Protection Against Autonomic Denervation Following Acute Myocardial Infarction by Preconditioning Ischemia

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To examine the effects of ischemic preconditioning on efferent autonomic responses following acute transmural myocardial ischemia/infarction (MI), the time course and extent of efferent sympathetic and vagal denervation were compared between control dogs that received a one-stage sustained coronary occlusion and preconditioned dogs that received four 5-minute coronary occlusions separated by 5 minutes of reperfusion before sustained occlusion. Effective refractory periods (ERP) basal and apical to MI were determined in the baseline state and during neural stimulation before and after preconditioning occlusions and 20, 60, 120, and 180 minutes after sustained occlusion by ligature ligation of diagonal branches of the left anterior descending coronary artery. In 10 control dogs with transmural MI, ERP shortening induced by bilateral ansae subclaviae stimulation (4-msec pulses, 2–4 Hz and 2–4 mA) was unchanged at basal sites but was attenuated at apical sites. Four of 40 apical test sites exhibited efferent sympathetic denervation (<2 msec shortening) 20 minutes after sustained occlusion. Thirteen of 40 apical sites became denervated during a 3-hour period. In 10 preconditioned dogs, ERP shortening at apical sites was unchanged after preconditioning occlusions and during the first 60 minutes of sustained ischemia but was attenuated at 120 minutes. Three of 40 apical test sites became denervated during a 3-hour period. The cumulative percentage of denervated apical test sites was significantly less in the preconditioned group compared with the control group (p=0.006) despite a comparable degree of subepicardial involvement in the MI (8.2±1.0% vs. 8.4±1.4%, the ratio to the left ventricular circumference, mean±SEM). In 11 control dogs tested for efferent vagal response after MI, ERP prolongation induced by bilateral vagal stimulation (4-msec pulses, 20 Hz with current strength 0.05 mA greater than that required to produce asystole) was unchanged at basal sites, but was attenuated at apical sites, and five of 44 test sites exhibited denervation (>1 msec prolongation) 20 minutes after sustained coronary occlusion. Fourteen of 40 apical sites became denervated during a 3-hour period. In 10 preconditioned dogs, vagally induced ERP prolongation was unchanged both at basal and apical sites, and none of 36 apical test sites exhibited denervation after preconditioning and during a 3-hour period of sustained coronary occlusion (p<0.001 vs. control group) despite a comparable degree of subendocardial involvement in the MI (11.8±0.8% vs. 11.9±1.3%). In preconditioned hearts, the blood flow reduction to the ischemic myocardium measured by radioactive microspheres during sustained coronary occlusion was comparable with that during the first preconditioning occlusion, indicating that an increase in collateral blood flow during MI cannot explain the protective effects of preconditioning. We conclude that preconditioning with brief episodes of ischemia preserves efferent sympathetic and vagal responses during the early period after coronary artery occlusion in the dog by mechanisms still to be determined. (Circulation Research 1989;64:437-448)
autonomic nerves traveling through the ischemic/infarcted myocardium. Heterogeneous loss of autonomic innervation can occur within several minutes after coronary occlusion and is reversible after short-term occlusions.1,4

Murry et al2 have shown that four 5-minute episodes of coronary occlusion and reperfusion dramatically limit infarct size of myocardium that is later subjected to 40 minutes of sustained ischemia. Although the mechanism of this protective effect of preconditioning is unknown, it is not due to an increase in collateral blood flow5 and may be related to reduced depletion of high-energy phosphates and/or to reduced catabolite accumulation.6 One suggested mechanism7 is that repeated episodes of ischemia cause a regional depletion of catecholamines so that the final ischemia takes place in "sympathectomized" myocardium that can withstand the effects of ischemia better than ischemic myocardium that has a normal catecholamine content.8 Therefore, the purpose of this study was to examine the effects of ischemia on efferent sympathetically and vagally induced changes in refractoriness of myocardium basal and apical to the site of ischemia/infarction during a 3-hour coronary occlusion in dogs with and without ischemic preconditioning.

**Materials and Methods**

**Surgical Procedures**

Sixty mongrel dogs of either sex weighing 16–28 kg were anesthetized with sodium secobarbital (30 mg/kg i.v.). Additional doses were injected as needed to maintain anesthesia. The dogs were intubated and ventilated with room air using a volume-cycled respirator (model 607, Harvard Apparatus, South Natick, Massachusetts). The chest was opened through a median sternotomy, and the heart was suspended in a pericardial cradle. A fluid-filled cannula placed in the right femoral artery was connected to a transducer (Statham p-23Db, Gould, Cleveland, Ohio) to monitor arterial blood pressure, and a femoral venous cannula was used to infuse normal saline at 100–200 ml/hr to replace spontaneous fluid losses. In 20 dogs used for measurement of myocardial blood flow, two additional fluid-filled cannulas were placed in left femoral and brachial arteries to withdraw blood, and a cannula was inserted into the left atrial appendage to inject microspheres. Dogs were placed on a heating pad, and the thoracotomy was covered by a plastic sheet. A thermistor (model 400, Yellow Springs Instrument, Yellow Springs, Ohio) was used to monitor epicardial temperature at 36–38°C. Arterial blood gases and pH were monitored and maintained within the physiological range.

A diagonal branch or multiple diagonal branches of the left anterior descending coronary artery were isolated, and silk sutures were passed around the branches for later occlusion. When visible anastomoses between a diagonal branch and a marginal branch of the circumflex coronary artery were seen, a marginal branch or two marginal branches were also isolated at their distal portions for later occlusion (Figure 1). The ansae subclaviae were isolated as they exited from the stellate ganglia, doubly ligated, and cut. The cervical vagi were also isolated, doubly ligated, and transected. Using a 22-gauge needle, two hook electrodes made from Teflon-coated wires, insulated except for their tips, were placed in midmyocardium basal to the isolated diagonal branch or branches, and four additional hook electrodes were inserted apically to the isolated diagonal branch or branches (Figure 1). The electrodes served as a cathode for unipolar stimulation. An anodal electrode consisting of a 33-mm metal disk was placed in the abdominal wall. A bipolar plunge electrode in the left ventricle was used to record the ventricular responses induced by extrastimuli.

**Measurement of Effective Refractory Period**

The effective refractory period (ERP) was determined at each electrode site by the extrastimulus technique, which employs a programmable stimulator (Krannert Medical Engineering, Indianapolis, Indiana) and a constant current isolator. Each ventricular test site was driven with a 2-msec rectangular stimulus twice the diastolic threshold, which was measured during each intervention. A train of nine stimuli (S1) was followed by a late premature stimulus (S2) that produced a propagated ventricular response. The S1-S1 interval was 240–250 msec in dogs tested for sympathetic innervation and 280–300 msec in dogs tested for vagal innervation and was kept constant throughout each experiment. The ventricular response to S2 was recorded in lead II electrocardiogram and from the bipolar plunge electrode placed in the left ventricle and displayed on a storage oscilloscope. The S1-S2 interval was shortened in steps of 2 msec until S2 failed to produce a propa-
gated response. The S1-S2 interval was then increased by 5 msec and was shortened by 1 msec decrements until S2 failed to produce a propagated ventricular response. The ERP was defined as the longest S1-S2 interval at which S2 failed to produce a propagated response. The ERP was determined twice, and values were within 1 msec of each other or the data were discarded and the determination was repeated.

Neural Stimulation

Bilateral ansae subclaviae stimulation. Shielded bipolar electrodes were placed on the right and left anterior and posterior ansae subclaviae to stimulate the efferent cardiac sympathetic nerves with separate constant current isolators driven by a programmable stimulator (Krahnert Medical Engineering). Stimuli were rectangular 4-msec pulses at a frequency of 2-4 Hz and a current of 2-4 mA. Determination of the ERP was started 2 minutes after the onset of stimulation.

Bilateral vagal stimulation. Two Teflon-coated wire electrodes were embedded in the cardiac end of each vagal nerve. Rectangular pulses of 4-msec duration were delivered at a frequency of 20 Hz by use of separate constant current isolators. The current strength was 0.05 mA greater than that required to produce asystole for the right vagus and asystole or complete atrioventricular block for the left vagus. Effects of vagal stimulation were determined during intravenous infusion of norepinephrine at constant rates of 0.125-0.25 μg/kg/min to achieve a constant background of sympathetic effect. The ERP during norepinephrine infusion served as control for the determination of efferent vagal effects on ventricular refractoriness. The conditions of neural stimulation were kept constant in each experiment.

Determination of Extent of Myocardial Infarction

After the experiment, the dog was killed by fibrillation of the ventricle, and the heart was removed and cut in five slices from base to apex with the electrodes still in place. Each slice was stained with nitro blue tetrazolium (NBT). With this technique, the presence of myocardial infarction could be confirmed macroscopically. The subepicardial and subendocardial widths of the myocardial infarction and the diameter of the left ventricle were measured in each slice. The ratio of the greatest width of subepicardial infarction to the epicardial circumference of the left ventricle was used to represent the involvement of infarction in each dog tested for sympathetic innervation, and the greatest width of subendocardial infarction relative to the epicardial circumference was used in each dog tested for vagal innervation, as reported previously.

Measurement of Myocardial Blood Flow

In 20 dogs, regional myocardial blood flow was measured before and after coronary occlusion by the microsphere technique. Approximately 2 million 15-μm carbonized plastic microspheres labeled with 53Co, 95Nb, 103Ru, or 113Sn (Biotechnology Systems, DuPont de Nemours, Boston, Massachusetts), as 1.0 mCi of nuclide suspended in 10 ml of 10% dextran with 0.01% Tween 80 added as a surfactant, were injected through the cannula in the left atrial appendage, followed by a 10-ml saline flush. Beginning 15 seconds before the injection, reference blood samples were drawn from the left brachial and femoral arteries at a rate of 2.06 ml/min until each sample volume reached 10 ml. Myocardial tissue samples of two or three slices were taken from the central ischemic/infarcted regions and the normal zone (core area) of the posterior left ventricular wall and subdivided into subendocardial, midmyocardial, and subepicardial thirds. Thus, 12-18 pieces were obtained. Each piece was weighed; the average weight of the pieces was 0.770 g. Blood and tissue sample radioactivities were measured for 1 minute per sample on a Packard Model 5530 gamma counter (Packard Instruments, Downers Grove, Illinois). Net counts-per-minute data for each isotope were provided after corrections of raw count data for interisotope interference, background, and decay. Myocardial blood flow was calculated according to the formula tissue flow = tissue counts × reference flow/reference counts × tissue weight, where reference counts was the mean of the values obtained from the left brachial and femoral artery samples.

Blood flow of ischemic/infarcted myocardium was expressed as a percentage of the mean core area flow. The values derived from the slice in which reduction of mean transmural flow was most prominent were used to represent the degree of ischemia for each dog.

Protocol

Dogs were randomized to preconditioned and control groups. Each group contained two subgroups in which sympathetic or vagal innervation was tested. Dogs in the preconditioned group received four 5-minute coronary occlusions induced by tightening snare around the branches and separated by 5 minutes of reperfusion. After preconditioning, a sustained coronary occlusion was produced by ligature ligation over a 3-hour period. The number of occluded branches during sustained occlusion was the same as that during preconditioning for each experiment. The control group received only a sustained 3-hour coronary occlusion produced by ligature ligation without preconditioning. Each subgroup tested for sympathetic innervation contained one dog in which sustained coronary occlusion was produced by injection of 0.3 ml latex solution using a PE-50 catheter to obtain essentially zero subepicardial flow.

The ERP was determined at six test sites before and at 20, 60, 120, and 180 minutes after sustained coronary occlusion in the baseline state and during neural stimulation. Sympathetically induced ERP shortening and vagally induced ERP prolongation served to indicate intact efferent autonomic innerva-
Analysis of Data

No data were used from electrode sites within the myocardial infarction. As reported previously, sites were considered to be sympathetically denervated if bilateral ansae subclaviae stimulation shortened the ERP ≥9 msec before coronary occlusion but ≤2 msec on at least one occasion after coronary occlusion.1,3 Sites were considered to be vagally denervated if bilateral vagal stimulation prolonged the ERP ≥3 msec before coronary occlusion but ≤1 msec on at least one occasion after coronary occlusion.2,3 Sites with <9 msec shortening of the ERP during bilateral ansae subclaviae stimulation or <3 msec prolongation of the ERP during bilateral vagal stimulation before coronary occlusion were excluded because of possibly insufficient effects of neural stimulation for accurate detection of denervation.3 For the comparison of efferent sympathetic responses in both groups, the data from dogs with nontransmural or no definite myocardial infarction were included for the comparison of efferent sympathetic responses.3 However, data from dogs with definite subendocardial or nontransmural myocardial infarction were excluded because of possibly insufficient effects of ischemia on cardiac sympathetic nerves, which are known to distribute mainly in the subepicardial layer.13,14 In contrast, the data from dogs with definite subendocardial or nontransmural myocardial infarction were included for the comparison of efferent vagal responses in both groups, since cardiac vagal fibers are distributed mainly in the subendocardial layer.10,14 For the correlation of efferent autonomic responses with myocardial blood flow reduction in the ischemic myocardium, data from all 20 dogs studied were included.

Statistical Analysis

The data in this study are expressed as mean±SEM. The difference among mean values was determined using an analysis of variance for repeated measurements. Paired and unpaired t test was performed when two measurements were com-
**Table 1. Baseline Effective Refractory Periods in Dogs With Transmural Myocardial Infarction Tested for Sympathetic Innervation**

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After IP</th>
<th>After permanent coronary occlusion (min)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td><strong>Control (N=10)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal sites (n=20)</td>
<td>162±2</td>
<td>NA</td>
<td>169±3</td>
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<tr>
<td>Apical sites (n=40)</td>
<td>167±2</td>
<td>NA</td>
<td>168±2</td>
</tr>
<tr>
<td><strong>Preconditioned (N=10)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal sites (n=20)</td>
<td>169±3</td>
<td>172±2</td>
<td>172±2</td>
</tr>
<tr>
<td>Apical sites (n=40)</td>
<td>171±2</td>
<td>173±1</td>
<td>173±1</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM in milliseconds. IP, ischemic preconditioning. *p<0.05 vs. before values.

Results

**Efferent Sympathetic Response**

Of 16 control dogs tested for efferent sympathetic response, four had nontransmural or intramural myocardial infarction and two others had no definite infarction at necropsy. Data from these six dogs were excluded from a comparison of efferent sympathetic responses. Of 16 preconditioned dogs tested for sympathetic response, four had nontransmural infarction and one had no definite infarction; one other dog became hypotensive (systolic blood pressure <80 mm Hg) immediately after sustained coronary occlusion. Data from these six dogs were excluded. Therefore, data from 10 control and 10 preconditioned dogs with transmural myocardial infarction were compared. The number of occluded branches (3.4±0.3 and 3.3±0.3, respectively) and the extent of subepicardial infarction (8.4±1.4% and 8.2±1.0%, respectively) were the same in both the control and preconditioned groups. Also, spontaneous heart rate (114±7 and 115±6 beats/min, respectively) and mean arterial blood pressure (116±6 and 122±8 mm Hg, respectively) immediately before sustained coronary occlusion were the same in both groups.

Baseline ERP values of test sites are shown in Table 1. Figure 3 shows the cumulative percentage of sympathetically denervated apical test sites. In control dogs, four of 40 apical test sites exhibited sympathetic denervation 20 minutes after sustained coronary occlusion and 13 of 40 apical sites became denervated over a 3-hour period; none of 20 basal sites became denervated (not shown). In preconditioned dogs, none of 40 apical sites became denervated after preconditioning or during the first hour after sustained coronary occlusion, although three of 40 apical sites became denervated at 2 hours. No basal sites became denervated (not shown). Cumulative percentage of denervated apical test sites in preconditioned dogs was significantly less than in control dogs (p=0.006). Figure 4 shows the shortening of ERP induced by bilateral ansae subclaviae stimulation at all basal and apical test sites. In control dogs, shortening of ERP at apical test sites became attenuated 20 minutes after sustained coronary occlusion and declined further thereafter. In preconditioned dogs, shortening of ERP at apical test sites was preserved after preconditioning and during the 1st hour after sustained coronary occlusion and became attenuated 120 minutes after sustained occlusion. At basal sites, shortening of ERP was not attenuated over a 3-hour period in either group.

Shortening of ERP induced by norepinephrine infusion did not differ between basal and apical test sites after the completion of preconditioning; that is, preconditioning did not cause a supersensitive

![Figure 3. Sympathetic denervation in dogs with transmural myocardial infarction. Cumulative percentage of sympathetically denervated apical sites is shown on ordinate as a function of time after permanent coronary occlusion. Solid line indicates data from 10 control dogs; dotted line indicates data from 10 preconditioned dogs. Figure indicates cumulative number of denervated test sites and (in parentheses) number of test sites that showed effective refractory period shortening of 2 msec or less during bilateral ansae subclaviae stimulation at moment of determination. Cumulative percentage of denervated apical test sites in preconditioned dogs was significantly less than in control dogs (p=0.006).](http://circres.ahajournals.org/asset/content/39/6/441/F3.large.jpg)
response of the apical myocardium to norepinephrine (Figure 5).

Efferent Vagal Response

Of 13 control dogs tested for efferent vagal response, two dogs had intramural or patchy myocardial infarction; data from these dogs were excluded. Data from two of 12 preconditioned dogs were also excluded because of patchy infarction. Therefore, data from 11 control and 10 preconditioned dogs with myocardial infarction were compared. The number of occluded branches (4.1±0.3 and 4.4±0.2, respectively) and extent of subendocardial infarction (11.9±1.3% and 11.8±0.8%, respectively) were the same in both the control and preconditioned groups. Spontaneous heart rate immediately before sustained coronary occlusion was 143±6 beats/min for control dogs and 164±7 beats/min for preconditioned dogs (p<0.05). Mean arterial blood pressure was the same in both groups (114±5 and 117±6 mm Hg, respectively). In control dogs, the current strength for vagal stimulation was 0.67±0.10 mA for the right vagus and 0.83±0.13 mA for the left vagus. In the preconditioned group, the current strength was 0.44±0.05 mA for the right vagus and 0.43±0.07 mA for the left vagus. Ventricular fibrillation was induced in one control dog during the determination of ERP 180 minutes after sustained coronary occlusion and in one preconditioned dog 120 and 180 minutes after sustained occlusion. Thus, the ERP data were not obtained at these determination points. In one control dog, one basal site was involved in myocardial infarction; therefore, data from this site were excluded.

Baseline ERP value of test sites are shown in Table 2. Figure 6 shows the cumulative percentage of vagally denervated apical test sites. In control dogs, five of 44 apical sites exhibited vagal denervation 20 minutes after sustained coronary occlusion. Fourteen of 40 apical sites became denervated over a 3-hour period. None of 21 basal test sites became denervated (not shown). In the preconditioned dogs, none of 36 apical test sites exhibited vagal denervation after preconditioning and during a 3-hour period after sustained coronary occlusion (p<0.001 vs. control) as did none of 18 basal sites (not shown).

Baseline ERP value of test sites are shown in Table 2. Figure 6 shows the cumulative percentage of vagally denervated apical test sites. In control dogs, five of 44 apical sites exhibited vagal denervation 20 minutes after sustained coronary occlusion. Fourteen of 40 apical sites became denervated over a 3-hour period. None of 21 basal test sites became denervated (not shown). In the preconditioned dogs, none of 36 apical test sites exhibited vagal denervation after preconditioning and during a 3-hour period after sustained coronary occlusion (p<0.001 vs. control) as did none of 18 basal sites (not shown).

Figure 7 shows the prolongation of ERP induced by bilateral vagal stimulation at all apical and basal test sites. In control dogs, prolongation of ERP was atten-
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TABLE 2. Baseline Effective Refractory Periods During Norepinephrine Infusion in Dogs With Myocardial Infarction Tested for Vagal Innervation

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After IP</th>
<th>After permanent coronary occlusion (minutes)</th>
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<tr>
<td></td>
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<td></td>
<td>20</td>
</tr>
<tr>
<td>Control (N=11)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Basal sites (n=21)</td>
<td>153±3</td>
<td>NA</td>
<td>157±4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(n=19)</td>
</tr>
<tr>
<td>Apical sites (n=44)</td>
<td>157±3</td>
<td>NA</td>
<td>158±3</td>
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<td></td>
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<td></td>
<td>(n=40)</td>
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<td>Preconditioned (N=10)</td>
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<tr>
<td>Basal sites (n=44)</td>
<td>154±2</td>
<td>158±3</td>
<td>155±3</td>
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<td></td>
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<td>(n=18)</td>
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<td>Apical sites (n=40)</td>
<td>153±1</td>
<td>156±2</td>
<td>154±2</td>
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<td></td>
<td></td>
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<td>(n=36)</td>
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</tbody>
</table>

Data are expressed as mean±SEM in milliseconds. IP, ischemic preconditioning.

uated at apical sites 20 minutes after sustained coronary occlusion and declined further thereafter. In preconditioned dogs, prolongation of ERP was not attenuated significantly after preconditioning and during a 3-hour period after sustained coronary occlusion at apical test sites. At basal sites, prolongation of ERP did not change over a 3-hour period in either group.

**Myocardial Blood Flow**

Myocardial blood flow was measured in five dogs from each of the following four subgroups (n=20): 1) control dogs tested for sympathetic innervation, 2) preconditioned dogs tested for sympathetic innervation, 3) control dogs tested for vagal innervation, and 4) preconditioned dogs tested for vagal innervation. Myocardial blood flow in the core areas before coronary occlusion was 103.6±8.6 ml/min×100 g (n=20). To correlate efferent autonomic responses with the degree of ischemia, ERP change

![Vagal denervation in dogs with transmural myocardial infarction. Cumulative percentage of vagally denervated apical sites is shown on ordinate as a function of time after permanent coronary occlusion. Solid line indicates data from 11 control dogs; dotted line indicates data from 10 preconditioned dogs. Figure indicates cumulative number of denervated test sites and (in parentheses) number of test sites that showed effective refractory period prolongation of 1 msec or less during bilateral vagal stimulation at moment of determination. Data at moment of determination when one dog in each group developed ventricular fibrillation were not included (•, •). In preconditioned dogs, none of 36 apical test sites exhibited vagal denervation during a 3-hour period after permanent coronary occlusion (p<0.001) vs. control.](http://circres.ahajournals.org/)

**Figure 6.**

![Vagal responses](http://circres.ahajournals.org/)

**Figure 7.** Prolongation of effective refractory period (ERP) induced by bilateral vagal stimulation at basal and apical sites. In both groups, prolongation of ERP at basal sites was not changed during a 3-hour period after permanent coronary occlusion. In control dogs, prolongation of ERP was attenuated at apical sites 20 minutes after permanent coronary occlusion and thereafter declined further. In preconditioned dogs, efferent vagal response at apical sites was preserved after ischemic preconditioning (IP) and during a 3-hour period after permanent coronary occlusion.
induced by neural stimulation at each apical test site was expressed as a percentage of the mean value of two basal sites (intact innervation). Among the four subgroups, there was no difference in the extent of ERP changes in 20 apical test sites before coronary occlusion (91.5±4.9%, 20 in five control dogs tested for sympathetic response; 95.4±3.4%, 20 in five preconditioned dogs tested for sympathetic response; 94.2±4.8%, 20 in five control dogs tested for vagal response; 99.6±5.4%, 20 in five preconditioned dogs tested for vagal response).

Figure 8 shows correlation of ERP shortening induced by bilateral ansae subclaviae stimulation in apical test sites determined 20 and 60 minutes after sustained coronary occlusion with the degree of blood flow reduction in the ischemic subepicardial layer measured 15 minutes after sustained occlusion, expressed as a percentage of the core area flow. In the control group, sympathetic denervation was noted at one site in the dog in which subepicardial blood flow was reduced to zero 20 minutes after sustained coronary occlusion. Attenuation of efferent sympathetic response (less than 50% of the basal sites) was observed at two sites in the dog in which subepicardial blood flow was reduced to 20% of the core area flow. In one dog with blood flow of 21% of the core area flow, there was no attenuation of sympathetic effect at 20 minutes. However, at 60 minutes, all three dogs in which subepicardial blood flow was reduced to less than 40% showed sympathetic denervation or attenuation in at least two apical sites. One of these three dogs was the dog that had showed no sympathetic attenuation with blood flow 21% of the core area flow at 20 minutes. There was a significant correlation between sympathetically induced ERP shortening at 60 minutes after sustained coronary occlusion and subepicardial blood flow in the ischemic myocardium (y=0.942x+26.0, r=0.723, p<0.001). In the preconditioned group, there was neither sympathetic denervation nor attenuation of ERP shortening 20 minutes after sustained coronary occlusion. Also, no site was denervated at 60 minutes, although one dog in which subepicardial blood flow was reduced to 1% showed attenuation of sympathetically induced ERP shortening. In one dog in which subepicardial blood flow was reduced to 12%, efferent sympathetic response was still normal at 60 minutes after permanent occlusion.

Figure 9 shows correlation of ERP prolongation induced by bilateral vagal stimulation in the apical

**Figure 8.** Correlation of shortening of effective refractory period (ERP) induced by bilateral ansae subclaviae stimulation at apical test sites with reduction of subepicardial blood flow. Extent of ERP shortening at 20 and 60 minutes after permanent coronary occlusion at each apical test site compared with mean value of two basal sites (intact innervation) is shown on ordinate as a percentage: degree of blood flow reduction in ischemic subepicardium measured 15 minutes after permanent occlusion is shown on abscissa as a percentage of core area flow. Each vertical column of data points indicating the same blood flow percentage represents data taken from the same dog. See text for details.
The major observations from this study were that 1) preconditioning ischemia (four 5-minute periods

Discussion

Major Findings

The major observations from this study were that 1) preconditioning ischemia (four 5-minute periods...
flow in three subdivided layers expressed as percentage of core area flow. Data are from nine preconditioned dogs. Myocardial blood flow was reduced significantly in all three layers either by the first preconditioning 5-minute coronary occlusion, MBF (2), or at 15 minutes after permanent coronary occlusion, MBF (3) (p<0.005 vs. control). However, there was no significant difference in collateral blood flow of each layer between MBF (2) and MBF (3).

Spontaneous Recovery of Function

In this study, as in our previous studies, some apical test sites that were designated initially as denervated by our arbitrary criteria subsequently showed responses to neural stimulation that exceeded the denervation cutoff value (Figures 3 and 6). This spontaneous return of response also suggested that early loss of autonomic responses might have been due to functional neural disturbances. The mechanism responsible for restoration of neural function did not seem to be due to alterations in blood flow. A possible related phenomenon has been observed electrophysiologically in cardiac tissues. Recovery of electrical activity during continued ischemia has been described previously and attributed to an increase in collateral blood flow or a diffusion of catabolites, such as potassium from the extracellular space in the ischemic zone. However, because a similar phenomenon having constant hypoxia, hyperkalemia, and acidosis has been noted in vitro during superfusion with altered Tyrode's solution, changes in blood flow are not a likely cause and the mechanism remains unexplained.

Cause of Denervation

Since little is known about the blood supply to the nerve fibers in the heart, it is unclear whether neural denervation results because the nerves themselves become ischemic, as do the contiguous myocardial fibers, or whether exposure to certain substances in the ischemic environment through which the axons pass causes functional denervation. For example, increased concentrations of K⁺ (10–20 meq/l) and H⁺ (pH 6.2–6.4) are known to inhibit norepinephrine release from sympathetic nerve terminals in noncardiac tissues. Adenosine also inhibits norepinephrine release both in noncardiac tissue and in rat heart. The concentration of these substances becomes elevated in the ischemic myocardium and could be plausible candidates for inhibiting neurotransmitter release and local neurotransmission in early ischemia. In the later stages of ischemia, norepinephrine accumulates within the extracellular space of the ischemic myocardium because of increased release from sympathetic nerve terminals following further increases in K⁺ concentration, increased leakage caused by the loss of structural integrity of cell membranes, or by decreased re-uptake of norepinephrine. This stage seems to correspond to the time at which the ischemic myocardium loses fluorescent sympathetic nerve terminals and more complete denervation occurs. Denervation of the non-ischemic myocardium situated apically to the infarcted myocardium is best explained by alteration of neurotransmission in the axons traveling through the ischemic/infarcted myocardium.

Protective Effects of Preconditioning

Several existing examples support the concept that reversible episodes of ischemia in some way...
Our data show that preconditioning ischemia preserved the efferent vagal response for a longer period of sustained ischemia than it preserved the efferent sympathetic response. This result is somewhat puzzling, considering the greater susceptibility of the subendocardial myocardium, where vagal fibers are mainly distributed, to blood flow reduction during ischemia compared with that of the subepicardial layer, where sympathetic fibers are mainly distributed. Less severe ischemia during the preconditioning occlusions might have prevented the subepicardium from being as well preconditioned as the subendocardium. Another explanation is that there may be differences in the accumulation of catabolites after acute myocardial infarction that affect neurotransmission in sympathetic versus vagal nerves, or there may be differences in the response of these two nerves. It is also possible that cavity blood helps protect the subendocardial vagal nerves.

**Methodological Consideration**

In the dog, the size of the ischemic vascular bed (area at risk), collateral blood flow to the ischemic region, and, less importantly, myocardial oxygen demand are the major modulators of myocardial viability after ischemia. In the present study, the number of occluded branches and subepicardial and subendocardial involvement in the infarction after the 3-hour period of sustained ischemia measured by the extent of the infarcted area and reduction in myocardial blood flow were similar in control and preconditioned dogs. Heart rate and arterial blood pressure were also similar in both groups tested for sympathetic denervation, and heart rate was slightly higher in preconditioned dogs than in control dogs tested for vagal denervation. Therefore, differences in these variables in both groups cannot account for the preservation of neural responses in the preconditioned dogs.

**Implication**

The importance of the observations on protective effects of preconditioning on autonomic activity, made in an animal model without preexisting coro-
nary disease, is unknown. However, it is possible that a heterogeneous loss of effenter sympathetic response after coronary occlusion increases the electrical heterogeneity between innervated and denervated regions and enhances the genesis of ventricular tachyarrhythmias. Therefore, protection by preconditioning ischemia against the heterogeneous development of effenter sympathetic and vagal denervation during the early period of acute myocardial infarction may help suppress ventricular arrhythmias that occur early after acute coronary occlusion.

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Key Words • effenter sympathetic stimulation • effenter vagal stimulation • denervation • myocardial infarction • ischemic preconditioning • refractory period • myocardial blood flow
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