Effects of \( \beta \)-Adrenergic Blockade on Papillary Muscle Function and the \( \beta \)-Adrenergic Receptor System in Noninfarcted Myocardium in Compensated Ischemic Left Ventricular Dysfunction

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**Background.** \( \beta \)-Adrenergic receptor blockade has been reported to improve hemodynamics and \( \beta \)-adrenergic receptor-adenylate cyclase function in idiopathic dilated cardiomyopathy. The purpose of this study was to determine the effects of \( \beta \)-adrenergic receptor blockade on the \( \beta \)-adrenergic receptor system and myocardial function in a model of compensated ischemic heart failure.

**Methods and Results.** We examined the effects of propranolol treatment on the \( \beta \)-adrenergic receptor-adenylate cyclase system and isolated papillary muscle isometric function in noninfarcted left ventricular myocardium in rats after coronary artery ligation. In untreated rats with large myocardial infarction (MI), developed tension (DT) (3.0±0.7 versus 5.1±1.1 g/mm²), peak rate of tension rise (+dT/dt) (40.3±9.5 versus 71.2±12.0 g/mm²/sec), and peak rate of tension fall (−dT/dt) (24.4±5.0 versus 38.2±6.0 g/mm²/sec) were decreased \((p<0.05)\). In addition, DT, +dT/dt, and −dT/dt of untreated MI rats demonstrated an impaired response to isoproterenol stimulation compared with controls. \( \beta \)-Adrenergic receptor density \((B_{max})\) measured by \( [\text{I}^{125}] \text{Iodocyanopindolol (ICYP)} \) binding was decreased 23% after infarction (9.3±0.6 versus 12.0±1.8 fmol/mg protein \([\text{prot}]\) \((p<0.05)\); however, the dissociation constant \((K_d)\) for ICYP was not changed (24.1±5.7 versus 33.2±12.1 pM). Adenylate cyclase activity in the presence of \( 10^{-2} \) M MgCl\(_2\) was reduced \((p<0.05)\) in MI rats (30.3±10.8 versus 45.9±12.5 pmol cAMP/min/mg prot). Maximal isoproterenol (52.5±7.3 versus 79.9±10.0 pmol cAMP/min/mg prot), guanyl-5'-imidodiphosphate (GppNHp) (95±8 versus 141±25 pmol cAMP/min/mg prot) and forskolin (503±76 versus 753±157 pmol cAMP/min/mg prot) stimulation of adenylate cyclase was also decreased \((p<0.05)\). In addition, manganese-stimulated adenylate cyclase activity was depressed \((p<0.05)\) in MI rats compared with controls (23.5±2.8 versus 52.1±9.0 pmol cAMP/min/mg prot). Chronic propranolol treatment in MI rats improved DT (4.1±0.9 versus 3.0±0.7 g/mm²) and +dT/dt (54.4±11.3 versus 40.5±9.5 g/mm²/sec) \((p<0.05)\); however, isoproterenol-stimulated isometric function remained impaired. Propranolol treatment normalized \(B_{max}\) (11.9±1.7 versus 9.3±0.6 fmol/mg prot) \((p<0.05)\), whereas adenylate cyclase activity remained depressed.

**Conclusions.** After large MI in rats, there is impaired papillary muscle function with decreased \( \beta \)-adrenergic receptors and adenylate cyclase activity in the noninfarcted myocardium. Propranolol treatment improved basal isometric muscle function and \( \beta \)-adrenergic receptor density in rats after myocardial infarction but did not improve adenylate cyclase activity or isoproterenol-stimulated muscle function. These data suggest that there is a primary defect in adenylate cyclase function that persists despite upregulation of receptors with propranolol treatment. (Circulation 1992;86:1584–1595)

**Key Words** • heart failure • myocardial infarction • contractility • propranolol • adenylate cyclase

Therapy with \( \beta \)-adrenergic receptor blockade has been shown to prolong survival after myocardial infarction (MI)\(^1\) and to improve hemodynamics, exercise tolerance, and survival in patients with idiopathic cardiomyopathy.\(^2,5\) Although \( \beta \)-blocker treatment is generally not administered to patients with decompensated heart failure, the beneficial effects of \( \beta \)-adrenergic receptor blockade after MI seem to be enhanced in those patients with more severe degrees of left ventricular (LV) dysfunction.\(^6\)

Studies in humans and in animal models of heart failure have demonstrated that alterations of the \( \beta \)-adrenergic receptor pathway accompany the various forms of heart failure.\(^7,8\) A decrease in \( \beta \)-adrenergic receptor function...
density, subsensitivity of these receptors to β-adrenergic stimulation, and abnormalities in receptor coupling have been documented. Investigators have also demonstrated that long-term β-adrenergic blockade in humans with dilated idiopathic cardiomyopathy results in increased myocardial β-adrenergic receptor density, with significant improvements in resting and dobutamine-stimulated hemodynamics.5 β-Adrenergic blockade in dogs with decompensated right ventricular failure secondary to pressure and volume overload results in improved myocardial isometric function and upregulation of β-adrenergic receptors.9 These and other studies lend support to the hypothesis that in severe heart failure, β-adrenergic receptor downregulation and contractile dysfunction occur and that β-adrenergic blockade may attenuate these abnormalities.

Despite these advances in our understanding of other forms of heart failure, relatively little is known of β-adrenergic receptor system function in ischemic LV dysfunction, the most common cause of heart failure in this country. Increasing data suggest that there is less downregulation and greater uncoupling of the β-adrenergic receptor system in patients with end-stage ischemic cardiomyopathy than in those with dilated idiopathic cardiomyopathy.10 Despite this new information, however, no studies to date have defined the effects of β-adrenergic blockade on myocardial and receptor function in heart failure secondary to MI. Studies from our laboratory11 and others12 suggest that β-adrenergic blockade may promote LV dilatation in this model of ischemic heart failure. Global LV function was neither improved nor impaired by propranolol treatment. In humans, data on the effects of β-adrenergic blockade in ischemic heart failure are limited.

The present study was undertaken to define the effects of chronic β-adrenergic receptor blockade on myocardial function and on the β-adrenergic receptor–adenylate cyclase system in rats with compensated heart failure after large MI. This widely studied model of ischemic heart failure allows analysis of β-adrenergic receptor function before evolution of decompensated LV failure, when extremes of circulatory adaptations can potentially distort the steady-state role of the β-adrenergic system in heart failure. Because of the segmental nature of ischemic heart disease, we chose to study muscle function of the noninfarcted papillary muscle to better elucidate changes in residual myocardial function. We hypothesized that in early compensated heart failure after MI, the β-adrenergic receptor system manifests abnormalities that are associated with impaired myocardial responsiveness to β-adrenergic stimulation. We also hypothesized that β-adrenergic blockade with propranolol may upregulate the β-adrenergic receptors, leading to improved muscle function in the noninfarcted myocardium with improved responsiveness to catecholamine stimulation.

**Methods**

MI was produced in male Sprague-Dawley rats (Harlan, Indianapolis, Ind.) (weight, 220–260 g) by techniques described below. Rats were screened for evidence of large MI 21 days after the procedure by surface electrocardiographic recording. Previous studies have shown that rats identified in this manner have infarctions of >35% of the left ventricle.11,13 Animals meeting ECG criteria for large infarctions and those without evidence for infarctions (sham-operated) were randomly assigned to receive either DL-propranolol hydrochloride (Sigma Chemical Co., St. Louis, Mo.) (500 mg/l) in their drinking water or drinking water alone for a period of 6 weeks. This dose and duration of propranolol treatment has previously been shown to produce β-adrenergic blockade with a significant decrease in basal heart rate and a rightward and downward shift in heart rate response to isoproterenol in both sham-operated and MI rats.12 Our laboratory has previously reported hemodynamics in a cohort of untreated and propranolol-treated rats after MI.11 In untreated rats with MI size of 36±2%, LV end-diastolic pressure was 27±1 mm Hg, and LV dP/dt was 6,188±496 mm Hg versus 6±1 mm Hg and 8,909±445 mm Hg, respectively, for control rats. Propranolol-treated MI rats with similar MI size (36±1%) likewise demonstrated elevated LV end-diastolic pressure (24±4 mm Hg) and depressed LV dP/dt (6,050±482 mm Hg/sec). Because anesthetics required for conscious hemodynamics may adversely affect β-adrenergic receptor analysis, hemodynamics were not repeated in this study. After 6 weeks of treatment, animals were killed by decapitation, and papillary muscle studies were performed. The remaining heart tissue was weighed and processed for β-adrenergic system analysis and infarct size determination. Because of technical considerations affecting tissue handling and storage, 34 of 44 animals studied had both muscle function and β-adrenergic studies performed. Hearts from the 10 remaining animals were used for either muscle function (five animals) or β-adrenergic studies (five animals).

**Production of MI**

Under anesthesia with acepromazine (2.4 mg/kg i.m.) and ketamine (125 mg/kg i.m.), a left anterior thoracotomy was performed under sterile conditions. The heart was expressed through the incision, and the left atrium was retracted to expose the proximal left anterior descending coronary artery. A 7-0 synthetic ligature was secured snugly around the artery, and the heart was replaced in the chest cavity. The thoracotomy was then sutured, and the rats were allowed to recover with free access to water and standard rat chow.

**Isometric Muscle Studies**

Noninfarcted posterior papillary muscles were rapidly dissected free from the LV wall in an oxygenated dissecting bath and suspended vertically from an isometric force transducer (Mettrigram, Gould Instruments, Cleveland, Ohio) in a tissue bath containing modified Krebs-Henseleit solution: 120 mM NaCl, 5.9 mM KCl, 5.5 mM dextrose, 25 mM NaHCO₃, 1.2 mM NaH₂PO₄, 1.2 mM MgCl₂, and 2.5 mM CaCl₂. The bath was maintained at a constant temperature of 30°C and bubbled with 95% O₂ and 5% CO₂. The muscle was stimulated (S44, Grass Instruments, Quincy, Mass.) to contract isometrically at 0.33 Hz by use of field stimulation delivered through a pair of platinum electrodes placed parallel to the muscle. Five-millisecond pulses at a voltage approximately 10% above threshold were used. The muscle was allowed to stabilize for 1–2 hours at a resting tension of approximately 1 g and then was stretched to the length at which maximum tension...
development occurs ($L_{\text{max}}$). Muscle length was measured at $L_{\text{max}}$ with a calibrated telemicroscope (M101AT, Gaertner Scientific Corp., Chicago, Ill.). Tension was recorded on a physiological recorder (Gould), and digitized data points recorded at 500 Hz were stored on-line onto an IBM AT computer with customized software. Isometric contractions were recorded before and after the addition of $\alpha$-isoproterenol HCl (Sigma), which was added serially to produce increasing final concentrations in the bath at logarithmic molar unit increments ranging from $10^{-9}$ to $10^{-4}$ M. All measurements were normalized to papillary muscle cross-sectional area (CSA) determined at $L_{\text{max}}$. Assuming cylindrical geometry and a specific gravity of 1.05, papillary muscle CSA was calculated as

$$\text{CSA} = \text{muscle mass}/1.05/L_{\text{max}}$$

**Myocardial Stiffness**

After the above measurements were completed, the muscle was passively stretched at a constant rate (1.4 mm/min) to a maximum stress of 3 g. A slow rate of stretch was chosen to avoid viscous effects. Tension was recorded as above, except the acquisition rate was 50 Hz. Natural strain (in L/Lo, where $L_o$=length at 0.1 g/mm² stress and L=instantaneous length) was calculated from digitized data points on the tension-versus-time curve. Three separate stretches of each muscle were done to confirm reproducibility. Muscle length was measured at a stress of 3 g. Muscle length at each digitized point was calculated from the known rate and duration of stretch. The muscle stiffness constant ($K_m$) was calculated using a modification of previously described methods.¹⁴

**Tissue Preparation**

Left ventricles with the septum intact were dissected from right ventricles, weighed, frozen rapidly on dry ice, and stored intact at $-80^\circ$C. While thawing, tissues were placed in ice-cold (0–4°C) buffer, and the following procedures were performed on ice until time of incubation or refreezing. The LV scar was removed, and the remaining tissue was bluntly minced with scissors. It should be noted that the scar is well formed and readily visible at this point after infarction. Tissues were placed in a calculated volume of 30 mM Tris HCl buffer, pH 7.5, so that the final concentration in the assay was 0.5% by weight. Tissues were homogenized by three repetitive 10-second cycles with 30-second pauses using a Brinkman Polytron at high speed (setting 8) and then filtered through four layers of Nitex dense-pore silk screen (Calcom Inc., Berkeley, Calif.). Assays for $\beta$-adrenergic receptors were performed immediately using whole homogenate tissue preparations. Binding assays were performed with a crude tissue preparation to minimize receptor loss and other artifacts caused by centrifugation. Homogenates used for the cyclic AMP (cAMP) assays were centrifuged twice at 30,000 g for 20 minutes and rinsed with 30 mM Tris HCl buffer, pH 7.5, between each spin. The resulting pellet was then stored at $-80^\circ$C until assays were performed.

**$\beta$-Adrenergic Receptor Binding Assay**

$\beta$-Adrenergic receptor density was determined by a method modified from Engel et al.¹⁵ using specific binding of [³²P]iiodocyanopindolol (ICYP) (2,200 Ci/mmol; NEN, Boston) (2–200 pM, eight concentrations) to the tissue homogenates from the noninfarcted left ventricle. Binding experiments were performed in triplicate in a 50 mM Tris HCl buffer, pH 7.5, containing 10 mM MgCl₂ in an assay volume of 100 μl for 30 minutes at 37°C. The binding reaction was terminated immediately by rapid vacuum filtration through glass fiber filters (Whatman GF/C) with a Brandel cell harvester. The filters were rinsed three times with cold 50 mM Tris HCl buffer, pH 7.5. The radioactivity remaining on the filters was counted at 72% efficiency in a gamma counter. Nonspecific binding was determined by the addition of 1 μM l-propranolol to paired assay tubes. Specific binding of ³²P-IcYP ranged from 80% to 90% of total binding at radioligand concentrations near the dissociation constant ($K_d$) values (20–30 pM). Receptor density and antagonist $K_d$ were determined by the nonlinear least-squares regression method. Receptor density was expressed per milligram of protein determined by a method modified from Lowry et al.¹⁶ using bovine serum albumin (BSA) as a standard.

**Adenylate Cyclase Activity**

The frozen pellet from the tissue preparation described above was resuspended in the original volume in fresh adenylate cyclase buffer. Adenylate cyclase activity was assayed in duplicate by modifications of methods previously described.¹⁷¹⁸ Preliminary assays were performed to verify tissue linearity, time course of incubation, and MgCl₂ dose response. In a final volume of 200 μl, the assay mixture contained 30 mM Tris-HCl, 30 mM Tris base, 10 mM MgCl₂, 1 mM 3-isobutyl-1-methylxanthine (IBMX), 100 mM NaCl, 50 mM sucrose, 0.5 mM EGTA, 0.5 mM dithiothreitol (DTT), and regenerating solution at a final pH 7.5. The regenerating solution consisted of the following (final concentration): 30 IU/ml creatine phosphokinase, 5 mM phosphocreatine, 1.3 mM ATP, and 0.5% BSA. The reaction was started by the addition of tissue (20–60 μg protein) to the assay tubes. Adenylate cyclase activity was stimulated with $\alpha$-isoproterenol ($10^{-9}$–$10^{-4}$ M), guanyl 5’-imidodiphosphate (GppNHp) ($10^{-3}$–$10^{-4}$ M), and forskolin ($10^{-3}$–$10^{-4}$ M). Isoproterenol stimulation was performed in the presence of 0.1 mM GppNHp. Incubations were carried out for 10 minutes at 37°C and terminated by addition of cold 50 mM Tris HCl buffer, pH 7.5, containing 4 mM EDTA followed by boiling for 7 minutes. cAMP levels were determined by a competitive protein binding assay according to a method modified from Gilman.¹⁹ In brief, the above assay solution was centrifuged at 5,600 g for 2 minutes. A 50-μl aliquot of the supernatant was incubated on ice for 2 hours with 50 μl (0.9 pmol) of [³²P]cAMP (31.3 Ci/mmol; NEN) and 100 μl of 50 mM Tris HCl buffer, pH 7.5, containing 4 mM EDTA, 6 μg of cAMP-dependent protein kinase (Sigma), and 0.1 mg of BSA. Standard solutions containing cAMP (0.13–64 pmol) were incubated under similar conditions in parallel. At the end of the incubation, free [³²P]cAMP was absorbed with 100 μl of ice-cold activated charcoal (Norit Ultra C, American Norit Co., Jacksonville, Fla.) in 50 mM Tris HCl buffer, pH 7.5, containing 4 mM EDTA and 2% BSA. The mixture was centrifuged at 5,600 g for 45 seconds to pellet the charcoal. Bound [³²P]cAMP in the 200 ml of
the supernatant was measured by scintillation counting. Again, protein was determined by a method modified from Lowry. Nonlinear least-squares regression analyses were used to calculate maximum velocity of the reaction ($V_{max}$), concentration of agent yielding half-maximal enzyme activity ($K_{act}$), and Hill slopes. Based on the analysis of the results obtained from the initial set of experiments, four or five samples from each group were analyzed for manganese-stimulated adenylate cyclase activity. Manganese ($10^{-2}$ M MnCl$_2$) stimulation was performed as above with the deletion of MgCl$_2$ from the assay conditions. Assays were performed in the presence of MnCl$_2$ alone and with the addition of propranolol ($10^{-6}$ M) and/or forskolin ($10^{-4}$ M).

**Histological Studies**

MI size was measured in a group of animals by use of techniques previously described by our laboratory. In brief, the separated left ventricle plus septum was dissected into four transverse slices from apex to base that were immersion-fixed in 10% formalin and embedded in paraffin. Thin sections of the ventricle were stained with Masson's trichrome, and infarct size was measured by tracing the outline of the infarcted and noninfarcted regions of the left ventricle at each of the four levels. Infarct size is reported as the mean percentage of epicardial and endocardial circumference occupied by scar tissue for the four sections. All hearts processed for MI sizing were examined histologically, demonstrating well-demarcated scar without focal scar in the remaining left ventricle. These findings have been reported previously by our laboratory.

All papillary muscles were processed for histological analysis to exclude presence of scar. Papillary muscles were immersion-fixed in 10% buffered formalin and embedded in plastic. Cross-sectional slices were made at multiple levels and stained with toluidine blue. Histological specimens were reviewed, with the operator blinded to the experimental group during analysis. Figure 1 demonstrates a cross section of a papillary muscle from an untreated infarcted rat. Note the absence of discrete scar tissue. Any animal with either gross or microscopic evidence of scar in the posterior papillary muscle was excluded from the study.

**Statistical Analysis**

All reported values are expressed as mean±SD except where specified. Isometric parameters are the means of three to five twitches, and muscle stiffness values are the means of at least three stretches for each muscle. All data were analyzed by a factorial one-way ANOVA. Intergroup comparisons were made with a Student-Newman-Keuls multiple comparisons test. For isoproterenol stimulation studies, two-way ANOVA was also used for intergroup comparisons to evaluate for possible interaction between infarction and propranolol treatment. Peak isoproterenol-stimulated isometric function was compared with basal unstimulated function by a paired t test. Significance was defined at the $p<0.05$ level.

**Results**

In total, 44 rats were studied. Two rats from the untreated infarct group and one rat from the treated infarct group were found to have moderate-sized rather than large infarction (<35%). Because of the small number of this group, these animals were not included in the analysis.

**Morphological Data**

There were no significant differences in body weight, LV weight, or LV-to-body weight ratio among the four
groups of animals (Table 1). Right ventricular weight and right ventricular-to-body weight ratio were increased significantly after MI \((p<0.05)\). Propranolol treatment had no significant effect on these parameters in MI or control animals. Infarct size was comparable in treated \((n=5)\) and untreated \((n=6)\) rats \((43\pm2\%\) versus \(44\pm8\%),\) with ranges of \(41\% - 46\%\) and \(38\% - 51\%,\) respectively. There were no significant differences in papillary muscle weight among the four groups of animals (Table 2). There was a trend for \(L_{\text{max}}\) to be decreased in infarct animals, with a significant decrease in untreated infarct rats compared with treated controls. Papillary muscle CSA was increased in MI animals compared with sham-operated animals; however, there were no differences in papillary CSA between treated and untreated infarct animals.

**Baseline Papillary Muscle Mechanics**

Animals with MI had significant alterations in papillary muscle mechanics (Table 3). Peak isometric developed tension \((DT)\), rate of tension rise \((+dT/dt)\), and rate of tension fall \((-dT/dt)\) were decreased \((p<0.05)\) in MI rats compared with controls. Time to peak tension \((TPT)\) showed a trend to increase in MI rats, but this difference was not significant. Although time to 50% tension decline, or relaxation half-time \((T\frac{1}{2}\text{R})\), was unchanged in infarct animals, passive muscle stiffness, as determined by \(K_m\), was increased in infarct rats compared with controls \((p<0.05)\). These changes in papillary muscle mechanics seen in the MI rats are consistent with those previously reported by our laboratory.\(^{14}\)

In propranolol-treated MI animals, DT, +dT/dt, and -dT/dt were significantly improved \((p<0.05)\) compared with untreated MI animals. This beneficial effect of propranolol was specific for infarct animals, with no demonstrable effect of treatment on sham-operated controls. Propranolol treatment had no significant effect on TPT, T\(\frac{1}{2}\text{R}\), or \(K_m\) in either control or infarct animals.

**Stimulated Papillary Muscle Function**

Dose-response curves for selected isometric parameters to cumulative doses of isoproterenol in the four groups of animals are shown in Figure 2. Data are shown as the increase over baseline to compensate for differences in baseline function. Spontaneous contractions developed in the papillary muscles of two of the untreated infarct animals and three of the propranolol-treated infarct animals; hence, these muscles were excluded from analysis. Comparing peak response with baseline, untreated and propranolol-treated sham-operated animals demonstrated increased peak +dT/dt and peak -dT/dt with isoproterenol stimulation \((p<0.005)\), whereas only untreated controls showed a significant increase in DT with isoproterenol stimulation. In contrast, MI rats showed an impaired response to isoproterenol stimulation in +dT/dt and -dT/dt and demonstrated a decline in DT with increasing isoproterenol concentrations. Analyzing maximal change from baseline (Table 4), this impaired response to isoproterenol stimulation was significant for DT, +dT/dt, and -dT/dt compared with controls. Propranolol treatment did not improve the response to isoproterenol stimulation, with treated MI rats demonstrating a depressed response in +dT/dt and -dT/dt and a decline in DT similar to untreated MI rats. There were no significant differences in maximal response to isoproterenol for any parameter comparing propranolol-treated rats with untreated MI rats. It should be noted that propranolol-treated control animals demonstrated an intact response to isoproterenol stimulation, with a trend for the dose-response curve to be shifted downward and rightward. However, there were no significant differences in peak responses in any parameters compared with untreated controls. This suggests that there is minimal residual propranolol

**Table 1. Body Weights, Heart Weights, Ratios of Heart Weight to Body Weight, and Infarct Sizes in Untreated and Propranolol-Treated Sham-Operated and Infarct Rats**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=10)</th>
<th>Control/prop (n=12)</th>
<th>Infarct (n=9)</th>
<th>Infarct/prop (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>415±52</td>
<td>393±31</td>
<td>410±28</td>
<td>403±37</td>
</tr>
<tr>
<td>RV (mg)</td>
<td>180±52</td>
<td>167±28</td>
<td>414±5*</td>
<td>376±95*</td>
</tr>
<tr>
<td>RV/BW (mg/g)</td>
<td>0.44±0.08</td>
<td>0.43±0.07</td>
<td>1.02±0.17*</td>
<td>0.94±0.28*</td>
</tr>
<tr>
<td>LV (mg)</td>
<td>821±65</td>
<td>791±49</td>
<td>856±65</td>
<td>844±76</td>
</tr>
<tr>
<td>LV/BV (mg/g)</td>
<td>1.99±0.16</td>
<td>2.01±0.24</td>
<td>2.09±0.11</td>
<td>2.10±0.21</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>. . .</td>
<td>. . .</td>
<td>43±2</td>
<td>44±8</td>
</tr>
</tbody>
</table>

Prop, propranolol; BW, body weight; RV, right ventricular weight; RV/BW, ratio of right ventricular to body weight; LV, left ventricular weight; LV/BW, ratio of left ventricular to body weight. Values are mean±SD. \(^{*}p<0.05\) vs. control.

**Table 2. Papillary Muscle Dimensions in Untreated and Propranolol-Treated Sham-Operated and Infarct Rats**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=9)</th>
<th>Control/prop (n=10)</th>
<th>Infarct (n=9)</th>
<th>Infarct/prop (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pap wt (mg)</td>
<td>5.5±1.8</td>
<td>6.1±2.3</td>
<td>6.5±1.7</td>
<td>6.8±2.3</td>
</tr>
<tr>
<td>(L_{\text{max}}) (mm)</td>
<td>5.7±1.1</td>
<td>6.0±1.2</td>
<td>4.5±1.1</td>
<td>4.9±1.4</td>
</tr>
<tr>
<td>CSA (mm(^2)</td>
<td>1.0±0.3</td>
<td>1.0±0.2</td>
<td>1.4±0.3*</td>
<td>1.3±0.3*</td>
</tr>
</tbody>
</table>

Prop, propranolol; Pap wt, papillary muscle weight; \(L_{\text{max}}\), length of papillary muscle at maximum developed tension; CSA, cross-sectional area of papillary muscle. Values are mean±SD. \(^{*}p<0.05\) vs. control.
in the tissue preparation. Hence, it is unlikely that the impaired isoproterenol response seen in treated MI rats was a result of the persistent β-adrenergic blockade effect of propranolol. Two-way analysis showed a significant effect of infarction (p<0.0001) for DT, +dT/dt, and −dT/dt but no significant effect of propranolol treatment alone. There was a trend toward an interaction (p=0.06) between propranolol treatment and infarction for +dT/dt only; however, this interaction was not significant. TPT and T½R shortened significantly (p<0.005) with isoproterenol stimulation in all groups, with no significant intergroup differences in maximal responses.

**β-Adrenergic Receptor Density and Adenylate Cyclase Activity**

-Adrenergic receptor density was decreased (p<0.05) by 23% in noninfarced myocardium after large MI (Table 5). The antagonist Kᵦ was not changed. Treatment with propranolol resulted in an upregulation of β-adrenergic receptors in MI rats (11.9±1.7 versus 9.3±0.6) (p<0.05), with no effect on β-adrenergic receptor density in control animals. Propranolol treatment had no significant effect on the Kᵦ in either infarct or control animals.

Adenylate cyclase activity in the presence of 10⁻² M MgCl₂ was reduced 34% in animals after infarction compared with sham-operated controls (Table 6). Dose–response curves for isoproterenol-, GppNHp-, and forskolin-stimulated adenylate cyclase activity are shown in Figure 3. Unfitted data are shown as the increase over baseline activity to compensate for the differences in the nonstimulated cyclase activity. For all three agents, the Vₘₐₓ was decreased in the infarct group compared with controls (p<0.05), with no differences in the slope of the curves or the Kᵦ (Table 6). The decrease in Vₘₐₓ for forskolin stimulation was 33% in MI rats. Interestingly, the magnitudes of the deficit seen in Vₘₐₓ with GppNHp and isoproterenol stimulation were similar. Manganese (MgCl₂)-stimulated adenylate cyclase activity was also depressed after infarction (Table 7). MnCl₂ stimulation alone and in the presence of propranolol, forskolin, or both was reduced approximately 50% in infarct rats compared with controls (p<0.05).

Propranolol treatment had no effect on adenylate cyclase activity in the presence of MgCl₂ in control or infarct animals. Likewise, propranolol treatment had no significant effect on isoproterenol-, GppNHp-, forskolin-, or manganese-stimulated adenylate cyclase activity, with treated infarct animals demonstrating an impaired response. Thus, despite an upregulation of β-adrenergic receptors, the adenylate cyclase activity remained impaired in the propranolol-treated infarct animals.

**Discussion**

The results of this study suggest that chronic propranolol treatment in rats after large MI improves resting isometric muscle function in the residual noninfarcted myocardium. There were significant improvements in DT and peak +dT/dt in propranolol-treated infarct rats. This beneficial effect of propranolol treatment was seen only in infarcted animals, because treatment had no effect in the sham-operated animals. To our knowledge, this is the first study to demonstrate that propranolol may improve muscle function in a model of ischemic LV failure.

**β-Adrenergic Receptor System: Effects of Propranolol**

We had hypothesized that the improved contractile function may occur with an upregulation of β-adrenergic receptors with propranolol treatment. Untreated heart failure animals demonstrated a significant decrease in β-adrenergic receptor density and decreased basal (10⁻² M MgCl₂) adenylate cyclase activity. In addition, isoproterenol-, forskolin-, GppNHp-, and manganese-stimulated adenylate cyclase activity were decreased. In agreement with our hypothesis, propranolol treatment increased β-adrenergic receptor density in heart failure rats. However, adenylate cyclase activity remained abnormal. In addition, papillary muscle function with isoproterenol stimulation in propranolol-treated MI rats remained impaired despite an increase in β-adrenergic receptor density. These findings suggest that there is an intrinsic defect in adenylate cyclase function that is not improved with β-adrenergic blockade. Hence, the improvement in muscle function after chronic propranolol treatment appears to involve mechanisms unrelated to the classic β-adrenergic receptor second messenger (cAMP).

Similar findings of impaired adenylate cyclase function have been reported in humans with ischemic cardiomyopathy and some experimental models of heart failure. Proposed mechanisms for decreased adenylate cyclase activity in heart failure include a possible defect in G-protein coupled activity or a primary defect.
in the catalytic subunit of adenylate cyclase. In this study, manganese-stimulated adenylate cyclase activity was decreased in untreated and treated MI rats, even in the presence of propranolol or forskolin. Manganese activation of adenylate cyclase is believed to be unaffected by the presence of G-proteins and hence, to reflect adenylate cyclase catalytic subunit activity. Also, in our studies, forskolin stimulation was performed in the absence of GppNHp, thus removing potential effects of G-protein interaction. These data suggest that there is a primary defect in the catalytic subunit of adenylate cyclase in this model of ischemic LV failure, which is unaffected by propranolol treatment.

A defect in the catalytic subunit of adenylate cyclase has been reported in pressure-overloaded human myocardium and in the pacing overdrive dog model. In humans with ischemic cardiomyopathy, adenylate cyclase function is also impaired; however, recent data suggest that the primary abnormality results from receptor uncoupling. Most important, data from Bristow and associates show that there are significant differences between patients with ischemic cardiomyopathy and those with dilated idiopathic cardiomyopathy, in whom downregulation of β-adrenergic receptors rather than receptor uncoupling predominates. Studies in different experimental models report various degrees of β-adrenergic receptor downregulation and receptor uncoupling. These differences in the β-adrenergic receptor system are of great importance in determining the role of β-adrenergic blocker treatment in various forms of heart failure.

Our findings correlate with those reported in humans with ischemic cardiomyopathy because adenylate cyclase dysfunction, rather than β-adrenergic receptor
downregulation, predominates. The effects of β-adrenergic blockade in heart failure has been evaluated primarily in humans with dilated idiopathic cardiomyopathy and in pulmonary artery–banded dogs.9 These data suggest that in heart failure where adenylate cyclase function is intact, upregulation of β-adrenergic receptors with chronic β-adrenergic blockade leads to improved hemodynamic response to catecholamine stimulation. Our data suggest that in ischemic LV failure, chronic β-adrenergic blockade may not improve myocardial responsiveness to catecholamine stimulation because of persistent adenylate cyclase dysfunction. Other investigators have reported that early treatment with metoprolol in guinea pigs prevented decreases in receptor density and stimulated adenylate cyclase activities in right ventricle three days after MI.22 Because of known regional variations in right ventricular and LV β-adrenergic receptor distribution and the lack of persistent abnormalities in receptor density and adenylate cyclase activities at 6 days in their study, it is difficult to compare these findings. It is possible that earlier treatment may attenuate changes in adenylate cyclase function. Nonetheless, the improvement seen in basal myocardial function suggests that these agents may be of benefit in ischemic heart failure.

**Mechanisms of Improved Contractility**

The improvements seen in baseline DT and +dT/dt in treated MI rats may be related to several possible mechanisms, including 1) increased calcium availability or responsiveness, 2) an increased number of myocardial crossbridges or contractile units, or 3) alterations in extracellular factors such as interstitial fibrosis. Because our study suggests that adenylate cyclase activity remains depressed, an improvement in contractility resulting from increased calcium availability would be via pathways independent of cAMP. Some data demonstrate that a stimulatory G-protein directly activates calcium channels in cardiac tissue, independent of cAMP-dependent protein kinases.23 Because this interaction still requires occupation of the β-adrenergic receptor, an upregulation of β-adrenergic receptor density with β-adrenergic blockade may enhance calcium channel function and hence, contractility, despite a persistent abnormality in adenylate cyclase function. Other investigators suggest that the depressed contractility seen in the noninfarcted myocardium is related to an altered responsiveness of the myofilaments to activator calcium rather than a decreased availability of calcium. Litwin and Morgan24 found that peak calcium, as detected by aequorin signalling, was not depressed in papillary muscles from MI rats, suggesting that the myofilaments are less responsive to activator calcium. It has been speculated that high circulating catecholamine levels may lead to “calcium overload” with subsequent altered sensitivity of the contractile apparatus to calcium. Our laboratory has shown that in this model, as in humans, there are high circulating levels of norepinephrine.25 In rats exposed to catecholamine excess with isoproterenol, it has been shown that there is an initial calcium overload, with subsequent depression of sarcoplasmic reticular calcium pump activities.26 There is also a concurrent decrease in contractility with longer exposure to isoproterenol. In the present study, papillary muscles from MI rats demonstrated a decline in DT with increasing concentrations of isoproterenol. Other investigators report that this decline in DT occurs in muscles from MI rats24 and in spontaneously hypertensive rats with heart failure27 despite an appropriate increase in activator calcium with isoproterenol stimulation. Again, this may suggest that the myofilaments have an altered responsiveness or sensitivity to calcium. The existing high catecholamine state after a large MI may lead to altered myofilament responsiveness that is exaggerated in the presence of exogenous isoproterenol. Of note, propranolol treatment did not prevent this decline in DT in MI rats, despite improving baseline function. This suggests that

### Table 4. Maximal Isometric Response to Isoproterenol in Papillary Muscles From Untreated and Propranolol-Treated Sham-Operated and Infarct Rats

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>Control/prop (n=7)</th>
<th>Infarct (n=7)</th>
<th>Infarct/prop (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT (g/mm²)</td>
<td>0.3±0.2</td>
<td>0.1±0.2</td>
<td>−0.4±0.2*</td>
<td>−0.5±0.3*</td>
</tr>
<tr>
<td>+dT/dt (g/mm²/sec)</td>
<td>24.9±4.4</td>
<td>18.6±5.9</td>
<td>2.8±4.0*</td>
<td>4.8±5.6*</td>
</tr>
<tr>
<td>−dT/dt (g/mm²/sec)</td>
<td>18.8±5.4</td>
<td>16.3±2.5</td>
<td>5.6±5.0*</td>
<td>7.2±5.3*</td>
</tr>
<tr>
<td>TPT (msec)</td>
<td>−29.4±5.7</td>
<td>−26.7±5.1</td>
<td>−27.0±6.0</td>
<td>−32.4±7.8</td>
</tr>
<tr>
<td>TV½R (msec)</td>
<td>−41.9±8.3</td>
<td>−43.2±9.3</td>
<td>−39.8±8.3</td>
<td>−40.5±14.3</td>
</tr>
</tbody>
</table>

Prop, propranolol; DT, developed tension; +dT/dt, peak rate of tension rise; −dT/dt, peak rate of tension fall; TPT, time to peak tension; TV½R, time to 50% tension fall from maximal tension. Values are mean±SD and represent maximal response minus baseline.

*p<0.05 vs. control.

### Table 5. β-Adrenergic Receptor Density and Antagonist Dissociation Constant in Noninfarcted Left Ventricular Myocardium From Untreated and Propranolol-Treated Sham-Operated and Infarct Rats

<table>
<thead>
<tr>
<th></th>
<th>Control (n=11)</th>
<th>Control/prop (n=11)</th>
<th>Infarct (n=7)</th>
<th>Infarct/prop (n=10)</th>
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</thead>
<tbody>
<tr>
<td>B&lt;sub&gt;m&lt;/sub&gt; (fmol/mg prot)</td>
<td>12.0±1.8</td>
<td>11.5±1.4</td>
<td>9.3±0.6*</td>
<td>11.9±1.7†</td>
</tr>
<tr>
<td>K&lt;sub&gt;d&lt;/sub&gt; (pM)</td>
<td>33.2±12.1</td>
<td>37.8±15.8</td>
<td>24.1±5.7</td>
<td>32.7±10.6</td>
</tr>
</tbody>
</table>

Prop, propranolol; B<sub>m</sub>, β-adrenergic receptor density; prot, protein; K<sub>d</sub>, antagonist dissociation constant. Values are mean±SD.

*p<0.05 vs. control; †p<0.05 vs. untreated infarct.
propranolol treatment does not attenuate the acute negative effects of isoproterenol in the MI rats and that mechanisms other than altered calcium responsiveness may be at play.

Another mechanism by which propranolol treatment may improve contractility is by prevention of myocyte injury and loss, thus increasing the numbers of contractile units. Some data suggest that high levels of systemic catecholamines have deleterious effects such as myocyte hypertrophy, necrosis, and death.28,29 Fishbein et al12 reported that propranolol treatment in this model attenuates myocyte hypertrophy in the noninfarcted myocardium of the MI rats. In addition, the number of myocytes per unit area was increased after propranolol treatment. Hence, propranolol treatment may prevent myocyte loss over time by mechanisms that remain uncertain. Eichhorn et al4 found that bucindolol treatment in patients with dilated cardiomyopathy increases stroke work without an increase in oxygen consumption. They postulated that β-adrenergic blockade may have favorable effects on myocardial energetics, possibly by suppressing catecholamine-induced fatty acid oxidation, permitting more efficient oxygen use.

Finally, unfavorable alterations in the extracellular matrix may be prevented by propranolol treatment. Our laboratory has previously shown that there is an increased passive collagen content and increased stiffness in the residual myocardium.14 The lack of improvement in passive muscle stiffness after propranolol treatment does not support an attenuation of the interstitial fibrosis; however, collagen analyses were not performed in this study.

Ventricular Remodeling

It should be noted that studies in this model have suggested that propranolol treatment promotes LV dilatation, which could be unfavorable or even deleterious.11,12 Concurrent hemodynamic studies in this model by our laboratory show that LV systolic function is preserved despite an increase in LV cavity size after propranolol treatment.11 Because of the segmental nature of ischemic heart failure, we chose to study isolated muscle preparation to better elucidate changes in the function of the noninfarcted myocardium and the relation of these changes to the β-adrenergic receptor system. The improvement seen in isometric function of the noninfarcted muscle after propranolol treatment in this model is not correlated with a change in overall systolic function. Studies in humans have not demonstrated promotion of LV dilatation after β-adrenergic receptor blockade, and indeed, LV dimensions have been improved in some studies; however, these studies have not included many patients with ischemic cardiomyopathy.4 Hence, it is possible that these agents may exert a cardioprotective effect without deleterious effects on ventricular remodeling in ischemic heart disease; however, further studies are necessary.

Myocardial Relaxation

In this study, there were no significant abnormalities in resting or isoproterenol-stimulated relaxation (T½R) in the noninfarcted papillary muscle in heart failure rats. In addition, propranolol treatment did not alter resting or isoproterenol-stimulated myocardial relaxation in control or MI rats. These findings of preserved lusitropic response to catecholamine stimulation in heart failure are consistent with those reported in other models27 and in humans.30,31 In addition, data in humans suggest that lusitropic response to catecholamine stimulation is preserved in patients with ischemic cardiomyopathy as well as those with dilated idiopathic cardiomyopathy. This divergence of cAMP-dependent inotropic and lusitropic functions has been evaluated by others.30 It has been suggested that the lusitropic path-

### Table 6. Adenylate Cyclase Activity in the Presence of 10⁻² M MgCl₂ in the Assay Buffer With Vₘₐₓ, Kₘₐₓ, and Hill Slopes for Isoproterenol-, GppNHP-, and Forskolin-Stimulated Adenylate Cyclase Activity in Untreated and Propranolol-Treated Sham-Operated and Infarct Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control/prop</th>
<th>Infarct</th>
<th>Infarct/prop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylate cyclase</td>
<td>(n=11)</td>
<td>(n=10)</td>
<td>(n=8)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>(pmol cAMP/min/mg prot)</td>
<td>45.9±12.5</td>
<td>44.4±15.5</td>
<td>30.3±10.8*</td>
<td>29.7±8.4*</td>
</tr>
<tr>
<td>(10⁻² M MgCl₂)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulated adenylate cyclase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>(n=10)</td>
<td>(n=9)</td>
<td>(n=7)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>Vₘₐₓ</td>
<td>79.9±10.0</td>
<td>76.3±11.8</td>
<td>52.5±7.3*</td>
<td>53.3±11.9*</td>
</tr>
<tr>
<td>Kₘₐₓ</td>
<td>0.34±0.27</td>
<td>0.27±0.34</td>
<td>0.28±0.27</td>
<td>0.16±0.10</td>
</tr>
<tr>
<td>Hill slope</td>
<td>0.58±0.08</td>
<td>0.54±0.12</td>
<td>0.57±0.10</td>
<td>0.60±0.12</td>
</tr>
<tr>
<td>GppNHP</td>
<td>(n=11)</td>
<td>(n=10)</td>
<td>(n=7)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>Vₘₐₓ</td>
<td>141±25</td>
<td>147±29</td>
<td>95.8±8*</td>
<td>112±29*</td>
</tr>
<tr>
<td>Kₘₐₓ</td>
<td>1.30±0.87</td>
<td>1.66±0.87</td>
<td>1.29±0.59</td>
<td>1.47±0.62</td>
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<tr>
<td>Hill slope</td>
<td>0.84±0.19</td>
<td>0.67±0.13</td>
<td>0.78±0.12</td>
<td>0.82±0.14</td>
</tr>
<tr>
<td>Forskolin</td>
<td>(n=11)</td>
<td>(n=10)</td>
<td>(n=7)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>Vₘₐₓ</td>
<td>753±157</td>
<td>709±176</td>
<td>503±76*</td>
<td>448±113*</td>
</tr>
<tr>
<td>Hill slope</td>
<td>6.78±2.91</td>
<td>6.69±3.70</td>
<td>6.25±1.55</td>
<td>5.68±2.20</td>
</tr>
</tbody>
</table>

Prop, propranolol; cAMP, cyclic AMP; prot, protein; Vₘₐₓ, maximum velocity of reaction (pmol cAMP/min/mg prot); Kₘₐₓ, concentration of agent yielding half maximal enzyme activity (μM); GppNHP, guanyl-5'-imidodiphosphate. Values are mean±SD and are calculated by linear least-squares regression.

*p<0.05 vs. control.
papillary muscle of MI rats. Propranolol treatment does not appear to improve this elevated muscle stiffness in MI rats. Both these active and passive muscle properties may contribute to alterations in diastolic function; however, it is not possible to make any conclusions regarding diastolic function in this isolated muscle preparation.

**Limitations**

There are several potential limitations of this study. First, we assume that papillary muscle function is representative of the remainder of the myocardium. Because of the segmental nature of ischemic LV dysfunction, we chose to study noninfarcted papillary muscle function to assess regional function of the noninfarcted myocardium. Morphometric studies have demonstrated that processes that cause hypertrophy of the ventricular free wall demonstrate qualitatively similar changes in the papillary muscle. Another potential criticism could be that persistent levels of residual propranolol may have blunted isoproterenol-stimulated function. However, propranolol-treated sham-operated animals showed no significant differences in muscle function compared with controls, suggesting that there was no significant level of propranolol in the papillary muscle preparation. In addition, the absence of any differences in $K_d$ values between treated and untreated controls also suggests that there was no significant tissue level of propranolol. We have also considered that the presence of muscle hypertrophy may lead to an apparent decline in $\beta$-adrenergic receptor density and adenylyl cyclase resulting from a dilutional factor. In this study, adenylyl cyclase activity was normalized to membrane protein content, which is standard. The persistent impairment of adenylyl cyclase activity despite an upregulation of $\beta$-adrenergic receptor density in our propranolol-treated MI rats argues against a dilutional factor. It should be noted that a filtered tissue homogenate was used for $^{3}H$ICYP binding studies. This was chosen to minimize receptor loss and other artifacts caused by centrifugation. Other investigators have reported the advantages of a crude preparation over a purified membrane preparation, in which the majority of $\beta$-adrenergic receptors are lost in the centrifugation process. Our normalized values for $\beta$-adrenergic receptor density are lower than those reported by other laboratories because of the higher protein yield with our preparation. The differences found in $\beta$-adrenergic receptor density in our tissue homogenates reflect absolute changes in the total $\beta$-adrenergic receptor population. In addition, our adenylyl cyclase activities are determined with a crude membrane preparation; hence, normalization of adenylyl cyclase activity to other marker sarcolemmal enzyme activities is not useful. It should be noted that G-protein function was not directly assessed in the study, although the impairment in manganese-stimulated adenylyl cyclase suggests an abnormality in the catalytic subunit. Future studies of G-protein alterations in treated and untreated groups would be of interest. Finally, there are limitations extrapolating findings from the rat model of ischemic heart failure to patients with heart failure after MI. As discussed, there appear to be differences in the $\beta$-adrenergic receptor--adenyl cyclase system in different models and etiologies of heart failure. Our data suggest

![Figure 3: Dose-response curves for (panel A): isoproterenol, (panel B): GppNHp, and (panel C): forskolin-stimulated adenylyl cyclase activity. Values are mean±SEM. Unfitted data are plotted as increase over basal activity to compensate for differences in baseline (MgCl2 stimulation) activity. Refer to Table 6 for group sizes and intergroup statistical comparisons. Control (○); control/propranolol (●); infarct (●); infarct/propranolol (●). cAMP, cyclic AMP; GppNHp, guanylyl-5′-imidodiphosphate.](image-url)
that there are important similarities, yet some differences, in the β-adrenergic receptor–adenylate cyclase system between this model and human ischemic heart failure. However, study of this model permits analysis of a homogeneous group of subjects at a defined stage of disease, which is not possible in human studies.

Conclusions

In summary, this study is the first to report the effects of β-adrenergic receptor blockade in a model of compensated ischemic LV dysfunction. Recent data support the concept that differences in the etiology of heart failure may produce a differential response to β-adrenergic receptor blockade. Our results suggest that in ischemic LV dysfunction, β-adrenergic receptor blockade is less likely to restore catecholamine responsiveness because of the persistent defect in adenylate cyclase function. However, it appears that β-adrenergic blocking agents may exert beneficial effects independent of adenylate cyclase activity, because basal myocardial function is improved in the noninfarcted myocardium. Further studies on the mechanisms of action of these agents and their effect on ventricular remodeling will better define the future role of β-adrenergic receptor blockade in the treatment of ischemic heart failure.

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Effects of beta-adrenergic blockade on papillary muscle function and the beta-adrenergic receptor system in noninfarcted myocardium in compensated ischemic left ventricular dysfunction.

A L Warner, K L Bellah, T E Raya, W R Roeske and S Goldman

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