Phenol Topically Applied to Canine Left Ventricular Epicardium Interrupts Sympathetic but Not Vagal Afferents

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SUMMARY. The intracardiac pathways carrying the cardiovascular reflex responses mediated by cardiac sympathetic and vagal afferent fibers were examined in this study. We investigated the response to epicardial applications of bradykinin (5 μg) and nicotine (50 μg) before and after topical applications of 85% phenol in chloralose anesthetized open-chest dogs. Bradykinin stimulated sympathetic afferents, while nicotine stimulated vagal afferents. Topical applications of phenol were used to interrupt these pathways. Before phenol encircling, bradykinin significantly increased—whereas nicotine significantly decreased—mean arterial blood pressure when applied at the same sites. After phenol, nicotine applied to all sites within and outside the phenol circle continued to decrease mean arterial pressure, whereas bradykinin applied to sites within the circle no longer increased mean arterial pressure. Removal of aortic and carotid baroreceptors did not significantly affect these responses. Painting horizontal stripes of phenol on the anterior and posterior left ventricular free wall basal to the site of bradykinin application eliminated the elevation in mean arterial pressure produced by bradykinin. Reapplication of bradykinin basal to the stripe restored its response. Phenol stripes eliminated the nicotine vasodepressor response only when the stripe was painted in the atrioventricular groove. When bradykinin and nicotine were injected via a nonocclusive intracoronary catheter, both drugs elicited an early depressor response (interrupted by vagotomy) and, in some animals a late pressor response (interrupted by stellectomy). Epicardial phenol encircling the flow distribution of the cannulated coronary artery interrupted most or all of the sympathetic afferents mediating pressor responses to bradykinin or nicotine, while leaving the depressor responses intact. The depressor responses were eliminated by applying phenol to the atrioventricular groove or by transecting the cervical vagi. These data suggest that sympathetic afferent fibers travel in the superficial subepicardium in an apex-to-base direction. Vagal afferent fibers travel deeper in the myocardium until they approach the superficial subepicardium. (Circ Res 55: 532-544, 1984)

AFFERENT nerve fibers have endings in the left ventricular wall. Some afferent fibers travel with sympathetic nerves and may be chemically sensitive, mechanosensitive, or both (Coleridge and Coleridge, 1979). These sympathetic afferent fibers mediate a pressor response and a pseudo-affective response in animals that can be elicited by epicardial application of bradykinin (Uchida and Murao, 1974a; Staszewska-Barczak et al., 1976), prostaglandins (Staszewska-Barczak et al., 1976; Baker et al., 1978), potassium in high concentrations (Uchida and Murao, 1974b; Nishi et al., 1977), or acid (Linden and Norman, 1969; Uchida and Murao, 1975). Sympathetic afferents also apparently mediate cardiac pain sensation in man (Lindgren and Olivecrona, 1947; Harken et al., 1955). Other afferent fibers travel with the vagus nerves and can be activated by epicardial application of nicotine (Kulaev, 1963; Sleight, 1964). The function of these nerves is not entirely clear, but they probably mediate depressor responses produced by ischemia or stretch of the left ventricular myocardium (Coleridge et al., 1964; Paintal, 1973; Coleridge and Coleridge, 1979).

It has been demonstrated previously that epicardial application of phenol on the anterior free wall of the left ventricle interrupts sympathetic but not vagal efferent nerves innervating epicardium and endocardium. This suggests that sympathetic efferent nerves travel in the epicardium, but vagal nerves travel deeper, possibly in the endocardium (Martins and Zipes, 1980; Takahashi et al., in press). We have also shown that acute and chronic transmural myocardial infarction interrupt sympathetic (Barber et al., 1983) or vagal (Browne et al., 1983) efferent transmission to noninfarced myocardium apical to the infarction, probably by damaging efferent nerves traversing the infarct. Little is known, however, of the intraventricular course of afferent sympathetic and vagal fibers whose nerve endings can be activated by epicardial application of bradykinin.
and nicotine, respectively. The purpose of this study was to determine if phenol applied to the epicardium of the left ventricular free wall interrupted afferent nerve impulses elicited by bradykinin and nicotine, to establish intraventricular pathways of these fibers, and to test the hypothesis that sympathetic afferent fibers travel in the subepicardium while vagal afferent fibers travel a deeper intramural or subendocardial course.

Methods

Animal Preparation

Mongrel dogs of either sex weighing 14–26 kg were anesthetized with α-chlorolose, 100 mg/kg, iv. Additional amounts (about 10 mg/kg per hr) were added as needed to maintain a level of light surgical anesthesia. Respiration was controlled with a cuffed endotracheal tube connected to a volume-cycled respirator. The dogs were placed on a heating pad to keep rectal temperature at 37°C. A left thoracotomy was performed through the 5th intercostal space, and the heart was supported by suturing the margins of the opened pericardium to the edges of the wound. The vagi were isolated high in the neck, and the stellate ganglia were isolated in the chest for later surgical interruption. A fluid-filled cannula was placed in a femoral artery was used to monitor arterial pressure, and a jugular venous cannula was used to administer drugs. A thermistor placed on the epicardium monitored temperature. Clear plastic cannula was used to administer drugs. A thermistor placed covering the wound and a light over the wound were used to maintain epicardial temperature between 36.5 and 38°C. Arterial blood gases and pH were monitored throughout the experiment at intervals of not more than 30 minutes, and NaHCO₃ was injected intravenously, or the tidal volume or respiratory rate was changed to maintain these parameters in the normal range. Arterial pressure and the electrocardiogram were recorded throughout the experiment on a physiologic recorder (Gould).

Five of these animals were used to test the influence of carotid and aortic baroreceptors on the responses to bradykinin and nicotine. These dogs were prepared in the manner described above, except that a region 2 cm above and below the carotid bifurcation was stripped of adventitia while the effect of the anesthesia dissipated until the dogs displayed conjunctival reflex and prominent muscle twitching in response to sudden jarring movement.

Phenol Circle Near the Apex

Twelve dogs were used to determine the effects of phenol applied around an epicardial site near the apex on the anterior wall of the left ventricle (Fig. 1). Bradykinin and nicotine were applied to two sites on the anterior wall of the left ventricle (site 1 near the apex and site 2 near the base). When control responses had been satisfactorily recorded, a 3-cm in diameter circle was painted around site 1 with a cotton-tipped applicator dipped in phenol (85%). The line of phenol was approximately 0.3 cm wide. Thirty minutes later, both sites were tested again with nicotine and bradykinin. In half the dogs (n = 6) the cervical vagal trunks then were cut, and both sites retested with nicotine and bradykinin. Finally, the left and right stellate ganglia were removed surgically, and both sites were retested with nicotine and bradykinin. In the remaining half of the dogs (n = 6), the order of stellate and vagal interruption was reversed.

Baroreceptor Denervation

Five dogs were used to test the effects of baroreceptor denervation. The protocol for these dogs was the same as described above, except that the two drugs were tested both before and after baroreceptor denervation. The phenol circle was placed around site 1 in all of these dogs. Autonomic ablation was done first by cutting the vagni and then by removing the stellate ganglia.

Mapping on the Anterior Wall

Ten dogs were used to map the responses to nicotine and bradykinin placed on the free wall of the left ventricle both before and after painting with phenol. This was done in two ways. In five of the dogs, an experiment similar to...
that described above was performed, with the exceptions that site 2 near the base on anterior wall was encircled with phenol, and six sites within and around the circle were tested with bradykinin and nicotine. After this series of drug applications, phenol was painted in the atrioventricular groove from the origin of the LAD to the obtuse marginal artery, avoiding the fat pad in the atrioventricular groove, and at least three sites on the anterior wall that had responded to both drugs previously were retested with nicotine and bradykinin.

In the remaining five dogs, the pattern of responses to bradykinin and nicotine on the anterior wall was tested by painting stripes of phenol 0.5–2.0 cm in length and roughly parallel to the atrioventricular groove across the left anterior descending artery or its diagonal branches. After obtaining control responses, the first stripes were painted across distal parts of the arterial tree, and after testing with bradykinin and nicotine, more proximal stripes were painted. Finally, phenol was painted on the anterior ventricular portion of the atrioventricular groove from the origin of the LAD to the obtuse marginal artery, and sites that had responded to both drugs up to this point were retested.

Mapping on the Posterior and Lateral Walls

In 10 dogs, the pattern of responses was tested on the posterior and lateral walls of the left ventricle. We prepared these dogs by performing a posterolateral thoracotomy and gaining access to the posterior and lateral walls of the left ventricle by removing the pericardium covering these regions. The remaining pericardium was then sutured to the wound margins so that the heart was rotated rightward, exposing the posterior and lateral surfaces. Multiple sites were tested with bradykinin and nicotine before and after painting with phenol.

After making control observations, we painted stripes of phenol, 0.5–2 cm long and roughly parallel to the atrioventricular groove, across the posterior descending, posterolateral, and obtuse marginal branches of the circumflex coronary artery. The first stripes were painted across the distal portions of the arterial tree, and later in the experiment, more proximal stripes were painted. Responses to bradykinin and nicotine were tested after each stripe was painted in the same manner as the experiments described for the anterior wall. Ultimately, phenol was painted from the obtuse marginal artery to the right coronary artery in the atrioventricular groove, and sites that continued to respond up to this point in the experiment were retested.

Intracoronary Injection of Bradykinin and Nicotine

In another 17 dogs, experiments were performed to define the effects of epicardial phenol application upon afferent fibers originating in the myocardium. These animals, prepared as described above, demonstrated hypertensive and hypotensive responses to epicardial bradykinin and nicotine application, respectively (see below). A diagonal branch of the LAD coronary artery was cannulated nonocclusively with a 20-cm PE-90 catheter having a total volume of 0.20 ml (Fig. 2). Prior to phenol applications, bradykinin (≤5 µg in 0.20 ml) and nicotine (≤50 µg in 0.20 ml) were injected directly into the coronary circulation via this catheter, while heart rate and blood pressure responses were being recorded. After control measurements, a circle of phenol approximately 4 cm in diameter was applied to surround the distribution of the cannulated diagonal artery. The preparation was allowed to stabilize, and measurements during bradykinin and nicotine injections were repeated. Phenol then was applied to the atrioventricular groove and the bradykinin and nicotine injections were repeated.

Because mixed pressor and depressor responses to intracoronary bradykinin and nicotine were obtained, 12 additional dogs were prepared as described above. Experiments then were performed to investigate the pathway of the depressor and pressor responses to intracoronary bradykinin and nicotine. In six animals, the cervical vagi were transected and the bradykinin and nicotine injected again. Bilateral stellectomy then was performed and the injections repeated. In the other six dogs, the order of nerve transections was reversed. No phenol applications were used in these latter experiments.

Statistics

The responses to nicotine and bradykinin applied topically to the anterior wall or injected via the intracoronary catheter were compared with responses after phenol application or nerve transections using analysis of variance and analysis of variance of repeated measures (Snedecor and Cochran, 1971). The mapping experiments are described qualitatively. Means are reported as ± standard error of the mean.

Results

Preliminary Experiments

The preliminary experiments revealed no evidence of tachyphylaxis when body temperature nicotine and bradykinin were applied repeatedly to the same site on the left ventricular wall every 10 minutes. A peak response to bradykinin continued to occur 50–60 seconds after the epicardial application of bradykinin, while the nadir of the hypotensive
response to nicotine was consistently seen 20 seconds after application (Fig. 3). Blood pressure after bradykinin application returned to control values in 120–150 seconds, and after nicotine in less than 100 seconds in this experiment. Temperature of the drug solution influenced the response to bradykinin, but appeared to have no effect on the nicotine response (Fig. 4). Bradykinin solution at room temperature produced a biphasic response with initial hypotension followed by hypertension. When the same dose of bradykinin, warmed to body temperature, was applied to the same site, only the hypertensive portion of the response was seen. Normal saline at room temperature applied to this same site produced a small hypotensive response, but when warmed to body temperature, no response was produced when applied to the epicardium.

The level of anesthesia also influenced the responses. When deep surgical anesthesia was produced, the drug responses were absent or highly attenuated. When the level of anesthesia was very light, frequently there were phasic oscillations of blood pressure and heart rate which were intensified by application of either nicotine or bradykinin. When bradykinin was applied during very light anesthesia, the pseudo-affective response consisting of stretching by the animal sometimes occurred (Staszewska-Barczak et al., 1976). Therefore, testing was done only when the anesthetic level depressed the conjunctival reflex and the pseudo-affective response, but some muscular twitching occurred when the dog was moved.

Despite careful control of the level of anesthesia, drug solution temperatures, epicardial and core temperature of the dogs, blood gases, and the time between drug applications, some dogs had no change in heart rate or blood pressure in response to nicotine, or had no hypertensive response to bradykinin. Because of the necessity to compare responses to nicotine and bradykinin and to be able to demonstrate abolition of a response, we excluded dogs from the study if they did not demonstrate a hypotensive response to nicotine within 40 seconds after application or a hypertensive response to bradykinin within 60 seconds after application under control conditions. In the course of the study, 12 of 52 dogs were rejected by these criteria in the epicardial drug application study, while 10 of 39 dogs were rejected in the intracoronary studies. Twelve of these dogs had obvious lobar pneumonia with consolidation of some lung segments.

**Phenol Circle Near the Apex**

Twelve dogs were studied successfully before and after application of a phenol circle around a site near the apex (site 1), as diagrammed in Figure 1. Mean arterial pressure and heart rate data from these dogs are shown in Figures 5 and 6. Bradykinin produced hypertension ($P < 0.001$) when applied at both sites prior to application of phenol (Fig. 5). After a circle of phenol was painted around site 1 (apex), bradykinin applied within the circle increased mean arterial pressure only $1 \pm 1$ mm Hg at 50 seconds, whereas bradykinin applied to site 2 (base) continued to produce an increase in pressure of $14 \pm 4$ mm Hg ($P < 0.001$, difference between responses at site 1 and 2). Nicotine application produced a biphasic response in arterial pressure in most dogs. All had a pressure decrease early after application, and 10 of the 12 had a subsequent increase in pressure, peaking approximately 100 seconds after nicotine application (Fig. 5). After encircling site 1 with phenol, nicotine applied to either site still produced a decrease followed by an increase in arterial pressure. Although responses at both sites were

**Figure 3.** Demonstration in a single experiment of multiple applications of bradykinin and nicotine applied to a single site on the anterior wall of the left ventricle at intervals of 10 minutes. These applications elicited reproducible responses in mean arterial blood pressure without any evidence of a tachyphylactic phenomenon.
TEMPERATURE RESPONSE

Saline

Room Temp.

168
148
128

Body Temp.

168
148
128

Bradykinin

Room Temp.

130
110
90

Body Temp.

106
86

FIGURE 4. This figure demonstrates the effect of temperature on 0.5 ml of saline or bradykinin applied to a 0.5-cm-square piece of surgical gauze on the anterior surface of the left ventricle. Saline at room temperature (24.5°C) caused a 5-mm decrease in mean arterial blood pressure, whereas saline at 38°C produced no change in pressure. Bradykinin at 24°C produced a biphasic response in arterial blood pressure, but when warmed to 38°C produced only a pressor response which was of greater magnitude than that produced by room temperature bradykinin. The temperature (24.5 vs. 38°C) of the nicotine solution did not significantly influence the response to nicotine.

slightly diminished compared to control responses, the responses after phenol were not significantly decreased from the pre-phenol responses (P = 0.1). Thus, encircling a region of epicardium on the anterior wall of the left ventricle near the apex ablated the pressor response to bradykinin applied within the circle, but did not abolish the response to bradykinin applied outside the circle near the base. In contrast, after phenol application, the nicotine response was preserved both at the apex and at the base.

Heart rate changes to nicotine and bradykinin applications are shown in Figure 6. Nicotine application prior to phenol at both sites produced a significant decrease in heart rate (P < 0.05) at 20 seconds, but heart rate returned to and exceeded control levels during the hypertensive phase. Heart rate responses to nicotine at both sites after phenol encircling of site 1 were slightly less pronounced but not significantly different (P > 0.05) than before phenol application. Bradykinin produced no significant change (P > 0.1) in heart rate.

Stellectomy eliminated the hypertension produced by bradykinin at both sites without affecting the response to nicotine in three dogs (Table 1, dogs 2, 4, and 5) in which stellectomy preceded vagotomy; vagotomy then eliminated the response to nicotine. Vagotomy eliminated the response to nicotine (Table 1, dogs 1, 3, and 6) but not to bradykinin in the three dogs that received vagotomy first; stellectomy then eliminated the response to bradykinin. Therefore, the nicotine depressor response required intact cardiac sympathetic nerves, but did not require intact cardiac sympathetic pathways. The bradykinin pressor response depended on intact cardiac sympathetic nerves, but not on intact vagi.

Baroreceptor Denervation

Baroreceptor denervation did not significantly alter the pattern of hypertensive response to bradykinin or hypotensive followed by hypertensive response to nicotine, (Table 2). Heart rate responses were also unchanged (Table 2). As before, the phenol circle abolished the response to bradykinin applied within the circle, but failed to abolish the nicotine response elicited from that site. Cutting the vagi abolished the depressor nicotine response but not the bradykinin response. After the stellate ganglia were removed, the bradykinin response could not be elicited.

Mapping on the Anterior Wall

In the group of five dogs in which the phenol circle was placed at site 2 near the base (Fig. 7), both drugs produced their usual response at each of six sites on the epicardium before phenol was applied. After making the phenol circle, nicotine continued to elicit characteristic responses at all sites. Bradykinin, however, failed to produce a response when applied within the circle or apical to it, but responses still occurred when bradykinin was applied basal, medial, or lateral to the circle. A typical example...
from one dog is shown in Figure 7. At the end of these experiments, phenol was painted in the anterior portion of the atrioventricular groove. After this procedure, neither nicotine nor bradykinin produced a response when applied anywhere on the anterior wall of the left ventricle.

In the second group of five dogs, sequential (A through D) stripes of phenol, 1 or 2 cm long, were painted across the left anterior descending artery and diagonal branches, first in their distal and then in their proximal portions. These applications abolished the bradykinin responses apical to the stripes (Fig. 8), while the nicotine response still could be elicited from all sites. Phenol painted on ventricular surface in the atrioventricular groove abolished all responses to either drug applied anywhere on the anterior wall.

**Mapping on the Posterior and Lateral Walls**

Phenol stripes across the posterior descending, posterolateral, and obtuse marginal branches of the circumflex coronary artery roughly parallel to the atrioventricular groove (Fig. 9; phenol stripe A) abolished responses to bradykinin applied apically to the stripes (sites 4 and 5), but not responses to nicotine applied at the same sites. When the posterolateral portions of the atrioventricular groove were painted with phenol (Fig. 9; phenol stripe B), responses to both nicotine (Fig. 9; phenol stripe B), responses to both nicotine and bradykinin applied

**TABLE 1**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Control Maximal ΔBP</th>
<th>Nerve transection</th>
<th>Maximal ΔBP</th>
<th>Nerve transection</th>
<th>Maximal ΔBP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>BK</td>
<td>SX</td>
<td>V</td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>-10</td>
<td>6</td>
<td>VX</td>
<td>-1</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>-6</td>
<td>11</td>
<td>SX</td>
<td>-9</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>-14</td>
<td>5</td>
<td>VX</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>-12</td>
<td>10</td>
<td>SX</td>
<td>-12</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>-9</td>
<td>21</td>
<td>VX</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

ΔBP = change in blood pressure measured in mm Hg; BK = epicardial bradykinin application; N = epicardial nicotine application; SX = bilateral stellectomy; VX = bilateral vagotomy.
TABLE 2
Effect of Baroreceptor Denervation on Maximal Blood Pressure and Heart Rate Changes to Epicardial Nicotine and Bradykinin

<table>
<thead>
<tr>
<th></th>
<th>Maximal ΔBP (n = 5)</th>
<th>Maximal ΔHR (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before BARO DNV</td>
<td>-20 ± 5</td>
<td>-30 ± 8</td>
</tr>
<tr>
<td>After BARO DNV</td>
<td>-18 ± 6</td>
<td>-21 ± 6</td>
</tr>
<tr>
<td>Bradykinin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before BARO DNV</td>
<td>11 ± 8</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>After BARO DNV</td>
<td>14 ± 5</td>
<td>5 ± 3</td>
</tr>
</tbody>
</table>

All data are expressed as mean ± SE. ABP = change in blood pressure measured in mm Hg; ΔHR = change in heart rate measured in beats/min; BARO DNV = baroreceptor denervation as described in text.

Circulation Research/Vol. 55, No. 4, October 1984

anywhere on the posterolateral surface of the left ventricle were abolished.

In two dogs there were slight variations in the distribution of the sympathetic afferent fibers. One of the sites on the posterolateral wall near the apex continued to respond to bradykinin when a stripe of phenol was painted above (basal to) it. When a second stripe was painted anterior to the site of bradykinin application, the response was still present. When, however, a stripe was painted on the inferior side of the site, no further responses to bradykinin could be obtained from this site. These findings suggest that this site was supplied with sympathetic afferent fibers that, in the apical portion of their course, traveled circumferentially on the epicardium rather than from base to apex. Therefore, there appears to be some variability in the course of left ventricular sympathetic afferents at the apex of

Figure 7. Example of one of the mapping experiments carried out on the anterior free wall of the left ventricle. Bradykinin and nicotine were applied to the six sites shown in the drawing with responses elicited at all sites. Site 2 was then encircled with phenol (interrupted circle) and all sites tested again. The responses shown are the changes in mean arterial blood pressure elicited by topical bradykinin (upper tracings) and nicotine (lower tracings) application before and after phenol circle. Numbers (right) beneath plotted responses refer to sites shown in the figure. Phenol abolished the response to bradykinin at sites 2 and 5 (site 2 is circled, site 5 is apical) while leaving nicotine responses intact. All remaining sites continued to respond to bradykinin and nicotine.
the left ventricle, but most travel from apex to base.

Intracoronary Injections of Bradykinin and Nicotine

The effects of intracoronary injections of bradykinin and nicotine were examined in 17 dogs. Of these 17 animals, one animal had no response to intracoronary bradykinin, while three dogs were unresponsive to intracoronary nicotine. Figure 10 demonstrates the mean data for these dogs. Prior to phenol, intracoronary bradykinin caused a 9 ± 1 mm Hg decrease in mean arterial pressure (P < 0.01 when compared to baseline) 30 seconds after injection, followed by a modest sustained increase in pressure of 4 ± 1 mm Hg. Intracoronary nicotine caused a maximal decrease in mean arterial pressure of 8 ± 1 mm Hg (P < 0.02) at 20 seconds after injection. After phenol circle (middle tracings, Fig. 10), a significant sustained decrease in mean arterial pressure (4 ± 1 mm Hg; P < 0.05) occurred after intracoronary bradykinin while the subsequent pressure increase was eliminated. This curve was significantly different (P < 0.05) from control. The depressor response to intracoronary nicotine was not significantly affected by the phenol circle. However, phenol applied topically to the atrioventricular groove eliminated the remaining depressor responses to intracoronary bradykinin while also preventing the decrease in pressure after intracoronary nicotine. One of the dogs continued to demonstrate a decrease in pressure to intracoronary bradykinin, while two animals had a decrease in mean arterial pressure with nicotine. In all of these animals, bilateral transection of the cervical vagi eliminated the remaining depressor responses to both bradykinin and nicotine.

In 12 additional dogs, intracoronary responses to bradykinin and nicotine were elicited under control conditions (Fig. 11) and followed by successive stellectomy then vagotomy (n = 6) or vagotomy then stellectomy (n = 6). In control conditions, both bradykinin and nicotine produced a statistically significant decrease in blood pressure, followed by an increase (not statistically significant) in blood pressure after nicotine (lower panels) and a slight increase in blood pressure after bradykinin (top right panel). Stellectomy did not significantly affect the depressor response to nicotine or bradykinin, but
Eliminated the previously seen pressor response to nicotine (left panel). Subsequent vagotomy after stellectomy produced neural decentralization and eliminated the remaining depressor response to both drugs. Conversely, when vagotomy was performed first (right panels), it eliminated the depressor response to both bradykinin and nicotine and unmasked a significant pressor response to both drugs. Subsequent sympathectomy eliminated this pressor response to the drugs.

**Discussion**

The major findings of this study are that epicardial application of phenol on the left ventricular free wall interrupted sympathetic afferent transmission and produced regions apical to the treated myocardium that no longer responded to chemical stimulation with bradykinin. Phenol did not interrupt vagal afferent transmission unless it was painted on the ventricular surface in the atrioventricular groove where it abolished sympathetic afferent reflexes as well. Sympathetic afferent fibers activated by topical bradykinin appeared to sweep over the epicardial surface of the left ventricular wall from apex to base, but did not travel only with the large, epicardial coronary arteries. There was some variability from animal to animal in the course of the sympathetic afferent fibers, particularly near the apex on the posterolateral wall. Intracoronary bradykinin activated a vagally mediated vasodepressor response, followed, in some animals, by a sympathetically mediated vasopressor response (see Fig. 10). A phenol circle significantly attenuated or eliminated the vasopressor response to bradykinin, whereas phenol in the atrioventricular groove totally eradicated the vasopressor response to bradykinin. Intracoronary nicotine produced primarily a vagally mediated vasodepressor response, but also demon-
Barber et al. / Phenol Interrupts Sympathetic but Not Vagal Afferents

BRADYKININ (N=16) NICOTINE (N=14)

CONTROL

PHENOL

CIRCLE

PHENOL IN AV-GROOVE

FIGURE 10. Responses to intracoronary injections of bradykinin (left tracings) and nicotine (right tracings) during control (top tracings) and after topical application of phenol circle around the cannulated coronary artery (middle tracings) and along the atrioventricular groove (bottom tracings). All data are expressed as mean ± SEM. ** = P < 0.01 compared to baseline; * = P < 0.02 compared to baseline.

strated a pressor component in some animals. The depressor responses were unaffected by a phenol circle but eliminated by phenol in the atrioventricular groove. A phenol circle eliminated the pressor responses to nicotine. These experiments suggest that the afferent nerves follow a course similar to their efferent counterparts (Ueda et al., 1968; Martins and Zipes, 1980; Takahashi et al., in press): sympathetic afferent fibers activated by epicardial bradykinin and intracoronary bradykinin appear to travel in the epicardium, while vagal afferent fibers activated by topical nicotine and intracoronary nicotine or bradykinin travel in deeper myocardial layers. However, both vagal and sympathetic afferent fibers appear to cross the atrioventricular groove in the epicardium.

Course of Nerves in the Left Ventricular Wall

The left ventricular course of efferent sympathetic and parasympathetic nerves has been investigated by several methods. Histological studies suggest that sympathetic efferent nerves travel over the epicardium, and the same fibers penetrate the myocardium to innervate the endocardium (Hirsch, 1971). Parasympathetic efferent fibers appear to reach the left ventricular myocardium via the interventricular septum and travel deeper within the myocardium (Hirsch, 1971). Szentivanyi et al. (1967), Randall et al. (1968), and Geis and Kaye (1968) used strain gauges affixed to the epicardium to monitor changes in contractility brought about by stimulation of sympathetic or parasympathetic efferent nerves. They found that removing thin strips of epicardium or painting phenol on the surface of the left ventricle abolished the contractile effects of stellate ganglion stimulation in localized epicardial regions apical to the epicardiectomy or phenol application. Martins and Zipes (1980) demonstrated that painting a circle of phenol with a radius of 2–3 cm around a bipolar electrode abolished shortening, induced by sympathetic efferent nerve stimulation, of the effective refractory period at both subendocardial and subepicardial poles of the electrode. Lengthening of the effective refractory period produced by vagal nerve stimulation was not affected at endocardial or epicardial sites by painting a circle of phenol around the electrode. We have also found abolition of effective refractory period shortening in response to stellate ganglion and lengthening in response to vagal stimulation in viable myocardium (both sub-

FIGURE 11. Effect of stellectomy followed by vagotomy (left side graphs) and vagotomy followed by stellectomy (right side graphs) on changes in mean arterial pressure following intracoronary bradykinin and nicotine injections. All data are expressed as mean ± SEM.
endocardium and subepicardium) apical to a region of transmural myocardial infarction (Barber et al., 1983; Browne et al., 1983). Takahashi et al. (in press) found that painting phenol in the atrioventricular groove interrupted the effects of parasympathetic afferent nerve stimulation on effective refractory period changes recorded in the subendocardium and subepicardium on the anterior wall of the left ventricle. A 2-mm-deep subendocardial left ventricular incision eliminated efferent vagal mediated prolongation of epicardial and endocardial effective refractory period on the apical side of the cut (Chilson et al., 1983). From these data it appears that, in dogs, sympathetic efferent nerves travel on the surface of the left ventricle and then penetrate into the myocardium to innervate the endocardial portions of the left ventricular wall. Sympathetic efferent transmission can be interrupted by surgical epicardectomy, superficial sclerosis produced by phenol, or transmural myocardial infarction. Vagal efferent fibers appear to travel in deeper levels of the left ventricular wall, possibly in the subendocardium, and are interrupted by transmural myocardial infarction or a shallow subendocardial incision. They may ascend near the atrioventricular groove where they seem to be located in the subepicardium, and can be interrupted by phenol applied to this region.

Little is known about the left ventricular course of afferent fibers that ultimately travel with the sympathetic or vagal nerves. Harken et al. (1955) used phenol on the surface of the heart to produce afferent denervation of the myocardium to treat intractable angina in patients. The sensation of myocardial pain is mediated by afferents traveling with the sympathetic fibers (White, 1957; Ueda et al., 1968; Uchida and Murao, 1974c; Nishi et al., 1977), so the assumption was made that phenol produced denervation. Mechanosensitive vagal afferents were studied by Thoren (1977) in cats. Using a technique of electrical stimulation of the fibers at various points in their course, he demonstrated that the fibers transmitting from these mechanosensitive endings traveled from apex to base. On the anterior wall, their course was generally toward the left main coronary artery. On the posterolateral wall, the fibers swept basally toward the posterior portion of the atrioventricular groove. Thoren did not comment on the depth of the vagal afferents within the left ventricular wall in this species.

Methodological Consideration

The interpretation of the data from these experiments depends upon the conclusion that epicardial application of bradykinin and nicotine activate sympathetic and parasympathetic afferents, respectively. Several lines of evidence suggest that the topical application of these substances may be used for this purpose. First, the pressor effect of bradykinin could be eliminated by removing the stellate ganglia, but not by sectioning the vagus nerves. The depressor effect of nicotine was abolished by vagotomy but not by interruption of the sympathetic pathways. The nicotine and bradykinin responses were affected in different ways by painting phenol on the surface of the heart, suggesting that the reflexes elicited by the two drugs required separate pathways. Carotid and aortic baroreflexes did not influence the pattern of responses elicited by nicotine or bradykinin.

Intracoronary injections of bradykinin and nicotine allowed us to examine responses of sympathetic and vagal afferents in regions other than the epicardium. To localize the distribution of these drugs, we performed nonocclusive cannulation of the first or second diagonal branch of the LAD coronary artery. This technique enabled us to examine myocardial responses with low doses of drugs that did not produce systemic effects. The disadvantages of this method were our inability to determine accurately the amount of myocardium being stimulated or the actual area of distribution of the drug. In addition, the use of intracoronary bradykinin and nicotine as selective activators of the sympathetic and vagal afferents, respectively, is less well defined.

Bradykinin injected as an intracoronary bolus in dogs has been noted to excite both sympathetic (Uchida and Murao, 1974a) and vagal (Kaufman et al., 1980) cardiac afferents. Kaufman et al. (1980) reported that bradykinin produced a marked transient increase in the activity of a population of cardiac vagal unmyelinated sensory fibers. This increase in activity lasted 20–50 seconds, with a gradual return to control activity. In dogs lightly or fully anesthetized with chloralose, intracoronary injection of bradykinin has resulted in pressor, depressor, or biphasic changes in blood pressure (Staszewska-Barczak et al., 1976; Staszewska-Barczak, 1983). Results from our experiments support these observations, inasmuch as variable responses to intracoronary bradykinin were seen from animal to animal, but the mean response was one of an early hypotension followed by a pressor response (see Figs. 10 and 11, top right panel). The pressor response to bradykinin was interrupted by phenol encircling the coronary artery, whereas the depressor response was eliminated with phenol in the atrioventricular groove, or by vagotomy (Fig. 10). The marked pressor response after vagotomy (Fig. 11, top right) was eliminated by stellectomy.

Intracoronary nicotine in most animals gave a depressor response (Figs. 10 and 11, bottom panels), whereas some dogs showed a biphasic response. The depressor response to nicotine was eliminated by vagotomy (Fig. 11) or phenol in the atrioventricular groove (Fig. 10), whereas the pressor response was eliminated by stellectomy (Fig. 11, bottom right panel). Dawes (1947) showed that small amounts of nicotine injected into the coronary arteries resulted in a hypotensive response. Sleight (1964) demon-
Barber et al. / Phenol Interrupts Sympathetic but Not Vagal Afferents

543

The use of phenol as a superficial sclerosing agent is well established (Geis and Kaye 1968; Martins and Zipes, 1980). Phenol applied to the epicardium produces necrosis only about 0.25 mm in depth (Kaye et al., 1968). Thus, phenol painted in very localized regions to necrose the epicardium served as a precise method to interrupt nerve fibers to small regions of the left ventricular wall. These studies were designed neither to investigate interactions between vagal and sympathetic afferent responses nor to delineate pathways other than functional intraventricular routes and whether the afferent limb was carried by vagal or sympathetic nerves. The data must be interpreted within these limitations.

Implications of the Experiments

These experiments suggest that chemically sensitive afferent nerve fibers follow a course similar to their efferent counterparts in the left ventricular wall, and that sympathetic and vagal afferents follow separate pathways in the left ventricular wall. Both types of afferents can be interrupted by painting phenol in the atrioventricular groove, and this procedure may be a method by which afferent autonomic interruption of the ventricles might be accomplished. Other means of producing epicardial damage, such as pericarditis (Harken et al., 1955), may also cause sympathetic afferent interruption. Transmural myocardial infarction could potentially destroy both sympathetic and parasympathetic afferent fibers passing through the infarcted region to innervate surviving myocardium apical to the infarcted region (Barber et al., 1982).

Such reflexes could be important modulators of blood pressure, myocardial and smooth muscle contractility, heart rate, and cardiac electrophysiological properties in a variety of situations such as myocardial infarction. The nontransmural infarction could interrupt one or the other autonomic limb and thus create a situation of autonomic imbalance that could be conducive to arrhythmia development (Randall et al., 1978). However, since these observations were obtained in anesthetized animals, it remains unproven whether our conclusions from these data can be applied to the conscious animal.

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strated that intracoronary and epicardial applications of nicotine elicited similar responses in the dog. In our experiments, all dogs had an initial hypotensive response to intracoronary nicotine, as described above, which was eliminated by phenol in the AV groove or by vagotomy. It appears, though, that intracoronary nicotine in some animals is not selective for activation of vagal afferents, and caution must be used in interpreting those data.

Bilateral vagotomy often unmasked a more marked response to intracoronary bradykinin and nicotine that was eliminated by subsequent stellatomy (Fig. 11, right panels). These data suggest that the response to vagal afferent stimulation elicited by these drugs modulated the vasopressor responses mediated by stimulation of sympathetic afferents. Interestingly, when stellatomy was performed first, it did not enhance the vasodepressor response mediated by vagal afferents (Fig. 11, left panels).

The use of topical nicotine and bradykinin as activators of specific reflexes that could be tested after experimental interventions was undoubtedly aided by excluding data from dogs that did not respond to epicardial nicotine or bradykinin. Several investigators have demonstrated that the responses to these substances can vary from animal to animal and also, with different doses (Kulaev, 1963; Sleight, 1964; Uchida and Murao, 1974a; Felder and Thames, 1982). We attempted to minimize the variability among animals by carefully controlling anesthetic level, body temperature, drug temperature, time between drug applications, blood gasses, and the manner of drug application. Twelve of 52 dogs in which we studied epicardial drug applications did not have a depressor response to nicotine or apressor response to bradykinin under control conditions, and were therefore excluded. Ten of 39 dogs studied were unresponsive to intracoronary injections of the drugs. A single dose of each drug was used throughout the experiments, so no attempt was made to demonstrate a dose-response relationship. Thus, under these restricted experimental conditions, the two drugs appeared to serve as selective activators of epicardial reflexes requiring afferent fibers traveling with different limbs of the cardiac autonomic nerves.

In addition to their ability to activate sympathetic or vagal afferent fibers selectively, epicardial application of nicotine and bradykinin on small pieces of surgical gauze provided the distinct advantage of more precise localization of the stimulus. This was important for the purpose of mapping, and it avoided the obvious difficulties attendant with intrapericardial (Sleight, 1964) or intravascular administration of the drugs. The disadvantages of this method were that only the pathways of afferent nerves subserving receptors on the epicardial surface where the drug was applied could be studied, and presumably only those fibers with chemically sensitive endings were activated by application of bradykinin and nicotine.
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