Denervation supersensitivity of refractoriness in noninfarcted areas apical to transmural myocardial infarction

JAMES J. KAMMERLING, M.D., FRANK J. GREEN, M.D., AUGUST M. WATANABE, M.D., HIROSHI INOUE, M.D., MICHAEL J. BARBER, M.D., PH.D., DAVID P. HENRY, M.D., AND DOUGLAS P. ZIPES, M.D.

ABSTRACT Denervation supersensitivity was demonstrated in anesthetized dogs 5 to 10 days after transmural myocardial infarction produced by latex embolization of a diagonal branch of the left anterior descending coronary artery. Sympathetic efferent denervation in noninfarcted myocardium apical to the infarction was demonstrated by a 90% depletion of myocardial norepinephrine content in the apical (45 ± 15 pg norepinephrine/g tissue) vs basal (437 ± 76 pg/g tissue) regions and by the lack of effective refractory period (ERP) shortening during bilateral ansae subclavieae stimulation in 34% of sites apical to the infarction. Supersensitivity in the area apical to the infarction was manifested by an exaggerated shortening of the ERP during both norepinephrine and isoproterenol infusions, with an upward and leftward shift in the dose-response curves in the apical vs basal regions (p < .001). The cellular mechanism for denervation supersensitivity did not involve detectable changes in the β-adrenergic receptor adenylate cyclase system. There was no difference in the density of β-adrenergic receptors ([125I]-cyanopindolol) in the apical (268.6 ± 22.7 fmol/mg protein) vs the basal (253.5 ± 24.8 fmol/mg protein) regions. Adenylate cyclase activity stimulated by guanosine triphosphate plus isoproterenol was slightly greater in the apical (58.7 ± 17.4%) than in the basal (49.6 ± 10.9%) region, but this difference did not reach statistical significance (p = .068). Muscarinic modulation of β-receptor coupling (oxotremorine attenuation of guanosine triphosphate plus isoproterenol-stimulated adenylate cyclase activity) also was not significantly different at the apical (31.6 ± 17.5% inhibition) and basal (21.4 ± 20.9% inhibition) sites. These data show that a transmural myocardial infarction produces denervation supersensitivity in areas apical to the infarction, but in this preparation no differences in the total number or a redistribution of β-adrenergic receptors or adenylate cyclase activity were detected.


TISSUE that is deprived of its nerve supply responds in an exaggerated fashion to certain agents, a phenomenon called denervation supersensitivity.1 Thus, sympathetically denervated tissue may become supersensitive to the effects of infused catecholamines. Sympathetic denervation supersensitivity has been demonstrated in a variety of tissues2–5 by the use of different denervation techniques.6–14 Data from several studies indicate that efferent sympathetic nerves of the left ventricle course in the superficial subepicardium before diving transmurally, and travel in a basal-to-apical direction.10, 15, 16 We have demonstrated that transmural myocardial infarction, presumably by affecting the postganglionic sympathetic nerves that traverse the area of infarction, produces sympathetic denervation in viable myocardium apical to the infarction.17 We therefore reasoned that this area of myocardium might exhibit an exaggerated response to infused norepinephrine. We chose to investigate an electrophysiologic end point, shortening of effective refractory period (ERP), because it may be important in the development of arrhythmias.18 The purpose of this study was to test the hypothesis that transmural myocardial infarction produces sympathetic denervation supersensitivity in noninfarcted myocardium apical to the infarction.
Methods

Surgical preparation. Healthy mongrel dogs of both sexes and weighing 18 to 31 kg were anesthetized with sodium secobarbital (30 mg/kg iv) and prepared as reported previously.\textsuperscript{16-18} Five to seven millimeters of a diagonal branch of the left anterior descending coronary artery (LAD) was isolated and two silk ligatures were placed under the vessel. The proximal ligature was tied and the distal ligature was maintained under traction to prevent bleeding due to retrograde arterial flow. The vessel was incised with scissors and cannulated with a PE-50 catheter. The catheter was secured to the artery and 0.3 to 0.7 ml of rapidly hardening vinyl latex solution (Carolina Biological Supply Co.) was injected into the artery to embolize the vasculature.\textsuperscript{17, 19} The catheter was removed, the proximal ligature was left in place, and the distal ligature was tied around the embolized artery. Four additional sham-operated dogs underwent left thoracotomy. After isolation of the diagonal branch of the LAD in the manner described above, the vessel was left intact and neither ligated nor embolized. The chest was closed in layers and the dogs were allowed to recover. Antibiotics were administered for 5 days postoperatively to prevent infection. Five to 10 days later, dogs were anesthetized with sodium secobarbital (30 mg/kg iv), intubated, and ventilated. Ventilation was adjusted and sodium bicarbonate was administered to maintain PO\textsubscript{2} at greater than 85 mm Hg and pH at between 7.35 and 7.45.

The right and left cervical vagi were isolated, doubly ligated, and transected. A median sternotomy was performed and the pericardium was opened and sutured to the wound edges. A banjo thermistor (YSI probe 425) was secured to the basal epicardial surface of the left ventricle to monitor temperature. A heating blanket and lamp were adjusted to maintain epicardial temperature between 37° and 38°C.

The ansae subclaviae were isolated as they exited from the stellate ganglia, and were doubly ligated and transected to produce neural decentralization of the heart.\textsuperscript{20} Shielded bipolar electrodes were placed on the right and left ansae subclaviae to stimulate the efferent cardiac sympathetic nerves. Stimuli were square-wave pulses (4 msec duration, 10 V, 2 to 4 mA) delivered at a rate of 3 to 5 Hz through a constant-current stimulus isolator and a Grass S88 stimulator.

The region of the sinus node was crushed in all dogs. This technique has been shown not to significantly alter the effects of sympathetic and vagal stimulation on ventricular refractoriness.\textsuperscript{21} Multiple bipolar plunge electrodes, constructed with two Teflon-coated stainless steel wires threaded through a 21-gauge needle and bent at the tip to form a hook, were placed in the midmyocardial basal and apical to the infarction (figure 1). The area of infarction was documented by subsequent staining with nitroblue tetrazolium (see below). In the sham-operated dogs, the electrodes were placed in similar positions. A plastic sheet covered the sternotomy to minimize drying of the cardiac surface.

Experimental protocol. The ventricles were paced at a basic cycle length of 300 msec throughout the experiment. The ERP was determined at each electrode site by the extrastimulus technique with a programmable stimulator (Krannert Medical Engineering) and a constant-current stimulus isolator, as described previously.\textsuperscript{16-18} The ERP was defined as the longest S\textsubscript{1}-S\textsubscript{2} interval that failed to produce a propagated ventricular response (capture). Each site was tested at least twice before moving to the next site. The repeat ERP determination yielded a value within 2 msec of the first or the data were discarded.

Effects of stimulation of the ansae subclaviae and infusion of norepinephrine. In 11 dogs, control ERPs were determined at each site 20 min after the placement of the bipolar plunge electrodes. The ERP determinations were repeated during bilateral stimulation of the ansae subclaviae. Stimulation was discontinued and the hearts were allowed to recover for 10 min. Control ERPs then were remeasured.

ERP was then determined during norepinephrine infusions that were administered in the following manner. Six different norepinephrine solutions were prepared and were infused in random order at a rate of 1 ml/min to deliver doses ranging from 0.01 to 1.50 μg/kg/min iv. ERPs were determined 5 min after the initiation of each infusion to allow for equilibration. The hearts were permitted to recover for 10 min between infusions and the process was repeated until all six doses were administered.

The hearts were then fibrillated and excised with the electrodes still in place. The left ventricle was separated from the rest of the heart and cut in breadloaf fashion from base to apex. The tissue slices were rinsed in tap water and placed in a solution of distilled water (288 ml), phosphate buffer (32 ml at pH 7.4), and the formazan stain nitro blue tetrazolium (100 mg, Sigma Chemical Company) at 37°C for 7 to 12 min.\textsuperscript{22} This method, which stains myocardial dehydrogenase, was used to verify the electrode position with respect to the infarction as well as the transmural extent of the infarction. The data presented here were obtained only from those sites that were located outside the area of infarction.

Effects of stimulation of the ansae subclaviae and infusion of isoproterenol. In 13 dogs, ERPs were measured before and during bilateral stimulation of the ansae subclaviae. Next, ERPs were determined before and during the infusion of isoproterenol (0.01 to 1.50 μg/kg/min iv) in a manner identical to that during the infusion of norepinephrine. The hearts were fibrillated and excised and the tissue encompassing the electrodes basal and apical to the infarction were assayed for myocardial norepinephrine content and β-adrenergic receptors (see below).

Effects of propranolol. Four dogs were given (dl) propranolol, 1 mg/kg iv, over 20 to 30 min with additional doses of 0.2 mg/kg given each hour. Five minutes after each dose, arterial
blood was drawn for determination of propranolol level. ERPs were measured before and during norepinephrine infusion (0.01 to 1.50 \( \mu \)g/kg/min iv) beginning 5 min after the loading dose of propranolol.

Sham-operated dogs. In the four sham-operated dogs ERP determinations were made before and during bilateral stimulation of the ansae subclaviae. The ERPs were then measured before and during norepinephrine infusion, as above. The hearts were fibrillated, excised, and stained with nitro blue tetrazolium.

Determination of myocardial norepinephrine content. Myocardial norepinephrine content was determined in tissue samples from areas basal and apical to the infarction in the dogs undergoing isoproterenol infusions. Immediately after the final intervention, the hearts were fibrillated and excised and the tissue was frozen in liquid nitrogen and stored at \(-30^\circ\) C until analysis. The samples were homogenized in 5 to 10 volumes (weight/volume) of 0\% 0.1N perchloric acid. An appropriate aliquot was then analyzed for norepinephrine content by use of a radioenzymatic assay. Each sample was assayed in duplicate, with an internal standard of 500 pg norepinephrine being used for each sample. Sensitivity of the assay was 1.5 pg and the coefficient of variation of the assay was 4.0%.

Determination of density and affinity of \( \beta \)-adrenergic receptors. Six dogs with hearts previously infarcted via the latex embolization method had tissue basal and apical to the infarction harvested for determination of the density and affinity of \( \beta \)-adrenergic receptors. These dogs did not receive catecholamine infusions. \( \beta \)-Receptor assays were also performed in 10 dogs after exposure to isoproterenol during the portion of the study in vivo.

Preparation of membrane vesicles. The harvested tissue was weighed and then frozen and fragmented in a percussion mortar cooled in liquid nitrogen. The samples, each weighing 1 to 3 g, were suspended in 10 ml of 10 mM NaHCO\(_3\) and disrupted in a polytron (Brinkman) three times for 30 sec each. The suspension was gradually accelerated for 10 min to 14,000 g and centrifuged (Beckman J2-21) for an additional 10 min. The supernatant was saved and the pellet resuspended, disrupted, and centrifuged as above. The pellet was discarded and the two supernatants were combined and centrifuged for 20 min at 14,000 g. The pellet was discarded and the supernatant was centrifuged for 60 min at 106,000 g (Beckman L5-50). The supernatant was discarded and the pellet was suspended in an aqueous solution of 0.6M KC1 plus 30 mM histidine and centrifuged for 60 min at 106,000 g. The supernatant was discarded and the pellet was resuspended in an aqueous solution of 0.25M sucrose plus 30 mM histidine in a concentration of 10 to 15 mg protein/ml, as determined by the method of Lowry et al. The membranes were stored at \(-70^\circ\) C until the assays were performed.

Radioligand binding

\(^{125}\text{I}-\text{cyanopindolol.}\) Membrane vesicles were suspended in Krebs phosphate buffer (120 mM NaCl, 5 mM KC1, 1.2 mM MgSO\(_4\), 200 \( \mu \)M CaCl\(_2\), 10 mM Na\(_3\) HPO\(_4\)) to a concentration of 0.066 mg protein/ml. Aliquots of the membrane suspension (0.150 ml) were incubated in duplicate or triplicate with ascending concentrations, 10 to 600 pM, of \(^{125}\text{I}-\text{cyanopindolol (New England Nuclear, 2200 Ci/mmol)}\) in a final volume of 0.250 ml at \(37^\circ\) C for 60 min in polypropylene tubes (Starstedt 55/511). Nonspecific binding was determined in the presence of 1 \( \mu \)M l-propranolol. The reaction was terminated by the addition of 10 ml of Krebs phosphate buffer (37\( ^\circ\) C). The suspension was immediately filtered over glass fiber filters (Whatman GF/C) and washed with 10 ml of buffer. The filters were counted at 80\% efficiency in a gamma counter (Packard Auto-gamma scintillation spectrophotometer). The saturation data were subjected to Scatchard analysis. Inhibition of \(^{125}\text{I}-\text{cyanopindolol (100 to 150 pM)}\) by isoproterenol, 0.1 nM to 100 \( \mu \)M, was also determined in the absence and presence of 100 \( \mu \)M 5\' guanylylimidodiphosphate after 60 min incubation at 37\( ^\circ\) C.

\(^{3}\text{H}-\text{CGP-12177. Fresh ventricular myocardium was homogenized three times for 30 sec (Brinkman Polytron, setting 6) in a 20% weight/volume solution of 0.25M sucrose and 30 mM histidine. The homogenate was diluted 1:5 and 100 \( \mu \)l was incubated with ascending concentrations (20 to 1500 pM) of \(^{3}\text{H}-\text{CGP-12177 (Amersham) in 1 ml volumes for 60 min. Non specific binding was defined in the presence of 0.3 \mu M l-propranolol. Non specific binding ranged from 10\% to 20\% at the highest concentration of \(^{3}\text{H}-\text{CGP-12177 used.}}\)

Adenylate cyclase activity assays. Adenylate cyclase activity in membrane vesicles was assayed in the presence and absence of guanosine triphosphate (GTP) plus isoproterenol by the method of Salomon et al. Modulation of isoproterenol-stimulated adenylate cyclase activity by the muscarinic agonist oxotremorine was also determined.

Data analysis. Data are given as the mean \pm\ SEM. Apical and basal values of variables were compared with a paired t test. If data were not normally distributed, the Wilcoxon signed-rank test was used. Dose-response curves were analyzed with repeated-measures analysis of variance. The within-dog repeated factors were site (apical vs basal) and dose levels. This analysis also provides an interaction term that indicates whether the curves are parallel.

Results

Evidence of transmural infarction. In each of the hearts stained with nitro blue tetrazolium 5 to 10 days after latex embolization of a diagonal branch of the LAD, a dense transmural myocardial infarction was demonstrated. All electrodes placed basal to the embolized diagonal branch and all but three of the electrodes placed apical to the vessel were found to be in tissue that stained normally with nitro blue tetrazolium. Data obtained from these three electrodes were discarded. This confirmed that the electrodes were placed in noninfarcted tissue both basal and apical to the transmural infarction. None of the four sham-operated dogs demonstrated myocardial infarction, as determined by nitro blue tetrazolium staining.

Myocardial norepinephrine content. Myocardial norepinephrine content obtained from tissue samples from 12 dogs given isoproterenol infusions are shown in table 1. There was a significantly lower norepineph...
rine concentration in the area apical to the infarction when compared with that in the area basal to the infarction (90% depletion; p < .001 by Wilcoxon signed-rank test).

**Ansae subclaviae stimulation.** ERP measurements were made in 22 dogs with previous myocardial infarction before and during bilateral stimulation of the ansae subclaviae. In 22 of 22 sites basal to the infarction, ERP shortened significantly during ansae subclaviae stimulation (mean change in ERP 11 ± 1 msec). In 20 of 58 (34%) sites apical to the infarction, ERP did not shorten during bilateral ansae subclaviae stimulation (mean change in ERP 1 ± 1 msec). Thirteen dogs had at least one site that failed to show ERP shortening during bilateral stimulation of the ansae subclaviae and in five dogs all sites tested were denervated. In 38 (66%) sites apical to the infarction, ERP shortened significantly during stimulation of the ansae subclaviae (mean change in ERP 10 ± 1 msec). In the sham-operated dogs, there was no difference in the amount of ERP shortening at basal and apical sites during ansae subclaviae stimulation (basal mean change in ERP of 11 ± 4 msec vs change in apical mean ERP of 11 ± 2 msec).

**Effects of norepinephrine.** In the dogs given norepinephrine infusions (table 2), the mean control ERP at sites basal to the infarction was not different from the mean ERP at apical sites (p > .1). For each concentration of norepinephrine, ERP shortened more at apical than basal sites. When the data are displayed as dose-response curves (figure 2), there is a leftward shift of the curve for ERPs obtained at the apex compared with the curve for ERPs obtained at the base (p < .001). Four of the 13 dogs did not demonstrate supersensitivity to isoproterenol infusion (table 3). In these nine dogs, the mean control ERP at the apical site was longer than at the basal site (p = .05). At each concentration of isoproterenol, ERP shortened more at apical than basal sites. When the data are displayed in the form of dose-response curves (figure 3), there is a leftward shift of the curve of ERPs obtained at the apex when compared with the curve for ERPs obtained at the base (p < .001). Four of the 13 dogs did not demonstrate supersensitivity to isoproterenol infusion (table 4). When the data from all 13 dogs are combined (table 5) for each concentration of isoproterenol, ERP shortened more at the apical than the basal sites. When the data for all 13 dogs are displayed as dose-response curves (figure 4), there is a leftward shift of the curve for ERPs obtained at the apex when compared with the curve for ERPs obtained at the base (p < .001).

**Effects of sham operation.** The four sham-operated dogs had similar control ERPs at the basal and apical sites (p > .1; table 6). There was also no significant difference in the response to each norepinephrine infusion in the basal vs apical sites. When the data are

### TABLE 2

Mean ERP shortening (msec) during infusion of norepinephrine (\(\mu g/kg/min \text{ iv}\))

<table>
<thead>
<tr>
<th>Site</th>
<th>Control</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ERP</td>
<td>0.01</td>
</tr>
<tr>
<td>Basal</td>
<td>151 ± 5</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>(n = 11)</td>
<td></td>
<td></td>
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<tr>
<td>Apical</td>
<td>148 ± 3</td>
<td>1 ± 1</td>
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<td>(n = 24)</td>
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</tbody>
</table>

p < .001, apical vs basal.

### TABLE 3

Mean ERP shortening (msec) during infusion of isoproterenol (\(\mu g/kg/min \text{ iv}\))

<table>
<thead>
<tr>
<th>Site</th>
<th>Control</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ERP</td>
<td>0.01</td>
</tr>
<tr>
<td>Basal</td>
<td>146 ± 4</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>(n = 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical</td>
<td>151 ± 2</td>
<td>3 ± 1</td>
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<tr>
<td>(n = 29)</td>
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</table>

p < .001, apical vs basal.
catecholamine infusions receive basal and or the formed in propranolol level was 411.6 ± 100.4 ng/ml.

Significant ERP shortening in myocardial infarction in nine dogs demonstrated supersensitivity to isoproterenol.

displayed as dose-response curves, the curves are essentially superimposable (figure 5).

Effects of propranolol administration. The four dogs given intravenous propranolol (table 7) did not have significantly different basal and apical control ERPs (p > .1). Norepinephrine infusions did not result in significant ERP shortening in the basal or apical sites after the administration of propranolol. The mean serum propranolol level was 411.6 ± 100.4 ng/ml.

Determination of β-adrenergic receptors. Assays of membrane vesicles for β-adrenergic receptors performed in 10 dogs after isoproterenol infusions (table 8) demonstrated no significant differences between the basal and apical sites in the dissociation constant (K_D) or the receptor number (B_max). The six dogs in which myocardial infarction was produced that did not receive catecholamine infusions had no significant differences in the K_D constant or in the B_max in the basal vs apical sites (table 8). Tissue was available from six dogs for study of isoproterenol inhibition of 125I-cyanopindolol binding. K_i of isoproterenol was 42 ± 34 nM in basal regions and 138 ± 125 nM in regions apical to the infarct. There was no systematic change in K_i of isoproterenol in either basal or apical areas in the presence of 5' guanylylimidodiphosphate (62 ± 72 vs 94 ± 42 nM, respectively).

Four other dogs were subjected to infarction and underwent subsequent stellate stimulation to identify development of denervation. Two exhibited characteristic findings of denervation; two did not. Fresh ventricular myocardium from these dogs was homogenized and assayed for β-adrenergic receptors with the use of the hydrophilic radioligand (3H)-CGP-12177 (table 8). In none of the four animals were differences noted in either K_D or B_max in myocardium harvested from regions basal and that from regions immediately apical to the infarction.

**TABLE 4**

Mean ERP shortening (msec) during infusion of isoproterenol (μg/kg/min iv) in four dogs without supersensitivity to the drug

<table>
<thead>
<tr>
<th>Site</th>
<th>Control</th>
<th>Dose</th>
<th>0.01</th>
<th>0.05</th>
<th>0.10</th>
<th>0.25</th>
<th>0.50</th>
<th>1.50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>ERP</td>
<td></td>
<td>161 ± 8</td>
<td>2 ± 1</td>
<td>9 ± 2</td>
<td>12 ± 3</td>
<td>18 ± 2</td>
<td>20 ± 3</td>
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<tr>
<td>(n=4)</td>
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<tr>
<td>Apical</td>
<td>ERP</td>
<td></td>
<td>163 ± 4</td>
<td>2 ± 1</td>
<td>10 ± 2</td>
<td>13 ± 3</td>
<td>19 ± 3</td>
<td>21 ± 4</td>
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<td>(n=8)</td>
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p = NS, apical vs basal.

**TABLE 5**

Mean ERP shortening (msec) during infusion of isoproterenol (μg/kg/min iv) in 13 dogs

<table>
<thead>
<tr>
<th>Site</th>
<th>Control</th>
<th>Dose</th>
<th>0.01</th>
<th>0.05</th>
<th>0.10</th>
<th>0.25</th>
<th>0.50</th>
<th>1.50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>ERP</td>
<td></td>
<td>149 ± 3</td>
<td>0 ± 1</td>
<td>4 ± 1</td>
<td>7 ± 1</td>
<td>13 ± 1</td>
<td>15 ± 1</td>
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<tr>
<td>(n=13)</td>
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<tr>
<td>Apical</td>
<td>ERP</td>
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<td>154 ± 2</td>
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<td>14 ± 1</td>
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</table>

p < .001, apical vs basal.

**FIGURE 3.** Dose-response curve. ERP shortening (y axis) in response to varying concentrations of isoproterenol (Iso, x axis) is shown for sites basal (circles) and apical (triangles) to the infarction in nine dogs demonstrating supersensitivity to isoproterenol.

**FIGURE 4.** Dose-response curve. ERP shortening (y axis) in response to varying concentrations of isoproterenol (Iso, x axis) is shown for sites basal (circles) and apical (triangles) to the infarction in all 13 dogs tested with isoproterenol.
Table 6
Mean ERP shortening (msec) during infusion of norepinephrine (μg/kg/min iv) in sham-operated dogs

<table>
<thead>
<tr>
<th>Site</th>
<th>Control ERP</th>
<th>Dose 0.01</th>
<th>Dose 0.05</th>
<th>Dose 0.10</th>
<th>Dose 0.25</th>
<th>Dose 0.50</th>
<th>Dose 1.50</th>
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</thead>
<tbody>
<tr>
<td>Basal</td>
<td>144±14</td>
<td>0±1</td>
<td>7±5</td>
<td>9±4</td>
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<td>24±5</td>
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<tr>
<td>Apical</td>
<td>144±9</td>
<td>0±1</td>
<td>5±3</td>
<td>7±4</td>
<td>11±4</td>
<td>17±5</td>
<td>22±5</td>
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</tbody>
</table>

p = NS, apical vs basal.

**Determination of adenylate cyclase activity.** Results of the assays of adenylate cyclase activity are depicted in Table 9. Membrane samples were available from both basal and apical regions of hearts from five dogs that had received prior catecholamine infusions and from six dogs that had not previously received catecholamines. For each group and for all 11 dogs combined, GTP plus isoproterenol significantly increased adenylate cyclase activity (p < .001). For each infusion group and for all 11 dogs combined, oxotremorine attenuated the GTP plus isoproterenol-induced stimulation of adenylate cyclase in the basal regions of the heart (p at least < .05 by paired t test); however, this difference did not reach statistical significance in the apical regions, perhaps due to the small number of observations. Comparison of the group that received prior catecholamine infusions with the group that did not revealed that the control adenylate cyclase activity was significantly higher in the basal regions of dogs that had received the infusion (p < .05). Yet between these two groups, there were no significant differences in the percent stimulation by GTP plus isoproterenol or the percent inhibition by oxotremorine. Paired t testing was performed to compare stimulation and inhibition of adenylate cyclase activity in the basal vs apical regions of the hearts. For the group of dogs that had not previously received catecholamines, the percent stimulation of adenylate cyclase by GTP plus isoproterenol was higher in the apical than basal regions; however, this difference did not reach statistical significance (p = .068). Similarly, in this same group, the percent inhibition by oxotremorine tended to be greater in the apical regions, but these differences were not statistically significant (p < .11).

**Discussion**

**Major findings.** The results of this study indicate that transmural myocardial infarction caused by latex embolization of a diagonal branch of the LAD produces denervation supersensitivity to infused catecholamines in noninfarcted myocardium apical to the infarction. In a previous study we presented biochemical, histochemical, and functional evidence of sympathetic denervation in this preparation.17 The present study confirms some of these results by demonstrating a 90% depletion of norepinephrine in the area apical to the infarction compared with the area basal to the infarction.

The concentration of norepinephrine in the normal area is only slightly less than we found previously,17 but is still within the range of normal values for canine myocardium as determined by the present method.23 High-pressure liquid chromatography with electrochemical detection yields higher values.30 Although myocardial norepinephrine content normally decreases from the base of the heart to the apex,30,31 the changes noted in those studies were not nearly as marked as those reported here.
The results of bilateral ansae subclaviae stimulation, during which 34% of the sites apical to the infarction failed to show significant ERP shortening, gave functional evidence of denervation in this preparation. This percentage compares favorably with our previous work (43%), and suggests that functional denervation apical to a transmural myocardial infarction tested by ansae subclaviae stimulation is heterogeneous. One might have predicted that only the sites at which ERP failed to shorten during ansae subclaviae stimulation would have demonstrated supersensitivity, while the other sites would not. This was not the case, since the response to stimulation of the ansae subclaviae did not predict the supersensitive response to catecholamine infusion. Of 61 apical sites sampled, 20 of 24 (83%) showed supersensitivity to norepinephrine infusion and 29 of 37 (78%) showed supersensitivity to isoproterenol infusion. Two potential mechanisms may explain why a site that appears "innervated" by ERP shortening during ansae subclaviae stimulation might still demonstrate supersensitivity. First, an area may undergo partial denervation that is sufficient to alter the properties of those cells responsible for supersensitivity yet still have enough intact sympathetic neurons to cause ERP shortening during bilateral ansae subclaviae stimulation. It has been demonstrated that supersensitivity can occur in incompletely denervated preparations. A second possibility is that a site may be totally denervated but norepinephrine released from neighboring intact sympathetic neurons during ansae subclaviae stimulation may reach that area via diffusion or the coronary circulation. In either situation, only a small amount of norepinephrine would be required to cause ERP shortening if the tissue were supersensitive.

In previous studies, supersensitivity has been demonstrated by a horizontal or vertical shift of the dose-response curve. The present study demonstrates a leftward shift of the apical vs basal ERP curves during norepinephrine and isoproterenol infusions, confirming supersensitivity at sites apical to the infarction. In these experiments we showed shortening of ERP in terms of absolute differences between control results and those obtained during drug infusion. Maximal shortening of ERP, defined as a plateau in the upper extent of the curves in figures 2, 3, and 4, was not

### TABLE 8

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Preparation (n)</th>
<th>Infusion</th>
<th>Site</th>
<th>$K_D$ (pM)</th>
<th>$B_{max}$ (fmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{125}$I-CYP</td>
<td>Membrane vesicles (10)</td>
<td>Isoproterenol</td>
<td>Basal</td>
<td>68.3 ± 8.2</td>
<td>186.9 ± 27.1</td>
</tr>
<tr>
<td>$^{125}$I-CYP</td>
<td>Membrane vesicles (6)</td>
<td>None</td>
<td>Basal</td>
<td>98.6 ± 21.6</td>
<td>253.5 ± 24.8</td>
</tr>
<tr>
<td>$^3$H-CGP-12177</td>
<td>Homogenate (2), nondenervated</td>
<td>None</td>
<td>Basal</td>
<td>266.4 ± 20.2</td>
<td>37.4 ± 12</td>
</tr>
<tr>
<td>$^3$H-CGP-12177</td>
<td>Homogenate (2), denervated</td>
<td>None</td>
<td>Basal</td>
<td>295 ± 2</td>
<td>21.9 ± 1</td>
</tr>
</tbody>
</table>

$CYP = cyanopindolol.$

### TABLE 9

<table>
<thead>
<tr>
<th>Adenylate cyclase activity</th>
<th>No prior infusion (n = 6)</th>
<th>Prior infusion (n = 5)</th>
<th>All (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ACA basal</td>
<td>142 ± 44</td>
<td>236 ± 72</td>
<td>185 ± 74</td>
</tr>
<tr>
<td>Control ACA apical</td>
<td>144 ± 40</td>
<td>171 ± 46</td>
<td>156 ± 43</td>
</tr>
<tr>
<td>GTP + ISO ACA basal</td>
<td>216 ± 78 (50.8 ± 12.6%)</td>
<td>348 ± 100 (48.2 ± 9.7%)</td>
<td>276 ± 109 (49.6 ± 10.9%)</td>
</tr>
<tr>
<td>GTP + ISO ACA apical</td>
<td>245 ± 91 (66.6 ± 18.5%)</td>
<td>253 ± 59 (49.2 ± 11.3%)</td>
<td>249 ± 75 (58.7 ± 17.4%)</td>
</tr>
<tr>
<td>GTP + ISO + OXO ACA basal</td>
<td>208 ± 74 (14.6 ± 16.2%)</td>
<td>311 ± 62 (29.6 ± 24.6%)</td>
<td>255 ± 85 (21.4 ± 20.9%)</td>
</tr>
<tr>
<td>GTP + ISO + OXO ACA apical</td>
<td>214 ± 80 (31.7 ± 12.6%)</td>
<td>227 ± 47 (31.5 ± 23.8%)</td>
<td>220 ± 64 (31.6 ± 17.5%)</td>
</tr>
</tbody>
</table>

$ACA = adenylate cyclase activity in pmol cAMP produced per min per mg protein; Control ACA = adenylate cyclase activity under basal conditions; GTP + ISO ACA = adenylate cyclase activity in the presence of GTP (10 μM), and isoproterenol (10 μM) ± SD and (% increase from control ± SD); GTP + ISO + OXO ACA = adenylate cyclase activity in the presence of GTP (10 μM), isoproterenol (10 μM), oxotremorine (10 μM) ± SD and (% attenuation of the GTP + ISO response ± SD).
obtained. Nevertheless, the shift leftward is clear evidence of supersensitivity.

Potential mechanisms of supersensitivity. A search for a mechanism of sympathetic supersensitivity in this animal preparation did not reveal any predictable changes in the β-adrenergic receptor adenylate cyclase system that could explain the physiologic observations. In previous studies various mechanisms have been suggested for denervation supersensitivity, including failure of neuronal reuptake or loss of "intraneuronal binding sites for norepinephrine," 34 postjunctional changes involving adenylate cyclase activity, 33 or an increase in number of β-adrenergic receptors. 34 Vatner et al. 13 demonstrated both a predominant prejunctional and a secondary postjunctional mechanism in the conscious surgically denervated dog. They also suggested altered "reflex buffering," although this mechanism would not be operative in our neurally decentralized preparation.

In a preparation of acute reversible myocardial ischemia and infarction, Mukherjee et al. 14 showed an increase in the number of functionally intact β-adrenergic receptors in a zone of ischemia after a 1 hr LAD occlusion. Gaide et al. 35 showed an increased response to sympathetic nerve stimulation in the normal area surrounding a transmural myocardial infarction in cats studied 3 to 15 months after infarction. Subsequently Maisel et al. 36 demonstrated in a guinea pig preparation that, as a function of increasing duration of ischemia, there is a redistribution of β-adrenergic receptors from intracellular light vesicles to the sarcolemma. In a canine preparation of chronic infarction, however, Karliner et al. 37 found no differences in total β-adrenergic receptor density.

In the preparation of transmural myocardial infarction used in this study, we demonstrated supersensitivity to both norepinephrine and isoproterenol. It is known that isoproterenol is not a substrate for reuptake into the prejunctional neuron. 38 Increased sensitivity to isoproterenol has been noted previously, 13, 39-41 in some instances due to an increased number of β-adrenergic receptors, which was not found in the present study. Increased sensitivity to isoproterenol may take longer to develop than supersensitivity to norepinephrine, 41 and may explain the failure of four dogs to develop supersensitivity to isoproterenol. We cannot completely exclude the role of altered norepinephrine reuptake since these four dogs did not undergo norepinephrine infusion to test for supersensitivity.

Although this study does not eliminate decreased enzymatic catabolism as a potential mechanism for supersensitivity, a prior study using autotransplanted dog hearts did not support this mechanism. 42 In these denervated hearts, there was a two- to sevenfold increase in total norepinephrine metabolism in vivo.

We did not measure norepinephrine diffusion in this study. However, we know from similar studies performed in our laboratory* that there is no significant difference in blood flow in the noninfarcted basal and that in the noninfarcted apical areas after infarction produced by latex embolization. Although this certainly does not prove that diffusion is not altered in this preparation, one of the main determinants of the catecholamine concentration gradient, namely blood flow, is not changed. We cannot exclude the possibility of increased release of norepinephrine from the prejunctional neurons, but it does not appear to be the major mechanism of supersensitivity since administration of catecholamines by intravenous infusion essentially bypassed this step.

The fact that pretreatment with propranolol abolished supersensitivity in this preparation suggests a receptor-mediated process. We did not detect an increase in total β-receptor number or affinity for the β-adrenergic antagonist 125I-cyanopindolol. There was also no apparent difference between apical and basal samples in the ability of isoproterenol to inhibit 125I-cyanopindolol binding. It has been shown that there can be rapid (1 hr) "downregulation" of receptors on exposure to catecholamines. 14 Therefore, the β-receptor assays were also conducted in tissue from dogs with infarctions that did not receive catecholamine infusions. In tissue from these animals, the density of β-receptors was slightly higher; however, there were still no differences between densities in the apical and basal samples, nor in affinity for isoproterenol. The myocardial homogenates were also examined with the use of [3H]-CGP-12177, a hydrophilic radioligand that binds to sarcomemal receptors but not to receptors sequestered on the inside of light buoyant density vesicles. 43 This approach should have detected altered distribution of receptors in the infarcted tissue. This radioligand also failed to detect any difference in β-adrenergic receptor density between areas basal and immediately apical to the infarction, whether in denervated or nondenervated animals. It does not therefore seem likely that an alteration in distribution of β-adrenergic receptors between the sarcolemma and these light vesicles occurred in this preparation.

The absence of changes in receptor density in this preparation contrasts with the results of other investigators. 13, 14, 38 Differences in the preparations examined may account for this discrepancy. For example, re-

*Browne KF, Barber MJ, Zipes DP: Unpublished data.
Regional denervation, as in the present study, may produce changes different from global denervation (e.g., stellate interruption). Second, although preparations of acute ischemia and infarction have documented a time-dependent increase in β-adrenergic receptors in sarcolemma,14, 36 this study as well as another study of chronic infarction37 did not show changes in β-adrenergic receptor density.

We also tested the possibility that in the denervated regions the receptors were more “efficiently” coupled to the catalytic subunit, and therefore the same amount of β-adrenergic agonist in this region generated a greater amount of cyclic AMP. Such a dissociation between changes in receptor number and coupling of receptors to adenylate cyclase has been observed in a number of other systems.44 There was a slightly greater increase in percent stimulation by GTP plus isoproterenol in the denervated apical regions (p = .068). Also, there was a slightly greater attenuation by oxotremorine of GTP plus isoproterenol stimulation of adenylate cyclase activity in the apical regions (p < .11). These differences were not statistically significant. It is unclear why no striking differences in either β-adrenergic receptor density or adenylate cyclase activity were apparent in a preparation in which supersensitivity of the effective refractory period to infused catecholamines clearly occurred. It is possible that this is a consequence of the heterogeneity of functional denervation noted during bilateral stimulation of the ansae subclaviae. These biochemical studies were of regions of myocardial tissue. If there were heterogeneous differences in β-receptor densities; i.e., some areas with reduced and other areas with normal or increased densities, it is possible that the size of our myocardial sample might have obscured such differences.

In conclusion, we have shown that sympathetic supersensitivity occurs in this preparation of transmural myocardial infarction. It does not appear to be due to lack of reuptake of norepinephrine or to an increase in the total number or a redistribution of β-adrenergic receptors. Small increases in adenylate cyclase activity suggested that more efficient coupling of the β-receptor to adenylate cyclase occurred in the denervated apical regions, but this difference did not reach statistical significance and must therefore be interpreted cautiously.

Limitations of the model. The vinyl latex coronary artery embolization method of producing a transmural myocardial infarction has been discussed in detail previously.17, 18 Since latex is chemically inert and the vehicle acetone has been shown not to cause sympathetic denervation when injected alone, we believe that latex embolization is a useful means of producing a transmural myocardial infarction in dogs. Even if the solution exerted a direct effect on the myocardium, rather than via creation of a myocardial infarction, such an event would still not explain the supersensitivity response of remote, noninvolved myocardium. Nor would it be expected to affect any of the biochemical assays performed a minimum of 5 days later. The presence of transmural myocardial infarction was demonstrated by nitro blue tetrazolium staining, which showed a dense area that did not stain and extended from endocardium to epicardium in the distribution of the injected vessel.

Dolezel et al.45 have shown that 2 weeks after surgical isolation of the LAD there is a 33% decrease in ventricular sympathetic neurons when compared with those in unoperated animals. Therefore, the possibility must be raised that the control group of sham-operated dogs represents a partially denervated group. However, this study demonstrated that there was not a sufficient degree of denervation to produce supersensitivity in this group. Thus, the denervation supersensitivity of the region apical to the infarction when compared with that of the basal region was not merely caused by surgical trauma produced during isolation of the diagonal branch of the LAD.

Recently, Janes et al.46 showed that neural activity could still be recorded from afferent nerves in response to mechanical stimulation of myocardium situated apically to a latex-induced myocardial infarction. That interesting observation does not negate our observations of functional denervation, however.

Implications of the study. Several studies have demonstrated that in man the use of β-blockers after myocardial infarction decreases the early postinfarction mortality.47–49 The mechanism of this protective effect is unclear and may include the prevention of reinfarction or a direct antiarrhythmic effect that may be due either to an anti–ischemic effect with secondary prevention of ischemia-induced arrhythmias or a primary antiarhythmic effect.

The present study demonstrates that transmural myocardial infarction in the dog produces efferent sympathetic denervation in an area apical to the infarction. Under conditions of enhanced sympathetic tone, the denervated area may demonstrate an exaggerated shortening of refractoriness. This may lead to dispersion of refractoriness between the denervated and neighboring innervated areas, giving rise to an arrhythmogenic milieu.18 The prior administration of β-adrenergic blockers, by abolishing the denervation supersensitivity, might prevent this dispersion of
refractoriness. Thus, the protective effect of \( \beta \)-blockers after myocardial infarction may be due at least in part to their ability to prevent this manifestation of denervation supersensitivity.

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