Efferent Sympathetic and Vagal Innervation of the Canine Right Ventricle

Makoto Ito, MD; Douglas P. Zipes, MD

**Background** The functional pathways of efferent sympathetic and vagal innervation to the right ventricle (RV) might be important in a variety of disease states that involve the RV wall. The purpose of this study was to investigate those pathways.

**Methods and Results** We determined the effects of phenol and endocardial radiofrequency ablation applied to the RV anterolateral wall and outflow tract on effective refractory period (ERP) shortening during bilateral ansae subclaviae stimulation and ERP lengthening during bilateral vagal stimulation. We found that efferent sympathetic axons to the RV are located in the superficial subepicardium and that lateral sites receive sympathetic innervation predominantly from the lateral margin of the RV near the AV groove. Medial sites close to the left anterior descending coronary artery (LAD) receive sympathetic innervation from both the right lateral atrioventricular (AV) groove and regions near the LAD. At the RV outflow tract, some sympathetic fibers are located intramurally. Efferent vagal fibers are located at the RV surface within 10 mm of the right lateral AV groove; they penetrate intramurally and reach to the medial sites of the RV anterior wall. Other vagal fibers originate near the LAD and are intramural. Vagal fibers to the RV outflow tract are located intramurally either from the lateral side (close to the right coronary artery) or medial side (close to the LAD).

**Conclusions** Efferent vagal and sympathetic innervation of the right ventricle resembles that of the left ventricle. A major difference is that efferent sympathetic fibers to the right ventricular outflow tract are located not only in the subepicardium but in the subendocardium as well. *(Circulation, 1994;90:1459-1468.)*

**Key Words** • nervous system • ventricles • innervation

Previous studies have explored the functional pathways of innervation to the left ventricle. Efferent sympathetic axons are located throughout the course in the superficial subepicardium, predominantly along with the larger coronary arteries. Most efferent vagal fibers as they cross the atrioventricular (AV) groove are found within 0.25 to 0.5 mm of the epicardial surface but then dive intramurally, at which point they are located in the left ventricular subendocardium.1,5

The functional pathways of efferent sympathetic and vagal innervation to the right ventricle (RV) have not been studied in detail and might be important in a variety of disease states, such as RV infarction, arrhythmogenic RV dysplasia, and ventricular tachycardia arising from the RV outflow tract. The RV consists of two major components, the trabecular zone and the outflow tract. Developmental studies of embryos show that the RV outflow tract consists of different anatomic substrates compared with the trabecular zone.6,7 Such anatomic changes may also create different autonomic innervation patterns.

The purpose of this study was to determine the functional pathways of efferent sympathetic and vagal nerves in the RV.

**Methods**

**Surgical Preparation**

Thirty-nine mongrel dogs of either sex weighing 20 to 36 kg were premedicated with morphine sulfate (2 mg/kg IM) and anesthetized with thiopental (20 mg·kg⁻¹ IV). Anesthesia was maintained with α-chloralose (75 mg·kg⁻¹ IV and 20 mg·kg⁻¹·h⁻¹ IV). The dogs were ventilated by means of a cuffed endotracheal tube and volume-cycled respirator (model 607, Harvard Apparatus). The chest was opened through a median sternotomy. A fluid-filled catheter was inserted into the left femoral artery and connected to a transducer (Statham P-23Db, Gould) to monitor arterial blood pressure. The left femoral vein was cannulated to infuse normal saline at 100 to 200 mL·h⁻¹ to replace spontaneous fluid losses. Dogs were placed on a heating pad, and the thoracotomy was covered by a plastic sheet. A thermistor (model 400, Yellow Springs Instrument) was used to monitor epicardial temperature. An operating room table lamp was used to maintain epicardial temperature at 36°C to 38°C. Arterial blood gases and pH were monitored and maintained within the physiological range.

**Electrode Placement for Measurement of Effective Refractory Period**

With a 25-gauge needle, two hook electrodes made from Teflon-coated wires, insulated except for their tips, were placed 2 to 3 mm beneath the epicardium in the right lateral RV myocardium (Fig 1, sites 1 and 2) close to the right lateral AV groove. Two electrodes were also inserted into the RV myocardium (Fig 1, sites 3 and 4) close to the left anterior descending coronary artery (LAD), and two additional electrodes were inserted into the myocardium of the anterior RV outflow tract (Fig 1, sites 5 and 6). The distance between electrodes 1 and 2 and electrodes 3 and 4 was 20 to 30 mm. The electrodes served as the cathode for unipolar stimulation, with the anodal electrode placed in the abdominal wall. A bipolar plunge electrode was inserted into the myocardium of the left ventricle to record the ventricular responses. Data collection began 30 minutes after the placement of the plunge electrodes.

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vagi also were isolated bilaterally, doubly ligated, and transected. Shielded bipolar electrodes were placed on the right and left anterior and posterior ansae subclaviae to stimulate the efferent cardiac sympathetic nerves with a stimulator (model SD-88, Grass Instrument Co). Stimuli were rectangular 4-millisecond pulses delivered at a frequency of 2 to 4 Hz and at 1.0 to 3.0 mA. Determination of the ERP was begun 2 minutes after the onset of ansae subclaviae stimulation. The cervical vagi were stimulated with rectangular pulses of 4-millisecond duration at a frequency of 20 Hz through two Teflon-coated wire electrodes embedded in the cardiac end of the cut nerve. The current strength was 0.05 mA greater than that required to produce asystole (>2 seconds) for the right vagus and asystole or complete AV block during spontaneous rhythm for the left vagus. The effects of efferent vagal stimulation on ventricular ERP were determined during intravenous infusion of norepinephrine at a rate of 0.125 to 0.25 μg·kg⁻¹·min⁻¹ to achieve a constant background of sympathetic effect. The conditions of neural stimulation were kept constant in each experiment.

Protocols

Efferent Sympathetic Innervation

Baseline ERP and ERP during bilateral ansae subclaviae stimulation were determined before (during control) and 20 minutes after each phenol application. Fig 1 shows the position of phenol strips and the plunge electrodes. Table 1 shows the different groups of animals studied.

To investigate the sympathetic innervation of the RV anterolateral wall, we applied vertical strips of phenol parallel to the right lateral AV groove, between the right lateral AV groove and the LAD (Fig 1, top left) in groups 1, 2, and 3: strip A, between the right lateral AV groove and test sites 1 and 2 (lateral sites); strip B, between test sites 1 and 2 and test sites 3 and 4 (medial sites); and strip C, between test sites 3 and 4 and the LAD. The sequence of phenol application in each group is shown in Table 1. A separate group of dogs (group 4, n=3) received phenol application perpendicular to strips A through C (Fig 1, top right): strip D, above electrodes 1 and 3; strip E, below electrodes 1 and 3; strip F, below electrodes 2 and 4; and strip G, above test sites 5 and 6 at the RV outflow tract. The sequence of phenol strips was E-F-D-G.

To determine the sympathetic pathways at the RV outflow tract, we applied strips of phenol around test sites 5 and 6 at the RV outflow tract (Fig 1, bottom left) in 11 dogs from groups 1, 2, and 3 (2 from group 1, 4 from group 2, and 5 from group 3): strip H, between test sites 5 and 6 and the right coronary artery (RCA); strip I, between test sites 5 and 6 and the LAD. The distance between the electrodes and strip H or I was 15 to 20 mm. The sequence of the phenol strips and the number of test dogs are shown in Table 1.

In group 5 (5 dogs), we applied phenol strips H and I on the RV outflow tract without applying phenol on the RV anterolateral wall (Fig 1, bottom left). There were four test sites at the RV outflow tract (electrodes 5 and 6 and two other electrodes 5 mm below electrodes 5 and 6 as in Fig 1, bottom left) and two test sites at the RV anterolateral wall (sites 2 and 4 as in Fig 1, bottom left).

To examine whether some of the sympathetic pathways to the RV outflow tract were concentrated in the subendocardium, we determined the sympathetic response before and after creating endocardial lesions in 3 dogs in group 5. Baseline ERP and ERP during bilateral ansae subclaviae stimulation were obtained after phenol strips H and I. Then a radiofrequency catheter (hexapolar or octapolar ablation catheter; each electrode length, 3 mm; interelectrode distance, 5 mm; EP Technologies Inc) was introduced into a femoral or a jugular vein and advanced to the RV outflow tract. Catheters were positioned on the medial (close to the LAD) or lateral (close to the origin of the RCA) sides of the RV outflow tract.

**Phenol Application and Measurement of Effective Refractory Period**

We applied strips of 88% phenol (4 to 6 cm long, 3 to 5 mm wide) with a wooden applicator stick to the RV epicardium. The effective refractory period (ERP) was determined at each electrode site by the extrastimulus technique by use of a programmable stimulator (Kranzert Medical Engineering) and a constant-current isolator as we have described previously.

**Neural Stimulation**

The ansae subclaviae were isolated bilaterally as they exited from the stellate ganglia, doubly ligated, and cut. The cervical

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**Fig 1.** Diagrams showing position of phenol strips on the right ventricular anterolateral wall and outflow tract and position of endocardial lesions induced by radiofrequency (RF) current in the sympathetic (top, bottom left) and vagal innervation studies (bottom). Closed circles indicate positions of the plunge electrodes. Top left, Stripped lines A through C indicate the phenol strips parallel to the right lateral atroventricular (AV) groove and the left anterior descending coronary artery (LAD). Stripped lines H and I indicate the strips of phenol placed around the plunge electrodes at the right ventricular outflow tract. Top right, Stripped lines D through G indicate the position of phenol strips perpendicular to the right lateral atroventricular groove and the LAD in group 4. Bottom left, Stripped lines H and I indicate the strips of phenol placed around the plunge electrodes at the right ventricular outflow tract. Lateral RF indicates the endocardial lesion close to the origin of the right coronary artery, and medial RF indicates the endocardial lesion close to the left anterior descending coronary artery. Striped line AV indicates the strip of phenol on the ventricular epicardium along the right and left antroventricular grooves. Bottom right, Striped lines J through M indicate the position of phenol strips on the right ventricular anterolateral wall and outflow tract in the vagal innervation studies. Striped line O indicates the phenol strip on the ventricular wall from the medial edge of strip M, across the LAD, along the atroventricular groove, to the interior end of strip J. Continuous strips J, M, and O create strip AV in the left panel. See text for details. RA indicates right atrium; RV, right ventricle; Ao, aorta; LV, left ventricle; LA, left atrium; PA, pulmonary artery.
Radiofrequency current (unmodulated continuous sine wave, 500 kHz) was generated by a power source (EP Technologies Inc) and delivered at 20 to 40 W. The sequence of application was at the medial (medial RF) and then at the lateral (lateral RF) sides of the RV outflow test sites (Fig 1, bottom left). Baseline ERP and ERP during bilateral ansae subclaviae stimulation were determined 30 minutes after delivery of each radiofrequency current. ERP response to norepinephrine infusion (0.125 or 0.25 µg·kg⁻¹·min⁻¹) was obtained at the end of the study.

In 2 dogs in group 5, we tested whether some of the sympathetic nerves to the RV outflow tract cross the AV groove. After phenol strips H and I, we applied an additional strip of phenol to the ventricular epicardium along the AV groove in a continuous band from the origin of the RCA, along the RV outflow tract near the origin of the pulmonary artery, across the origin of the LAD, beneath the left atrial appendage, and along the left circumflex coronary artery to the origin of the RCA (AV in Fig 1, bottom left).

**Efferent Vagal Innervation**

The epicardium of the right lateral AV fat pad was incised along its ventricular limits, and the fat pad was dissected (2 to 5 cm long and 5 to 15 mm wide) away from the RV wall without damaging vessels or myocardium. Such a dissection did not reach the atrial wall and did not alter ERP prolongation in response to vagal stimulation. In all dogs (n=12) of groups 6 and 7, we removed part of the fat pad along the right lateral AV groove in this manner before applying phenol directly on the RV wall at the AV groove.

The plunge electrodes for groups 6 and 7 were positioned at sites 1 through 6 noted earlier (Fig 1, bottom right). We applied phenol strips as follows (Fig 1, bottom right): strip J, on the RV wall of the right lateral AV groove ≤10 mm from the AV groove; strip K, between the AV groove and test sites 1 and 2 >10 to 15 mm from the AV groove; strip L, a continuous band of phenol from the upper end of strip J, above electrodes 1 and 3, between test sites 3 and 4 and the LAD, and along the left posterior descending branch of the left circumflex coronary artery to the inferior end of strip J. The sequence of strips in each group is shown in Table 1. In 2 dogs in group 7, the number of test sites was four for the lateral sites, none for the medial test sites, and two for the RV outflow tract.

To determine the vagal pathways to the RV outflow tract, additional strips of phenol were applied to the RV epicardium (Fig 1, bottom right) after strips K, L, and J in 3 dogs in group 6: strip M, from the upper end of strip J through the origin of the RCA and the base of the pulmonary artery to the site between the RV outflow test sites and the LAD.

We applied an additional strip of phenol to the ventricular epicardium along the AV groove in 3 dogs in group 6: strip O, a phenol strip in a continuous band from the medial end of strip M, across the origin of the LAD, beneath the left atrial appendage, and along the circumflex coronary artery to the inferior end of strip J (Fig 1, bottom right). In 5 dogs (2 from group 6, 3 from group 7), phenol was applied at strips M and O after phenol applications on the anterolateral wall.

In a separate group of 3 dogs (group 8), to create an endocardial lesion around the test sites at the RV outflow tract, we applied radiofrequency current as described above in the “Efferent Sympathetic Innervation” section. Radiofrequency current was applied endocardially to the lateral sides of the RV outflow tract first, then to the medial sides of the RV outflow tract (Fig 1, bottom left). There were four plunge electrodes for the RV outflow tract (electrodes 5 and 6 and two other electrodes 5 mm below electrodes 5 and 6) and two (electrodes 2 and 4 in 1 dog) or three (electrodes 1, 2, and 4 in 3 dogs) for the RV anterolateral wall. ERPs were determined.

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**Table 1. Sequence of Phenol Strips and Radiofrequency Current Application in Efferent Sympathetic and Vagal Innervation Studies**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sympathetic innervation</th>
<th>Vagal innervation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RV Anterolateral Wall</td>
<td>RV Outflow Tract</td>
</tr>
<tr>
<td>Group 1</td>
<td>B-A-C</td>
<td>I-H (N=2)</td>
</tr>
<tr>
<td>Group 2</td>
<td>B-C-A</td>
<td>H-I (N=1)</td>
</tr>
<tr>
<td>Group 3</td>
<td>C-A</td>
<td>I-H (N=3)</td>
</tr>
<tr>
<td>Group 4</td>
<td>E-F-D</td>
<td>H-I</td>
</tr>
<tr>
<td>Group 5</td>
<td></td>
<td></td>
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</tbody>
</table>

**AV (N=2)**

**Lateral-medial (N=3)**

**Medial-lateral**

| RV indicates right ventricular; N, number of test dogs; and AV, phenol application on the ventricular epicardium along the right and left atrioventricular groove (see text and Fig 1). Phenol strips A through O and AV, see Fig 1.
before and 30 minutes after delivery of each radiofrequency current. ERP response to methacholine chloride infusion (12.5 \( \mu \)g \( \cdot \) kg\(^{-1}\) \( \cdot \) min\(^{-1}\)) was determined during norepinephrine infusion (0.125 or 0.25 \( \mu \)g \( \cdot \) kg\(^{-1}\) \( \cdot \) min\(^{-1}\)) at the end of the study in 2 dogs in group 10.

### Analysis of Data

As reported previously,\(^8\)\(^{-10}\) sites were considered to be sympathetically denervated if bilateral ansae subclaviae stimulation shortened the ERP \( \geq 9 \) milliseconds before phenol application but \( \leq 2 \) milliseconds after phenol or radiofrequency current application. Sites were considered to be vagally denervated if bilateral vagal stimulation prolonged ERP \( \geq 3 \) milliseconds before phenol or radiofrequency current application but \( \leq 1 \) millisecond after phenol or radiofrequency current application. Sympathetic attenuation was defined as present when ERP shortening induced by bilateral ansae subclaviae stimulation was reduced compared with control value after phenol or radiofrequency current application but still was \( > 2 \) milliseconds. Vagal attenuation was defined as present when ERP prolongation induced by bilateral vagal stimulation was reduced compared with control value after phenol or radiofrequency current application but still was \( \geq 1 \) millisecond.

Data from ventricular test sites with \( < 9 \)-millisecond shortening of the ERP elicited by bilateral ansae subclaviae stimulation or \( < 3 \)-millisecond lengthening of ERP induced by bilateral vagal stimulation during the first control determination were excluded because of insufficient effects of neural stimulation at those particular sites.\(^8\)\(^{-9}\) A total of 10 sites of 243 (4%) met these exclusion criteria.

After completion of the study, hearts were fibrillated and excised with electrodes still in place. The positions of the electrodes were measured, and the hearts were cut into slices and stained with triphenyltetrazolium chloride to measure the lesion size made by radiofrequency current.

Data were expressed as mean\(\pm\)SEM. One-way repeated-measures ANOVA and Newman-Keuls comparisons were used to compare the baseline ERPs and ERP response to ansae subclaviae stimulation or vagal stimulation. Statistical significance was set at a value of \( P<.05 \).

### Results

#### Efferent Sympathetic Innervation of the RV Anterolateral Wall

Baseline ERPs before and after phenol application on the RV epicardium are shown in Table 2. Fig 2 shows the effects of the phenol strips on ERP shortening induced by bilateral ansae subclaviae stimulation. In group 1 (Fig 2, top), strip B alone showed no effects on ERP shortening at the lateral test sites (sites 1 and 2). However, addition of strip A attenuated ERP shortening at lateral sites (\( P<.001 \)). No lateral sites showed denervation after strip B alone, but 8 of 9 sites exhibited denervation after addition of strip A. In contrast, at medial test sites (sites 3 and 4), strip B alone attenuated ERP shortening (\( P<.001 \)). Addition of strip A showed no effects on ERP shortening, whereas addition of strip C further reduced ERP shortening at medial sites compared with strip A (\( P<.001 \)). No medial sites showed denervation after strip B alone, but 9 of 9 sites exhibited denervation after addition of strip C.

In group 2 (Fig 2, middle), phenol strips B and C did not affect ERP shortening at lateral test sites. Addition...
Sympathetic Innervation of the RV Outflow Tract

Phenol strips below and above the test sites at the RV outflow tract did not affect bilateral ansae subclaviae stimulation–induced ERP shortening (group 4, see above). Baseline ERPs are shown in Table 2 for the 11 dogs (2 group 1 dogs and all group 2 and 3 dogs) that received phenol strips H and I (Fig 1, left) around the electrodes at the RV outflow tract. Phenol painting on the RV anterolateral wall did not affect ERP shortening at sites 5 and 6 in the RV outflow tract (A+B+C or A+C in Fig 3, top), but phenol strip H attenuated ERP shortening at the RV outflow test sites and denervated 1 of 10 sites (Fig 3, top left). Addition of strip I further attenuated ERP shortening and denervated 2 additional sites in 5 dogs (Fig 3, top left). In 6 other dogs, strip I attenuated ERP shortening and strip H further reduced ERP shortening (Fig 3, top right); strip I denervated no sites, and addition of strip H denervated 3 of 12 sites.

Fig 3, bottom left, shows the effects of phenol strips H and I on the ERP shortening at the RV outflow test sites in group 5 that did not receive phenol painting on the RV anterolateral wall. Strips of phenol at H attenuated ERP shortening and denervated 1 of 20 sites, and addition of strip I further attenuated the ERP shortening and denervated 1 additional site. Thirty-four of 42 RV outflow test sites were still sympathetically innervated after phenol application of strips H and I, ie, phenol encircling the RV outflow test sites in 16 dogs. In 2 dogs in group 5, phenol on the ventricular epicardium at both the right and left AV grooves (AV in Fig 1, bottom left) did not denervate any RV outflow test sites (data not shown).

Phenol strips H and I did not affect sympathetic response at the RV anterolateral sites in group 5 dogs; bilateral ansae subclaviae stimulation–induced ERP shortening was 12.4±0.9 milliseconds control, 13±1.0 milliseconds after H, and 12.2±0.8 milliseconds after I.

Table 2 shows baseline ERP data for the 3 group 5 dogs that received phenol strips and radiofrequency current to the endocardium of the RV outflow tract. Phenol strips H and I did not affect the ERP response at RV anterolateral test sites 2 and 4 but attenuated the sympathetic response at the RV outflow tract test sites and denervated 1 of 12 sites (Fig 4, left).

The lesions created by radiofrequency current were 30 to 43 mm long, 3 to 13 mm wide, and 2 to 7 mm deep from the endocardium (three were transmural and three were nontransmural subendocardial lesions). The distance from the edges of the lesions to the electrodes ranged from 9 to 17 mm. No RV outflow test sites were located in the lesions made by radiofrequency current. These lesions did not affect the ERP response at RV
SYMPATHETIC INNERVATION

RV Outflow Tract

With Phenol on Anterolateral Wall

Without Phenol on Anterolateral Wall

Fig 3. Bar graphs show effects of phenol strips to the right ventricular (RV) anterolateral wall and along the plunge electrodes at the RV outflow tract. Diagram at bottom right shows the position of plunge electrodes and phenol strips. Top left shows effects of phenol application to the RV anterolateral wall (A+B+C or A+C) and outflow tract (H and I) on effective refractory period (ERP) shortening induced by bilateral ansae subclaviae stimulation at the RV outflow test sites. Data were obtained in 5 dogs: 1 from group 2 and 4 from group 3. Top right shows effects of phenol strips on the ERP shortening at the RV outflow test sites. Data were obtained from 6 dogs: 2 from group 1, 3 from group 2, and 1 from group 3. Bottom left shows effects of phenol strips H and I on ERP shortening at the RV outflow test sites in the dogs that did not receive phenol strips on the RV anterolateral wall. Data were obtained from group 5 dogs. Bottom right shows the position of plunge electrodes and phenol strips. Both phenol strips H and I attenuated ERP shortening. *P<.001 vs control; tP<.01 vs H; tP<.001 vs I. Format and abbreviations are as in Fig 2.

Efferent Vagal Innervation of the RV Anterolateral Wall

Baseline ERPs during norepinephrine infusion are shown in Table 3. Removing the fat pad over the RV along the right lateral AV groove did not affect ERP prolongation induced by vagal stimulation at lateral and medial test sites in the anterolateral RV wall (7.3±0.4 milliseconds during control versus 7.6±0.4 milliseconds after the fat pad was removed, n=32) and at the RV outflow tract test sites (7.3±0.6 milliseconds control versus 7±0.4 milliseconds after the fat pad was removed, n=16).

In group 6, ERP prolongation induced by bilateral vagal nerve stimulation was not attenuated after strip K or L but was attenuated after strip J (Fig 5, top left) at lateral test sites 1 and 2. No lateral test sites in group 7 were vagally denervated after strips K and L, but 7 of 10 sites were denervated after addition of strip J. At medial test sites 3 and 4, ERP prolongation was unchanged after strips K and L but was attenuated after strip J in group 7 (Fig 5, top middle). However, none of 10 medial test sites were denervated after strips K, L, and J.

In two dogs that received strips K, J, and L in group 7, no lateral sites were denervated after strip K, whereas 4 of 4 sites were denervated after addition of strip J. None of 4 medial sites were denervated after strips K, J, or L. In 5 dogs (Fig 5, bottom) that received strips J and L in group 7, strip J alone attenuated ERP prolongation (P<.001) at lateral test sites and denervated 11 of 14 sites (Fig 5, bottom left). Strip J alone reduced ERP prolongation (P<.05) at medial sites but denervated only 1 of 6 sites. Addition of a phenol strip at L did not affect ERP prolongation or denervate at
medial sites (Fig 5, bottom right). Phenol strips J, K, and L did not affect the ERP response to vagal stimulation at the RV outflow tract: ERP prolongation induced by bilateral vagal stimulation was 7.9±0.8 milliseconds control, 8.1±0.9 milliseconds after K, 7.9±0.9 milliseconds after L, and 8.3±0.8 milliseconds after J in group 6 and 7.3±0.6 milliseconds control, 6.3±0.5 milliseconds after J, and 6.2±0.5 milliseconds after L in group 7 (P>.1).

Fig 6, top, shows the effect of the phenol strips at the RV wall and the AV groove on bilateral vagal stimulation–induced ERP prolongation in the RV medial sites. Strips K+L did not affect ERP prolongation at medial test sites, whereas strip J attenuated the ERP response in 3 dogs in group 6 (Fig 6, top left). Addition of phenol strips at M showed no additional effects on ERP prolongation at medial test sites (Fig 6, top left). However, addition of phenol strip O further attenuated ERP prolongation and denervated 4 of 6 medial sites (Fig 6, top left). In another 5 dogs (2 from group 6, 3 from group 7), phenol strips J, K, and L or strips J and L reduced ERP prolongation at 10 medial sites (Fig 6, top right). Addition of phenol strips M+O further reduced ERP prolongation and denervated 4 of 10 medial sites (Fig 6, top right).

**Vagal Innervation of the RV Outflow Tract**

Phenol strips at the RV anterolateral wall (strips J, K, and L) did not affect ERP prolongation induced by vagal stimulation at the RV outflow test sites in 3 dogs

<p>| Table 3. Changes in Baseline Effective Refractory Periods During Intravenous Infusion of Norepinephrine |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Phenol Application</th>
<th>Radiofrequency Current Application (RV Outflow)</th>
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</thead>
<tbody>
<tr>
<td>Group 6 (N=5)</td>
<td></td>
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<tr>
<td>Lateral sites (n=10)</td>
<td>146±4</td>
<td>146±3</td>
<td>146±4</td>
</tr>
<tr>
<td>Medial sites (n=10)</td>
<td>149±4</td>
<td>147±3</td>
<td>148±3</td>
</tr>
<tr>
<td>Outflow tract (n=10)</td>
<td>149±4</td>
<td>146±4</td>
<td>147±4</td>
</tr>
<tr>
<td>Group 7 (N=7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral sites (n=18)</td>
<td>138±4</td>
<td>145±2</td>
<td>140±3</td>
</tr>
<tr>
<td>Medial sites (n=5,n=10)</td>
<td>147±4</td>
<td>142±2</td>
<td>149±4</td>
</tr>
<tr>
<td>Outflow tract (n=14)</td>
<td>144±3</td>
<td>151±2</td>
<td>142±4</td>
</tr>
<tr>
<td>Group 8 (N=3)</td>
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<tr>
<td>Anterolateral sites (n=8)</td>
<td>148±3</td>
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<tr>
<td>Outflow tract (n=12)</td>
<td>151±2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**VAGAL INNERSATION**

Fig 5. Bar graphs show effects of various strips of phenol on effective refractory period (ERP) prolongation induced by bilateral vagal stimulation (ordinate) in group 6 (top) and group 7 (bottom). Diagram at top right shows the position of plunge electrodes and phenol strips. Strips K and L did not affect ERP prolongation, but strip J attenuated the ERP response at lateral test sites (left). Strip J attenuated ERP prolongation at medial test sites (middle), but strips K and L showed no significant effect on ERP response. Cont indicates control. *P<.05, **P<.001 vs control.
in group 6 (Fig 6, bottom left). Addition of strip M showed no significant effects on ERP prolongation at the RV outflow test sites (Fig 6, bottom left). Phenol application at strip O attenuated ERP prolongation and denervated 1 of 6 outflow test sites (Fig 6, bottom left). In 5 dogs (2 from group 6, 3 from group 7), phenol application to the RV anterolateral wall (strips J, K, and L) or strips J and L) did not affect ERP prolongation induced by vagal stimulation. Addition of phenol strips M+O attenuated ERP prolongation and denervated 1 of 10 RV outflow test sites (Fig 6, bottom right).

The lesions created by radiofrequency current (lateral and medial RF) were 27 to 42 mm long, 3 to 17 mm wide, and 2 to 7 mm deep from the endocardium (four transmural and two nontransmural lesions). The distance from the edges of lesions to the electrodes ranged from 6 to 19 mm. No electrodes were located in the lesions created by radiofrequency current. Lateral RF attenuated the ERP response to vagal stimulation and denervated 2 of 12 sites (Fig 7, left). Addition of medial RF further reduced the vagal response and denervated an additional 7 sites. Three of 12 sites still showed vagally induced ERP prolongation > 1 millisecond (Fig 7, left) after the lesions were created at both sides of RV outflow tract test sites. Application of radiofrequency current did not affect the vagal response at the 8 RV anterolateral test sites; ERP prolongation was 5.8 ± 0.3 milliseconds control, 6.1 ± 0.4 milliseconds after medial RF, and 5.8 ± 0.5 milliseconds after lateral RF. ERPs were prolonged at the RV outflow tract test sites during methacholine infusion in 2 dogs.

**Discussion**

**Major Findings**

**Efferent Sympathetic Innervation**

The major conclusions from this study are that efferent sympathetic axons to the RV are located in the superficial subepicardium and are oriented perpendicular to the right lateral AV groove or the LAD. Myocardial sites close to the RCA (sites 1 and 2) receive efferent sympathetic innervation predominantly from the lateral margins of the RV near the AV groove. Myocardial sites close to the LAD (sites 3 and 4) receive efferent sympathetic innervation from both the right lateral AV groove and regions near the LAD (Fig 8). Efferent sympathetic innervation to the RV outflow tract is both from the right lateral AV groove near the origin of the RCA and from regions near the LAD. Some axons are located in the superficial subepicardium, but other pathways are located in the deep myocardium and represent the only intramural cardiac sympathetic pathways that we have found as yet (Fig 8). This innervation differs from the rest of the RV and from the LV.
**Efferent Vagal Innervation**

Vagal fibers are located superficially at the right lateral AV groove and penetrate the myocardium quickly, so that at sites >10 to 15 mm from the AV groove they are intramural. Myocardial sites close to the RCA (sites 1 and 2) receive efferent vagal innervation predominantly from the lateral margins of the RV near the AV groove. Myocardial sites close to the LAD (sites 3 and 4) receive vagal innervation partially from the right lateral AV groove as well as from regions near the LAD. Pathways of vagal innervation to the RV outflow tract are located deep in the myocardium, originating from the right lateral AV groove near the origin of the RCA and from regions near the LAD (Fig 8).

**Previous Studies**

Our results are consistent with the previous conclusion\(^\text{15-17}\) that efferent sympathetic axons are located in the superficial subepicardium (<0.5 mm) in the anterolateral RV wall. Our data do not support the conclusion that the efferent sympathetic nerves innervate the RV outflow and anterolateral wall through the base of the pulmonary artery\(^\text{11}\) because the phenol strip on the RV outflow tract near the base of pulmonary artery (Fig 1, top right, G) did not affect the ERP response at the RV anterolateral myocardiun and the RV outflow tract. The length and the extent of the surgical excision or the phenol strips over the pulmonary conus may importantly affect the sympathetic response; these data were unclear in the study by Randall et al.\(^\text{11}\)

Phenol application to the RV along the right AV groove showed marked reduction of contractile response induced by stellate ganglion stimulation in both the RV anterior wall and outflow tract.\(^\text{11}\) However, the extent of phenol strips along the right AV groove might importantly affect the sympathetic response, because in our study the ERP response at the RV outflow tract was not attenuated after a phenol strip between the right lateral AV groove and lateral test sites but was attenuated after a strip between the origin of the RCA and RV outflow in our experiment.

**Methodological Considerations**

Some lateral and medial sites did not show sympathetic denervation after phenol applications on the RV epicardium close to the right lateral AV groove and LAD. It is possible that some sympathetic axons might penetrate the myocardium obliquely from outside the phenol strips to reach the lateral and medial test sites.\(^\text{1}\) Another possible reason is that the fat pad on the epicardium may have protected some of the nerves against phenol-induced necrosis.

Nontransmural subendocardial lesions created by radiofrequency current induced sympathetic and vagal denervation at the RV outflow test sites. However, from our results, it was difficult to determine the exact intramural sympathetic and vagal nerve routes. Some radiofrequency current–induced lesions were transmural. It is possible that these transmural lesions might have damaged the subepicardial sympathetic fibers >0.5 mm deep from the epicardial surface. However, they were applied after the phenol strips were administered.

Pharmacological and histological studies show that the fat pad along the AV groove contains vagal nerves\(^\text{5,14-16}\) and that mechanical damage to the fat pad attenuates vagal function.\(^\text{5}\) In the present study, we dissected the fat pad not from its atrial side but from the ventricular side toward the AV groove, and these procedures did not affect ERP prolongation induced by vagal stimulation. This result suggests that vagal fibers are located in the superficial atrial wall or in the fat pad over the atrial wall.\(^\text{17}\) Painting phenol on the RV wall <10 mm from the right lateral AV groove did not entirely denervate lateral RV test sites. It is possible that other vagal nerves originating from regions near the LAD may be located in the deep myocardium and reach the lateral test sites.

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