Increased $\beta$-Receptor Density and Improved Hemodynamic Response to Catecholamine Stimulation During Long-term Metoprolol Therapy in Heart Failure From Dilated Cardiomyopathy

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Severe heart failure is associated with a reduction in myocardial $\beta$-adrenergic receptor density and an impaired contractile response to catecholamine stimulation. Metoprolol was administered during a 6-month period to 14 patients with dilated cardiomyopathy to examine its effects on these abnormalities. The mean daily dose of metoprolol for the group was 105 mg (range, 75–150 mg). Myocardial $\beta$-receptor density, resting hemodynamic output, and peak left ventricular dP/dt response to dobutamine infusions were compared in 9, 14, and 7 patients, respectively, before and after 6 months of metoprolol therapy while the patients were on therapy. The second hemodynamic study was performed 1–2 hours after the morning dose of metoprolol had been given. Myocardial $\beta$-receptor density increased from 39±7 to 80±12 fmol/mg ($p<0.05$). Resting hemodynamic output showed a rise in stroke work index from 27±4 to 43±3 g/m/m$^2$, $p<0.05$, and ejection fraction rose from 0.26±0.03 to 0.39±0.03 after 6 months of metoprolol therapy, $p<0.05$. Before metoprolol therapy, dobutamine caused a 21±4% increase in peak positive left ventricular dP/dt; during metoprolol therapy, the same dobutamine infusion rate increased peak positive dP/dt by 74±18% ($p<0.05$). Thus, long-term metoprolol therapy is associated with an increase in myocardial $\beta$-receptor density, significant improvement in resting hemodynamic output, and improved contractile response to catecholamine stimulation. These changes indicate a restoration of $\beta$-adrenergic sensitivity associated with metoprolol therapy, possibly related to the observed up-regulation of $\beta$-adrenergic receptors. (Circulation 1989;79:483–490)

Congestive heart failure is associated with activation of the sympathetic nervous system. Circulating levels and urinary excretion of norepinephrine are increased, whereas myocardial stores of norepinephrine are depleted.1–5 In patients with heart failure, net release of norepinephrine into the coronary sinus6 is partly due to reduced norepinephrine reuptake.7 Evidence exists that this heightened activity of the adrenergic nervous system is associated with a loss of myocardial cell surface $\beta$-adrenergic receptors and a resultant selective loss in the myocardial contractile response to $\beta$-adrenergic stimulation.8–11 Subsensitivity to $\beta$-adrenergic stimulation in patients with heart failure may, therefore, be related to long-term catecholamine exposure.

Clinical trials have suggested an apparent benefit after the use of $\beta$-adrenergic blocking drugs in the treatment of congestive heart failure.12–16 These studies have reported a benefit in left ventricular function, including improvement in New York Heart Association functional class, mean exercise capacity, and radionuclide ejection fraction, and have suggested a favorable impact on patient survival. Recently, we developed methods for quantifying

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β-adrenergic receptor density in human right ventricular endomyocardial biopsy tissue with the radioligand (-)[125]iodocyanopindolol (ICYP). In patients with severe left ventricular dysfunction, β-adrenergic receptor down-regulation was associated with pharmacologically specific impairment of the β-agonist (dobutamine)–mediated contractile response. The present study was designed to evaluate the effect of long-term metoprolol therapy on myocardial β-receptor density and on hemodynamic function as assessed by invasive measures. Our goal was to relate any changes in β-receptor density to the contractile response to exogenous catecholamines by comparing the peak positive left ventricular dP/dt response to a graded dobutamine infusion before and during long-term metoprolol therapy.

Methods

Patients

The study population consisted of 16 patients referred to Stanford University Medical Center, Stanford, California, for evaluation of heart failure secondary to idiopathic dilated cardiomyopathy. The mean age of the patients was 48 years with a range of 29 to 68 years. Patients were eligible for this study if a prior cardiac catheterization or non-invasive investigation showed significant left ventricular dysfunction. Coronary artery disease was not present in any of the patients as shown by prior coronary angiography or angiography at the time of the initial baseline study. No patient with significant lung disease, primary valvular heart disease, or history of alcoholism was studied.

The mean duration of symptoms in the 16 patients initially enrolled in the study was 17 months (range, 1–48 months). Three patients were classified as NYHA Functional Class III, seven as Class II, and five as Class I. All patients had angiographically determined left ventricular ejection fractions of less than 0.45 at entry (range, 0.42–0.1). All were being treated with diuretics, 11 were receiving digoxin, and 13 were receiving vasodilators (seven of whom were receiving angiotensin converting inhibitors, four were receiving nitrates, and two were receiving hydralazine).

Fourteen patients completed the baseline and 6-month invasive evaluation. Two patients enrolled after the initial catheterization experienced worsening symptoms of heart failure and were withdrawn from the study. One of these patients subsequently refused cardiac transplantation and died shortly after initial evaluation. The second patient underwent successful cardiac transplantation.

Endomyocardial Biopsy

Right ventricular endomyocardial biopsy specimens were obtained from all subjects at initial and 6-month follow-up invasive evaluation. Briefly, as previously described, a 9F sheath was inserted percutaneously into the right internal jugular vein under local anesthesia. A 50-cm right ventricular Caves-Schutz biopsy was then inserted through the sheath and advanced to the intraventricular septum. Three to six specimens were obtained in nine patients for β-adrenergic receptor quantification.

Membrane Preparation and Receptor Assay

The procedure of membrane preparation and myocardial β-adrenergic receptor determination was recently described. Fifteen to 25 mg of right ventricular tissue were placed in a buffer solution containing ice-cold 10 mM Tris buffer, 1 mM ethyleneglycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, pH 8.0. The sample was homogenized with a polytron followed by extraction of contractile proteins with ice-cold potassium chloride. The crude membrane preparation was then incubated in an assay buffer consisting of 20 mM Tris buffer, 150 mM NaCl, 1 mM ascorbate, pH 7.5, at 30°C for 120 minutes with seven increasing concentrations of ICYP between 3.25 and 150 pM in the presence and absence of 1 M (-)propranolol. Membranes were trapped by vacuum filtration and counted on a Micromedic Systems gamma counter (San Clemente, California). Receptor binding variables B max (maximum bound ICYP) and K d (radio-ligand dissociation constant) were determined by computer analysis of saturation isotherm data. Satisfactory assays were obtained at baseline and after 6 months of metoprolol therapy in nine patients. In four patients, consent for serial receptor measurements was not obtained. In one patient, insufficient tissue was obtained for a satisfactory assay.

Drug Protocol

Metoprolol, at an initial dose of 6.25 mg b.i.d., was administered after the initial catheterization. The dose was subsequently titrated slowly during a period of 8 weeks; a mean dose of 103 mg (range, 75–150 mg daily) was achieved in all patients. The dose was gradually increased until either the heart rate fell to below 70 beats/min or a maximum dose of 150 mg daily was achieved. The particular combination of conventional medications, including digoxin, diuretics, vasodilators, and anticoagulants, was maintained in all patients and not changed during the treatment protocol.

Hemodynamic Variables

Fourteen patients completed the baseline and 6-month invasive evaluation. After right ventricular biopsy and procurement of tissue for histologic receptor analysis, a 7F Edwards Swan-Ganz catheter was advanced to the pulmonary artery for recording mean right atrial, pulmonary artery, and pulmonary capillary wedge pressures; cardiac output was measured by the thermodilution method with triplicate 10 ml injections of 5% dextrose at room temperature. Left ventricular angiography was performed in the 30° right anterior oblique projection with a 7.2F angiopigtail catheter posi-
tioned in the left ventricular apex. Ejection fraction was calculated by digitizing end-systolic and end-diastolic images with a dedicated computer system. A 6F fiberoptic transducer-tipped catheter (Model 110-4; Camino Laboratories, San Diego, California) was inserted through a long sheath (9F; Cook Catheter) and placed into the left ventricular apex to measure left ventricular end-systolic and end-diastolic pressures and peak positive left ventricular dP/dt. All pressures were recorded on paper with a Honeywell-Meddars chart recorder.

**Inotropic Response to Dobutamine**

A 7F bipolar pacemaker was placed in the right atrium, and patients were paced at a fixed rate of 110 beats/min for 2 minutes before each set of hemodynamic measurements. Hemodynamic measurements were repeated at 5 minutes after starting dobutamine infusions of 2.1, 4.2, and 8.2 μg/kg/min. After the paced hemodynamic variables were obtained, the pacemaker was turned off for recording heart rate response after each infusion. The entire hemodynamic response to dobutamine was repeated after 6 months of metoprolol therapy. All patients received their maintenance dose of metoprolol 1–2 hours before the second catheterization.

Of the 14 patients undergoing serial baseline and 6-month invasive evaluation of resting hemodynamic output, contractile responses to dobutamine infusions were measured in 11 patients at the time of the initial catheterization. Three patients refused to participate in this portion of the study protocol. At the time of the second catheterization, four patients developed atrial fibrillation at the first dobutamine infusion rate and were, therefore, unsuitable for further evaluation. Seven patients who completed the entire sequence of dobutamine infusions (2.1, 4.2, and 8.2 μg/kg/min) at baseline and 6 months were used in the data analysis.

**Statistical Methods**

Baseline and 6-month β-adrenergic receptor density and hemodynamic measurements were compared by paired t tests. A p value less than 0.05 was considered significant. All values are expressed as mean ± SEM. Dose-response curves to dobutamine were constructed by excluding baseline values, calculating percent increments, and analyzing the data points by one-way analysis of variance (ANOVA).

**Results**

**Alteration in β-Receptor Density**

Myocardial β-receptor densities were measured in nine patients at entry and after 6 months of metoprolol therapy. As shown in Figure 1, a highly significant change in receptor density was noted during the treatment period. The mean entry receptor density of 39 ± 7 fmol/mg protein rose to 80 ± 12 fmol/mg protein after 6 months of therapy. The radioligand dissociation constant (Kd) did not change with metoprolol therapy (27.7 ± 3.5 vs. 21.8 ± 4.8).

**Hemodynamic Analysis**

Table 1 compares resting hemodynamic values of 14 patients obtained at entry with those after 6 months of metoprolol therapy. As shown in Figure 2, the mean baseline ejection fraction in 13 patients (the left ventricular angiogram could not be adequately digitized in one patient) rose from 0.26 ± 0.03 to 0.39 ± 0.03. The increase in cardiac index from 2.8 ± 0.2 to 3.2 ± 0.1 l/min/m² was not significant. The mean arterial and right atrial pressures remained unchanged. Small changes in systemic vascular resistance (1,123 ± 71 to 1,004 ± 56 dynes/sec/cm²), left ventricular end-diastolic pressure (22 ± 3 to 17 ± 2 mm Hg), and resting peak left ventricular dP/dt (750 ± 69 to 804 ± 90 mm Hg/sec/sec) after 6 months of metoprolol therapy were not statistically significant. The mean heart rate fell from 94 ± 5 to 69 ± 4 beats/min. Stroke work index improved from 27 ± 4 to 43 ± 3 g/m² (p < 0.05) during metoprolol therapy.

**Changes in Contractile Response to Dobutamine Stimulation**

Table 2 lists the hemodynamic responses at entry and after 6 months of metoprolol therapy in the seven patients who received serial (2.1, 4.2, and 8.2 μg/kg/min) dobutamine infusions.

The percent increase from baseline in peak left ventricular dP/dt recorded at each infusion of dobutamine is shown in Figure 3. The percent increment...
TABLE 1. β-Adrenergic Receptor Density and Hemodynamic Values at Entry and After 6 Months of Metoprolol Therapy

<table>
<thead>
<tr>
<th>Hemodynamic value</th>
<th>Entry</th>
<th>p</th>
<th>6 Mo metoprolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-adrenergic receptor density (fmol/mg protein)</td>
<td>39 ± 7</td>
<td>0.005</td>
<td>80 ± 12</td>
</tr>
<tr>
<td>K_d</td>
<td>27.7 ± 3.5</td>
<td>NS</td>
<td>21.8 ± 4.8</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>0.26 ± 0.03</td>
<td>0.005</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td>Cardiac index (l/min/m²)</td>
<td>2.8 ± 0.2</td>
<td>NS</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>82 ± 3</td>
<td>NS</td>
<td>83 ± 3</td>
</tr>
<tr>
<td>Systemic vascular resistance (dynes/sec/cm²)</td>
<td>1,123 ± 71</td>
<td>NS</td>
<td>1,004 ± 56</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td>22 ± 3</td>
<td>NS</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Right atrial pressure (mm Hg)</td>
<td>5 ± 1</td>
<td>NS</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>94 ± 5</td>
<td>0.001</td>
<td>69 ± 4</td>
</tr>
<tr>
<td>Peak left ventricular dP/dt (mm Hg/sec/sec)</td>
<td>750 ± 69</td>
<td>NS</td>
<td>804 ± 90</td>
</tr>
<tr>
<td>Stroke work index (g/m/m²)</td>
<td>27 ± 4</td>
<td>0.002</td>
<td>43 ± 3</td>
</tr>
</tbody>
</table>

Data are unpaced mean ± SEM; n=14 patients.

in left ventricular dP/dt after 6 months of metoprolol therapy was 22 ± 5%, 41 ± 10%, and 74 ± 18% (at 2.1, 4.2, and 8.2 µg/kg/min dobutamine) compared with 11 ± 2%, 21 ± 4%, and 21 ± 4% (at the corresponding doses) at the time of initial catheterization. The heart rate response (after the atrial pacemaker was turned off) after each infusion of dobutamine compared with baseline heart rate is shown in Figure 4. No significant increase in heart rate response occurred after 6 months of therapy. Three patients were not included in the heart rate analysis because of the development of atrial fibrillation (two patients) and supraventricular tachycardia (one patient) before dobutamine infusion.

Figures 5 and 6 show percent change of mean arterial and left ventricular end-diastolic pressures at each infusion of dobutamine. Minimal change in arterial pressure occurred after infusions of 2.1 or 4.2 µg/kg/min dobutamine, either at entry or after 6 months of therapy. A significant difference in percent change of left ventricular end-diastolic pressure occurred at 8.2 µg/kg/min dobutamine. At this dosage, the left ventricular end-diastolic pressure fell to 44 ± 9% compared with the baseline pressure, in contrast to a decrease to 20 ± 9% from baseline after 6 months of therapy.

Discussion

Chronic heart failure is associated with a reduction in myocardial β-adrenergic receptors and subsensitivity to β-adrenergic stimulation in vitro and in vivo studies in humans.9-11 This phenomenon may be related to a reduction in myocardial β-adrenergic surface receptors in response to increased long-term exposure to the sympathetic neurotransmitter norepinephrine. Clinical studies appear to have shown a paradoxical improvement in some patients with chronic heart failure who are treated with the specific β₁-adrenoreceptor antagonist metoprolol.12-16 This study was designed to test the hypothesis that at least some of the benefit from β-blocker therapy in chronic heart failure is related to an effect of the myocardial surface β-adrenergic receptor density and of the subsequent response to β-adrenergic agonist stimulation in those patients with persistent or worsening symptoms.

To test this hypothesis, left and right heart hemodynamic output at rest and during a graded intravenous infusion of the full agonist, dobutamine, was compared at entry and again during metoprolol therapy 6 months later. Thus, we were able to
evaluate any change in inotropic response to exogenous catecholamine stimulation. Myocardial β-adrenergic receptor density was also measured at entry and during long-term β-blocker therapy. Our results show that long-term therapy with the β1-selective blocker metoprolol is associated with a doubling of the β-adrenergic receptor density to a level similar to that in patients free of significant heart failure.10 Resting hemodynamic output improved in 14 of the 16 patients originally entered into the study. Although these patients were selected on the basis of having severe symptomatic heart failure from idiopathic dilated cardiomyopathy, they were not considered immediate candidates for heart transplantation because of relative stability in their condition. Nevertheless, they had moderate-to-severe cardiac dysfunction with a mean ejection fraction at entry of only 0.26 and a mean left ventricular end-diastolic pressure of 22 mm Hg (n = 16 patients originally entered into the study). Our study would have been more conclusive if a control group had been included. Although each patient acted as his own

**TABLE 2. Hemodynamic Response to Dobutamine at Entry and After 6 Months of Metoprolol Therapy**

<table>
<thead>
<tr>
<th>Hemodynamic value</th>
<th>Dobutamine (μg/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak left ventricular dP/dt (mm Hg/sec/sec)</td>
<td></td>
</tr>
<tr>
<td>Entry</td>
<td>0</td>
</tr>
<tr>
<td>6 mo metoprolol therapy</td>
<td>2.1</td>
</tr>
<tr>
<td>8 mo metoprolol therapy</td>
<td>4.2</td>
</tr>
<tr>
<td>10 mo metoprolol therapy</td>
<td>8.2</td>
</tr>
<tr>
<td>Stroke work index (g/m/m²)</td>
<td></td>
</tr>
<tr>
<td>Entry</td>
<td>23.6±2.4</td>
</tr>
<tr>
<td>6 mo metoprolol therapy</td>
<td>31.8±2.8</td>
</tr>
<tr>
<td>Cardiac index (/l/min/m²)</td>
<td></td>
</tr>
<tr>
<td>Entry</td>
<td>2.9±0.3</td>
</tr>
<tr>
<td>6 mo metoprolol therapy</td>
<td>3.3±0.1</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
</tr>
<tr>
<td>Entry</td>
<td>87±3</td>
</tr>
<tr>
<td>6 mo metoprolol therapy</td>
<td>89±4</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td></td>
</tr>
<tr>
<td>Entry</td>
<td>23±3</td>
</tr>
<tr>
<td>6 mo metoprolol therapy</td>
<td>13±2</td>
</tr>
</tbody>
</table>

Data are paced absolute mean±SEM; n = seven patients.

**Figures 3 and 4.** Plot of percent increment in peak left ventricular (LV) dP/dt compared with baseline at entry and baseline at 6 months of metoprolol therapy during dobutamine infusion. Values were obtained during atrial pacing at 110 beats/min and represent mean±SEM; n = eight patients. **Figure 4.** Plot of percent change in heart rate (HR) response compared with baseline at entry and baseline at 6 months of metoprolol therapy during dobutamine infusion. Values represent unpaced, mean±SEM chronotropic response to dobutamine infusion; n = seven patients.
Control, our findings could have been the result of spontaneous improvement and a "secondary" increase in β-adrenergic receptor density and catecholamine responsiveness. We believe this is unlikely because of the severe chronic heart failure in all of our patients with dilated cardiomyopathy.

Dobutamine was used to evaluate the contractile response to β-adrenergic stimulation before and during long-term metoprolol therapy. Dobutamine appears to have predominantly myocardial β-agonist effects, although the compound probably also exerts weak peripheral β-receptor (vasodilatory) and opposing α-receptor (vasoconstrictor) effects. We used peak positive dP/dt to determine the contractile response to dobutamine. The effect of heart rate on this variable of left ventricular performance was negated by comparing the values at a fixed heart rate. Peak positive dP/dt is also influenced by left ventricular loading conditions. Although dobutamine infusion caused a greater increase in mean arterial pressure after metoprolol, the small difference is unlikely to explain the very different response in left ventricular dP/dt at entry and after 6 months of metoprolol therapy. Similarly, the greater reduction seen in left ventricular end-diastolic pressure at the peak dobutamine dose during the entry study does not account for the marked increase in dP/dt response during the study with metoprolol.

The chronotropic response to dobutamine was not altered by long-term metoprolol therapy. Newman\textsuperscript{18} has shown this apparent separation between the inotropic and chronotropic subsensitivity in a dog model of experimental heart failure. This may be related to the finding by Bristow et al,\textsuperscript{19} of selective down-regulation of the β\textsubscript{1}-receptor population in heart failure, with relative preservation of the β\textsubscript{2}-receptor population such that the β\textsubscript{1} : β\textsubscript{2} ratio changes from a normal 80:20 to approximately 60:40 in severe heart failure. If metoprolol up-regulates the β\textsubscript{1}-receptor, but not the β\textsubscript{2}-receptor fraction, and heart rate is more of a β\textsubscript{2} response,\textsuperscript{20} the chronotropic effect of dobutamine would not be expected to change after metoprolol therapy. Alternatively, the failure of the chronotropic response to improve significantly may be related to the relative sensitivity of dobutamine for β\textsubscript{1} compared with β\textsubscript{2}-adrenoceptors, although recent studies suggest the β\textsubscript{1}-adrenoceptor selectivity of dobutamine is minimal.\textsuperscript{21} In the present study in which only milligram quantities of myocardial tissue were obtained from right ventricular biopsies, it was not possible to perform assays to determine the β-adrenergic subpopulation before or after metoprolol therapy.

The most straightforward explanation for our findings is that the increased β-adrenergic receptor density after 6 months of metoprolol is related to reduced catecholamine exposure caused by receptor occupancy. This increase in receptor density induced by β-blockers was previously observed clinically in nonfailing hearts and may be the cause of so-called supersensitivity phenomena after abrupt β-blocker withdrawal.\textsuperscript{22} The augmented contractile response to dobutamine during long-term metoprolol therapy implies that the observed increase in β-adrenergic receptors or the competitive nature of receptor occupancy with metoprolol or both were responsible for improved catecholamine sensitivity. However, we were not able to measure concentrations of guanine nucleotide regulatory proteins (G,
and $G_s$) in the amount of myocardial membrane prepared from the myocardial biopsy tissue. Thus, an alternative explanation related to concentration changes of these proteins may have been overlooked.

Alternatively, the long-term administration of $\beta$-blockers may benefit the failing myocardium by a still undefined mechanism: prevention of calcium overload, inhibition of free radical formation, or peripheral effects that result in a secondary myocardial improvement or decreased sympathetic activation, or both, that may also result in an increased $\beta$-adrenergic receptor density. Large placebo-controlled studies that examine the time course of these phenomena after metoprolol therapy may answer some of these questions.

The improvement in basal resting hemodynamic output in our study is in agreement with one other report of the hemodynamic changes after long-term metoprolol therapy in dilated cardiomyopathy. In this previous study of 21 patients with idiopathic dilated cardiomyopathy, a similar improvement in ejection fraction and stroke work index was noted. These hemodynamic improvements likely resulted from augmented contractility, as opposed to a response to reduced afterload because systemic vascular resistance did not fall. In addition, our group has shown a significant reduction in end-systolic dimension derived echocardiographically (at a time when mean systemic pressure was higher) in patients serially evaluated during treatment with long-term metoprolol therapy.

This study provides evidence that long-term metoprolol therapy for dilated cardiomyopathy is associated with $\beta$-receptor up-regulation. Furthermore, restoration of myocardial $\beta$-receptor density is associated with improved catecholamine responsiveness and,
hence, the ability of the adrenergic nervous system to provide inotropic support to the failing heart.

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KEY WORDS • myocardial β-receptor density • dobutamine
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