Effects of Sympathetic and Vagal Nerves on Recovery Properties of the Endocardium and Epicardium of the Canine Left Ventricle

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SUMMARY The purpose of this study was to determine if autonomic nerve interventions exerted quantitatively dissimilar effects on recovery properties of endocardium compared with epicardium. Effective refractory periods (ERP) were measured by the extrastimulus technique in the endocardium and epicardium of the canine left ventricular anterior wall. The basic train and premature stimuli were administered to the endocardium and overlying epicardium via different poles on the same multipolar needle electrode, using cathodal stimuli. Sympathetic augmentation produced via bilateral carotid arterial occlusion or electrical stimulation of right, left, and both sympathetic nerves shortened ERP. Bilateral sympathetic denervation prolonged ERP. The changes in ERP of the endocardium were no different than were changes in the ERP of overlying epicardium. In separate studies, electrical stimulation of the cervical vagi prolonged ERP similarly in epicardium and endocardium. Pacing at slower rates or physostigmine administration potentiated the ERP prolongation in endocardium similar to epicardium. Augmented sympathetic tone produced by carotid occlusion also potentiated prolongation of ERP by vagal stimulation. The percent change in endocardial sites was slightly but significantly less than in epicardial sites. ERP prolongation due to vagal stimulation was attenuated markedly after sympathectomy and abolished with both propranolol and atropine. We conclude that, in the normal anterior left ventricular myocardium of the dog, sympathetic augmentation shortens ERP in epicardial sites equivalent to that in the underlying endocardial sites, that vagal nerve stimulation prolongs ERP in epicardial sites equal to or slightly greater than in the underlying endocardial sites, and that vagal stimulation antagonizes background sympathetic activity. *Circ Res 46: 100-110, 1980*

THE recovery of excitability in the normal left ventricle occurs almost simultaneously in endocardium and epicardium (van Dam and Durrer, 1961; Burgess et al., 1972). The duration of this recovery process is influenced by many factors including both limbs of the autonomic nervous system; sympathetic influences shorten duration by activation of β receptors (Yanowitz et al., 1966; Giotti et al., 1973), whereas parasympathetic influences prolong duration, presumably by modulating the concurrent sympathetic tone (Kolman et al., 1976; Bailey et al., 1979). It is not known whether either sympathetic or parasympathetic nerves influence endocardial recovery differently than epicardial recovery.

Regional differences in innervation occur between and within cardiac chambers (Angelakos et al., 1969; Schmid et al., 1978) and are reflected in variations of functional responses to electrical stimulation (Randall et al., 1972; Randall and Armour,1974; Yanowitz et al., 1966; Kralios et al., 1975). Histological studies suggest that transmural differences in innervation may exist also; the endocardium might be innervated less well with sympathetic nerves (Dahlstrom et al., 1965) and innervated better with parasympathetic nerves (Kent et al., 1974) compared to the epicardium.

The purpose of this study was to measure the effective refractory period (ERP) of the anterior left ventricular epicardium and underlying endocardium to test the following hypotheses: first, that sympathetic nerve interventions produce greater changes in ERP of the epicardium compared to the endocardium and, second, that vagal nerve interventions produce greater changes in ERP of the endocardium compared to the epicardium. The present study presents data to refute both hypotheses.

Methods

Surgical Preparation

Healthy mongrel dogs (n = 51) weighing 10–22 kg were preanesthetized with morphine (4 mg/kg im) and anesthetized with α chloralose (80 mg/kg iv). Additional amounts of chloralose were given to

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maintain anesthesia during the study. No measurements were obtained for at least 10 minutes after each additional dose of chloralose, and intervening dosages were avoided when serial measurements of refractory periods were obtained. The dogs were ventilated by means of a cuffed endotracheal tube and volume-cycled respirator. Arterial P_{CO_2}, pH, and P_{O_2} were monitored throughout the experiment and were adjusted by varying the tidal volume. Positive expiratory pressure was used to maintain P_{O_2} in the normal range. Sodium bicarbonate was given intravenously to some dogs to maintain pH above 7.30.

The sternum was split, and the open pericardium was sutured to the wound edges to support the heart. The cervical vagi were isolated, doubly ligated, and cut in all dogs. The left femoral vein was cannulated to infuse drugs and normal saline at 200 ml/hour during the surgical procedure. A fluid-filled cannula placed in the left femoral artery was connected to a Statham P-23 transducer to monitor pressure. Mean arterial pressure was determined by electrical filtering and recorded on an oscillographic recorder. To continuously monitor epicardial temperature, a temperature probe was sutured to an anterior epicardial site located 1–2 cm from the decapolar electrode. Normal body temperature was maintained by a heating blanket. In addition, the sternotomy was covered by a plastic sheet and, by adjusting the distance of the operating table lamp to the cardiac surface, epicardial temperature was maintained at 37.5°C. In five dogs, an endocardial temperature probe also was placed into the left ventricular apex through a stab incision.

Tests of ERP

The region of the sinus node was crushed in all experiments except two. The latter were used to determine whether interrupting autonomic nerves coursing over the right atrium in the sinus nodal region might alter the results. Since the effects of autonomic nerve interventions on the left ventricle were no different in the two dogs in which the sinus node was not crushed, they are therefore considered together with experiments in which the sinus node was crushed. The atria and ventricles were paced simultaneously at a basic cycle length of 280–300 msec to maintain control of ventricular rate during all interventions and to eliminate hemodynamic changes from varying ventricular filling owing to different timing of atrioventricular contractions. We paced the right atrial appendage with a bipolar hook electrode and the left ventricle with unipolar cathodal stimuli delivered through one pole of a multipolar plunge needle (22-gauge) electrode. The anode was an electrode, 3.5 cm in diameter, located in the subcutaneous tissue of the abdomen.

The ERP was measured by the extrastimulus technique (Krayer et al., 1951) employing a programmable stimulator with separate constant current outputs. The right atrial appendage and each ventricular test site were paced at one and a half to two times late diastolic threshold with a stimulus duration of 2 msec. Late diastolic thresholds of ventricular sites, measured during each intervention, averaged 29 ± 2 (SEM) μA and, if they varied by more than 10 μA, the data were discarded. Current intensity was kept constant.

The train of eight basic stimuli (S_1) was followed by a late premature stimulus (S_2) that produced a propagated ventricular response (capture). The ventricular response to S_2 was recorded in lead II and from an adjacent pair of electrodes on the multipolar needle and displayed on a storage oscilloscope at rapid sweep speed. The S_1-S_2 interval was shortened by 2-msec decrements (e.g., 160, 158, 156 failed) until S_2 failed to elicit a propagated response. An irregular rhythm was produced owing to the shorter S_1-S_2. However, the irregularity was present throughout the entire experiment and, therefore, its effect on ERP was consistent.

After failure to capture, the S_1-S_2 interval was increased by 9 msec, and the shortening of the S_1-S_2 was repeated (e.g., 165, 163, 161, 159, 157 failed) 30 seconds later (Han and Moe, 1969; Janse et al., 1969). Each test site was stimulated at least twice before moving to another test site. Repeat ERP determination resulted in a value within 1 msec of the first or the data were discarded. The ERP was defined as the longest S_1-S_2 which just failed to capture the ventricle and, therefore, the longer ERP was used for analysis. Serial testing after a single anesthetic dose without intervention in this dog model demonstrated ERP shortening of 0–1 msec in endocardial and epicardial sites over a 15-minute interval. Control values and the effects of interventions reported in this study were generally obtained in <10 minutes.

Strength-Interval Curve Testing

Strength-interval curves were performed in four experiments. The premature stimulus (S_2) was delivered to each ventricular site (van Dam and Durrer, 1961) via the same electrode as the basic train (S_1) but through a separate constant current output. The current intensity of the basic train was one and a half to two times late diastolic threshold, whereas the S_2 current intensities were varied at predetermined levels of 200, 400, 800, and 1600 μA. Stimulus durations were all 2 msec. The lowest level was usually 4 times the highest late diastolic threshold. Currents exceeding 1600 μA were not used because they occasionally produced tissue damage, detected by S-T segment elevation and a permanent increase in late diastolic threshold. Experiments in which either alteration occurred were discarded to eliminate the effects of tissue damage on electrophysiological response to autonomic intervention (Singer et al., 1967). At the onset of testing at each level of current intensity, the S_1-S_2 interval was shortened

by 1-msec decrements until failure to capture occurred (Krayer et al., 1951).

Ventricular Test Sites

The ERP was measured in the anterior left ventricular epicardium and underlying endocardium (1–2 cm lateral to the basal half of the left anterior descending coronary artery) with the multipolar electrode (interelectrode distance = 1 mm) placed in the myocardium perpendicular to the epicardial surface.

The upper half of the left septum was paced in six experiments by means of a fine Teflon-coated wire hook electrode placed in a 22-gauge needle and inserted through the right ventricular wall and cavity into the left side of the septum. The needle was removed, leaving the hook electrode in place on the left ventricular septum.

The sites of all electrodes were confirmed at necropsy when the experiment was terminated. In addition, the location of the endocardial and epicardial sites to be paced by the multipolar needle were confirmed by recording bipolar electrograms from adjacent poles of the multipolar needle during spontaneous supraventricular rhythm. After recording all electrogram combinations between the left ventricular cavity and epicardium, the endocardial pole chosen for ventricular stimulation was one from which the earliest transmural ventricular activation was recorded and, if possible, one from which a Purkinje spike also was recorded (Durrer and van der Tweel, 1956). The epicardial pole chosen for ventricular stimulation was one from which the latest transmural ventricular activation was recorded. The distance between endocardial and epicardial test sites ranged from 5 to 9 mm.

Temperature Studies

To demonstrate the sensitivity of ERP determined by the extrastimulus technique, the effects of limited changes in temperature on epicardial and underlying endocardial refractory periods were determined in 11 dogs. ERP was measured in the endocardium and epicardium with a plastic sheet covering the sternal incision. The plastic sheet then was removed and the heart was allowed to cool. ERP measurements were repeated at both sites.

Sympathetic Interventions

Bilateral Carotid Occlusion

After control ERP measurements had been made, both common carotid arteries were occluded (n = 14) at their midpoints with bulldog clamps producing an increase in mean arterial pressure by the baroreceptor reflex. ERP measurements were performed when blood pressure was stable for 1 minute and again 10 minutes after reperfusion of the carotid arteries.

Surgical Interruption

In 12 dogs, ERP measurements were performed before and 5 minutes after the proximal communications of both stellate ganglia were cut, leaving only attachments to the anterior and posterior ansa subclavia intact.

Electrical Stimulation

After sympathectomy, shielded stainless steel bipolar electrodes were placed around the anterior and posterior ansa subclavia. The electrode nearest the heart was always the cathode. Stimulation of right and left sympathetic nerves was carried out with separate constant current isolators driven by a single stimulator at 1–8 Hz with pulses 4 msec in duration. Each nerve was tested separately to determine the current intensity at which the maximal stable heart rate or blood pressure was obtained. That current, ranging from 1 to 7 mA, was used at constant frequency for the remainder of the experiment. In seven dogs, ERP measurements were performed before and 1 minute after onset of bilateral sympathetic nerve stimulation. Responses to individual right and left sympathetic stimulation at the same frequency done in a random order also were measured in eight dogs.

Vagal Nerve Interventions

Surgical Interruption

Effects on ERP of surgically interrupting both cervical vagi were determined in seven dogs. Control ERP measurements were obtained in endocardium and epicardium and repeated 5 minutes after the vagi were doubly ligated and cut.

Vagal Stimulation

The cervical vagi were stimulated via two Teflon-coated hook electrodes imbedded in the peripheral cut nerve (Lazzara et al., 1973). Pulses were rectangular, 4 msec in duration, and delivered at a frequency of 20 Hz. The current strength was 0.05 mA greater than that required to produce asystole or a ventricular escape rhythm (during spontaneous rhythm) and ranged from 0.2 to 1.5 mA. During each experiment, between interventions, vagal stimulation was retested without varying the current strength to be certain that the ventricular rate response to stimulation of either right or left vagus remained constant. Data were excluded if ventricular rate responses were not constant.

ERP measurements were obtained prior to bilateral vagal stimulation (control), after 1 minute of continuous vagal stimulation, and 3 minutes following cessation of vagal stimulation.

Vagal Stimulation following Drug Administration

The ERP response to vagal stimulation was determined in selected dogs 5–20 minutes after an

intravenous injection of a single bolus of one of three drugs. Physostigmine, an acetylcholinesterase inhibitor prepared daily from the hydrochloride salt, was given to augment ERP response to vagal stimulation. Doses of 0.5, 5.0, and 50 μg/kg were given to each of 10 dogs. The highest dose produced muscle twitching which prevented administration of higher doses.

To determine if ERP prolongation produced by vagal stimulation was dependent on sympathetic activity, propranolol (0.7 mg/kg) was given by an intravenous bolus injection to three dogs with intact cardiac sympathetic nerves. In preliminary experiments, this dose was found to block 90% of the shortening of ERP produced by bolus injection of isoproterenol (0.5 μg/kg) given 10–20 minutes after propranolol administration. The effective dose of propranolol, which blocked 50% of isoproterenol-induced ERP shortening, was 0.15 μg/kg. To determine if prolongation of ERP by vagal nerve stimulation was mediated via muscarinic cholinergic receptor activation, atropine (0.4 mg/kg, iv) was administered to two selected dogs.

Statistical Methods

The data were expressed as mean ± standard error in all cases. Whenever two statistical comparisons were to be made on one control or intervention data group, two-way analysis of variance was performed, employing Duncan's multiple range test for comparisons when F values were significant (Steele and Torrie, 1960). When only one statistical comparison was employed between a control and an intervention in sympathetic studies, Student's t-test for paired data was employed. The probability of missing a 25% difference between endocardium and epicardium (type II error) was estimated and was always less than 7.5% (Colton,1974).

Results

Temperature Studies (Fig. 1)

With the plastic sheet covering the sternotomy, the mean epicardial temperature was within 0.2°C of the endocardial temperature. As expected, epicardial ERP was shorter than endocardial ERP ($P < 0.01$). When the plastic was removed from the incision, epicardial temperature fell by 2.9 ± 0.3°C, whereas the underlying endocardial temperature fell only by 1.0 ± 0.4°C. This temperature change significantly prolonged the duration of the epicardial ERP but not the endocardial ERP, which still exceeded the epicardial ERP ($P < 0.05$).

Sympathetic Interventions (Figs. 2 and 3)

Bilateral Carotid Occlusion

Reflex augmentation of sympathetic activity increased mean arterial pressure from 76 ± 3 to 106 ± 5 mm Hg ($P < 0.01$). Both epicardial and endocardial ERP decreased significantly by a mean of 4 msec (Fig. 2A). The percent change of ERP in endocardium was not significantly different than in epicardium.

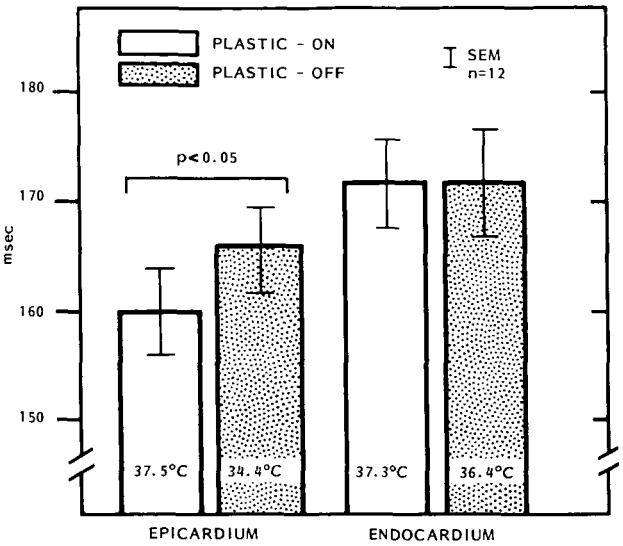

FIGURE 1 *With a plastic sheet covering the sternal incision, temperatures were nearly equivalent and epicardial ERP was shorter than endocardial ($P < 0.01$). When the plastic was removed, temperature stabilized at 34.4°C at the epicardium where ERP significantly prolonged. ERP of endocardium did not change where temperature fell to 36.4°C. n = pairs of endocardial and epicardial sites in 11 dogs.*

Surgical Interruption

After bilateral sympathectomy, mean arterial pressure fell from 73 ± 3 to 64 ± 4 mm Hg ($P < 0.01$). The ERP of both epicardium and endocardium prolonged by a mean of 10 msec. There was no difference between the percent change of ERP between endocardium and overlying epicardium (Fig. 2B). In five dogs without further intervention, ERP values 30 minutes after sympathectomy were no different than values obtained at 5 minutes.

Electrical Stimulation

Stimulation of both right and left sympathetic nerves increased mean arterial pressure from 66 ± 2 to 97 ± 3 mm Hg ($P < 0.01$). This was associated with a 19-msec mean decrease in endocardial ERP and an 18-msec mean decrease in ERP of epicardium. There was no difference in the percent change of ERP in endocardium vs. epicardium (Fig. 2C).

To uncover possible differences in effects of sympathetic nerve stimulation on duration of epicardial and endocardial ERP, we also stimulated the right and left sympathetic nerves individually as well as simultaneously (Fig. 3). Right sympathetic nerve stimulation increased mean arterial pressure from 64 ± 4 to 91 ± 5 mm Hg ($P < 0.01$). Left sympathetic nerve stimulation at identical frequency increased

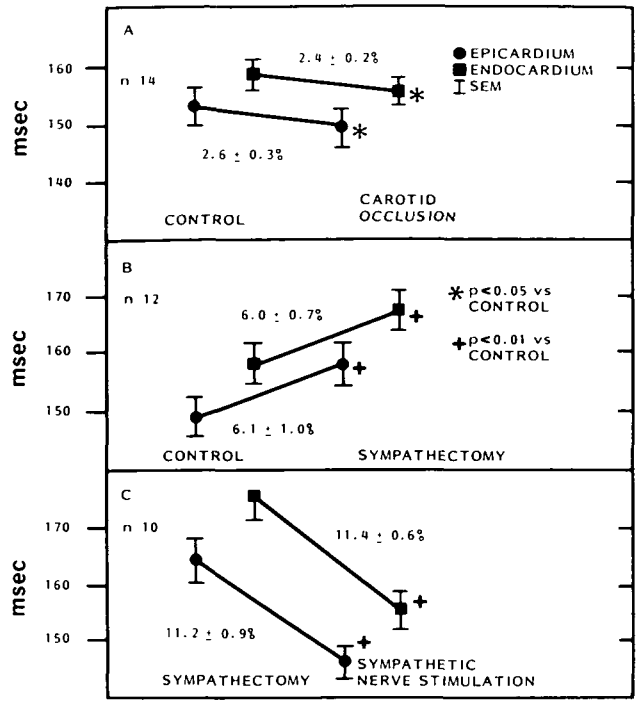

FIGURE 2 *ERP (msec) is shown during control and sympathetic interventions. The percent change in the epicardium (below the dots) and in the endocardium (above the squares) is shown. ERP shortened equivalently during bilateral carotid occlusion (panel A), prolonged equivalently after bilateral sympathectomy (panel B), and shortened equivalently after electrical stimulation of both sympathetic nerves (panel C). n = pairs of sites in 14 (A), 12 (B), and 7 (C) dogs.*

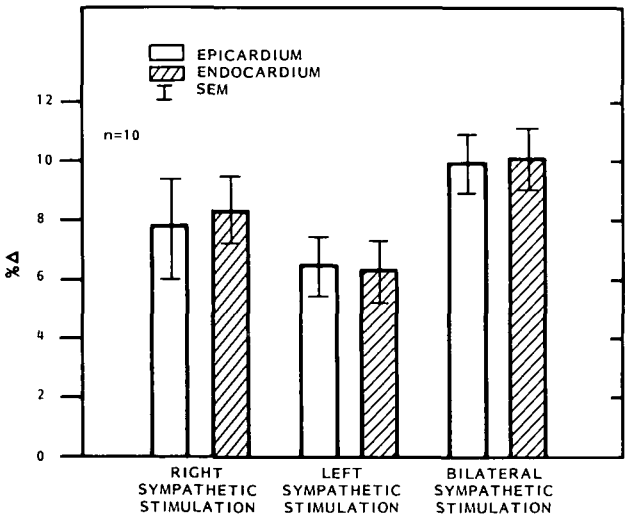

FIGURE 3 *This graph shows the percent change in ERP determined in epicardium and endocardium during selective electrical stimulation of right sympathetic nerves, left sympathetic nerves, and both right and left sympathetic nerves in eight dogs. No statistically significant differences resulted between endocardium and epicardium. n = pairs of sites.*

mean arterial pressure from 68 ± 4 to 97 ± 6 mm Hg ($P < 0.01$). There were no differences in percent shortening of endocardial compared to epicardial ERP during unilateral right or left or bilateral sympathetic stimulation. Right sympathetic stimulation shortened ERP in endocardium by 14 ± 2 msec, which was not different from the shortening of ERP in epicardium (14 ± 3 msec $P > 0.25$). Similarly, left sympathetic stimulation shortened ERP in endocardium by 11 ± 2 msec, which was not different from the shortening of ERP in epicardium (11 ± 2 msec, $P > 0.25$).

We also calculated the range of ERP between endocardium and epicardium by subtracting the value of the epicardial ERP from the endocardial ERP before and during sympathetic nerve stimulation in the same dogs. Range of ERP was unchanged from control, during bilateral stimulation (from 13 ± 2 to 11 ± 3 msec), left sympathetic stimulation (from 11 ± 1 to 11 ± 2 msec), and right sympathetic stimulation (from 11 ± 1 to 11 ± 2 msec).

Vagal Nerve Interventions

Surgical Interruption

Bilateral cervical vagotomy was associated with insignificant changes in endocardial (160 ± 3 to 159 ± 3 msec, $P > 0.09$) and epicardial (155 ± 3 to 154 ± 3 msec, $P > 0.1$) ERP.

Vagal Stimulation

Vagal stimulation performed in eight dogs during atrial and ventricular pacing at a basic cycle length of 300 msec decreased mean arterial pressure from 74 ± 4 to 65 ± 5 mm Hg ($P < 0.01$). ERP lengthened at almost every test site (Fig. 4). The percent change in ERP varied from 1.6 in left septum to 2.5 in epicardium (Table 1). The former was significantly less than the latter. After cessation of vagal stimulation, ERP returned to a level slightly but significantly lower ($P < 0.05$) than control prior to vagal nerve stimulation.

Vagal Stimulation during Varied Sympathetic Activity

Since the endocardium and left septum did not develop greater prolongation of ERP due to vagal nerve stimulation than did the epicardium, three approaches were used to potentiate the effects of vagal stimulation to uncover differences in responses suggested by our second hypothesis. These were augmentation of sympathetic activity, pacing at slower rates, and administration of physostigmine. Bilateral carotid occlusion increased mean arterial pressure from 73 ± 4 to 106 ± 5 mm Hg ($P < 0.01$). This intervention alone shortened ERP in all areas by 1.4–2.7% (Table 1), although the change was not statistically significant in the left septum. When bilateral vagal nerve stimulation was performed simultaneously with carotid occlusion at a

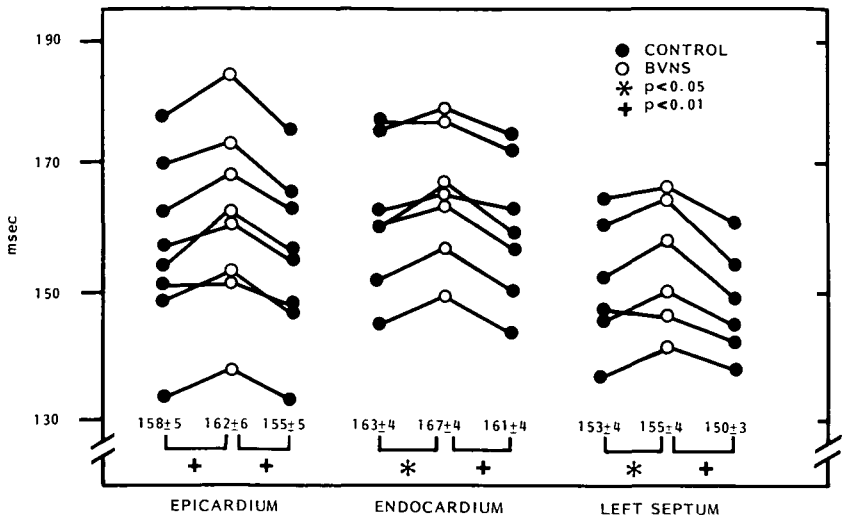

FIGURE 4 *This graph shows the ERP changes of individual epicardial, endocardial, and left septal sites during bilateral vagal nerve stimulation (BVNS). Vagal stimulation prolonged ERP significantly in each site. Three minutes after vagal stimulation, ERP returned to values slightly lower than that of the prestimulation control. n = pairs of sites in eight dogs.*

separate time, mean arterial pressures rose from 74 ± 4 to only 87 ± 7 mm Hg ($P < 0.01$). ERP was prolonged in each site which reflected a reversal rather than equalization (no additive effects, see Table 1) of the expected ERP shortening due to augmented sympathetic activity. The percent prolongation ERP of the endocardium and left septum was significantly less than that of the epicardium (Table 1).

After bilateral sympathectomy in four dogs. vagal nerve stimulation prolonged ERP in eight test sites (by 2-4 msec) while producing 1-4 msec shortening in three sites. As a result, the mean values of ERP during vagal stimulation were not significantly different from those obtained following stellectomy (Table 2).

Vagal Stimulation at Slower Heart Rate

In eight dogs, the atria and ventricles could be paced at a basic cycle length of 500 msec in additon to 300 msec (Fig. 5). The magnitude of ERP prolongation produced by vagal stimulation at both epicardium and endocardium was significantly increased. However, no significant difference was found between the percent change in endocardium compared with the change in the epicardium.

Vagal Nerve Stimulation following Drug Administration

We also potentiated the effects on ERP of vagal stimulation with physostigmine. After administration of physostigmine, 5 and 50 μg/mg, iv, vagal nerve stimulation increased the absolute value and percent change in ERP compared to effects of vagal nerve stimulation performed prior to physostigmine administration (Fig. 6). At each dose level, no difference in percent change was found between epicardium vs. endocardium.

To elucidate further the mechanism by which vagal stimulation prolonged recovery in the left ventricle, the effects of atropine or propranolol administration during vagal stimulation were studied (Table 2). Propranolol prolonged ERP in all sites, determined 10-20 minutes after administration. Vagal stimulation no longer prolonged ERP. In separate experiments after atropine administration, va-

TABLE 1 *Effects of Vagal Stimulation on ERP during Augmented Sympathetic Activity*

	Control	BVNS	Control	BCO	Control	BVNS + BCO
Epicardium ERP (msec)	158 ± 5	162 ± 6*	158 ± 6	153 ± 5*	157 ± 5	161 ± 5*
%Δ	2.5 ± 0.4		−2.7 ± 0.4		2.8 ± 0.7	
Endocardium ERP (msec)	163 ± 4	167 ± 4†	161 ± 4	157 ± 4*	162 ± 4	164 ± 3
%Δ	2.3 ± 0.5		−2.4 ± 0.3		1.4 ± 0.9‡	
Left septum ERP (msec)	153 ± 4	155 ± 4†	151 ± 4	149 ± 3	152 ± 3	155 ± 4†
%Δ	1.6 ± 0.6‡		−1.4 ± 0.2‡		2.0 ± 0.4‡	

BVNS = bilateral vagus nerve stimulation; BCO = bilateral carotid occlusion; n = eight pairs of endocardial and epicardial sites in eight dogs, data evaluated by two-way analysis of variance.

* $P < 0.01$ vs. Control; † $P < 0.05$ vs. Control; ‡ $P < 0.05$ vs. EPI.

TABLE 2 *Effects of Vagal Stimulation on ERP after Sympathectomy or during Autonomic Blockade*

	Control	BVNS	Intervention	After intervention	BVNS after intervention
ERP (msec)	152 ± 4	156 ± 4*	Sympathectomy $n = 11$	159 ± 4*	160 ± 4
%Δ	2.4 ± 0.3				0.9 ± 0.5†
ERP (msec)	192 ± 5	199 ± 4*	Propranolol (0.7 mg/kg) $n = 8$	208 ± 5*	208 ± 5
%Δ	4.0 ± 0.9				0.1 ± 0.3†
ERP (msec)	165 ± 6	172 ± 6*	Atropine (0.4 mg/kg) $n = 6$	165 ± 7	163 ± 6
%Δ	4.3 ± 1.2				−1.3 ± 0.4†

n = the total number of sites tested in four (sympathectomy), three (propranolol), and two (atropine) dogs; data evaluated by two-way analysis of variance.
* $P < 0.01$ vs. control; †$P < 0.025$ vs. preintervention value.

gal stimulation failed to prolong ERP and actually resulted in shortening of ERP in all sites tested; this change was not statistically significant ($P = 0.10$).

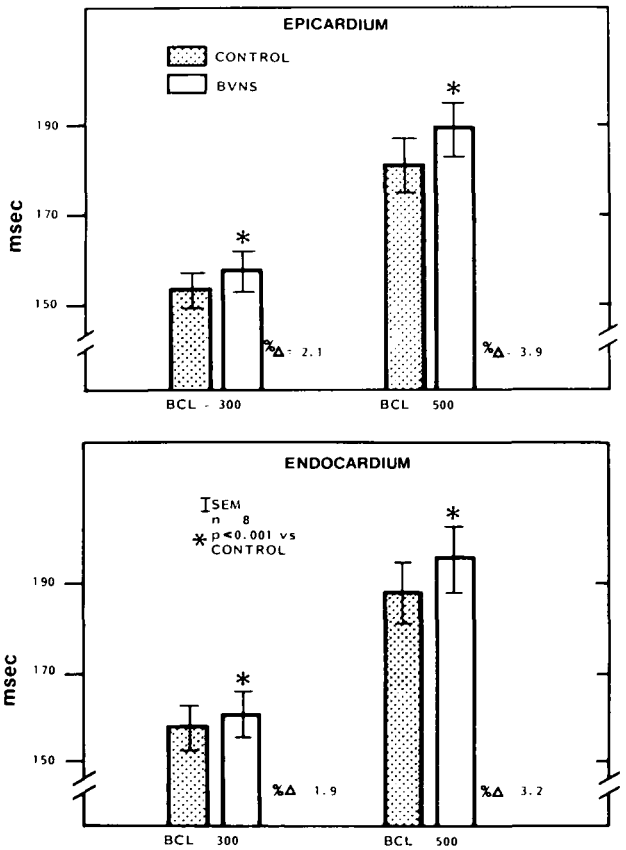

FIGURE 5 *These graphs show the effect of a change in basic cycle length on ERP before and during bilateral vagal nerve stimulation in epicardium and endocardium. At a basic cycle length of 500 msec, the ERP of epicardium and endocardium prolonged to a greater extent with vagal nerve stimulation than at a cycle length of 300 msec. The percent change in epicardium due to vagal stimulation was not statistically different from the percent change in endocardium. n = pairs of sites in eight dogs.*

Strength-Interval Curves

Strength-interval curves were constructed in separate experiments. The entire strength-interval curve was shifted to the right in both epicardium and endocardium during vagal stimulation (Fig.7). The percent change at each level of current intensity in the epicardium was no different from that in the endocardium

Discussion

Major Findings

The results of this study indicate that: (1) sympathetic nerves to the left ventricle exert equivalent effects on the duration of epicardial and underlying endocardial ERP during reflex augmentation, denervation, and unilateral or bilateral electrical stimulation; (2) electrical stimulation of the vagus nerves prolongs ERP similarly in the epicardium, endocardium, and left septum; (3) by activating muscarinic cholinergic receptors, vagal stimulation prolongs ventricular ERP by antagonism of sympathetic activity.

Sympathetic Interventions

One of the aims of the study was to determine whether sympathetic nerves exerted nonuniform effects on ERP of left ventricular epicardium and endocardium, as might be predicted from previous reports. Nonuniform effects of right or left sympathetic nerve stimulation over large areas of epicardial surface of both ventricles have been demonstrated by changes in regional contractile force (Randall et al., 1972) and refractory period (Yanowitz et al.,1966; Kralios et al., 1975). A nonuniform dispersion of refractory periods also has been noted over an 8-mm area of the epicardial surface during left sympathetic nerve stimulation (Han and Moe, 1964). This dispersion of refractoriness may provide one mechanism by which varying sympathetic neural input to the heart might result in ventricular arrhythmias. Histological evidence supports these functional observations. The distance from cate-

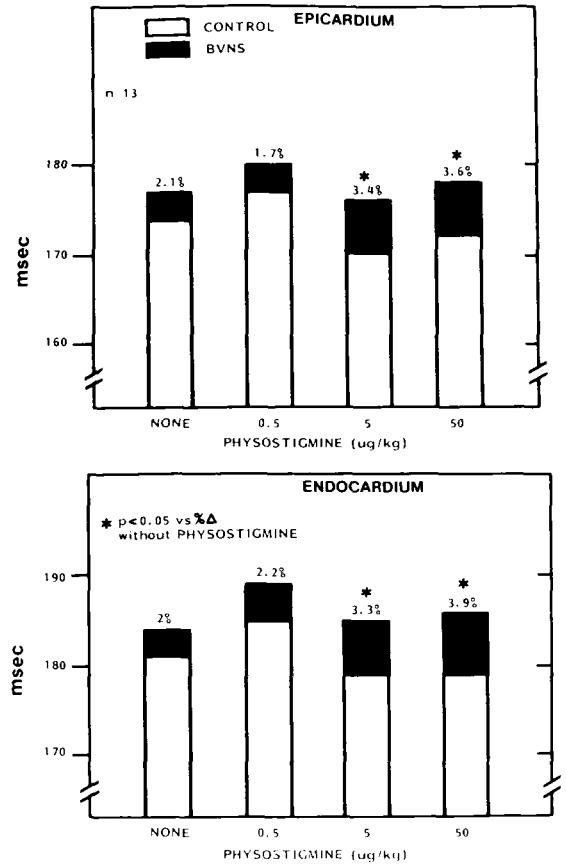

FIGURE 6 *These graphs show the effects of three doses of physostigmine on the ERP response to vagal stimulation in epicardium and endocardium. The absolute magnitude of ERP prolongation due to vagal stimulation is shown by the black portion of each bar and is expressed as a percent change above each bar. Doses of 5 and 50 μg/kg significantly potentiated the ERP response to vagal stimulation compared to vagal stimulation before physostigmine was given. No statistical differences between epicardial and endocardial responses to vagal stimulation were uncovered at any dose of physostigmine. n = pairs of sites in 10 dogs.*

cholamine-containing nerves to cardiac cells may vary greatly by an average of 30 μm (Angelakos, et al., 1969), so that it is possible that some cell groups may be under greater neural influence than are others. A comparable observation has been noted in Purkinje fibers; they are associated with fewer sympathetic nerves as compared to muscle (Dahlstrom, et al., 1965), and thus Purkinje fibers and / or endocardial muscle may show less electrophysiological effect of sympathetic nerve interventions compared to epicardial muscle.

Our findings demonstrated an equal effect of sympathetic nerves on ERP in the endocardium compared to the epicardium whether bilateral or individual right or left sympathetic nerve stimulations were performed. These data are consistent with the findings of Angelakos et al. (1969), who demonstrated equivalent concentrations of norepinephrine in endocardium and epicardium in the dog. Our data also confirm and expand upon the findings of Yanowitz et al. (1966), who showed that left stellectomy produced directionally similar prolongation of refractory period in endocardium and epicardium.

Several theoretical and statistical reasons support our conclusion that we did not fail to uncover real (hypothesized) differences between the two layers of the myocardial wall. First, we evaluated the statistical probability of committing a type II error, i.e., falsely accepting the null hypothesis of no difference between endocardium vs. epicardium. In each case, the probability of missing a 1-3% lesser or greater change in the endocardium compared to the epicardium was always less than 7.5%. Second, the temperature studies demonstrated that the extrastimulus technique detected at least a 4% prolongation in the epicardium and no change in the underlying endocardium. Therefore, this method of ERP testing is sufficiently sensitive to recognize differential changes in endocardium and epicardium, since the changes due to stellectomy or sympathetic nerve stimulation were >6%. Last, our data exclude the possibility that the increased metabolic requirements of the endocardium rendered it

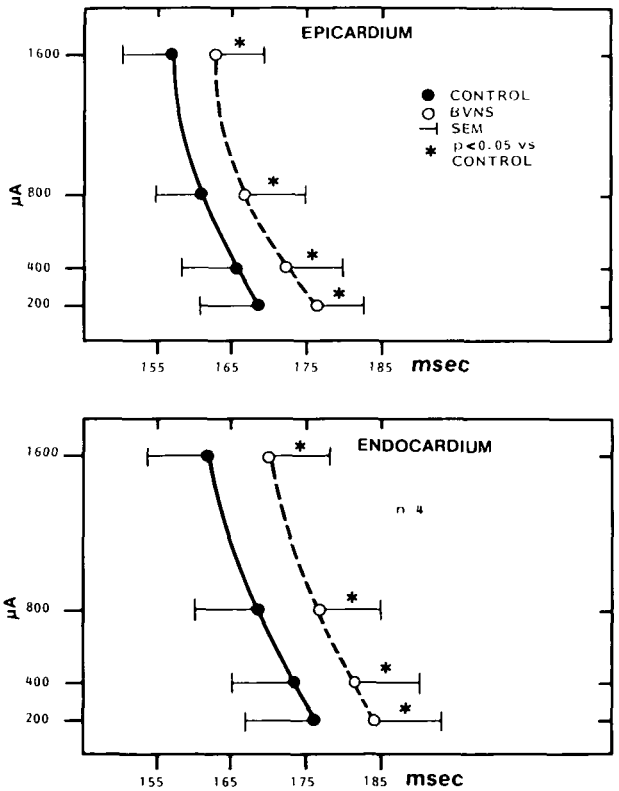

FIGURE 7 *These graphs show strength interval curves for epicardium and endocardium before and during vagal nerve stimulation. At each current strength, the curve was shifted later into diastole. There was no significant difference in the percent change at any current strength between epicardium and endocardium.*

relatively ischemic during sympathetic stimulation (and thereby prevented a maximal ERP response), since equivalent ERP changes in both sites also were demonstrated with sympathectomy. Therefore, we believe that the results reported in the present study reflect a true uniform effect of sympathetic innervations on the ventricular endocardium and epicardium.

There are several implications of these data. First, since the duration of recovery of epicardium and the underlying endocardium of the anterior left ventricle are affected equally by sympathetic nerve interventions, T-wave changes produced by the latter more likely result from other causes, such as differences in sympathetic neural control of recovery in the anterior compared to the posterior surface of the heart (Yanowitz et al., 1966). Second, these data for normal animals do not rule out the possibility that sympathetic nerve interventions produce unequal effects on recovery of endocardium and epicardium in disease states such as the long Q-T interval syndrome in patients. Moreover, myocardial ischemia, infarction or other diseases may differentially modulate the electrophysiological response of either endocardium or epicardium to sympathetic nerve influences which, under normal conditions, would be equivalent. Third, effects of parasympathetic intervention on endocardial and epicardial ERP will be influenced by the sympathetic tone since the vagal nerves lengthen ventricular recovery by antagonizing the sympathetic system (Bailey et al., 1979). Equivalent sympathetic effects on ERP of endocardium and overlying epicardium provide the substrate that allows a comparison of effects of vagal nerve interventions on ERP of the two layers.

Vagal Nerve Interventions

Recent studies have defined clearly the presence of choline acetyltransferase, the enzyme that catalyzes the production of acetylcholine in parasympathetic nerves (Schmid et al., 1978), and the presence of muscarinic cholinergic receptors in mammalian ventricles (Fields et al., 1978). This parasympathetic innervation exerts a negative inotropic effect on the ventricle, the magnitude of which is augmented by an increase in sympathetic tone (Levy, 1971; Watanabe and Besch, 1975).

The electrophysiological effects of parasympathetic innervation on the ventricle only recently have been clarified. In vitro studies on Purkinje fibers have demonstrated that acetylcholine decreases automaticity by depressing the slope of diastolic depolarization (Bailey et al., 1972) and that this is concentration dependent (Danilo et al., 1978). It is apparent that vagal stimulation or acetylcholine administered intravenously in intact animals can slow the discharge rate of ventricular automatic foci (Eliakim et al., 1961; Danilo et al., 1978).

Vagal effects on ventricular action potential duration and recovery of excitability are not settled. Previous in vitro studies failed to demonstrate any major effect of acetylcholine on Purkinje fiber action potential duration (Hoffman and Suckling, 1953). In recent studies, only very high dose levels (10^{-6} to 10^{-4} M) of acetylcholine shortened action potential duration (Danilo et al., 1978; Gadsby et al., 1978). However, during isoproterenol-induced shortening of action potential duration, acetylcholine (10^{-6} M) prolonged the action potential duration (Bailey et al., 1979). These effects of acetylcholine were blocked by atropine. Thus, it appears that Purkinje fiber recovery is modulated by parasympathetic influences indirectly by antagonism of the degree of sympathetic influence.

In intact animals, vagal stimulation has been shown to prolong right ventricular endocardial ERP, and this result may have depended upon an antagonism of the prevailing sympathetic tone (Kolman et al., 1976), since the effects of vagal stimulation were abolished with propranolol. However, vagal effects were not accentuated in that study during left sympathetic nerve stimulation, possibly because the left sympathetic nerves exert little control over the ERP of the right ventricle and septum (Kralios et al., 1975).

Our results in intact anesthetized dogs are consistent with this parasympathetic-sympathetic interaction. During baseline sympathetic tone bilateral vagal nerve stimulation prolonged ERP by 1.6–2.5% (Table 1). This effect appeared to be augmented during carotid occlusion since ERP was still prolonged 1.4–2.7% by vagal stimulation; these changes reflected reversal of the expected ERP shortening produced by carotid occlusion alone. Vagal stimulation, performed after interruption of sympathetic nerves to the heart, did not significantly prolong the ERP, although in eight of 11 sites tested, ERP prolonged slightly (overall change was 0.9%); this small change presumably reflected antagonism of the remaining circulating catecholamine from adrenal sources. Propranolol or atropine pretreatment essentially eliminated vagal-induced shortening of ERP. Thus, in intact animals the prolonging effect of vagal nerve stimulation on ERP was mediated via muscarinic cholinergic receptors and seems related to the prevailing sympathetic tone.

We augmented the ERP response to vagal nerve stimulation to test the hypothesis that vagal stimulation prolonged the ERP of the endocardium more than of epicardium. We uncovered either no difference or a significantly greater prolongation of ERP in the epicardium compared to the endocardium during vagal stimulation. At the present time, we are unable to be certain of the basis for or physiological significance of this difference found only during augmented sympathetic activity. It may relate to hemodynamic changes or complex parasympathetic-sympathetic interactions which our data cannot reconcile.

Our data show that vagal stimulation produced no greater effects on ERP in the endocardium compared with epicardium and suggest that the parasympathetic innervation to the anterior left ventricular myocardium may be relatively homogeneous. Our data do not allow us to compare ERP of Purkinje fibers with ventricular muscle and, therefore, it is possible that the former may show a much greater effect of vagal stimulation than the present data demonstrate in muscle as suggested by histochemical data.

However, the presence of nerves that stained for acetylcholinesterase in the endocardium (Kent et al., 1974) does not necessarily indicate that these nerves all terminate and release acetylcholine only into the endocardium. The endocardium may be the site of the major neural pathways by which parasympathetic nerves travel to the entire ventricular wall. Thus, histological staining of the ventricular wall might reveal a greater density of parasympathetic nerves in the endocardium compared to the epicardium. Functional data from other studies would substantiate our findings of a more homogeneous distribution of vagal innervation. Indeed, the negative inotropic effects of vagal stimulation appear too prominent to be a result of parasympathetic innervation confined to the endocardium alone (DeGeest et al., 1965; Randall and Armour, 1974).

The small changes in ERP with electrical stimulation of cervical vagal nerves, although comparable in magnitude to effects of reflex sympathetic augmentation, may be due to several factors. First, electrical stimulation of the cervical vagus, a vagosympathetic trunk in the dog, may also activate sympathetic fibers (Randall et al., 1972) that could mitigate vagal effects. This could explain our observation that ERP tended to shorten during vagal stimulation after atropine administration (Table 2). Such interactions may have been one reason why we demonstrated that vagotomy alone produced no significant change in ERP; vagotomy not only removed efferent parasympathetic inhibitory nerves but also sympathetic efferents which oppose the former. Second, cycle length importantly influences the magnitude of vagal effects. Slower, more physiological cycle lengths may have increased vagal-induced prolongation of ERP. Third, the effects of anesthesia in this animal model cannot be evaluated on the basis of the present data, but it is clear that anesthesia may affect the vagal as well as sympathetic tone, and under conditions of more normal autonomic tone, the effects of vagal intervention might have been greater (Peiss and Manning, 1964).

Possible Significance for Development of Arrhythmias

These data provide understanding of a mechanism to explain the observations that activation of muscarinic cholinergic receptors by vagal maneuvers may terminate ventricular tachycardia in man (Waxman and Wald, 1977) or prevent death in cats from ventricular arrhythmia after coronary artery occlusion (Corr and Gillis, 1974). If prolongation of ERP in ventricular myocardium can prevent or interact with arrhythmias, these data may be one mechanism for these effects. One might expect the magnitude of vagal effects on recovery to be quite accentuated in the presence of arrhythmia associated with increased sympathetic activity (Jewitt et al., 1969).

Kent et al. (1974), on the basis of histochemical studies, concluded that vagal intervention must act on arrhythmias via effects on the specialized conduction system. From the present study, it is clear that the vagus could act on arrhythmias involving ventricular muscle as well.

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