Long-term Propranolol Administration Alters Myocyte and Ventricular Geometry in Rat Hearts With and Without Infarction

Michael C. Fishbein, MD, Li-Quan Lei, MD, and Stanley A. Rubin, MD

To determine the effects of long-term β-adrenergic receptor blockade on adult rats with myocardial infarction, we studied 24 female Sprague-Dawley rats with myocardial infarction induced at 20–22 weeks of age. Two days after surgery, the animals were randomized to receive either propranolol (750 mg/l) in their drinking water or water alone for 5 weeks. Plastic, embedded, longitudinal and cross sections of septum (1 µm thick) were prepared for morphometric measurements. In untreated rats, infarction was followed by myocardial hypertrophy, as shown by significant increases in septal area (23%), myocyte length (19%), cross-sectional area (20%), and volume (43%) (p=0.05). In rats with and without infarction, β-blockade resulted in decreased myocyte dimensions and increased left ventricular cavity dimensions. Propranolol had special effects in rats with infarction, resulting in significant blunting of increased cross-sectional area (15% less, p=0.04) and a greater increase in left ventricular cavity dimensions (38% more, p=0.04). Thus, propranolol blunts myocardial hypertrophy and increases left ventricular cavity dimensions in rats with myocardial infarction. (Circulation 1988;78:369–375)

Materials and Methods

Animals

Female Sprague-Dawley rats (n=24) (Hilltop Laboratories, Scottsdale, Pennsylvania) obtained at 6 weeks of age were retained by our laboratory until they were 20–22 weeks old. Animals were caged in proportion to size, were given water and rat chow ad libitum, housed in a climate-controlled environment, and subjected to 12-hour light/dark cycles. The animals were housed and cared for in a vivarium that is accredited by the American Association for Accreditation of Laboratory Animal Care. The use and care of laboratory animals was according to National Institutes of Health guidelines.

Infarction

Myocardial infarction was produced by ligation of the left coronary artery by a suture technique. Briefly, while the animal was under isoflurane anesthesia, the thorax was opened by incision, and the heart was exteriorized by pulling the apex with a 7-0 suture. The left coronary artery was ligated a few millimeters from its origin with a 5-0 prolene suture. The chest was closed, and the animal was allowed to recover. Control rats were subjected to a sham operation with the intention of producing infarction by ligation; however, the procedure randomly resulted in a failed occlusion of the left coronary artery without an infarction. Mortality after success-
ful ligation was 50%, with almost all deaths occurring within 24 hours of occlusion.

**Treatment**

Beginning 2 days after surgery, the animals were randomized to receive either propranolol (Sigma Chemical, St. Louis, Missouri) 750 mg/l in their drinking water or drinking water alone for 5 weeks. In pilot studies involving control rats, we tested the effects of this drug dosage to produce β-blockade during long-term treatment. A standardized dose-response test of the effects of isoproterenol on heart rate showed the expected competitive antagonist effects of β-blockade (Figure 1). Water bottles were changed three times each week. Water consumption was monitored throughout the study, and it averaged 15–20 ml/day for each rat. There was no difference in the water consumption of the propranolol- or drinking-water-treated animals. No deaths occurred after treatment randomization of the animals.

**Fixation Procedure**

Five weeks after initiating drinking-water treatment, the rats were killed. The rats were placed under ketamine (150 mg/kg) anesthesia, endotracheally intubated, and mechanically ventilated. A catheter was placed in the right carotid artery, the thorax was opened, the descending aorta was clamped, and the heart was arrested with a 2-ml injection of 1 meq/ml KCl. A perfusion of ice-cold fresh saline containing 5,000 units/l heparin was started at a pressure of 100 mm Hg, and the right atrium was cut to allow the drainage of blood and perfusate. After perfusion with cold saline for 2 minutes, the myocardium was perfused for 15 minutes with ice-cold 1.5% glutaraldehyde in 0.1 M cacodylate buffer. The heart was excised, the right ventricle was dissected free, and the left ventricle was cut into four transverse slices. Blocks of tissue 1–2 mm² were cut from the middle layer of the septum and stored overnight in 3% glutaraldehyde. The small septal tissue blocks were placed in 0.2 M cacodylate buffer and then postfixed in 1% osmium tetraoxide in 0.1 M phosphate buffer at pH 7.4. The tissue was dehydrated in increasing concentrations of ethyl alcohol and placed into propylene oxide and mixtures of propylene oxide and Epon before final embedding in Epon 812.

The remaining left ventricular slices, including septum, were postfixed in 10% neutral-buffered formalin and processed for light microscopy studies. After processing in graded concentrations of alcohol, clearing in xylene, and embedding in paraffin, 5-µm thick sections were cut from each slice and stained with hematoxylin and eosin.

**Determination of Infarct Size and Left Ventricular Dimensions**

The complete transverse sections were projected onto a screen at a ×10 magnification, and a planimeter was used to make the following measurements: infarct extent; average length of endocardial circumference of the middle ventricle, determined from the two midventricular slices; the cross-sectional area of the septum obtained as an average of the two midventricular slices; and the average wall thickness of septum in the middle left ventricle. The latter measurement was performed by dividing the septum into three equal parts between its attachments to the right ventricle, by measuring the

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**Figure 1.** Plot of competitive β-adrenergic receptor antagonism by propranolol. Dose-response curve shows the effect of isoproterenol infusion on heart rate. Rats were treated for 5 weeks with either tap water or propranolol (750 mg/l). Propranolol shifted the dose-response curve in the typical manner of a competitive antagonist without partial agonist activity.

**Figure 2.** Photomicrographs of cross (Panel A) and longitudinal (Panel B) sections of 1-µm section used for morphometric studies. Arrows in Panel B indicate nuclei (methylene blue and azure II, magnification ×300 [Panel A] and ×60 [Panel B]).
thickness of each, and by averaging the three measurements. Infarct thickness was measured at three sites directly from the histological sections at the midventricular level, at ×20 magnification with a calibrated eyepiece grid. Internal radius, r(i), was calculated from the circumference of the middle ventricle (C_{endo}) by assuming a spherical model:

\[ r(i) = \frac{C_{endo}}{2 \cdot \pi} \]

**Morphometric Measurements**

Methods and equations used for morphometric studies have been published previously in detail. Epon-embedded tissue blocks from the septum were sectioned longitudinally and transversely at a thickness of 1 μm with an LKB Ultratome III (Gaithersburg, Maryland) and were stained with methylene blue and azure II (Figure 2). Under the light microscope at ×1,000 magnification, the total number of myocyte nuclear profiles in 0.01-mm² transverse tissue sections (myocytes in cross section) was delineated with an ocular reticle (10×10, AO 477, American Optical, Buffalo, New York) by standardized rules of point counting. Thirty such fields were evaluated in each animal to determine the mean number of nuclear profiles per unit area of myocardium N(n)A.

The average nuclear length, L(n), was determined in each animal from 30 measurements of longitudinally-oriented myocytes under the light microscope at a magnification of ×1000 and viewed with a calibrated microscope with an ocular micrometer accurate to 0.5 μm. Only those nuclei were measured in which the nuclear envelope was sharply defined at both ends and in which clusters of mitochondria were clearly visible in the areas adjacent to the nuclear edges.

Measurements of the number of myocyte nuclei per unit volume of myocardium, N(n)v, were obtained with the equation

\[ N(n)v = N(n)A / L(n) \]

The volume fraction of myocytes in the myocardium, V(m)v, was determined by the point-counting technique in the same fields and by the same technique as used for determination of N(n)A. The myocyte cell volume per nucleus, V(m)n, was calculated as

\[ V(m)n = V(m)v / N(n)v \]

In addition, the number of myocyte profiles per unit tissue area, N(n)A, was determined by the same methods as described above for the transverse fields. The average cross-sectional area of myocytes, A(m), was calculated from

\[ A(m) = V(m)v / N(m)A \cdot \pi \]

**TABLE 1. Weights and Infarct Size**

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th></th>
<th>Propranolol-treated</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sham (n = 6)</td>
<td>MI (n = 6)</td>
<td>Sham (n = 6)</td>
<td>MI (n = 6)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>265 ± 24</td>
<td>257 ± 19</td>
<td>258 ± 21</td>
<td>252 ± 12</td>
</tr>
<tr>
<td>Infarct size (% of LV)</td>
<td>&lt;5</td>
<td>26.9 ± 10.9</td>
<td>&lt;5</td>
<td>29.9 ± 15.8</td>
</tr>
<tr>
<td>LV weight (mg)</td>
<td>894 ± 111*</td>
<td>. . .</td>
<td>777 ± 90†</td>
<td>. . .</td>
</tr>
<tr>
<td>RV weight (mg)</td>
<td>241 ± 24*</td>
<td>. . .</td>
<td>236 ± 45</td>
<td>. . .</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
Sham, sham-operated; MI, myocardial infarction; LV, left ventricular; RV, right ventricular.

*_{n}=4; †_{p}<0.05.

**FIGURE 3. Photomicrographs of cross sections of infarcted hearts of untreated (Panels A and B) and propranolol-treated (Panels C and D) rats. Note the increased cavity size and decreased septal thickness (asterisks) in the propranolol-treated rat hearts (Panels C and D) (hematoxylin and eosin stain; magnification ×5).**
The length of myocyte per nucleus, L(m)n, was calculated as

\[ L(m)n = V(m)n/A(m) \cdot \pi \]

Mean myocyte diameter, D(m), was calculated assuming a circular cross section of the myocyte with area A(m):

\[ D(m) = 2 \cdot (A(m)/\pi)^{0.5} \]

**Statistical Methods**

Results are presented as mean ± SD and were computed from the average measurements obtained from each rat. The main statistical analysis was made with a two-way analysis of variance. Three statistical hypotheses were tested: there were no differences between disease condition (infarct vs. no infarct); there were no differences between treatments (propranolol vs. no propranolol); and there were no interactions between disease and treatment (a special or predominant effect of propranolol on infarct animals). When the third hypothesis was rejected, that is, when a significant interaction was present, additional tests were performed between individual groups. A modified unpaired t test, which included Bonferroni’s correction, was performed to establish the significant effects of infarction or propranolol treatment on specific variables.

**Results**

**Cardiac Weight and Dimensions (Table 1)**

All rats had similar body weights initially and at the time of killing. However, in rats without infarcts, left ventricular weights at the time of death averaged 13% less in propranolol-treated rats than in controls. Right ventricular weights were similar in these two groups.

Mean infarct size was similar in the two groups of rats with infarcts. Cross-sectional area of septum was greatest in untreated rats with infarcts, being 23% more than in untreated rats without infarcts. Septal area and thickness were greater in propranolol-treated rats with infarcts when compared with rats without infarcts (Figure 3). However, in both groups of propranolol-treated rats (with and without infarct), septal area was less than that of both groups of untreated rats. In rats with infarcts, the mean scar thickness in untreated rats was 0.23 ± 0.06 mm and was 0.18 ± 0.03 mm in propranolol-treated rats (p = 0.103). When only the thinnest portion of the scar was considered (minimum scar thickness), the difference in scar thickness became significant (0.20 ± 0.05 vs. 0.13 ± 0.03, p = 0.025). Left ventricular cavity radius was greatest in propranolol-treated rats with infarcts, being 48% greater than in propranolol-treated rats without infarcts and 38% greater than in untreated rats with infarcts (Figures 3 and 4). In untreated rats, cavity radius was 12% greater in rats with infarcts than in rats without infarcts.

**Morphometric Measurements of Myocytes (Table 2)**

In sham-operated rats, myocyte volume per nucleus was 10% less in propranolol-treated than in untreated rats (Figure 5). In rats with infarcts, myocyte volume per nucleus was 20% less in propranolol-treated rats. In untreated rats, infarction resulted in 43% increase in myocyte volume per nucleus, while in propranolol-treated rats, infarction resulted in a 25% increase in myocyte volume per nucleus.

In sham-operated rats, cross-sectional area of myocytes was 6% less in propranolol-treated animals than in untreated animals (Figure 6). In rats with infarcts, cross-sectional area was 15% less in the propranolol-treated group.

In sham-operated rats, myocyte length was 4% less in propranolol-treated rats than in untreated rats. In rats with infarcts, myocyte length was 6% less in the propranolol-treated animals than in the untreated animals. In untreated rats with infarcts, myocyte length was 19% greater than in sham-operated rats. In the propranolol-treated group, myocyte length was 16% greater in rats with infarcts than in those with sham operation.

Two-way analysis of variance indicated that there were several morphometric changes that occurred in rats with infarcts in which propranolol had a special effect; that is, the effects of propranolol...
were greater in the diseased rats (with infarcts) than in sham-operated rats. These were increased endocardial circumference and resulting cavity radius calculations, increased number of myocytes per area, and decreased cross-sectional area of myocytes and resulting calculation of mean myocyte diameter \((p < 0.05\) for each variable). When the two groups without infarcts were compared by the modified unpaired \(t\) test, differences in left ventricular radius, myocyte cross-sectional area, and myocyte volume per nucleus did not reach statistical significance \((p > 0.10\) for each variable). However, when the two groups with infarcts were compared, those differences were significant \((p < 0.01, < 0.01, \text{and} < 0.02, \text{respectively})\).

**Discussion**

This study shows that long-term propranolol administration in rats causes significant structural changes in the heart. This is true not only in rats with myocardial infarcts but in sham-operated rats as well. In mature rats without infarcts, there was a trend toward atrophy of myocytes in the propranolol-treated animals. In rats with infarcted hearts, propranolol blunted the compensatory hypertrophic response that normally occurs in the myocardium without infarcts. In addition, rats treated with propranolol had an increased left ventricular volume. This was true in rats without infarcts but was present to a much greater extent in the rats with infarcts. Interestingly, two-way analysis of variance indicated that the effects of propranolol were exceptional in the rats with infarcts. That is, propranolol caused greater cavity dilation and greater limitation of myocyte volume in animals with infarcts in comparison with those without infarcts. Myocyte length increased (19% vs. 16%) in both untreated and propranolol-treated rats with infarcts. However, in light of the marked dilation of the hearts of propranolol-treated animals, there appears to be a relative limitation of the myocytes to grow in the longitudinal and transverse directions. Increased myocyte length has been shown to contribute to enlarged chamber volume in dilated hearts. \(\beta\)-Receptors are important in the regulation of cardiac contractile function. It is well established that long-term \(\beta\)-adrenergic receptor blockade has negative inotropic and chronotropic effects and

**Table 2. Morphometric Data for Untreated and Propranolol-Treated Rats**

<table>
<thead>
<tr>
<th>Morphometric data</th>
<th>Untreated</th>
<th>Propranolol-treated</th>
<th>Statistics ((p) values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham ((n=6))</td>
<td>MI ((n=6))</td>
<td>Difference (%)</td>
</tr>
<tr>
<td>Septal area ((\text{mm}^2))</td>
<td>16.7±2.5</td>
<td>20.5±4.3</td>
<td>+23</td>
</tr>
<tr>
<td>Septal thickness ((\text{mm}))</td>
<td>2.0±0.4</td>
<td>2.2±0.4</td>
<td>+10</td>
</tr>
<tr>
<td>Minimum scar thickness ((\text{mm}))</td>
<td>. . .</td>
<td>0.20±0.05</td>
<td>. . .</td>
</tr>
<tr>
<td>Endocardial circumference ((\text{mm}))</td>
<td>16.6±3.8</td>
<td>18.1±3.7</td>
<td>+9</td>
</tr>
<tr>
<td>LV cavity radius ((\text{mm}))</td>
<td>2.6±0.6</td>
<td>2.9±0.6</td>
<td>+12</td>
</tr>
<tr>
<td>Nuclei/(\text{mm}^2) ([\text{N(n)A}])</td>
<td>851±55</td>
<td>628±81</td>
<td>-26</td>
</tr>
<tr>
<td>Nuclear length ([\mu\text{m}, L(n)])</td>
<td>16.3±0.4</td>
<td>17.3±1.1</td>
<td>+6</td>
</tr>
<tr>
<td>Nuclei/(\text{mm}^2) ([\text{N(n)v}])</td>
<td>52,209±4,085</td>
<td>36,648±6,716</td>
<td>-30</td>
</tr>
<tr>
<td>Volume fraction of myocytes ([%%, V(m)v])</td>
<td>81.8±1.0</td>
<td>80.3±3.4</td>
<td>-2</td>
</tr>
<tr>
<td>Myocyte volume/nucleus ([\mu\text{m}^3, V(m)n])</td>
<td>15,748±1,182</td>
<td>22,487±3,969</td>
<td>+43</td>
</tr>
<tr>
<td>Myocytes/(\text{mm}^2) ([\text{N(m)A}])</td>
<td>2,869±123</td>
<td>2,365±238</td>
<td>-18</td>
</tr>
<tr>
<td>Cross-sectional area of myocytes ([\mu\text{m}^2, A(m)])</td>
<td>286±11</td>
<td>342±35</td>
<td>+20</td>
</tr>
<tr>
<td>Myocyte diameter ([\mu\text{m}, D(m)e])</td>
<td>19.1±0.4</td>
<td>20.9±1.1</td>
<td>+9</td>
</tr>
<tr>
<td>Myocyte length/nucleus ([\mu\text{m}, L(m)n])</td>
<td>55.0±2.7</td>
<td>65.3±5.3</td>
<td>+19</td>
</tr>
</tbody>
</table>

Sham, sham-operated; MI, myocardial infarction; LV, left ventricular.
reduces overall contractile function of the heart. Our study now demonstrates that striking changes in structure also occur. The decreased function of the heart with long-term \( \beta \)-blockade documented in rats\(^7\) is associated with a limitation of hypertrophy in the heart rendered abnormal in this model of anterior myocardial infarction.

Sen et al\(^6\) have demonstrated that 6 weeks of \( \beta \)-adrenergic blockade begun in spontaneously hypertensive rats (SHR) that are 12–14 weeks old caused a 9% reduction in the hypertrophy that occurred in untreated SHR. Pfeffer et al\(^7\) treated SHR and Wistar-Kyoto (WKY) rats with propranolol from conception until 12 weeks old. Although propranolol-treated rats had reduced central venous pressure, cardiac index, and maximum acceleration of aortic flow, there were no blood pressure reductions and no alterations in cardiac weight in the SHR or WKY. Our model is considerably different because myocardial infarction does not result in the pure pressure overload seen in the SHR. Hypertrophy after myocardial infarction has been termed "reactive hypertrophy"\(^16\) and involves a loss of contractile structures and increased volume load. Also, the rats studied by Pfeffer et al\(^7\) were younger, and perhaps at these ages, the effects of potent stimulants of normal growth, such as growth hormone,\(^17\) outweighed the effects of \( \beta \)-blockade. We have shown previously that even in adult rats growth hormone causes cardiac growth with 6 weeks of growth hormone excess, resulting in a 46% increase in left ventricular mass.\(^17,18\)

These data raise several questions worthy of consideration and study. First, how does propranolol limit myocyte volume? The structural changes may all be secondary to decreased contractile function or blood pressure, a type of disuse atrophy. On the other hand, \( \beta \)-receptors may have a direct trophic function and may be necessary for the maintenance of normal cell size and for compensatory hypertrophy of the myocardium under increased work load. Our study does not provide insight into the mechanism of the observed effects of propranolol.

Second, how do the observed increases in chamber dimensions and scar thinning come about? It has been shown in this rat model\(^9\) that global cardiac remodeling occurs after infarction, which involves both expansion of the infarcted segment and overall change in ventricular shape. Thus, the exaggerated cavity enlargement and scar thinning we observed in propranolol-treated animals could be due to increased expansion of the infarcted zone, greater remodeling of the noninfarcted myocardium, or even a direct effect of propranolol on scar tissue. There is no evidence that propranolol alters the inflammatory response or collagen deposition during healing, so the third possibility seems unlikely. Propranolol depresses cardiac contractility and heart rate. Both effects would tend to increase cardiac volume and wall tension. These pharmacological effects of propranolol could lead to secondary effects on infarcted scar formation as well as residual myocardium. Additional effects on septal thinning by propranolol, in part due to the limitation of myocyte hypertrophy, also could promote dilation. The final shape of the ventricle when dilated would be the net result of all the individual effects. Thus, one cannot unravel the relative contributions of these mechanisms on septal thinning. In any case, it is likely that infarct expansion and ventricular remodeling are related phenomena because the extent of these changes correlates with each other and is independent of infarct size and age.\(^19\)

Other important questions relate to the clinical implications of these findings if they can indeed be extrapolated from this rat model to humans. \( \beta \)-Blockers are widely used in patients with myocar-
dial infarction because they clearly reduce mortality.1–3 How do the structural alterations that we observed coincide with the pharmacological effects of propranolol, and are these changes beneficial or detrimental? One could argue that decreased cell size may have a beneficial effect by limiting contractile function and oxygen demands of the noninfarcted myocardium, an increase in metabolic-mechanical efficiency as suggested by Swynghedauw et al.20 On the other hand, limiting the normal compensatory hypertrophy of the remaining myocardium could have long-term adverse effects on the functional reserve of the heart. This is a difficult question to resolve in humans. Humans with infarcts often have hypertension, arrhythmias, papillary muscle dysfunction, aneurysms, congestive heart failure, and other abnormalities that may be affected by β-blockade and that complicate the unraveling of positive versus negative effects of β-blockade in such patients. For example, in hypertensive patients, the benefit of a reduced wall stress if blood pressure is lowered might offset the detriment of a reduced capacity for hypertrophy.

It is debatable whether limitation of hypertrophy is beneficial or detrimental in patients with myocardial infarction; however, it is difficult to imagine that increased left ventricular cavity volume could be beneficial. Ventricular enlargement is associated with a reduced survival after myocardial infarction.21 Attenuation of ventricular enlargement may improve prognosis after infarction.22 We are not aware of any studies that have attempted to determine specifically whether β-blockade potentiates ventricular dilation in patients with myocardial infarction. However, a preliminary report of drug treatment after myocardial infarction suggested that cardiac volume increased in a control group of patients, of whom some were receiving β-blockade.22

Last, β-blockade is sometimes used to treat noncardiac disorders, such as migraine headaches.23 This raises concerns about the cardiac changes that may be occurring in normal hearts in such patients and also provides the opportunity to confirm our observations in experimental animals and in humans. This probably could be accomplished by noninvasive serial echocardiographic studies in such patients.

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References


Key Words • hypertrophy • atrophy • morphometry • β-blockade
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