Evidence against Significant Resting Sympathetic Coronary Vasoconstrictor Tone in the Conscious Dog

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SUMMARY The primary objective of this study was to ascertain whether resting coronary blood flow is under tonic restraint due to sympathetically mediated α-adrenergic coronary vasoconstriction. To accomplish this, we first developed and verified a technique for selectively sympathectomizing the posterior region of the canine left ventricle. This technique entailed the topical application of phenol in a thin line to specific sites on the myocardium and epicardial vessels. As part of the verification, we demonstrated that left stellate nerve stimulation caused increases in the myocardial extraction ratios for oxygen and lactate in the normally innervated region (I) of the ventricle, but not in the sympathectomized region (Sx). We then measured regional myocardial blood flow with microspheres in phenol-treated animals under conscious, resting conditions. The animals were acclimated to the laboratory environment, and their arterial plasma norepinephrine levels averaged 135 ± 37 pg/ml. Heart rate (81 ± 3 bpm) and mean aortic pressure (100 ± 2 mm Hg) were not significantly affected by α-adrenergic blockade or combined α- and β-adrenergic blockade in these animals. Blood flow in I and Sx averaged 0.87 ± 0.08 ml/min per g and 0.85 ± 0.07 ml/min per g, respectively, and the difference was not statistically significant. The endocardial-to-epicardial blood flow ratio in I and Sx averaged 1.23 ± 0.03 and 1.29 ± 0.04, respectively, and the difference was not statistically significant. The results were not significantly affected by β-adrenergic blockade or combined α- and β-adrenergic blockade. We were unable to confirm previous evidence in the literature of significant resting sympathetic coronary vasoconstrictor tone in the conscious animal. Circ Res 49: 866-876, 1981

WITHIN the last 15 years, investigators from a number of laboratories have demonstrated that cardiac sympathetic nerve stimulation can produce coronary vasoconstriction by activation of coronary vascular α-adrenoceptors (Ross, 1976). Earlier studies in anesthetized animals were primarily concerned with the demonstration of this coronary vasoconstrictor mechanism, but more recent studies in conscious animals have been aimed at elucidating its physiological significance.

One of the impressions that has emerged from the conscious animal studies is that resting coronary blood flow is under tonic restraint due to sympathetic nerve activity at the coronary arteriolar level. Holtz et al. (1977), performing a study in conscious, resting dogs in which a portion of the left ventricle had been sympathectomized by the intracoronary administration of 6-hydroxydopamine, observed that blood flow was approximately 40% lower in the normally innervated region of the ventricle than in the sympathectomized region. Since the coronary perfusion pressure, preload, afterload, and frequency of contraction were the same in both regions, the lower blood flow in the normally innervated region was attributed to resting sympathetic coronary vasoconstrictor tone. This conclusion was reinforced by the additional observation that blood flow in the normally innervated region rose to equal that in the sympathectomized region following the administration of phentolamine, an α-adrenergic blocking agent. Additional evidence of significant resting sympathetic coronary vasoconstrictor tone has also been reported by others (Vatner et al., 1970; Vatner and McRitchie, 1975; Orlick et al., 1978; Murray and Vatner, 1979).

The possibility that efferent sympathetic nerve activity at the level of the coronary arterioles is an important determinant of resting coronary blood flow challenges the generally accepted metabolic hypothesis, i.e., that coronary blood flow is regulated locally by the extracellular concentration of vasoactive metabolites released from surrounding myocytes (Berne, 1964). The question raised over the primacy of extrinsic vs. intrinsic regulation of resting coronary blood flow is a fundamentally important one which deserves further investigation.

In the present study, an experimental approach...
similar to that described by Holtz et al. (1977) was employed, but a technique which entailed less exposure of the myocardium and coronary vasculature to a toxic chemical substance was used to produce a regional sympathectomy of the canine left ventricle (Martins and Zipes, 1980). Regional myocardial blood flow was measured in the conscious state under normal resting conditions, both before and after the administration of adrenergic blocking agents.

Methods

Surgical Preparation

Male mongrel dogs (25-40 kg) were screened for microfilaria, medicated against rabies and intestinal parasites, and maintained on a nourishing diet for 30 days. Dogs were fasted overnight and anesthetized with sodium pentobarbital (30-35 mg/kg, iv), intubated, and ventilated with room air using a mechanical respirator (Harvard Apparatus Co., model 607). Using sterile surgical procedures, a left thoracotomy was performed, the pericardium was incised, and the heart was exposed for the topical application of 85% phenol (Fig. 1). The region of the left ventricle to be enclosed by phenol was identified and the heart was exposed for the topical application of 85% phenol (Fig. 1). The region of the left ventricle to be enclosed by phenol was identified and the segment of the left circumflex artery entering this region along with accessible distal branches were carefully dissected. Umbilical tape, wetted with phenol, was passed under and wrapped around the vessels and then quickly removed. The next step was the application of a 3- to 5-mm-wide line of phenol, using the wooden end of a cotton applicator stick dipped in phenol. The path to be painted was dried prior to the application of the phenol. Phenol was painted from the base to the apex in a series of interconnecting lines along the anterior boundary of the area to be sympathectomized. Contact between the painted line and the pericardium was prevented for several minutes to avoid smearing of the line. Subsequently, the posterior boundary was painted from the apex (connecting to the anterior line) to the base. The base of the free wall, connecting the anterior and posterior lines was painted next. Phenol was applied in the atroventricular groove above the basilar line with the cotton end of the applicator dipped in phenol. In dogs to be used for conscious myocardial blood flow studies, a catheter with a flanged Teflon end, connected to Silastic, which in turn was connected to EVA tubing (Thermoplastics Scientifics, Inc.), was secured in the left atrium with a purse-string suture and exteriorized through the nape of the neck via a subcutaneous passage. The pericardium and chest were closed. An incision was then made in the neck and the right carotid artery was isolated. A catheter, 16 cm of Silastic connected to EVA tubing, was advanced into the aorta (confirmed by autopsy) via the right carotid artery, which was ligated. This aortic catheter was advanced subcutaneously and exteriorized through the same wound as the left atrial catheter. After surgery, the animal was treated with antibiotics (Bicillin; Wyeth) for approximately 1 week. During a 2-week period, the animal was trained to lie quietly on a table, and the catheters were flushed and filled with heparin (1000 U/ml) 2-3 times/week.

Assessment of Regional Ventricular Sympathectomy

The extent and completeness of the regional ventricular sympathectomy was evaluated in dogs prepared specifically for that purpose and also confirmed in several of the dogs used for the conscious myocardial blood flow studies. Approximately 2 weeks after phenol treatment, an animal was fasted overnight, sedated with morphine sulfate (2.5 mg/kg, sc), and approximately 1 hour later was anesthetized with a-chloralose (100 mg/kg, iv) dissolved in polyethylene glycol 400 (200 mg/ml). Additional anesthesia was given as required during the experiment. The animal was intubated and ventilated with a mechanical respirator. Supplemental oxygen was added to the inspired air to maintain a normal arterial oxygen tension. Blood gases and pH were determined on an Instrumentation Laboratory blood gas analyzer (model 113-S1) and, if needed, the respiratory volume or rate was changed to maintain PaO2 and PaCO2 within normal ranges. If required, sodium bicarbonate was administered to animals to maintain pH above 7.30. The right femoral vein and artery were isolated and catheterized, with the catheters advanced to the inferior vena cava and aortic arch. Arterial pressure was monitored with a Statham pressure transducer (P23Db) and an Electronics for Medicine (DR-8) recorder. A left thoracotomy was performed and heparin (500 U/kg) was administered.

![Diagram of the heart, illustrating the sites of phenol application on the left ventricle, epicardial vessels, and atrioventricular groove in a typical experiment. The sites of application varied slightly depending upon the location of the branches of the circumflex artery.](http://circres.ahajournals.org/)

**Figure 1** Diagram of the heart, illustrating the sites of phenol application on the left ventricle, epicardial vessels, and atrioventricular groove in a typical experiment. The sites of application varied slightly depending upon the location of the branches of the circumflex artery.
In six dogs, regional cardiac sympathetic nerve function was assessed by the uptake of \textsuperscript{3}H-norepinephrine (Amersham, 30-40 Ci/mmole). Using the basic method described by Kaye and Tyce, \(1978\), \textsuperscript{3}H-norepinephrine (2.5 \(\mu\)Ci/kg) was infused iv over a 5-minute period to prevent a change in systemic hemodynamics. After a 20-minute incubation period, which enabled the \textsuperscript{3}H-norepinephrine to become incorporated into neuronal pools as well as clear the extracellular space, the heart was rapidly excised and immediately cooled in saline chilled at 0\(^\circ\)C. In a controlled temperature room (4\(^\circ\)C), epicardial blood vessels were dissected free from surrounding tissues, minced, and placed in 2 ml 0.4 N perchloric acid at 0\(^\circ\)C, and glass homogenized. This homogenate was centrifuged at 10,000 \(g\) for 20 minutes, and the supernatant was decanted and analyzed for \textsuperscript{3}H-norepinephrine, using the steps described below on the myocardial tissue supernatant. The left ventricle was divided into 33 sections, the free wall into 25 sections, and the septum into 8 (Fig. 2). Each section was minced and pieces of tissue totaling in weight between 0.5 and 2.0 g were placed into a tube containing 10 ml of 0.4 N perchloric acid chilled at 0\(^\circ\)C. The tissue was homogenized with two 10-second bursts of a polytron, centrifuged at 10,000 \(g\) for 2 minutes, and the supernatant was decanted and frozen. Two ml of the supernatant were used for tissue \textsuperscript{3}H-norepinephrine analysis and were neutralized to a pH of 8.6 with 3 ml of 2 M Tris-0.5 M EDTA buffer. Alumina, activated according to Anton and Sayre \(1962\) (150 mg), was added to the neutralized supernatant, and thoroughly vortexed. At this pH, norepinephrine is bound to alumina, whereas the \(O\)-methylated metabolites are not. The supernatant was aspirated, and the alumina was washed 3 times with distilled water, vortexing and aspirating after each wash. This procedure removes the \(O\)-methylated metabolites. The \textsuperscript{3}H-norepinephrine was displaced from the alumina by the addition of 1 ml of 0.05 N perchloric acid and thorough vortexing. The perchloric acid was pipetted from the alumina and added to a liquid scintillation tube containing 10 ml of a Toluene based cocktail (3a20, Research Products International). Samples were counted on a Packard Tri-carb Liquid Scintillation Spectrometer for 10 minutes, with a counting efficiency for tritium as calculated by quenching standards of 44%. Using \textsuperscript{3}H-norepinephrine standards, the average recovery of the alumina purification procedure was 41%. All values reported are corrected for tissue weight, efficiency, and percent of yield of the alumina extraction, yielding results in disintegrations/min per g. When duplicate uptake analyses were regressed against one another, the correlation coefficient was 0.95.

In another group of eight dogs, studies were performed to compare metabolic responses of the normally innervated and sympathectomized regions to left stellate stimulation. The left stellate ganglion was isolated and both ansae were stimulated with a bipolar platinum electrode at 4-7 V, 10 Hz, for approximately 3 minutes. In these studies, the myocardial extraction ratios (A-V/A) for lactate and oxygen were determined in venous blood draining the normally innervated and sympathectomized regions during control conditions and at least 30 seconds after beginning left stellate stimulation. Medium-sized epicardial veins were catheterized using 22-g "intracaths" anchored with sutures. The veins were not obstructed by the intracaths and during sampling blood was removed at a rate of 2 ml/min. Blood was collected simultaneously from veins in the innervated and sympathectomized regions and from the aorta. Arterial and venous oxygen content was determined on an Instrumentation Laboratories co-oximeter (model 182), which was calibrated against oxygen content as determined by the manometric technique of Van Slyke and Neill \(1924\). Blood lactate samples were drawn and precipitated with 0\(^\circ\)C 6% perchloric acid and lactate was assayed enzymatically \(\text{Hohorst, 1963}\).

For a further metabolic comparison between the normally innervated and sympathectomized re-
Measurements of Regional Myocardial Blood Flow

Six dogs prepared with chronically implanted left atrial and aortic catheters at the time of phenol treatment were brought to the laboratory for study. The room was quiet and dimly lit and the recording equipment was located outside the room. The only person in the room was the operator who had trained the animal. The dog lay on a padded table throughout the procedure. Aortic blood pressure was monitored via the aortic catheter and heart rate was determined from the phasic pressure curve. After aortic pressure and heart rate had stabilized, and the animal was considered to be in a resting steady state, arterial blood was withdrawn for the determination of plasma norepinephrine content according to the radioenzymatic method of Henry et al. (1975). A previously placed in the apex of the left ventricle. The frozen tissue samples were divided into inner, middle, and outer thirds. Each third was weighed, pulverized, homogenized in 0.3 N perchloric acid, and centrifuged. After centrifugation, the supernatant was decanted and analyzed for creatine phosphate, adenosine triphosphate, and lactate (Hohorst, 1963). The frozen tissue samples were divided in 25 sections (Fig. 2), and each section was divided into equal outer and inner halves and weighed to the nearest milligram on an electrobalance (Cahn, model 7500). Tissue samples were counted in a Packard Auto-Gamma Spectrometer with three channels set for the three different energy levels of the isotope. The myocardial blood flow values were calculated by computer, using a program in which the isotope overlaps were also corrected.

The effects of adrenergic blockade were studied using the following protocol: Within a few minutes after the first blood flow measurement was completed, propranolol-HCl (INDERAL, Ayerst) was administered in a dose of 1 mg/kg via the left atrial catheter over a 1- to 2-minute period. Twenty minutes later, the second blood flow measurement was made, and 5 minutes after that, phentolamine mesylate (REGITTINE-Ciba) was administered in a dose of 1 mg/kg via the left atrial catheter over a 1- to 2-minute period. Five minutes later, the third blood flow measurement was made. In three of the animals, the adequacy of the a-adrenergic blockade was assessed by the heart rate response to a 20 μg/kg bolus of isoproterenol and the adequacy of the a-adrenergic blockade was assessed by the pressor response to a 20 μg/kg bolus of methoxamine. Either no response or a barely detectable response to these agonists was observed. In one animal, dose-response data were obtained on different days for each agonist before and after administration of the respective blocking agent. The threshold dose of isoproterenol was shifted two orders of magnitude to the right 20 minutes after the administration of propranolol. For methoxamine, the highest dose used in the unblocked state (100 μg/kg) produced no pressor response 5 minutes after the administration of phentolamine, and the shift in the threshold dose was not determined.

Following the regional myocardial blood flow studies three of the six animals were immediately after completion of the microsphere injection. This withdrawal rate was shown to be adequate in a separate evaluation of the method. Myocardial blood flow was calculated from the myocardial tissue radioactivity (Cr), the withdrawal rate of the pump (W), and the total radioactivity of the reference sample (Cr), as shown in the following expression:

MBF (ml/min per g) = \( \frac{Cm \text{ (counts/time per g)}}{Cr \text{ (counts/time)}} \times W \text{ (ml/min)} \)

Three blood flow measurements were made during an experiment, utilizing microspheres with different isotopes (35Sc, 57Co, 113Sn) which were randomized for each dog. After the animal was killed, the free wall of the left ventricle was systematically divided in 25 sections (Fig. 2), and each section was divided into equal outer and inner halves and weighed to the nearest milligram on an electrobalance (Cahn, model 7500). Tissue samples were divided into inner, middle, and outer thirds. Each third was weighed, pulverized, homogenized in 0.3 N perchloric acid, and centrifuged. After centrifugation, the supernatant was decanted and analyzed for creatine phosphate, adenosine triphosphate, and lactate (Hohorst, 1963). Additional unfrozen tissue samples were obtained in four animals not receiving H-norepinephrine for analysis of norepinephrine content under the radioenzymatic method of Henry et al. (1975).
Statistical Analysis

Differences in $^{3}$H-norepinephrine uptake, oxygen extraction ratios, and lactate extraction ratios between the innervated and sympathectomized regions of the ventricle were analyzed by Student's paired $t$-test. In the conscious animal studies, differences in heart rate and mean aortic pressure in the control, $\beta$-adrenergically blocked, and combined $\alpha$- and $\beta$-adrenergically blocked states were analyzed by a one-way analysis of variance. Differences in myocardial blood flow between the innervated and sympathectomized regions in each of the states were analyzed by a one-way analysis of variance. Differences in myocardial blood flow in any given region or ventricular layer between the control state and the $\beta$-adrenergically blocked state or between the control state and the combined $\alpha$- and $\beta$-adrenergically blocked state were analyzed by a two-way analysis of variance. A $P$ value $<0.05$ was considered to be statistically significant.

Results

Regional $^{3}$H-Norepinephrine Uptake and Tissue Norepinephrine Content

The $^{3}$H-norepinephrine uptake data obtained in six dogs that had previously undergone phenol treatment are summarized in Figure 2. In Figure 2, each square in the grids of the left ventricular free wall and septum contains the mean $^{3}$H-norepinephrine uptake value for tissue in that area. The two lefthand columns of the ventricular free wall (anterior region) contained the tissue samples with the highest uptake values, whereas the two righthand columns (posterior region), excluding the two apical samples, contained the tissue samples with the lowest uptake values. The middle column (mid-region) contained the tissue samples with intermediate uptake values. Based on this pattern, which was the same in every animal, the data from the individual sample areas were grouped into three larger areas for further analysis, as shown in Figure 3. The mean uptake value of 20,600 dpm/g in the high uptake area was essentially the same as the 20,000 dpm/g value obtained for the same area in eight normal animals previously studied in an identical manner (Chilian et al., 1980). On this basis, tissue samples obtained from the two posterior columns, excluding the two apical samples, were considered to be in a completely sympathectomized region.

The mean $^{3}$H-norepinephrine uptake value of 1,600 dpm/g in the low uptake area was markedly less than the 19,075 dpm/g value obtained for the same area in normal animals, and only 8% of the 20,600 dpm/g uptake value obtained in the high uptake area of the same ventricle. The difference between these values was statistically significant ($P < 0.01$). From data obtained in normal animals pretreated with cocaine to block the neuronal uptake mechanism (Chilian et al., 1980), in which the mean $^{3}$H-norepinephrine uptake value was 4,000 dpm/g, the $^{3}$H-norepinephrine uptake in this low uptake area could be accounted for entirely by non-neuronal uptake mechanisms. Thus, tissue samples obtained from the two posterior columns, excluding the two apical samples, were considered to be in a normally innervated region.
value of 8,800 and 11,300 dpm/g in the two tissue samples comprising this area suggested that the area was incompletely denervated. The interventricular septum was not included in the study of regional myocardial blood flow in the present study.

Also shown in Figure 3 are the mean ³H-norepinephrine uptake values for the epicardial conductance vessels in the anterior and posterior ventricular wall regions. The mean uptake value of 5,000 dpm/g in branches of the left circumflex artery distal to the site of dissection and phenol application was markedly lower (83%) than the 30,300 dpm/g uptake value obtained for the nontreated left anterior descending artery. The difference between these values was statistically significant (P < 0.01).

Tissue norepinephrine content data were obtained in four dogs not receiving ³H-norepinephrine. The norepinephrine content of tissue samples removed from the epicardial and endocardial halves of the posterior ventricular wall region was extremely low, averaging 29 ± 9 ng/g in the epicardium and 22 ± 8 ng/g in the endocardium. This was in sharp contrast to the norepinephrine content of tissue samples removed from the normally innervated region, which averaged 838 ± 243 ng/g in the epicardium and 1136 ± 420 ng/g in the endocardium. With respect to the epicardial conductance vessels, the norepinephrine content of segments of the left circumflex artery distal to the point of phenol application averaged 85 ± 7 ng/g in two animals. In contrast to this, the norepinephrine content in segments of the normally innervated left anterior descending artery averaged 852 ± 263 ng/g. These data provide additional proof of the absence of a functioning sympathetic nerve supply in the area identified as the low ³H-norepinephrine uptake area after phenol treatment.

Myocardial Oxygen and Lactate Extraction Ratios

Figure 4 contains data on myocardial extraction ratios for oxygen and lactate in the normally innervated (I) and sympathectomized (Sx) regions of the ventricle under control conditions and during left stellate nerve stimulation. Under control conditions, the mean and standard error of the mean values for the oxygen extraction ratios in the normally innervated and sympathectomized regions were identical (0.69 ± 0.02). The lactate extraction ratio values in the two regions were also virtually identical (I = 0.49 ± 0.03, Sx = 0.49 ± 0.04) under control conditions.

During left stellate nerve stimulation sufficient to increase aortic systolic pressure 23% (control value = 128 ± 9 mm Hg) and heart rate 17% (control value = 132 ± 12 beats/min), the oxygen extraction ratio increased significantly in the normally innervated region (0.78 ± 0.02; P < 0.05), but not in the sympathectomized region (0.73 ± 0.03; P = NS).

Likewise, the lactate extraction ratio increased significantly in the normally innervated region (0.57 ± 0.03; P < 0.05), but not in the sympathectomized region (0.47 ± 0.03; P = NS). The proportional increases in the oxygen and lactate extraction ratios in the innervated region were the same (16%).

Myocardial Tissue Metabolite Levels

Mean data obtained in eight animals on the levels of creatine phosphate, adenosine triphosphate, and lactate in the normally innervated and sympathectomized regions of the ventricle are summarized in Table 1. The values for the outer, middle, and inner thirds of the tissue sample were averaged to yield the mean transmural value. No significant differences between the normally innervated and sympathectomized regions were noted for any of these metabolites.

Regional Myocardial Blood Flow

The results obtained in six conscious, resting dogs studied 2 weeks after phenol treatment are presented in Table 2 and Figure 5. Table 2 contains data on heart rate, mean aortic pressure, and mean transmural blood flow (average of epicardial and endocardial halves) in the normally innervated and sympathectomized regions for each of the three conditions under which myocardial blood flow was determined, i.e., control conditions, after β-adrenergic blockade, and after combined α- and β-adrenergic blockade. Summarizing the results presented in Table 2: under control conditions, blood flow in
**TABLE 1**  Tissue Metabolite Levels in Normally Innervated and Sympathectomized Regions of the Left Ventricle

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Normally innervated region</th>
<th>Sympathectomized region</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine phosphate</td>
<td>8  9.97 ± 0.37</td>
<td>9.93 ± 0.36</td>
<td>NS</td>
</tr>
<tr>
<td>Adenosine triphosphate</td>
<td>8  5.45 ± 0.15</td>
<td>4.72 ± 0.32</td>
<td>NS</td>
</tr>
<tr>
<td>Lactate</td>
<td>8  0.72 ± 0.24</td>
<td>0.67 ± 0.20</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. NS = not significant.

The innervated region was not significantly different from that in the sympathectomized region. Blood flow in both regions was not significantly different from that in their respective regions under control conditions. The addition of α-adrenergic blockade to these β-adrenergically blocked animals resulted in no significant changes in heart rate or mean aortic pressure. Once again, blood flow in the innervated region was not significantly different from that in the sympathectomized region, and blood flow in both regions was not significantly different from that in their respective regions under control conditions. In Figure 5, blood flow data for the endocardial and epicardial halves of the ventricular wall are presented separately. Endocardial blood flow in the innervated and sympathectomized regions was virtually identical under each of the three experimental conditions. Epicardial blood flow showed a tendency to be slightly higher in the innervated than in the sympathectomized region under each of the three experimental conditions, but in no case was the difference statistically significant. The endocardial-to-epicardial blood flow ratios in the innervated and sympathectomized regions under control conditions were 1.23 ± 0.03 and 1.29 ± 0.04, respectively, and not significantly different. These ratios were not significantly altered during β-adrenergic blockade or combined α- and β-adrenergic blockade.

Arterial blood data obtained in these animals under basal conditions prior to the first blood flow measurement were: plasma norepinephrine, 135 ± 37 pg/ml; Po2, 86 ± 7 mm Hg; Pco2, 31 ± 3 mm Hg; pH, 7.40 ± 0.04; and hematocrit, 39 ± 4%.

**Discussion**

The feasibility of producing a regional ventricular sympathectomy by applying phenol to the surface of the heart was suggested by earlier studies in which the technique was used to demonstrate the pattern of sympathetic nerve projections on the heart (Randall et al., 1968; Geis and Kaye, 1968), and by a more recent study in which the technique was used to eliminate sympathetic nerve influences on the effective refractory period in the left ventricular myocardium (Martins and Zipes, 1980). In the latter study, phenol was applied in a circle near the ventricular apex. Tissue samples taken from the center of this circle 3 days later revealed marked
depletion of norepinephrine content in the epicardial and endocardial regions and disappearance of catecholamine histofluorescence normally caused by adrenergic neurons. The effects of parasympathetic nerve stimulation on the effective refractory period were preserved within the phenol-encircled area, indicating that the epically applied phenol produced, in effect, a selective regional sympatheticotomy. This lack of an effect on parasympathetic nerve function was considered to be consistent with other evidence that significant efferent parasympathetic innervation of the left ventricle occurs via the interventricular septum (Hirsch, 1971; Kent et al., 1974).

In view of evidence that there are sympathetic nerve fibers in the adventitial layer of the coronary conductance arteries (Denn and Stone, 1976), which presumably could innervate the coronary arterioles, a procedure additional to that of Martins and Zipes (1980) that we performed in the present study was the perivascular application of phenol to the epicardial arteries entering the phenol-encircled area. Although this procedure may have also destroyed parasympathetic nerve fibers, there is evidence that interruption of these fibers would not have eliminated parasympathetic innervation in the phenol-treated region because of additional innervation via the septum (Denn and Stone, 1976). One further procedure was the application of phenol in the posterior atrioventricular groove to assure denervation of the basilar portion of the posterior ventricular wall. From preliminary studies it was apparent that these additional procedures increased the extent and completeness of the sympatheticotomy, as judged by regional "H-norepinephrine uptake data.

Validation of "H-norepinephrine uptake as an index of sympathetic innervation was provided by Kaye and Tyce (1978) who correlated data on the myocardial uptake of "H-norepinephrine with other parameters of sympathetic reinnervation at various times after total surgical cardiac denervation in the dog. In that study, the uptake of "H-norepinephrine by the left ventricle after cardiac denervation was noted to be reduced to 4% of the normal value at a time when inotropic and chronotropic responses to sympathetic nerve stimulation were absent. Subsequently, there was an increase in "H-norepinephrine uptake by the myocardium which correlated well with the reappearance of inotropic and chronotropic responses. Tissue norepinephrine content, which decreased to negligible levels after cardiac denervation, did not increase in a similar manner, leading the investigators to conclude that development of the norepinephrine storage mechanism lagged behind that of the uptake mechanism during cardiac reinnervation. Evidence that norepinephrine uptake may be a more reliable index of sympathetic innervation than norepinephrine content has also been provided by others (Duckles and Rapaport, 1979). In the present study, the relative reductions in both "H-norepinephrine uptake and norepinephrine content in the posterior ventricular wall after phenol treatment were similar to those obtained in the left ventricle after surgical cardiac denervation (Kaye and Tyce, 1978). Also, the "H-norepinephrine uptake values in this area were not above those previously noted in normal animals pretreated with cocaine to block the neuronal mechanism (Chilian et al., 1980). Thus we concluded that an essentially complete sympatheticotomy was achieved in the low "H-norepinephrine uptake area of the left ventricle in the present study.

Functional evidence of a difference in the intactness of the sympathetic nerve supply to the anterior and posterior regions of the phenol-treated ventricle was obtained from the stellate nerve stimulation studies. Increases in the myocardial extraction ratios for oxygen and lactate were noted in the anterior, but not the posterior, region during stellate nerve stimulation. In the dog, the anterior cardiac veins contain blood only from the anterior ventricular region, but the posterior cardiac veins contain an admixture of blood from both the anterior and posterior regions (Nakazawa et al., 1978). Thus, during stellate nerve stimulation, the extraction ratios obtained for the posterior region could have been overestimated due to the presence of blood from the anterior region, and the true differences between the two regions may have been even greater than the observed differences. The finding of an increased oxygen extraction ratio in the an-

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**Table 2: Heart Rate, Mean Aortic Pressure and Regional Myocardial Blood Flow in Conscious, Resting Animals before and after Adrenergic Blockade**

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate</th>
<th>Mean Aortic Pressure</th>
<th>Regional Blood Flow</th>
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<tbody>
<tr>
<td></td>
<td>HR (beats/min)</td>
<td>MAP (mm Hg)</td>
<td>Normally innervated region (I)</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>81 ± 3</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>β-Blocked</td>
<td>6</td>
<td>82 ± 3</td>
<td>105 ± 2</td>
</tr>
<tr>
<td>α- &amp; β-Blocked</td>
<td>6</td>
<td>86 ± 5</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>P(β-Blocked vs. Cont.)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P(α- &amp; β-Blocked vs. Cont.)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.
The regional myocardial blood flow measurements were made in conscious animals that had been trained to lie quietly. Plasma norepinephrine levels in arterial blood samples drawn after the heart rate and mean aortic pressure had stabilized were low and similar to those obtained in normal resting human subjects (Henry et al., 1979). It has been shown in conscious human studies that the plasma norepinephrine level is a useful index of sympathetic neural activity (Lake et al., 1976), and that the resting plasma level is approximately only one-eighth of that needed to produce measurable hemodynamic or metabolic changes via a circulating hormonal action (Silverberg et al., 1978). Evidence of minimal sympathetic neural activity in the conscious, resting dog was reported over a decade ago (Pitt et al., 1968). The findings in the present study of a low plasma norepinephrine level and of insignificant changes in heart rate and mean aortic pressure after \( \beta \)-adrenergic blockade or combined \( \alpha \)- and \( \beta \)-adrenergic blockade provide further evidence of minimal sympathetic neuronal activity in the conscious animal under normal resting conditions, and they are contrary to the concept of significant sympathetic neural influences on the heart under these conditions.

The finding in the present study of an insignificant difference in blood flow between the normally innervated and sympathectomized regions of the ventricle is not in agreement with the previous results of Holtz et al. (1977), who reported a significant difference in blood flow between a normally innervated region and a region chemically sympathectomized with 6-hydroxydopamine. In that study, 6-hydroxydopamine, mixed with saline and ascorbic acid, was administered into the circumflex artery of the conscious dog via a chronic indwelling catheter and held in place for 45 seconds by infusing a cuff occluder on the vessel. Two treatments were performed 48 hours apart, and regional myocardial blood flow measurements were made 48 hours after the second treatment. Blood flow in the normally innervated region was noted to be approximately 40% lower than that in the 6-hydroxydopamine-treated region, and following \( \alpha \)-adrenergic blockade with phenolamine, this difference was essentially abolished, primarily because of a rise in blood flow in the innervated region.

The reason for the conflicting findings in the two regional ventricular sympathectomy studies is not readily apparent. A negative finding was obtained in the present study, and one reason for a false negative finding would be a masking of coronary vasodilation in the sympathectomized region because of a simultaneous reduction in myocardial and coronary \( \beta \)-adrenergic stimulation. However, this would appear to be ruled out by the lack of a unilateral reduction in blood flow in the normally innervated region following \( \beta \)-adrenergic blockade, i.e., unmasking of \( \alpha \)-adrenergic tone. Another reason for a false negative finding in the present study would be an inhibition of coronary blood flow in the sympathectomized region because of a post denervation \( \alpha \)-adrenergic supersensitivity to circulating catecholamines (Trendelenburg, 1963). This would appear to be ruled out by the lack of an increase in blood flow in the sympathectomized region following \( \alpha \)-adrenergic blockade.

The study by Holtz et al. (1977) provided positive evidence of a regional blood flow difference, and one reason for a false positive finding would be greater blood flow in the sympathectomized region because of a post denervation \( \beta \)-adrenergic supersensitivity. However, as shown in that study, this would appear to be ruled out by the lack of a decrease in blood flow in the sympathectomized region following \( \beta \)-adrenergic blockade. Another reason for a false positive finding in that study would be vasodilation in the sympathectomized region because of vascular or myocardial damage, secondary to such factors as microthrombi from the
chronic indwelling coronary catheter or the direct intracoronary instillation of 6-hydroxydopamine. According to the authors, this possibility of nonspecific damage in the sympathectomized region was not completely ruled out. In the final analysis, the authors defended their results on the basis of a finding in the normally innervated region, namely, an increase in blood flow in that region following α-adrenergic blockade with phentolamine. However, it has been demonstrated that phentolamine augments the release of norepinephrine from adrenergic neurons due to blockade of presynaptic α2 adrenoceptors (Hoffman and Lefkowitz, 1980). Thus, the increase in blood flow in the normally innervated region could be explained by β-adrenergic stimulation in that region. In the present study, α-adrenergic blockade was preceded by β-adrenergic blockade, and no increase in blood flow in the normally innervated region occurred. This finding constitutes further evidence against significant resting sympathetic α-adrenergic coronary vasoconstriction. We believe, therefore, that the discrepancy between the two studies could be due to a false positive finding in the study by Holtz et al. (1977).

Results bearing on the question of resting sympathetic coronary vasoconstrictor tone have also been obtained in conscious animal studies in which coronary blood flow has been measured continuously with a chronically implanted flow probe. Schwartz and Stone (1977) reported that α-adrenergic blockade with phentolamine caused no significant changes in resting heart rate, blood pressure, dP/dt max, or mean coronary blood flow in seven conscious dogs. They did report, on the other hand, that reactive hyperemic blood flow following a 10-second circumflex artery occlusion was significantly increased after α-adrenergic blockade or after performing a left stellate nerve section on the animal, suggesting that physiologically significant sympathetic coronary vasoconstriction developed reflexly in response to the circumflex artery occlusion. In the same report, evidence was presented of a slight, but statistically significant, increase in the endocardial-to-epicardial blood flow ratio after left stellate nerve section, but the results were not obtained in the conscious animal. The results obtained in the present study were negative with respect to significant sympathetic neural regulation of transmural blood flow distribution under conscious, resting conditions.

Murray and Vatner (1979) reported that α-adrenergic blockade with phentolamine caused a significant decrease in late diastolic coronary vascular resistance in nine quietly standing dogs. However, the ventricles were being paced in these animals and α-adrenergic blockade caused a significant decline in mean aortic pressure. This decline in mean aortic pressure, which represented a 32% reduction below the control level, indicates that sympathetic neural activity was elevated above a normal resting level in these animals. Furthermore, when α-adrenergic blockade was preceded by β-adrenergic blockade, no decrease in late diastolic coronary vascular resistance occurred, suggesting that the decrease in resistance produced by α-adrenergic blockade alone was due to an increase in β-adrenergic stimulation, secondary to the blockade of presynaptic α2 adrenoceptors. In this same study by Murray and Vatner (1979), significant α-adrenergic coronary vasoconstrictor tone was detected during severe exercise, but the extent to which this could be ascribed to increased coronary sympathetic nerve stimulation vs. increased plasma catecholamine levels (Silverberg et al., 1978) was not determined.

Studies on the reflex behavior of the coronary circulation in the conscious dog have provided evidence of a reflex withdrawal mechanism for sympathetic coronary vasoconstrictor tone. A transient coronary vasodilation has been demonstrated during carotid sinus nerve stimulation (Vatner et al., 1970), intracarotid nicotine administration, and pulmonary hyperinflation (Vatner and McRitchie, 1975) which persists after both β-adrenergic and muscarinic receptor blockade, but not after α-adrenergic blockade. Since the reflex response was elicited in conscious, resting animals, the results were interpreted as evidence of significant resting sympathetic coronary vasoconstrictor tone. However, this conclusion is open to question because administration of the α-blocking agent did not itself result in a reduction in resting coronary resistance to the level normally observed during the reflex maneuver.

The major findings and conclusions of this study are summarized as follows: First, a regional sympathectomy of the canine left ventricle was produced by applying a thin line of phenol to the surface of the myocardium and the epicardial coronary arteries. This finding extends the results of Martins and Zipes (1980), who previously used the phenol technique, and it supports other evidence that the cardiac sympathetic nerves traverse and penetrate the myocardium via the epicardial surface. Second, in this animal model, left stellate nerve stimulation caused an increase in the myocardial extraction ratios for oxygen and lactate in the normally innervated region of the ventricle. These findings support previous evidence (Feigl, 1975) that, during cardiac sympathetic nerve stimulation, myocardial oxygen tension is reduced due to α-adrenergic coronary vasoconstriction. New evidence is provided that this reduction in myocardial oxygen tension is not accompanied by the development of myocardial anaerobic metabolism. Third, in this animal model, myocardial blood flow and its transmural distribution were not significantly different in the normally innervated and sympathectomized regions of the ventricle under conscious resting conditions. Furthermore, blood
flow was not significantly altered in either region following adrenergic blockade. Thus, these findings do not support previous evidence of significant resting sympathetic coronary vasoconstrictor tone in the conscious animal.

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Evidence against significant resting sympathetic coronary vasoconstrictor tone in the conscious dog.

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