ± 1.5</td>
<td>36.0 ± 3.4</td>
</tr>
<tr>
<td>50</td>
<td>16.9 ± 1.4</td>
<td>36.8 ± 1.7*</td>
</tr>
</tbody>
</table>

* P < .05, ** P < .01, significantly different from value for the vehicle-treated BIO 53.58 hamsters (control).

Elevation in plasma catecholamine (mainly norepinephrine and epinephrine) concentrations. This was accompanied by a significant decrease in myocardial norepinephrine levels of these hamsters. Essentially similar alterations in myocardial (-)-[125I]CYP binding sites and in plasma catecholamine concentrations were also elicited in 11-week-old BIO 53.58 hamsters, whereas little significant differences in these biochemical parameters were observed between F1B and BIO 53.58 hamsters at 5 weeks of age. These data provide biochemical evidence for a developmental decrease in myocardial beta adrenoceptor densities, accompanied by a concomitant increase in catecholaminergic neuronal activity, in BIO 53.58 hamsters. Taken together, they suggest that the lowered densities of myocardial adrenoceptors in BIO 53.58 hamsters can be attributed to the chronic exposure of receptors to a high concentration of catecholamines subsequent to an increased activity of catecholaminergic neurons, reflecting a physiological down-regulation of adrenoceptors. Inasmuch as there was a significant decrease in the number of myocardial [3H]prazosin binding sites in BIO 53.58 hamsters at 11 and 18 weeks of age but not at 5 weeks of age, not only beta but also alpha-1 adrenoceptors in the myocardium of cardiomyopathic hamsters are down-regulated. Such down-regulation seems to be a specific change to adrenoceptors, because the density of myocardial AII receptors ([125I]AII binding sites) in 18-week-old BIO 53.58 hamsters was not altered.

Because an abnormality in catecholaminergic neuronal activity and in myocardial beta adrenoceptors of BIO 53.58 hamsters was not present early in life (at 5 weeks of age) but appeared at 11 and 18 weeks of age, i.e., with the onset of heart failure, it is likely that such a defect trigger partly off the development of cardiomyopathy. Specifically, a continuous elevation in circulating catecholamines in BIO 53.58 hamsters might cause myocardial cell injury by an induction of intracellular calcium overload and/or an increase in peripheral vascular resistance. These effects might result in worsening congestive heart failure.

It has been shown that the hearts from 100-day-old BIO 53.58 hamsters exhibited a diminished contractile response to beta adrenoceptor stimulation. Feldman et al. (1990) found that the activation of adenyl cyclase by isoproterenol in myocardial membranes from young (30-day-old) BIO 53.58 hamsters was not different from that in age-matched F1B controls, but that from 100-day-old hamsters was significantly decreased. In myocardial membranes from these hamsters, the adenyl cyclase activation by forskolin and fluoride ion was also decreased. Therefore, they have suggested that diminished beta adrenoceptor responsiveness and hemodynamic changes in BIO 53.58 hamsters are associated with a functional abnormality of the guanine nucleotide-binding regulatory protein (Gs) that stimulates adenyl cyclase in hearts. Our study indicates that a down-regulation of beta adrenoceptors, in addition to a functional abnormality in the guanine nucleotide-binding regulatory protein (Gs), may underlie, at least in part, the decreased hemodynamic responsiveness to the receptor stimulation in hearts of BIO 53.58 hamsters. The data obtained here were not in an agreement with previous observations by Feldman et al. (1990), who found no change in the number of myocardial (-)-[125I]CYP binding sites in 100-day-old BIO 53.58 as compared with F1B hamsters. The reason for this discrepancy is unclear, although it may be due to some differences in binding param-
ters and in binding assay conditions. We obtained lower values of both \( K_d \) and \( B_{\text{max}} \) for myocardial \((-\)\[^{125}\text{I} \)CYP binding in BIO 53.58 hamsters at 11 and 18 weeks of age (table 1) than their data \( K_d = 33.2 \pm 9.1 \text{nM}, B_{\text{max}} = 65.4 \pm 10.7 \text{fmol/mg protein}, n = 4 \). This may mean predominant labeling of higher-affinity \( \beta \) receptor sites with lower capacity in the myocardium. Further, we used freshly prepared myocardial membranes, whereas they used frozen membranes. Previous study has suggested that \( \beta \) adrenoceptor binding parameters in frozen cardiac tissues of rats are altered, compared with those in fresh tissues (Yamada et al., 1980b).

Heilbrunn et al. (1989) and Waagstein et al. (1983, 1989) reported that in patients with DCM, treatment with metoprolol caused a significant increase (62–105%) in myocardial \( \beta \) adrenoceptor density as assessed in right ventricular endomyocardial biopsy samples, and this was accompanied by a marked improvement in cardiac hemodynamics. In addition, an increase in the number of these \( \beta \) adrenoceptors was associated with a significant enhancement of positive inotropic response to the dobutamine infusion. Subsequently, Ishida et al. (1993) and Yamada et al. (1996) confirmed that an improvement in cardiac function by long-term therapy with metoprolol and bisoprolol in patients with DCM may be ascribed to the recovery of \( \beta \) adrenoceptor density due to the induction of a sustained up-regulation. In the present study, the repeated p.o. administration of metoprolol for 7 weeks in 11-week-old BIO 53.58 hamsters has been demonstrated to induce an increase in myocardial \( \beta \) adrenoceptors \((-\)\[^{125}\text{I} \)CYP binding sites) with a marked decrease in the norepinephrine, calcium deposition and fibrosis in the heart. These improvements in metoprolol-administered cardiomyopathic hamsters were accompanied by a substantial reduction in plasma catecholamine levels, i.e., a significant recovery to the F1B control levels, and also by a concomitant increase in myocardial catecholamine levels. Likewise, Tomita et al. (1994) have reported that plasma levels of norepinephrine and epinephrine were markedly reduced in arotinolol (a nonselective \( \beta \) adrenoceptor antagonist)-treated BIO 53.58 hamsters compared with nontreated hamsters, although the mechanism of this reduction is not known. Therefore, long-term treatment with \( \beta \) adrenoceptor antagonists in BIO 53.58 hamsters has been shown to restore catecholaminergic neuronal function to the F1B control levels.

A presynaptic mechanism appears to be involved in the autoregulation of norepinephrine release during sympathetic nerve stimulation. Presynaptic \( \beta \) adrenoceptors mediate a positive feedback mechanism that increases neurotransmitter release (Langer, 1974; Vizi, 1980). The observed reduction in plasma catecholamine levels by repeated administration of metoprolol in BIO 53.58 hamsters may be associated with a blockade of presynaptic \( \beta \) adrenoceptors, leading to the inhibition of catecholamine release. Consequently, long-term therapy with metoprolol in patients with DCM seems to exert a prolonged blockade of both pre- and postsynaptic \( \beta \) adrenoceptors, which may account for the ameliorating effect against heart failure. However, one cannot exclude the possibility of inhibition of catecholaminergic neuronal activity through a central effect, because metoprolol is moderately lipophilic and crosses the blood-brain barrier easily (Cruckshank et al., 1980; Neil-Dwyer et al., 1981; Dimanes et al., 1990). In this connection, it is interesting to note that the concentration of metoprolol in CSF from hypertensive patients treated with the drug was similar to that in the plasma (Kaia and Marttila, 1993).

The repeated p.o. administration of metoprolol to BIO 53.58 hamsters caused significant ameliorating effects on the fibrosis of hearts, on the density of \( \beta \) adrenoceptors and on the level of plasma and myocardial catecholamines. Maximal effects were already seen at the lowest dose \((1 \text{mg/kg/day}) \) of metoprolol examined; this suggests that there is no dose-response relationship at dosages of 1, 10 and 50 mg/kg/day. It may be assumed, therefore, that relatively low doses of metoprolol are enough to produce a maximal recovery of myocardial function in BIO 53.58 hamsters, perhaps because of a moderate blockade of \( \beta \) adrenoceptors. In relation to this finding, it is of some interest that long-term treatment with a relatively low dose of metoprolol is more effective for ameliorating heart failure in patients with DCM (Yamada et al., 1996).

In summary, we have found a reduced number of myocardial \( \alpha \)-1 and \( \beta \) adrenoceptors in relation to the increased catecholaminergic neuronal activity in BIO 53.58 hamsters, compared with F1B controls. Furthermore, the biochemical changes in BIO 53.58 hamsters were effectively reversed by the repeated administration of metoprolol, which concomitantly caused a marked reduction in norepinephrine, calcium deposition and fibrosis in the heart. This is the first to clarify the characteristics of adrenoceptors in relation to the catecholaminergic neuronal activity with aging and further to demonstrate a significant amelioration by long-term therapy with \( \beta \)-1 adrenoceptor antagonist in a dilated model of cardiomypathy. Results of the present study may offer further support for the use of \( \beta \)-1 adrenoceptor antagonists in patients with DCM.

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References


Feldman, A. M., Tenia, R. G., Kessier, P. D., Weisman, H. F., Schulman, S. P., Blumenthal, R. S., Jackson, D. G. and DOP, C. V.: Diminished \( \beta \)-adrenergic receptor responsiveness and cardiac dilation in hearts of myopathic Syrian...
Metoprolol in Dilated Cardiomyopathy

1395


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