Time Course of Denervation of Efferent Sympathetic and Vagal Nerves After Occlusion of the Coronary Artery in the Canine Heart

Hiroshi Inoue and Douglas P. Zipes

To determine the time course of efferent sympathetic denervation after transmural myocardial infarction (TMI) and of efferent vagal denervation after transmural or nontransmural myocardial infarction (NTMI), we measured effective refractory periods (ERP) basal and apical to TMI or NTMI in secoarbital-anesthetized, open-chest dogs. In eight dogs with latex-induced TMI, bilateral ansa subclaviae stimulation shortened ERP at all 45 apical and basal test sites before latex injection. After latex injection, ERP shortening was unchanged at all 15 basal sites but was eliminated (±2 msec shortening) at three apical sites in 5–20 minutes and at 14 of 30 apical sites 30–180 minutes after latex injection. At the remaining 13 apical sites, ERP shortening was not eliminated but attenuated significantly in 5–180 minutes. ERP shortening induced by infused norepinephrine (0.20–0.25 μg/kg/min) did not differ between basal and apical test sites 3–4 hours after latex injection; that is, no supersensitivity occurred. Of six dogs with TMI produced by ligation of multiple coronary arteries without latex injection, ERP shortening induced by efferent sympathetic neural stimulation was eliminated at 10 apical sites in four dogs over a period of 3 hours. At 14 apical sites that did not show denervation in these six dogs, ERP shortening was unchanged. In seven dogs with latex-induced TMI, bilateral vagal stimulation lengthened ERP at all 40 apical and basal test sites before latex injection. Vagally induced ERP lengthening was unchanged at all 13 basal sites after latex injection. ERP lengthening was eliminated (±1 msec lengthening) at four of 27 apical sites in 5–20 minutes and at 13 apical sites 30–180 minutes after latex injection. At the remaining 10 apical sites, ERP lengthening was not eliminated but decreased significantly 3 hours after latex injection. Of nine dogs with ligation-induced NTMI, five dogs showed elimination of vagally induced ERP lengthening at eight apical sites in 3 hours after ligation. ERP lengthening induced by vagal stimulation was unchanged at all 17 basal sites in nine dogs with NTMI. We conclude that TMI produced by latex injection and ligation of multiple coronary arteries produces heterogeneous loss of efferent sympathetic innervation in noninfarcted apical sites as early as 5–20 minutes after coronary occlusion with more complete denervation occurring over time. Loss of efferent vagal innervation also occurs heterogeneously in 5–20 minutes after coronary occlusion by latex injection (TMI) and by ligation of a diagonal branch (NTMI) and continues with more sites losing efferent responsiveness over time. (Circulation Research 1988; 62:1111–1120)

Transmural myocardial infarction induced by latex injection into a diagonal branch of the left anterior descending coronary artery in the dog heart interrupts efferent sympathetic fibers innervating the myocardium apical to the infarction by 90 minutes. Efferent vagal fibers are also interrupted by transmural myocardial infarction induced by latex injection into a diagonal branch 90 minutes after embolization and by nontransmural myocardial infarction induced by ligation of the left anterior descending coronary artery followed by reperfusion in canine hearts. Nontransmural myocardial infarction following ligation of a coronary artery spares the epicardium and does not result in sympathetic denervation. Despite these studies, little is known about how quickly efferent sympathetic and vagal denervation occur after coronary occlusion. If denervation occurred at the onset of ischemia and was heterogeneous, it might contribute to the genesis of ventricular arrhythmias during the acute stage of myocardial infarction.

The purpose of the present study was to determine the time course of loss of efferent sympathetic and vagal innervation in noninfarcted, normal myocardium apical to an infarction produced by latex injection or ligature occlusion of a coronary artery in the canine heart.

Materials and Methods

Mongrel dogs of either sex weighing 14–29 kg were anesthetized with sodium secobarbital (30 mg/kg i.v.). Additional amounts of secobarbital were injected as needed to maintain anesthesia. The dog was 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
femoral artery was connected to a Statham P23 ID transducer (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. The dog was placed on a heating pad, and the thoracotomy was covered by a plastic sheet. An operating table lamp was used to maintain epicardial temperature between 37° and 39° C. Arterial blood gases and pH were monitored and maintained within the physiological range.

One or two diagonal branches of the left anterior descending coronary artery were isolated for later occlusion. The cervical vagi were isolated, doubly ligated, and transected. The ansae subclaviae were also isolated as they exited from the stellate ganglia, doubly ligated, and cut. 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 apical to the isolated diagonal branch or branches (Figure 1). The electrodes served as a cathode for unipolar stimulation; the anodal electrode was a 33-mm-diameter metal disk placed in the abdominal wall. A bipolar plunge electrode in the ventricle was used to record the ventricular responses induced by extrastimuli. Data were recorded beginning 30 minutes after the placement of the plunge electrodes.

Measurements of Effective Refractory Period

The effective refractory period was determined at each electrode site by the extrastimulus technique employing 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 diastolic threshold, which was measured during each intervention. A train of nine stimuli (S1) was followed by a late threshold, which was measured during each intervention isolator. Each ventricular test site was driven with a 2-msec rectangular stimulus twice diastolic threshold, which was measured during each intervention. A train of nine stimuli (S1) was followed by a late threshold, which was measured during each intervention.

Ten seconds later, the S1-S2 interval was increased by 5 msec and the S1-S2 interval was shortened by 1-msec decrements until S2 failed to produce a propagated ventricular response. Repeated S1-S2 interval determinations yielded values within 1 msec of the first, or the data were discarded. The effective refractory period was defined as the longest S1-S2 interval at which S1 failed to produce a propagated ventricular response.

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 (Krannert Medical Engineering). Stimuli were rectangular 4-msec pulses (frequency 2 Hz, 2 mA). Determination of the refractory period was started 2 minutes after the onset of stimulation. After the experiment, norepinephrine was infused at doses of 0.20–0.25 μg/kg/min, and effective refractory periods were determined.

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 using 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 rates of 0.10–0.25 μg/kg/min to achieve a constant background of sympathetic tone. The amount of norepinephrine infused was kept constant in each experiment. Effective refractory period during norepinephrine infusion served as control for determination of vagal effects on ventricular refractoriness.

Protocol

Measurements during myocardial infarction. Ten minutes after obtaining the baseline values, coronary occlusion was performed by latex injection or ligation as described below. The isolated diagonal branch was cannulated with a PE-50 catheter, and 0.5 ml latex solution was injected in 16 dogs to produce transmural myocardial infarction as in our previous study. In nine dogs, two diagonal branches were ligated in a one-stage manner along with ligations of visible collaterals from the posterior descending coronary artery to produce transmural myocardial infarction. In the remaining 11 dogs, a diagonal branch (or two diagonal branches, if necessary) was ligated in a one-stage manner to produce nontransmural myocardial infarction. In the last group of dogs, visible collaterals from the posterior descending coronary artery were also ligated.

FIGURE 1. Diagram of heart. Solid circles, sites of electrode placement; shaded area, region of infarction; AO, aorta; LA, left atrium; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; LV, left ventricle; PA, pulmonary artery; RA, right atrium; RV, right ventricle.
Refractory periods were determined at 5, 30, 60, 120, and 180 minutes after coronary occlusion. Because it took about 15 minutes to determine the effective refractory period at six test sites both before and during neural stimulation, the data presented at 5 minutes after coronary occlusion, for example, were collected from 5 to about 20 minutes after coronary occlusion.

Determination of myocardial infarction involvement. After the experiment, the heart was removed and cut in four slices from base to apex with the electrodes still in place. Each slice was stained with nitro blue tetrazolium (NBT). The width of the myocardial infarction and the diameter of the left ventricle were measured in each slice (Figure 2). For the experiment dealing with sympathetic denervation, it was important to know the extent of epicardial involvement because cardiac sympathetic fibers are distributed in the subepicardial layer. Therefore, the width of subepicardial infarction to the epicardial circumference of the left ventricle was determined in each slice (Figures 2A and 2B). In contrast, for the experiment of vagal denervation, it was important to know the extent of endocardial involvement because cardiac vagal fibers are distributed in the subendocardial layer. Therefore, the width of subendocardial infarction relative to the epicardial circumference of the left ventricle was determined in each slice (Figures 2A and 2C). The greatest value was selected to represent the involvement of infarction for each dog. While this technique may not accurately provide an estimate of the total amount of myocardium that is infarcted, for the purpose of the present study, it was more important to know the extent of epicardial and endocardial involvement.

Analysis of Data

No data were used from electrode sites located within myocardial infarction. Sites were considered to be sympathetically denervated if stimulation of bilateral ansae subclaviae shortened the effective refractory period. Sites that were considered to be vagally denervated exhibited 3-8 msec lengthening of effective refractory period during bilateral vagal stimulation before coronary artery occlusion and demonstrated <1-msec prolongation of effective refractory period during vagal stimulation after coronary artery occlusion. In three sites of dogs receiving coronary ligature and two sites of dogs receiving coronary ligation, vagal stimulation during the 3 hours after infarction lengthened effective refractory period <1 msec on one occasion and 2-3 msec on one or more different occasions, representing the cutoff value. These sites were considered to be vagally denervated. In an additional four sites of dogs receiving latex injection and two sites of dogs receiving coronary ligation, vagal stimulation during the 3 hours after infarction lengthened effective refractory period <1 msec on one occasion and 2-3 msec on one or more different occasions, representing some variation in response around the cutoff value. These sites were considered to be vagally denervated.

The data in this study are expressed as mean ± SEM. Both analysis of variance and an analysis of variance for repeated measures were performed to compare the data. When multiple comparisons were made, a modified Bonferroni test was used. An unpaired t-test was used to compare the difference between two mean values. Cumulative rate of denervation was compared using the survival analysis of Lee-Desu. Statistical significance was set at p<0.05.

Results

Sympathetic Denervation

Dogs with latex injection of a diagonal branch. In all eight dogs receiving intracoronary latex injection, transmural myocardial infarction was demonstrated by NBT staining, and sympathetic denervation was achieved in at least one apical site in each dog over a period of 3 hours. Baseline effective refractory periods of test sites are shown in Table 1. Figure 3 shows the cumulative rate of sympathetically denervated apical test sites. Three of 30 apical test sites exhibited sympathetic denervation as early as 5-20 minutes after latex injection. Seventeen of 30 apical sites became denervated over a period of 3 hours after latex injection (Figure 3), but none of 15 basal sites became denervated.
TABLE 1. Baseline Effective Refractory Period in Dogs Tested for Sympathetic Denervation

I. Eight dogs with transmural myocardial infarction from latex injection of coronary artery

<table>
<thead>
<tr>
<th>Before</th>
<th>After coronary occlusion (minutes)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Basal sites (n = 15)</td>
<td>169 ± 2</td>
</tr>
<tr>
<td>Apical sites without denervation (n = 13)</td>
<td>172 ± 3</td>
</tr>
<tr>
<td>Apical sites with denervation (n = 17)</td>
<td>170 ± 2</td>
</tr>
</tbody>
</table>

II. Six dogs with transmural myocardial infarction from ligation of multiple coronary arteries

<table>
<thead>
<tr>
<th>Before</th>
<th>After coronary occlusion (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Basal sites (n = 12)</td>
<td>163 ± 2</td>
</tr>
<tr>
<td>Apical sites without denervation (n = 14)</td>
<td>163 ± 1</td>
</tr>
<tr>
<td>Apical sites with denervation (n = 10)</td>
<td>172 ± 3</td>
</tr>
</tbody>
</table>

* n = 10, † n = 6 (see text for details).
Values are mean ± SEM in milliseconds.

When the data of these 17 denervated sites were pooled, shortening of effective refractory period declined progressively after latex injection (Figure 4). At the remaining 13 apical sites without apparent denervation (i.e., effective refractory period still shortened 3 msec or more during ansae subclaviae stimulation), shortening of effective refractory period became attenuated after latex injection, while it remained unchanged at 15 basal sites (Figure 4). The shortening of effective refractory period induced by norepinephrine infusion was not different among the basal sites, the apical sites without apparent denervation, and the apical denervated sites (11.2 ± 1.2, 10.7 ± 1.6, and 8.0 ± 0.8 with latex).
8.8 ± 1.1 msec, respectively; Figure 4). The subepicardial width of infarction relative to the left ventricular circumference was 10 ± 1%.

Dogs with ligation of multiple coronary arteries. Of nine dogs receiving ligation of multiple coronary arteries (4.6 ± 0.5 ligations), three dogs had nontransmural myocardial infarction and six dogs had transmural myocardial infarction as demonstrated by NBT staining. The data from the six dogs with transmural myocardial infarction were included in the following analysis. Baseline effective refractory periods of test sites are shown in Table 1. Sympathetic denervation was achieved in at least one apical site in each of four dogs but not at any site in the remaining two dogs with transmural myocardial infarction. In one of the four dogs showing sympathetic denervation, ventricular fibrillation was induced during refractory period measurement 5 minutes after ligation. Ventricular fibrillation was achieved promptly by DC shock (20 J), and all data except that collected 5–20 minutes after coronary ligation were obtained and included in the analysis. Sympathetic denervation occurred at one apical site 5–20 minutes after ligation and at 10 apical sites in four dogs with transmural myocardial infarction over a period of 3 hours (Figure 3). These 10 denervated sites had shorter baseline effective refractory periods after coronary ligation than before coronary ligation (p < 0.005; Table 1).

As a group, shortening of effective refractory period induced by ansae subclaviae stimulation declined progressively at these 10 denervated apical sites (Figure 5). At the remaining 14 innervated apical sites and 12 basal sites, responses were not attenuated over time (Figure 5). Shortening of effective refractory period induced by norepinephrine infusion was not different among the basal sites, apical sites without apparent denervation, and apical denervated sites (11.8 ± 0.7, 10.4 ± 1.3, and 9.3 ± 1.0 msec, respectively; Figure 5). In four dogs with sympathetic denervation, the subepicardial width of infarction relative to the left ventricular circumference was 7 ± 4%. In the remaining two dogs without apparent sympathetic denervation, the subepicardial width of infarction was only 1–2% of the left ventricular circumference.

In the three dogs with nontransmural myocardial infarction, sympathetic denervation was not achieved. Shortening of effective refractory period at 10 apical sites tended to decline over time, but the difference was not significant in these three dogs (Table 2).

The width of subepicardial infarction relative to the left ventricular circumference tended to be greater in eight dogs with transmural myocardial infarction produced by latex embolization (10 ± 4%) than in six dogs with transmural myocardial infarction produced by ligation of multiple coronary arteries (5 ± 3%), but the difference was not significant.

Vagal Denervation

Dogs with latex injection. In one of eight dogs receiving latex injection, all apical test sites were involved in myocardial infarction; therefore, data from this dog were excluded. In the remaining seven dogs, the current strength of vagal stimulation was 0.55 ± 0.03 mA for the right vagus and 0.48 ± 0.07 mA for the left vagus. Baseline effective refractory periods of test sites are shown in Table 3. Vagal denervation was achieved in at least one apical test site in six of seven dogs and was not achieved in all 13 basal sites in seven dogs. Cumulative rate of denervated apical sites in seven dogs is shown in Figure 6. Seventeen apical test sites were vagally denervated over a period of 3 hours after latex injection (Figure 6). Four test sites were denervated as early as 5–20 minutes after latex injection, and so were another six sites 30–45 minutes after injection. The response to vagal stimulation, however, reappeared in four of these 10 sites at 60–75 minutes after latex injection and was close to the

Table 2. Changes in Effective Refractory Period Induced by Ansae Subclaviae Stimulation in Three Dogs With Nontransmural Myocardial Infarction

<table>
<thead>
<tr>
<th>Basal sites (n = 6)</th>
<th>5</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before coronary occlusion</td>
<td>-17.0</td>
<td>-14.5</td>
<td>-16.3</td>
<td>-16.2</td>
<td>-16.7</td>
</tr>
<tr>
<td>± SEM</td>
<td>1.6</td>
<td>1.0</td>
<td>0.9</td>
<td>0.8</td>
<td>0.9</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Apical sites (n = 10)</th>
<th>5</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>-14.2</td>
<td>-13.1</td>
<td>-11.8</td>
<td>-12.0</td>
<td>-10.7</td>
</tr>
<tr>
<td>± SEM</td>
<td>1.1</td>
<td>1.0</td>
<td>1.0</td>
<td>1.4</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Values in milliseconds.
TABLE 3. Baseline Effective Refractory Period During Norepinephrine Infusion in Dogs Tested for Vagal Denervation

<table>
<thead>
<tr>
<th></th>
<th>Before coronary occlusion</th>
<th>After coronary occlusion (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>I. Seven dogs with transmural myocardial infarction from latex injection of a coronary artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal sites (n = 13)</td>
<td>158±4</td>
<td>155±4</td>
</tr>
<tr>
<td>Apical sites without denervation (n = 14)</td>
<td>159±3</td>
<td>157±3</td>
</tr>
<tr>
<td>Apical sites with denervation (n = 13)</td>
<td>160±3</td>
<td>159±4</td>
</tr>
<tr>
<td>II. Nine dogs with nontransmural myocardial infarction from ligation of coronary artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal sites (n = 17)</td>
<td>159±1</td>
<td>158±1</td>
</tr>
<tr>
<td>Apical sites without denervation (n = 27)</td>
<td>157±1</td>
<td>158±1</td>
</tr>
<tr>
<td>Apical sites with denervation (n = 8)</td>
<td>162±2</td>
<td>161±1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM in milliseconds.

Baseline values at 3 hours after injection (5.8 ± 0.3 vs. 4.3 ± 0.5 msec, p<0.02). In Figure 7, the time course of changes in refractory period is shown. The four test sites at which vagal response recovered were excluded entirely from the analysis in Figure 7. Lengthening of the refractory period remained unchanged at all 13 basal test sites over a period of 3 hours after latex injection. At 13 apical sites with vagal denervation, lengthening of refractory period was significantly decreased as early as 5–20 minutes after latex injection (Figure 7). At 10 apical sites without apparent vagal denervation, responses were decreased 3 hours after injection (p<0.005).

In six dogs with vagal denervation, the subendocardial width of infarction relative to the left ventricular circumference was 14 ± 3%, and in the remaining dog without apparent vagal denervation, the relative width was 10%.

Dogs with ligation of multiple coronary arteries. Diagonal branches and collaterals were ligated in 11 dogs (average 2.2 ± 0.3 ligations). Fewer coronary arteries were ligated in this group of dogs than in the nine dogs tested for sympathetic denervation (p<0.001). In two of 11 dogs, myocardial infarction was not demonstrated by NBT staining, and in nine dogs, nontransmural myocardial infarction was present. No dog showed transmural myocardial infarction. Current strength of vagal stimulation was 0.59 ± 0.09 mA for the right vagus and 0.64 ± 0.11 mA for the left vagus in these nine dogs with myocardial infarction. Baseline effective refractory periods of test sites are shown in Table 3. Vagal denervation did not occur at all 17 basal test sites in nine dogs. Four dogs did not show vagal denervation at any apical test sites, but five dogs showed vagal denervation in at least one apical test site. Eight apical sites were vagally denervated as shown in Figure 3 and was greater in dogs receiving latex injection than in dogs with ligation of coronary arteries (p<0.002).
over a period of 3 hours after ligation, but denervation developed more slowly when compared with dogs undergoing latex injections \((p<0.002, \text{Figure 6})\). In Figure 8, the time course of changes in effective refractory period is shown. Seventeen basal test sites in nine dogs did not show significant changes. Eight apical test sites with vagal denervation showed progressive decrease in lengthening of effective refractory period and significant decrease at 30–45 minutes after ligation (Figure 8). Vagal responses did not return at any of these eight sites. At 27 apical sites without apparent vagal denervation in nine dogs, lengthening of effective refractory period was also significantly decreased over time after coronary ligation \((p<0.005)\).

In five dogs with vagal denervation, the subendocardial width of infarction relative to the left ventricular circumference was 7 ± 1% and was not different from that in four dogs without apparent vagal denervation \((11 ± 2\%)\). The relative subendocardial width of infarction in all nine dogs with coronary ligation was not different from that in seven dogs with latex injection \((9 ± 1\% \text{ vs. } 11 ± 2\%)\).

**Discussion**

**Major Findings**

Some areas of noninfarcted myocardium apical to the transmural myocardial infarction lost sympathetic neural responsiveness 5–20 minutes after coronary occlusion with latex and after ligation of multiple coronary arteries. In both types of transmural myocardial infarction, sympathetic denervation developed heterogeneously and gradually over a period of 3 hours. Sympathetic denervation did not occur in dogs with nontransmural myocardial infarction. Sympathetic denervation supersensitivity to infused norepinephrine did not occur 3–4 hours after coronary occlusion. The response to efferent vagal stimulation was also interrupted by transmural myocardial infarction produced by latex injection and by nontransmural myocardial infarction produced by coronary ligation. Vagal denervation occurred as early as 5–20 minutes after coronary occlusion and developed gradually and heterogeneously over a period of 3 hours, as did sympathetic denervation.

**Sympathetic Denervation**

Cardiac sympathetic fibers run along with large coronary arteries and are then distributed in the subepicardial layer. Our previous data showed that transmural myocardial infarction interrupted both efferent and afferent sympathetic fibers innervating the noninfarcted myocardium apical to the infarction 90 minutes after latex injection into the diagonal branch of the left anterior descending coronary artery. Sympathetic denervation was confirmed 7–21 days later biochemically, histochemically, and electrophysiologically. In these studies, we did not test systematically to determine whether responsiveness was lost earlier than 90 minutes or attempt to determine a time course of denervation. However, a study on efferent sympathetic responses produced by epicardial application of bradykinin solution indicated that some afferent responses were eliminated as early as several minutes after injection of a diagonal branch with latex and suggested that neural responses might be lost considerably sooner than 90 minutes.

Schmid et al. showed that the activity of tyrosine hydroxylase, a biochemical marker for sympathetic innervation, decreased significantly in the ischemic left ventricle 5 hours after ligation of the left anterior descending coronary artery but not in 2½ hours. The activity of this enzyme decreased further over a period of 170 hours after coronary ligation. In contrast, the data recorded in noninfarcted myocardium apical to the site of infarction from the present study (Schmid et al. did not study the noninfarcted myocardium) indicate that efferent sympathetic neural responses were interrupted 5–20 minutes after coronary occlusion. In addition to technical differences between the study of Schmid et al. and the present study, differences may be explained by a disparity between time courses for functional and biochemical changes in response to myocardial infarction.

One possible cause of early functional denervation in our model is the rapid development of hyperkalemia and acidosis in the extracellular fluid of the ischemic zone. Regional hyperkalemia exceeding 10 meq/l and pH reduction to 6.5–7.0 could depolarize the nerves located in the ischemic zone and result in functional block of transmission, possibly following an initial potassium-induced release of norepinephrine. Subsequent denervation may be due to actual nerve death, consistent with the observations of Schmid et al.

In dogs with transmural myocardial infarction produced by latex injection, apical test sites without apparent sympathetic denervation showed gradual decrease in the shortening of the effective refractory
period induced by ansae subclaviae stimulation, suggesting partial denervation. This partial denervation may have been progressive and might have become more complete if we had observed the dogs for a longer time period. Partial denervation in this manner is a new observation. It is consistent with our previous observation that, after transmural myocardial infarction produced by latex injection, some dogs demonstrated shortening of refractory period in apical sites in response to ansae subclaviae stimulation (no apparent sympathetic denervation) yet showed a supersensitive response of refractory period shortening to infused norepinephrine. It is possible that these sites became partially denervated from the infarction (e.g., Figure 4 middle panel) and that this partial denervation was sufficient to permit development of sympathetic supersensitivity. The question of why only partial denervation occurs needs to be explored in the future. Correlation with changes in myocardial blood flow would be helpful.

Dogs with ligation of multiple coronary arteries tended to have infarctions with less epicardial involvement than did dogs with latex infarction, and partial denervation did not appear to occur. Nondenervated apical test sites had stable shortening of effective refractory period induced by ansae subclaviae stimulation. This observation may be related to the fact that latex injection generally eliminates collateral flow, while dogs with ligation of multiple coronary arteries still might develop collaterals that might preserve marginally ischemic areas.

In dogs with ligation of multiple coronary arteries, 10 denervated apical sites had shorter baseline effective refractory periods after coronary ligation than before coronary ligation. Although the reason for this shortening is not clear, it is not likely that the shortening of baseline effective refractory period affected the response to ansae subclaviae stimulation. If shortening of the baseline effective refractory period were the reason for the decreased response to ansae subclaviae stimulation with time, we would have expected shortening of the baseline effective refractory period to parallel a decrease in the response to ansae subclaviae stimulation. Instead, the baseline effective refractory period remained constant.

Vagal Denervation

Vagal fibers appear to be located in the subendocardial layer after crossing the atrioventricular groove in the dog. An encircling endocardial incision 2 mm deep can interrupt efferent vagal fibers innervating the region apical to the incision. Transmural myocardial infarction produced by injection of latex into a diagonal branch interrupts efferent vagal fibers innervating the noninfarcted apical myocardium 90 minutes after injection and also decreases choline acetyltransferase activity, a marker for vagal innervation, in the normal apical myocardium 7 days after myocardial infarction. Nontransmural myocardial infarction produced by ligation of the left anterior descending coronary artery followed by reperfusion also interrupts vagal innervation in the normal myocardium overlying the infarction 24 hours after ligation. Schmid et al found that choline acetyltransferase activity did not decrease significantly in the ischemic canine left ventricle 5 hours after ligation of the left anterior descending coronary artery, but it did decrease at 25 and 170 hours. Data from the present study, however, demonstrate that functional efferent vagal denervation occurs 5–20 minutes after coronary occlusion and develops heterogeneously and gradually over a period of 3 hours, as does sympathetic denervation. This seems compatible with our previous observation demonstrating that the afferent vagal response to epicardial application of nicotine solution was significantly attenuated 13–15 minutes after ligation of a diagonal branch. As we suggested above, a measurable decrease in transmitter or enzyme activity may lag behind the loss of functional response after coronary occlusion. Functional changes may be related to regional extracellular hyperkalemia and acidosis.

Methodological Considerations

Secobarbital sodium, rather than α-chloralose was used as an anesthetic, and the ansae subclaviae were stimulated at a slightly lower frequency (2 vs. 3–5 Hz) than before. Despite these minor differences, effective refractory period shortening induced by ansae subclaviae stimulation in the present study was comparable to previous results. Our previous studies showed that in the canine heart 4–21 days after transmural myocardial infarction or dissection of the atrioventricular groove, elimination of responses in effective refractory period to ansae subclaviae stimulation or vagal stimulation, respectively, were accompanied by the biochemical evidence of denervation of sympathetic or vagal fibers. Therefore, it seems reasonable to use the changes in effective refractory period in response to stimulation of ansae subclaviae and vagi as a marker of efferent sympathetic and vagal innervation. However, because only a limited number of sites were sampled, more extensive denervation may exist than suggested by the data presented.

It took about 15 minutes to determine the duration of the effective refractory period both in the control state and during nerve stimulation. While it is possible that minor spontaneous alterations in effective refractory period might take place during those 15 minutes, this does not appear to be an important source of error. Refractory periods in the basal sites were quite constant throughout the study.

The criteria of denervation were arbitrary in the present study as they were in our previous study. In dogs with latex injection, the extent of effective refractory period shortening induced by stimulation of ansae subclaviae decreased gradually after latex injection at apical test sites that were considered to be innervated using the present definition. This finding may be explained in several ways. First, these sites may simply have exhibited a slower rate of denervation progression. If we had waited longer, these sites might
shown more complete loss of response to stimulation. Second, these test sites may have been partially denervated, consistent with data from our other studies. Finally, the criteria to establish the presence of denervation may have been too strict.

Some test sites that were designated as denervated by the present criteria (over a 3-hour period after infarction on one or more occasions) showed some variation in responses to neural stimulation exceeding the cutoff value (Figures 3 and 6). These sites were considered to be denervated and, for the group as a whole, showed a gradual decline of responses in effective refractory period to neural stimulation (Figures 4, 5, 7, and 8).

Restoration of vagal responses occurred at four of 17 apically denervated sites in dogs receiving latex injection. Although denervation tended to occur more slowly in dogs with coronary ligation than in dogs with latex injection, restoration of vagal responses did not occur in the group with coronary ligation. Collaterals do not provide an adequate answer for restoration of vagal responses because dogs with latex infarctions generally do not develop collaterals. That is the reason for using the latex method. The effects of cavity blood may not explain the restoration of vagal responses because it occurred only in dogs with latex injection. We do not have plausible explanations for recovery of the vagal response that occurred 60 minutes after latex injection at the four apical test sites.

Latex was injected to embolize collaterals and to produce a transmural myocardial infarction in the present study as in previous studies. Because of its inert properties, it is not likely that latex itself interrupted both sympathetic and vagal responses. We have shown that injection of acetone, the vehicle in which latex is suspended, into the diagonal branch does not interrupt sympathetic responses. Of importance is the fact that myocardial infarction produced by coronary artery ligation alone also interrupted both sympathetic and vagal responses in the present study. Therefore, it is reasonable to attribute the loss of sympathetic and vagal responses in dogs with latex injection to myocardial infarction, not to the latex itself. We have also shown that a careful dissection of the adventitia over the left anterior descending coronary artery or its diagonal branch does not interrupt sympathetic responses, despite recently published observations. Control measurements during ansae subclaviae stimulation in the present study were obtained after the dissection, indicating intact sympathetic innervation.

Myocardial blood flow was not determined in the present study. The site of electrode placement with respect to the infarction was determined using NBT staining. This determination plus the fact that infused norepinephrine shortened effective refractory periods equally at the basal sites and the apical denervated sites suggest that there were still functioning adrenergic receptors at the apical test sites and that there appeared to be enough blood flow to distribute infused norepinephrine to the apical test sites.

It has been shown that cardiac sympathetic and vagal fibers are distributed in the subepicardial and subendocardial layers, respectively, in the canine heart. Based on these facts, it is reasonable to assume that the subepicardial and subendocardial extent of myocardial infarction may be one of determinants that affect the efferent sympathetic and vagal responses, respectively. Therefore, the subepicardial and subendocardial width relative to the left ventricular circumference was used as an indicator of extent of infarction in dogs tested for sympathetic and vagal denervation, respectively. While there are inherent problems using this method as an indicator of the extent of myocardial infarction, the approach seemed reasonable for the questions we wanted to answer.

Clinical Implications

We showed that sympathetic denervation was arrhythmogenic in dogs with myocardial infarction 4-22 days after denervation. Although arrhythmia induction was not tested in the present study, it is quite possible that the heterogeneous development of sympathetic and vagal denervation following myocardial infarction may contribute the development of ventricular arrhythmias that occur early after coronary occlusion.

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References


**KEY WORDS** • denervation • efferent sympathetic nerve • efferent vagal nerve • effective refractory period • myocardial infarction
Time course of denervation of efferent sympathetic and vagal nerves after occlusion of the coronary artery in the canine heart.

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