

# Activation of Cardiac Sympathetic Afferents During Coronary Occlusion

## Evidence for Reflex Activation of Sympathetic Nervous System During Transmural Myocardial Ischemia in the Dog

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**Background.** Left ventricular sympathetic afferent nerves are located mainly in superficial epicardial layers. Reflex excitatory responses mediated by sympathetic afferent nerves have been observed during myocardial ischemia in cats and humans but not in dogs. Previous canine studies have induced ischemia by occlusion of a coronary artery. Extensive collateral circulation in the canine heart may limit ischemia of epicardial layers during simple coronary occlusion, resulting in little stimulation of sympathetic afferent nerves and minimal reflex excitatory responses.

**Methods and Results.** In anesthetized dogs with sinoaortic and vagal deafferentation, we determined whether reflex sympathoexcitatory responses mediated by sympathetic afferents occurred during transmural myocardial ischemia. Reflex sympathoexcitation was quantitated by direct recording from either efferent renal ( $n=20$ ) or cardiac ( $n=5$ ) sympathetic nerves. Responses of arterial pressure and efferent sympathetic nerve activity were measured during simple occlusion of the anterior descending artery (LAD alone) and during LAD occlusion with a circumflex stenosis (LAD+CIRC). This circumflex stenosis was adjusted to abolish coronary vasodilator reserve without reducing basal flow. We observed significantly greater reflex increases in renal ( $32\pm5\%$ ) and cardiac ( $58\pm15\%$ ) nerve activity during LAD+CIRC than during LAD alone ( $14\pm6\%$  and  $8\pm7\%$ , respectively). Reflex changes in renal nerve activity during LAD+CIRC were abolished by interruption of cardiac sympathetic afferent pathways ( $n=5$ ). In eight experiments, myocardial blood flow was measured during the two coronary occlusions. These experiments confirmed that LAD+CIRC elicited more transmural ischemia in the LAD distribution than did LAD alone. However, these experiments also revealed that LAD+CIRC elicited endocardial ischemia in the circumflex distribution. In five additional experiments, regional sympathetic deafferentation of the posterior left ventricle by epicardial application of 88% phenol along the atrioventricular groove had no significant effect on renal nerve responses to LAD+CIRC ( $36\pm5\%$  increase before phenol versus  $31\pm3\%$  increase after phenol). These results indicate that endocardial ischemia in the circumflex distribution did not contribute to the reflex increases in nerve activity that were noted during LAD+CIRC.

**Conclusions.** Reflex sympathoexcitation mediated by cardiac sympathetic afferents can be elicited in dogs. However, these responses are significant only during ischemia that is transmural and involves the superficial epicardial layers of the left ventricle. (*Circulation* 1991;84:357-367)

**R**eflex sympathoexcitatory responses are elicited by coronary occlusion and myocardial ischemia in cats.<sup>1-4</sup> These responses are the result of activation of sensory endings in the ischemic left ventricle, whose afferent fibers travel to the

central nervous system with the sympathetic nerves (sympathetic afferents). In humans, excitatory responses characterized by hypertension and tachycardia frequently are associated with myocardial ischemia involving the anterior wall of the left ventricle.<sup>5</sup>

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These excitatory responses may also represent the reflex effects of cardiac sympathetic afferent activation. In contrast, coronary occlusion in dogs with sinoaortic baroreceptor denervation evokes cardioinhibitory, vasodepressor, and sympathoinhibitory responses.<sup>6,7</sup> These inhibitory responses are abolished by vagotomy. After vagotomy, no significant reflex excitatory responses are observed during coronary occlusion.<sup>6,7</sup> This lack of an excitatory response during ischemia is surprising, since activation of sympathetic afferents by direct epicardial application of bradykinin results in hypertension, tachycardia, and sympathoexcitation.<sup>8</sup>

Recent studies<sup>9-11</sup> suggest that left ventricular sympathetic afferent fibers are located mainly in the superficial epicardial layers and that vagal afferent fibers are located in the deeper endocardial layers. Because of extensive collateral circulation in the canine heart, simple occlusion of a coronary artery may result in ischemia involving mainly the endocardium. The epicardial layers may receive sufficient blood flow by collateral vessels to prevent or limit ischemia. As a result, there may be minimal stimulation of sympathetic afferents located in these superficial epicardial layers. Thus, it is possible that the failure to evoke reflex excitatory responses during simple coronary occlusion in dogs is merely the result of a failure to provide an adequate stimulus to sympathetic afferent endings due to their location in relation to the ischemic myocardium.

The purpose of our study was to determine whether reflex sympathoexcitatory responses mediated by cardiac sympathetic afferents are greater during transmural ischemia than during subendocardial or nontransmural ischemia.

### Methods

Experiments were performed in anesthetized, mechanically ventilated dogs with cervical vagotomy and sinoaortic denervation. The dogs were anesthetized with thiamylal sodium (15 mg/kg i.v.) followed by  $\alpha$ -chloralose (80 mg/kg i.v.). Additional doses of  $\alpha$ -chloralose (10 mg/kg i.v.) were administered hourly. The dogs were ventilated with a mixture of oxygen and room air. Arterial blood gases were determined at intervals, and either the respiratory settings were adjusted or sodium bicarbonate was administered to maintain pH between 7.35 and 7.45. Arterial and venous cannulas were placed in the femoral vessels, and arterial pressure was monitored continuously during the protocol. In 10 experiments, a cannula was positioned in the left atrium for continuous monitoring of left atrial pressure. Body temperature was maintained by external warming. During the recording of nerve activity, muscular movement was eliminated with pancuronium bromide (2 mg i.v.).

### Surgical Preparation

A midline cervical incision was made to expose the carotid arteries and cervical vagi bilaterally. The

vagal cardiopulmonary receptors and the aortic arch baroreceptors were denervated by sectioning the cervical vagi. The carotid baroreceptors were denervated by ligating and sectioning all of the structures that course between the internal and external carotid arteries. Carotid sinus denervation was confirmed by assessment of nerve traffic and arterial pressure changes during bilateral carotid occlusion. Previous studies<sup>12-14</sup> indicate that bilateral cervical vagotomy acutely abolishes aortic arch reflexes.

An incision was then made in the fifth left intercostal space to expose the heart. A small opening was made in the pericardium. The proximal segment of the left anterior descending coronary artery was carefully isolated, and a snare occluder was placed around the vessel. Then the proximal left circumflex coronary artery was isolated, and a hydraulic occluder and Doppler velocity transducer were placed on this vessel. Care was taken to keep the epicardial surface moist with warm saline. Epicardial temperature was monitored, and external warming was used to maintain the temperature between 36°C and 38°C.

### Nerve Recordings

Activity was recorded from either renal or cardiac efferent sympathetic nerves. An incision was made in the left flank to expose the left renal artery. A small branch of the renal sympathetic nerves was dissected free from the renal artery and the surrounding connective tissue. The nerve was sectioned, and the nerve sheath was removed. The nerve was immersed in mineral oil and placed on bipolar platinum-iridium electrodes for the recording of action potentials as previously described in detail.<sup>6</sup> In brief, the signal was amplified by a band-pass amplifier (model P511, Grass Instrument Co., Quincy, Mass.) with high-frequency cutoff set at 1,000–3,000 Hz and low-frequency filter at 30–100 Hz. The output of this amplifier was fed into an audio amplifier and a spike counter, which counted and integrated all nerve spike activity whose amplitude exceeded a preselected voltage level (just above noise). The ventrolateral cardiac sympathetic nerve was identified adjacent to the left thoracic vagus and was dissected free from surrounding connective tissue. This particular nerve was selected because it has been shown to contain mainly efferent sympathetic nerve fibers rather than sensory fibers.<sup>15</sup> The nerve was cut distally and placed on a dissecting stage in a mineral oil pool. The nerve sheath was removed, and the nerve was placed on hook electrodes for action potential recording as described above for the renal nerves.

### Experimental Protocols

Measurements of arterial pressure, left atrial pressure ( $n=10$ ), and either renal ( $n=20$ ) or cardiac ( $n=5$ ) sympathetic nerve activity were made during 2-minute occlusions of the left anterior descending coronary artery (LAD). Coronary occlusion was performed with circumflex flow unimpeded (LAD alone) and in the presence of a stenosis that was applied to the circum-

flex coronary artery to limit collateral flow to the anterior descending vascular bed (LAD+CIRC). The order in which these occlusions were performed was randomized, and 45 minutes was interposed between occlusions for stabilization. The circumflex stenosis was adjusted to abolish coronary vasodilator flow reserve without reducing the basal level of coronary flow. Coronary flow reserve was assessed by measurement of the hyperemic flow velocity responses, which were induced by a 5-second occlusion of the circumflex artery. A separate snare was used to create these temporary occlusions. The circumflex stenosis was tightened until the hyperemic flow velocity response noted on release of the transient circumflex occlusion was eliminated. All adjustments of the stenosis were made before occlusion of the LAD, and adequate time was allowed to insure that circumflex flow was stable.

In five of these experiments, responses during LAD+CIRC were recorded before and after interruption of all cardiac sympathetic afferent fibers. Bilateral cardiac sympathetic deafferentation was performed by removal of both stellate ganglia, by section of the thoracic sympathetic chains caudal to T-4, and by section of the T-1 through T-4 white rami.

In eight separate experiments, the ability of the circumflex stenosis to limit collateral flow and to elicit greater epicardial/transmural anterior ischemia during LAD occlusion was assessed using radiolabeled microspheres to measure myocardial blood flow. Regional blood flow measurements were performed using conventional techniques.<sup>16</sup> For each blood flow determination, 1.2–2.0 million microspheres of 15- $\mu\text{m}$  diameter were injected into the left atrium. Four different isotopes were used: <sup>113</sup>Sn, <sup>141</sup>Ce, <sup>46</sup>Sc, and <sup>103</sup>Ru. Microsphere suspensions were vortex-agitated and sonicated for 2 minutes before injection. Baseline blood flow determinations were made before each coronary occlusion. After coronary occlusion, adequate time (approximately 15–30 seconds) for stabilization of arterial pressure was allowed before microsphere injection. An arterial reference sample was withdrawn at a constant rate by pump from the right common carotid artery. Reference sample withdrawal was begun 10 seconds before microsphere injection and continued for 110 seconds thereafter.

Blood flow to the entire left ventricle was analyzed. After the dog was killed, the heart was removed and the left ventricle was separated from the atria and right ventricle. The ventricle was divided into five slices of equal thickness from base to apex. Each of these slices then was divided into eight equal pieces using an external reference to guide the first cut. This external reference extended from the bifurcation of the left main coronary artery to the ventricular apex. Finally, all of the ventricular sections were divided into three pieces of equal thickness corresponding to epicardial, midmyocardial, and endocardial layers. All blood and tissue samples were weighed and placed in counting vials for assay of radioactivity

using a gamma counter (Compugamma 1282, Pharmacia LKB Nuclear Inc., Gaithersburg, Md.). All counts were corrected for background and overlapping activity. Myocardial blood flow was calculated using the formula  $Q_m = (C_m \times Q_x)/Cr$ , where  $Q_m$  is myocardial blood flow (ml/min),  $C_m$  is corrected counts per minute in the tissue sample,  $Q_x$  is the withdrawal rate of the arterial reference sample, and  $Cr$  is the corrected counts per minute in the reference sample. Myocardial blood flow was then divided by the weight of the corresponding tissue sample to obtain flow per gram of tissue.

Because these blood flow studies revealed that ischemia of the posterior endocardial layers was elicited by LAD+CIRC, an additional set of experiments ( $n=5$ ) was performed to assess whether this posterior endocardial ischemia was contributing to the reflex nerve traffic changes that were observed during this experimental maneuver. In these experiments, renal nerve responses during LAD+CIRC were recorded before and after regional sympathetic denervation of the posterior left ventricle by application of 88% phenol solution along the left atrioventricular groove. Denervation was considered to be adequate when the reflex increases in renal nerve activity, which were induced by application of bradykinin to the posterior epicardial surface, were abolished. The doses of bradykinin used to assess the adequacy of denervation ranged from 50 to 200  $\mu\text{g}$ . This method has been shown in a previous study<sup>9</sup> to interrupt afferents that originate from both the endocardium and epicardium.

#### Data Analysis

Arterial pressure, left atrial pressure ( $n=10$ ), phasic and mean circumflex blood flow velocity, raw nerve activity, and integrated nerve activity were recorded continuously on an electrostatic recorder (model ES 1000, Gould, Cleveland, Ohio). In each experiment, measurements of mean arterial pressure and sympathetic nerve activity (impulses/sec) were made at baseline before coronary occlusion and during each 30-second period of the 2-minute coronary occlusion. Recovery observations were made 5 minutes after release of the occlusion. Because we recorded from multiunit nerve preparations, nerve activity changes were expressed as percentage changes from control values. In multiunit preparations, the absolute value of nerve activity is dependent on the number of active fibers placed on the recording electrode. This number of fibers may differ widely from one experiment to another. Statistical comparisons require that nerve activity be normalized to basal values. Observations from all dogs in each group were combined, and values (mean  $\pm$  SEM) were computed.

A repeated measures analysis of variance was used to determine if the addition of a circumflex stenosis during LAD occlusion had a significant effect on the changes in mean arterial pressure and sympathetic nerve activity. Similar analysis was used to determine if sympathetic afferent denervation had a significant

effect on these parameters during sequential LAD occlusions with circumflex stenosis. In addition, the baseline nerve activity levels and the maximal renal nerve activity changes that were observed during LAD alone and during LAD+CIRC were compared by a paired *t* test.

Myocardial blood flow values during coronary occlusions were normalized to their respective baseline flow values and expressed as a percentage of this basal flow. The purpose of these experiments was to determine whether the addition of a circumflex stenosis during LAD occlusion had a significant impact on blood flow to the region supplied by the LAD. This ischemic "risk region" was identified by analysis of endocardial flow changes during LAD occlusion with circumflex flow unimpeded. Based on the effects of LAD alone on endocardial flow, the left ventricle was divided into ischemic and nonischemic regions. The ischemic region was defined as any segment in which endocardial flow was reduced by 20% during LAD alone (i.e., when blood flow value was  $\leq 80\%$  of baseline blood flow). Paired *t* tests were used to compare the effects of the two coronary occlusions on percent blood flow reduction in endocardial and epicardial layers. The masses of myocardial tissue in which various levels of reduced flow were noted during the two coronary occlusions were also compared by paired *t* tests. A value of  $p < 0.05$  was considered statistically significant.

## Results

### *Renal Sympathetic Nerve Activity*

Figure 1 illustrates the effects of LAD occlusion with and without a circumflex stenosis on mean arterial pressure and renal sympathetic nerve activity ( $n=20$  dogs). During LAD alone, there were small increases in renal nerve activity. During LAD+CIRC, a greater degree of sympathoexcitation was observed. Statistical analysis indicated that the addition of a circumflex stenosis during LAD occlusion elicited significantly greater increases in renal nerve activity than did LAD alone. There were no significant differences in the baseline level of nerve activity measured immediately before each coronary occlusion (LAD alone,  $83 \pm 9.8$  impulses/sec; LAD+CIRC,  $105 \pm 10.3$  impulses/sec;  $p=0.14$ ).

Decreases in mean arterial pressure were observed during both coronary occlusions. The fall in arterial pressure was significantly greater during LAD+CIRC, probably reflecting a greater degree of mechanical ventricular dysfunction during this maneuver.

Since each individual dog reached its maximal change in nerve traffic at different times during the 2-minute occlusions, these maximal changes also were analyzed. The effect of a circumflex stenosis on the reflex increases in nerve activity during LAD occlusion is more striking when these maximal changes are examined (Figure 2). During LAD alone, renal nerve activity increased  $14 \pm 6\%$ . The peak response was noted at an average of  $71 \pm 7.1$

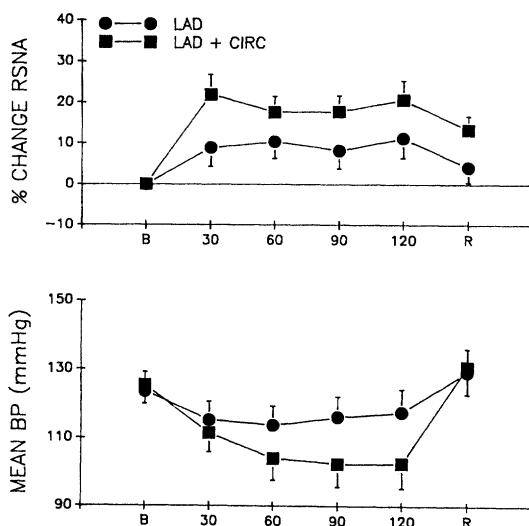


FIGURE 1. Plots showing percent change in efferent renal sympathetic nerve activity (RSNA, top panel) and change (mm Hg) in mean arterial blood pressure (mean BP, bottom panel) for left anterior descending coronary artery occlusion alone (LAD) and left anterior descending coronary artery occlusion with circumflex stenosis (LAD+CIRC). Observations were made at baseline (B) before each coronary occlusion and during each 30-second period of the 2-minute occlusion. A recovery observation (R) was made 5 minutes after release of occlusion. Changes in nerve activity and arterial pressure were significantly greater during LAD+CIRC than during LAD. Values shown represent mean  $\pm$  SEM for 20 dogs.

seconds after occlusion. During LAD+CIRC, nerve activity increased by  $32 \pm 5\%$  ( $p=0.002$ , LAD alone versus LAD+CIRC). The peak response occurred at an average of  $91 \pm 8.3$  seconds after occlusion. Figure 3 shows a representative example of the nerve traffic changes that were observed during the 2 coronary occlusions. In this experiment, the largest increases in renal nerve activity during LAD+CIRC occurred during the second minute of occlusion.

The effect of the two coronary occlusions on changes in left atrial pressure was determined in 10

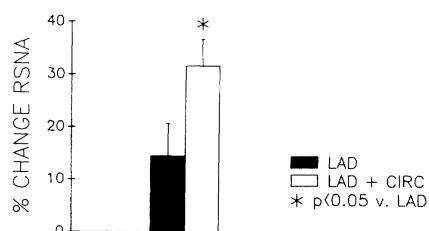


FIGURE 2. Bar graph showing maximal percent changes in renal sympathetic nerve activity (RSNA) that were observed during coronary occlusion. Maximal values observed during left anterior descending coronary artery occlusion alone (LAD) are shown on the left, and the maximal values observed during left anterior descending coronary artery occlusion with a circumflex stenosis (LAD+CIRC) are shown on the right. Values shown represent mean  $\pm$  SEM for 20 dogs.

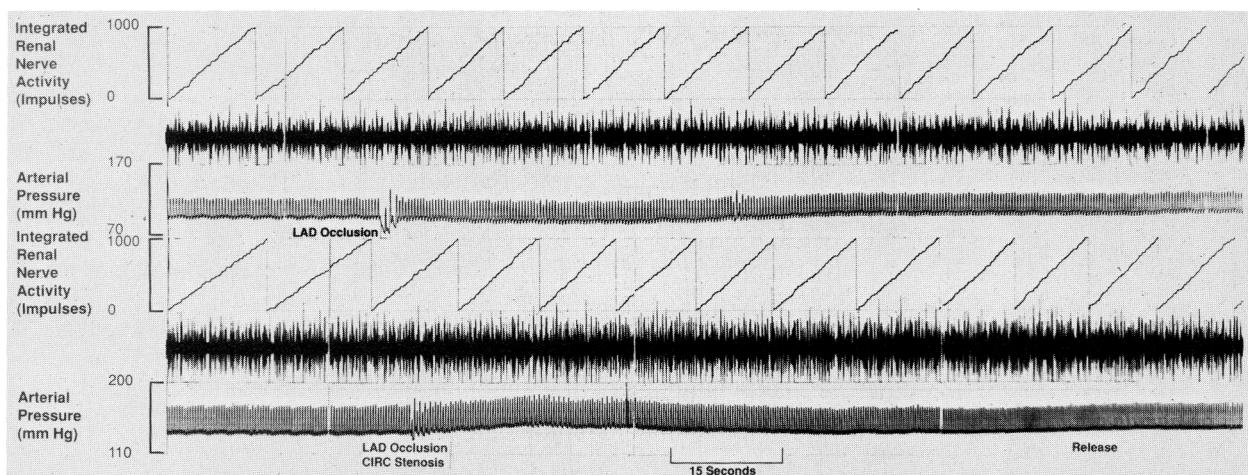


FIGURE 3. Original recording from renal nerve experiment illustrating changes in nerve activity (raw electroneurogram and integrated nerve activity) and arterial pressure during left anterior descending coronary artery (LAD) occlusion alone and during LAD occlusion with circumflex (CIRC) stenosis. In this experiment, maximal increase in renal nerve activity was 19% during LAD occlusion alone and 40% during LAD occlusion with CIRC stenosis.

experiments. A significantly greater increase in left atrial pressure was observed during LAD+CIRC than during LAD alone (LAD+CIRC,  $5.3 \pm 1.3$  mm Hg; LAD alone,  $2.6 \pm 0.4$  mm Hg;  $p=0.04$ ). However, this greater increase in left atrial pressure during LAD+CIRC was not uniformly associated with a greater reflex change in nerve activity. Figure 4 illustrates the changes in these two variables during both coronary occlusions. There was no correlation between these variables. Thus, the augmented reflex sympathoexcitation observed during LAD+CIRC was not solely related to the greater increase in cardiac filling pressure.

#### Cardiac Sympathetic Nerve Activity

Reflex changes in cardiac sympathetic nerve activity that were observed during the two coronary occlusions were qualitatively similar to those noted in

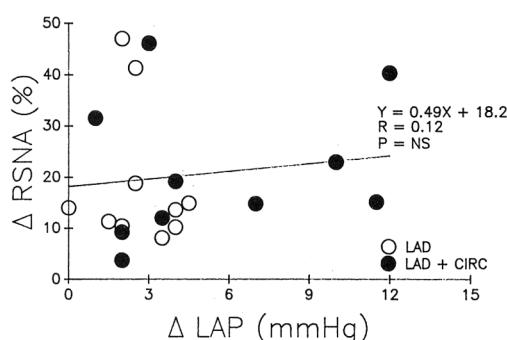


FIGURE 4. Plot showing percent changes in efferent renal sympathetic nerve activity (RSNA) plotted against changes in left atrial pressure (LAP) that were observed during left anterior descending coronary artery occlusion alone (LAD) and during left anterior descending coronary artery occlusion with circumflex stenosis (LAD+CIRC). Although greater increases in LAP occurred during LAD+CIRC, there was no significant correlation between these changes in LAP and the changes in RSNA.

renal sympathetic nerves. These results are illustrated in Figure 5 ( $n=5$  dogs). LAD alone elicited virtually no change in cardiac sympathetic nerve activity. During LAD+CIRC, significant reflex sympathoexcitation was observed. As in the renal nerve experiments, baseline nerve activity levels measured before each occlusion were not significantly different (LAD alone,  $169 \pm 60$  impulses/sec; LAD+CIRC,

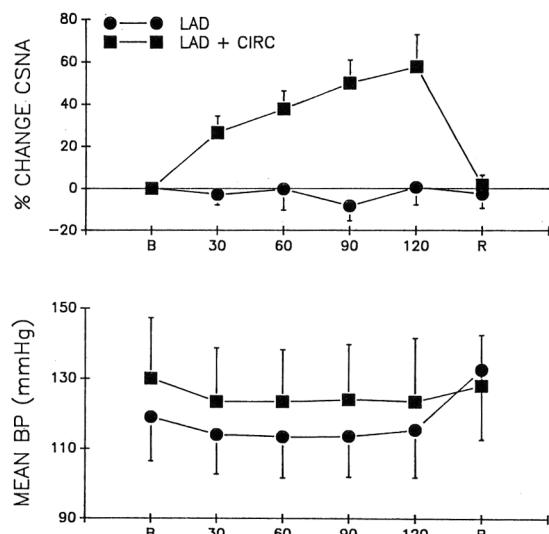


FIGURE 5. Plots showing percent changes in efferent cardiac sympathetic nerve activity (CSNA, top panel) and changes (mm Hg) in mean arterial pressure (mean BP, bottom panel) for left anterior descending coronary artery occlusion alone (LAD) and left anterior descending coronary artery occlusion with a circumflex stenosis (LAD+CIRC). Observations were made at baseline (B) before each coronary occlusion and during each 30-second period of the 2-minute occlusion. A recovery observation (R) was made 5 minutes after release of the occlusion. Changes in nerve activity and arterial pressure were significantly greater during LAD+CIRC than during LAD. Values shown represent mean  $\pm$  SEM for five dogs.

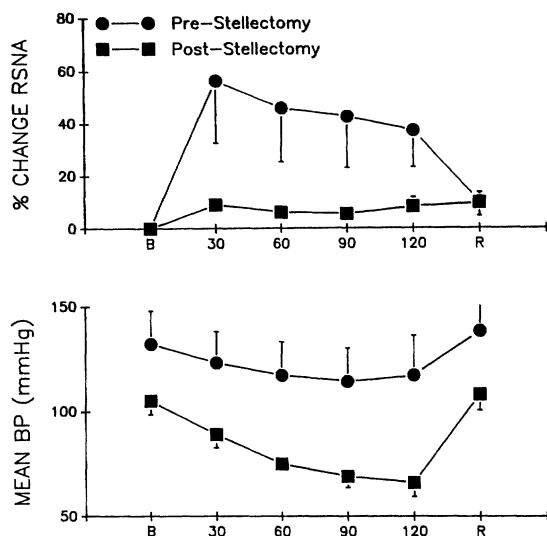


FIGURE 6. Plots showing percent changes in efferent renal sympathetic nerve activity (RSNA, top panel) and changes (mm Hg) in mean arterial pressure (mean BP, bottom panel) for left anterior descending coronary artery occlusion with a circumflex stenosis before (filled circles) and after (filled squares) interruption of cardiac sympathetic afferent pathways. Observations were made at baseline (B) before each coronary occlusion and during each 30-second period of the 2-minute occlusion. A recovery observation (R) was made 5 minutes after release of the occlusion. Values shown represent mean  $\pm$  SEM for five dogs.

121  $\pm$  35 impulses/sec;  $p=0.44$ ). Unlike the renal nerve experiments, there were no significant differences in the responses of arterial pressure during the two coronary occlusions. We speculate that the intense reflex sympathoexcitation that occurred in these experiments may have increased systemic vascular resistance and prevented a greater fall in arterial pressure during LAD+CIRC.

#### Total Cardiac Sympathetic Afferent Denervation

In five experiments, responses of renal sympathetic nerve activity and mean arterial pressure during LAD+CIRC were assessed before and after total

cardiac sympathetic denervation. These results are illustrated in Figure 6. With sympathetic afferent pathways intact, LAD+CIRC elicited reflex sympathoexcitation. After interruption of all sympathetic afferent pathways, the reflex changes in renal nerve activity during LAD+CIRC were abolished.

#### Coronary Blood Flow Analysis

Table 1 lists baseline blood flow values in the ischemic and nonischemic regions for eight experiments. The measurements made before LAD+CIRC demonstrate that the stenosis could be adjusted so that basal myocardial blood flow in the nonischemic region was not reduced.

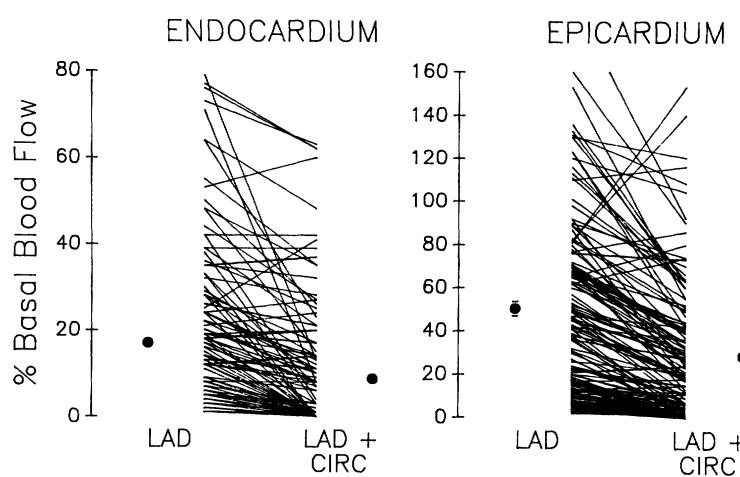
Blood flow values (individual and mean  $\pm$  SEM) that were observed in the ischemic region are shown in Figure 7. In this figure, there are 52 pairs of data points that share common lines for endocardial flow and four pairs of data points that share common lines for epicardial flow. As described above, each observation is expressed as a percentage of its corresponding baseline flow value. By definition, flow in all endocardial segments of this region was reduced by at least 20% during LAD alone (i.e., flow was no greater than 80% of basal flow levels). Analysis of these data indicates that severe flow reductions occurred in the endocardial layers during both coronary occlusions. During LAD alone, endocardial flow was reduced to 17.0  $\pm$  1.6% of basal values. During LAD+CIRC, endocardial flow was reduced to 8.8  $\pm$  1.5% of basal values ( $p < 0.0001$ , LAD alone versus LAD+CIRC). The majority of endocardial segments in the ischemic region (99 of 141) had severe blood flow reductions during LAD alone ( $\leq 20\%$  of basal flow). During LAD+CIRC, an additional 19 segments had flow reduced to less than 20% of basal levels. This resulted in a small but significant increase in the mean mass of endocardial tissue in which severe flow reductions occurred during LAD+CIRC (Figure 8).

In the epicardial layers of the ischemic region, blood flow changes during LAD alone were less pronounced and more variable than in the endocardial layers. The mean flow value for the ischemic

TABLE 1. Baseline Myocardial Blood Flow Values in Dogs

Experiment	MBF in ischemic region (ml/min/g)				MBF in nonischemic region (ml/min/g)			
	Endo		Epi		Endo		Epi	
	LAD	L+C	LAD	L+C	LAD	L+C	LAD	L+C
1	0.76 $\pm$ 0.11	0.79 $\pm$ 0.12	0.70 $\pm$ 0.12	0.75 $\pm$ 0.13	0.75 $\pm$ 0.11	0.82 $\pm$ 0.08	0.61 $\pm$ 0.13	0.70 $\pm$ 0.16
2	1.76 $\pm$ 0.28	1.37 $\pm$ 0.32	1.56 $\pm$ 0.16	1.46 $\pm$ 0.18	1.60 $\pm$ 0.27	1.47 $\pm$ 0.27	1.54 $\pm$ 0.17	1.64 $\pm$ 0.27
3	1.02 $\pm$ 0.12	1.04 $\pm$ 0.27	1.16 $\pm$ 0.26	1.27 $\pm$ 0.21	0.98 $\pm$ 0.16	1.66 $\pm$ 0.46	0.84 $\pm$ 0.17	1.09 $\pm$ 0.22
4	1.05 $\pm$ 0.32	0.89 $\pm$ 0.27	0.78 $\pm$ 0.13	0.68 $\pm$ 0.13	0.96 $\pm$ 0.12	0.88 $\pm$ 0.13	0.71 $\pm$ 0.12	0.62 $\pm$ 0.08
5	0.84 $\pm$ 0.27	1.17 $\pm$ 0.15	1.04 $\pm$ 0.15	1.41 $\pm$ 0.22	0.91 $\pm$ 0.19	1.14 $\pm$ 0.15	0.96 $\pm$ 0.19	1.13 $\pm$ 0.23
6	0.66 $\pm$ 0.06	0.89 $\pm$ 0.12	0.51 $\pm$ 0.12	0.77 $\pm$ 0.19	0.62 $\pm$ 0.10	0.97 $\pm$ 0.22	0.57 $\pm$ 0.09	0.89 $\pm$ 0.20
7	1.25 $\pm$ 0.25	1.28 $\pm$ 0.53	1.31 $\pm$ 0.25	1.28 $\pm$ 0.26	1.03 $\pm$ 0.23	0.85 $\pm$ 0.21	1.06 $\pm$ 0.18	0.98 $\pm$ 0.19
8	1.04 $\pm$ 0.19	1.51 $\pm$ 0.39	0.76 $\pm$ 0.19	1.30 $\pm$ 0.34	1.08 $\pm$ 0.18	1.05 $\pm$ 0.27	0.88 $\pm$ 0.18	1.24 $\pm$ 0.27

Values are mean  $\pm$  SEM. MBF, myocardial blood flow; Endo, endocardial; Epi, epicardial; LAD, left anterior descending coronary artery occlusion alone; L+C, left anterior descending coronary artery occlusion with a circumflex stenosis.



**FIGURE 7.** Mean $\pm$ SEM (filled circles) and individual myocardial blood flow values for the endocardial (left panel) and epicardial (right panel) layers of the ischemic region. Differences in the blood flow scales for endocardial and epicardial flow should be noted. All blood flow values are expressed as a percent of the basal flow that was measured immediately before either left anterior descending coronary artery occlusion alone (LAD) or left anterior descending coronary artery occlusion with circumflex stenosis (LAD+CIRC). For endocardial flow data, there are 52 sets of values that share common lines. For epicardial flow data, there are four sets of values that share common lines. For some mean values, the standard error was very small, and as a result, error bars are included in the symbol and do not appear.

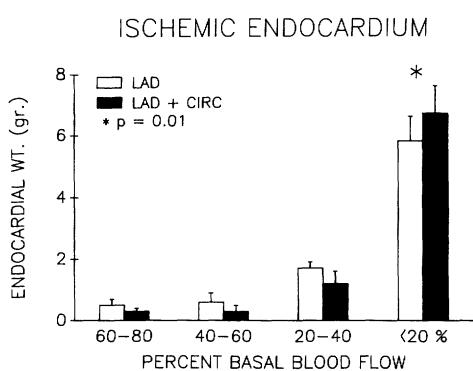
epicardium was reduced to only  $50.4\pm3.4\%$  of basal flow values during LAD alone (Figure 7). Analysis of the individual values observed during LAD alone revealed that flow in 53 of the 141 epicardial segments remained at levels greater than 60% of basal flow. In contrast, only nine of 141 ischemic endocardial segments had levels of flow greater than 60% of basal values during LAD alone.

The influence of a circumflex stenosis on blood flow changes during LAD occlusion was greater in the epicardial layers of the ischemic region than in the endocardial layers. Like the endocardial layers, epicardial blood flow reductions were significantly greater during LAD+CIRC ( $28.6\pm2.6\%$ ) than during LAD alone ( $p<0.0001$ ). Most important, 32 of the 53 epicardial segments with flow greater than 60% of basal values during LAD alone had flow reductions to less than 60% of basal values during LAD+CIRC. As a result, there was a significant

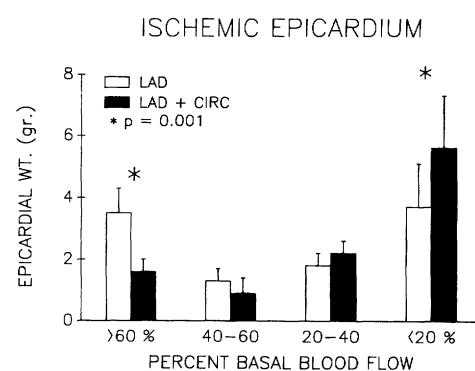
decrease in the mean weight of myocardial tissue in which flow was "preserved" during LAD+CIRC (Figure 9).

Unlike the endocardial layers, severe reductions in flow ( $\leq 20\%$  of basal values) were observed in only 46 of the 141 epicardial segments during LAD alone. During LAD+CIRC, the number of epicardial segments with severely reduced flow increased to 74. Thus, the amount of epicardial tissue with flow reduced to less than 20% of basal values was significantly greater during LAD+CIRC than during LAD alone (Figure 9).

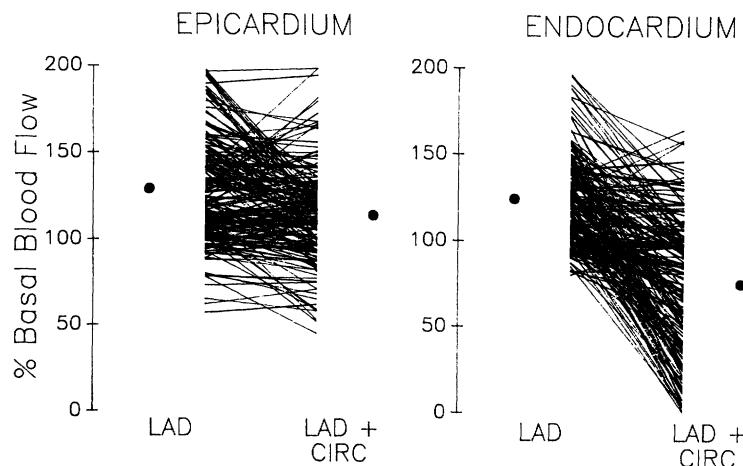
Individual and mean blood flow values that were observed in the nonischemic regions are shown in Figure 10. During LAD alone, blood flow increased in both the epicardial ( $129\pm2.4\%$ ) and endocardial ( $124\pm3.1\%$ ) layers of the nonischemic region. During LAD+CIRC mean flow in the epicardial layers was reduced toward baseline levels ( $114\pm2.1\%$ ;



**FIGURE 8.** Bar graph showing effect of coronary occlusions on blood flow in endocardial layers of ischemic region. Percent blood flow reduction compared with basal flow values is shown on horizontal axis. Weight of endocardial tissue in which each flow level was observed is shown on vertical axis. Data are shown for left anterior descending coronary artery occlusion alone (LAD) and for left anterior descending coronary artery occlusion with circumflex stenosis (LAD+CIRC). Values represent mean $\pm$ SEM.



**FIGURE 9.** Bar graph showing effects of coronary occlusions on blood flows in epicardial layers of ischemic region. Percent blood flow reductions compared with basal flow values are shown on horizontal axis. Weights of epicardial tissue in which each flow level was observed are shown on vertical axis. Data are shown for left anterior descending coronary artery occlusion alone (LAD) and left anterior descending coronary artery occlusion with circumflex stenosis (LAD+CIRC). Values represent mean $\pm$ SEM.



**FIGURE 10.** Mean $\pm$ SEM (filled circles) and individual myocardial blood flow values for epicardial (left panel) and endocardial (right panel) layers of nonischemic region. All blood flow values are expressed as percent of basal flow that was measured immediately before either left anterior descending coronary artery occlusion alone (LAD) or left anterior descending coronary artery occlusion with circumflex stenosis (LAD+CIRC). No error bars appear because standard errors are so small that error bars are included within the symbol for mean values.

$p<0.0001$ , LAD+CIRC versus LAD alone). Only seven of 179 epicardial segments in the nonischemic region had significantly reduced flow (<60% of basal levels) during LAD+CIRC. Thus, the stenosis had minimal impact on epicardial segments that were remote from those supplied by the occluded LAD.

Conversely, blood flow in the endocardial layers of the nonischemic region was reduced below baseline levels during LAD+CIRC ( $75.0\pm2.0\%$ ;  $p<0.0001$ , LAD+CIRC versus LAD alone). This experimental maneuver lowered blood flow below 80% of basal values in 88 of 179 "nonischemic" endocardial segments. Twenty-four percent of these 88 segments had mild flow reductions (60–80% of basal values), whereas 20% of these segments had severe flow reductions ( $\leq20\%$  of basal values). Thus, the circumflex stenosis did have a significant impact on blood flow to endocardial segments that were remote from the segments supplied by the occluded LAD.

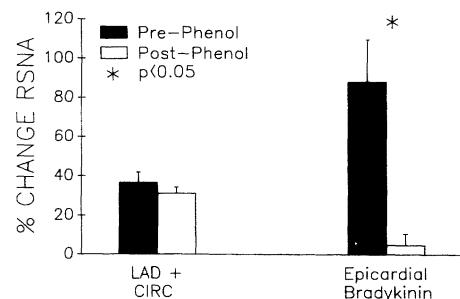
#### Regional Posterior Left Ventricular Denervation

Since posterior endocardial blood flow was reduced during LAD+CIRC, additional experiments were performed to determine whether this posterior endocardial ischemia activated sympathetic afferents and contributed to the reflex increases in renal nerve activity. The results of these experiments are illustrated in Figure 11. Before treatment with phenol, application of bradykinin to the epicardial surface of the posterior left ventricle elicited large increases in renal nerve activity ( $89\pm22\%$ ). After application of phenol to the atrioventricular groove, the reflex increases in renal nerve activity during application of bradykinin were abolished ( $5\pm6\%$ ), indicating successful sympathetic deafferentation of the posterior or nonischemic region. Despite this denervation of the posterior left ventricle, phenol application had no significant effect on the reflex increases in renal nerve activity that occurred during LAD+CIRC ( $36\pm5\%$  before phenol versus  $31\pm3\%$  after phenol).

#### Discussion

Canine and feline experiments that use direct recordings of cardiac sympathetic afferent activity

have demonstrated clearly that these fibers are stimulated during coronary occlusion.<sup>1,2</sup> This activation would be expected to produce reflex excitatory responses, since direct electrical stimulation of the sensory fibers or chemical stimulation of these sensory endings usually elicits such responses.<sup>8,17–20</sup> However, studies that have evaluated the reflex responses associated with ischemia-induced activation of sympathetic afferents have not yielded consistent results. In cats, reflex sympathoexcitation during coronary occlusion has been observed in both renal and cardiac efferent sympathetic nerves.<sup>3,4</sup> Although these excitatory responses have been noted when the vagi are intact,<sup>3</sup> they are most apparent after interruption of the arterial baroreflexes and vagal cardiac



**FIGURE 11.** Bar graph showing maximal percent changes in renal sympathetic nerve activity (RSNA) that were observed during either left anterior descending coronary artery occlusion with circumflex stenosis (LAD+CIRC) or application of bradykinin to posterior surface of left ventricle. Two-minute coronary occlusions and epicardial bradykinin application were done before and after administration of 88% phenol solution along the left atrioventricular groove. Values shown are mean $\pm$ SEM for five dogs. After phenol administration, reflex nerve traffic responses to epicardial bradykinin were significantly diminished, indicating successful sympathetic deafferentation of posterior left ventricle. Reflex nerve traffic responses to LAD+CIRC were not significantly different after administration of phenol, indicating that sympathetic afferent input from the posterior left ventricle did not contribute to the reflex nerve traffic changes elicited by LAD+CIRC.

reflexes.<sup>4</sup> In humans, an apparent sympathoexcitatory state characterized by hypertension and tachycardia is encountered frequently in the early stages of acute anterior myocardial infarction.<sup>5</sup> In dogs, however, excitatory reflexes mediated by cardiac sympathetic afferents during simple occlusion of a coronary artery were detected only after section of the spinal cord.<sup>6</sup> Myocardial ischemia elicited no reflex sympathoexcitation when the spinal cord was intact. The experimental model used in those studies was similar to ours in that the potential confounding influences of the arterial baroreflexes and vagal cardiac reflexes were controlled or eliminated to detect the independent effects of sympathetic afferent activation. On the basis of those findings, it was suggested that cardiac sympathetic afferent input to the canine spinal cord and brain is modulated significantly by descending inhibitory supraspinal influences.

Our experimental results indicate that the importance of sympathetic afferent activation during myocardial ischemia has been underestimated in dogs. Several lines of evidence suggest that sympathetic and vagal afferent fibers may not be uniformly distributed to all layers of the left ventricular myocardium. First, epicardial application of bradykinin elicits reflex cardioaccelerator, vasopressor, and sympathoexcitatory responses characteristic of those mediated by cardiac sympathetic afferents.<sup>8</sup> Delivery of bradykinin to the deeper endocardial layers by intracoronary administration produces reflex depressor responses characteristic of those mediated by cardiac vagal afferents.<sup>11</sup> Second, selective interruption of superficial nerves in the left ventricle by epicardial application of phenol abolishes reflexes mediated by sympathetic afferents in response to topical bradykinin.<sup>9</sup> Reflexes mediated by vagal afferents in response to topical nicotine are preserved unless phenol is applied to the atrioventricular groove. These data indicate a specific and selective anatomic distribution of ventricular afferent fibers with sympathetic afferents being located mainly in superficial epicardial layers and vagal afferents located in the deeper endocardial layers.

This epicardial distribution of sympathetic afferent fibers may be important when evaluating reflex responses to coronary occlusion and myocardial ischemia. Previous animal studies that evaluated the role of ventricular sympathetic afferents in these reflex responses used the method of simple coronary occlusion to elicit ischemia. In the cat, simple coronary occlusion likely produces transmural ischemia, since the feline coronary arterial bed has limited collateral circulation.<sup>21</sup> However, the canine heart has the potential for extensive coronary collateral flow. This collateral flow may restrict the degree of epicardial ischemia that develops in response to simple coronary occlusion. As a result, there may be minimal activation of epicardial afferent fibers. Therefore, failure to detect reflex excitatory responses in previous canine studies may have been related to limited ischemic stimulation of afferent fibers that are located mainly in superficial epicardial layers.

Our results provide direct evidence to support this hypothesis. We have demonstrated that reflex excitatory responses mediated by cardiac receptors with sympathetic afferents can be elicited by myocardial ischemia in the dog with an intact spinal cord. However, these excitatory responses were only apparent when a stenosis was used to limit the ability of the circumflex artery to provide collateral flow to the anterior left ventricle. Myocardial blood flow analysis indicated that flow increased dramatically in the circumflex artery during simple occlusion of the LAD. This increased flow may have provided collateral support that significantly diminished the amount of anterior epicardial ischemia elicited by simple occlusion of the LAD. By limiting this collateral flow, the circumflex stenosis facilitated the production of greater transmural ischemia in the region supplied by the occluded LAD. This greater epicardial ischemia provided a more intense stimulus to sympathetic afferent fibers located in these layers. As a result, a significant reflex response was observed. Based on our results, we would predict that even greater degrees of epicardial ischemia than we were able to achieve in our experiments would give rise to even larger reflex increases in sympathetic nerve activity than we observed. Finally, the reflex excitatory responses observed during transmural anterior ischemia were abolished after bilateral removal of the stellate ganglia and interruption of the first through fourth dorsal white rami. This finding definitively establishes cardiac sympathetic receptors as the origin of these reflexes.

How do our present findings in dogs with the spinal cord intact relate to those previously reported<sup>6</sup> in dogs with the cervical spinal cord transected? In the previous studies, sympathoexcitatory responses were observed during simple coronary occlusion only after cord transection. It was suggested that removal of descending inhibitory influences by spinal cord transection "unmasked" reflex excitatory responses mediated by sympathetic afferents. An alternate interpretation is that the extent to which sympathetic afferents are activated by simple coronary occlusion is inadequate to overcome descending inhibitory influences on sympathetic neurons. Creation of transmural ischemia, as was done in the present study, elicited sufficient activation of cardiac sympathetic afferents to overcome descending inhibition. As a result, there was reflex sympathoexcitation even though the spinal cord was intact.

In interpreting our findings, several possible limitations should be considered. We defined an ischemic risk region as the area in which endocardial flow was reduced by 20% during LAD alone. This definition was by no means an arbitrary one. The rationale for this definition was based on previous findings<sup>22</sup> in which myocardial blood flow and regional myocardial function were measured during graded coronary occlusions. In these experiments, endocardial wall thickening abnormalities could be detected when endocardial blood flow was reduced to 80% of basal

values. The use of this functional parameter facilitated the analysis of our blood flow data. We acknowledge that these parameters may not accurately reflect the extent to which ventricular receptors are activated during myocardial ischemia. The precise degree of blood flow reduction that will elicit chemical activation of ventricular receptors is unknown. Nevertheless, there is evidence to suggest that the metabolic derangements that are associated with myocardial ischemia are linked closely to mechanical alterations.<sup>23</sup> The majority of endocardial segments (133 of 141) in the ischemic region had flow reductions below 60% of basal values during LAD alone. Thus, our conclusions concerning the effect of a circumflex stenosis on the amount of anterior transmural ischemia during LAD occlusion would be unchanged regardless of the method used to define the ischemic risk region.

In several of our experiments, the circumflex stenosis caused significant reductions in blood flow to the endocardial layers of the nonischemic region when the LAD was occluded. It could be suggested that the greater nerve traffic changes that we observed during LAD+CIRC were related to more extensive endocardial rather than epicardial ischemia. We consider this to be unlikely. No reflex sympathoexcitation was apparent after simple occlusion of the LAD or circumflex artery in prior studies<sup>6,7</sup> in dogs with vagotomy and sinoaortic denervation. This observation indicates that there is no augmented activation of sympathetic afferents during marked anterior or inferoposterior endocardial ischemia. Since the circumflex artery usually supplies blood flow to a greater portion of the canine left ventricle than does the LAD, this finding also illustrates that the reflex sympathoexcitation is not dependent on the amount of endocardial tissue that is ischemic. Furthermore, the results of our experiments in which we selectively denervated the posterior left ventricle indicate that afferent input from the posterior endocardial layers does not contribute to the reflex sympathoexcitation that was observed during LAD+CIRC. Based on these data, we conclude that the greater reflex responses that we observed during LAD+CIRC are related to the effects of the stenosis on anterior epicardial blood flow rather than on posterior endocardial flow.

We observed reflex increases in the activity of both renal and cardiac efferent sympathetic nerves during transmural myocardial ischemia. This indicates that activation of cardiac sympathetic afferents elicits similar reflex changes in sympathetic outflow to the kidney and the heart. These results are consistent with the results of other studies that have shown similar responses in renal and cardiac sympathetic nerves during activation of vagal cardiopulmonary receptors<sup>7,24</sup> and during unloading of the sinoaortic baroreceptors.<sup>25</sup> In our study, reflex changes in cardiac sympathetic nerve activity appeared to be larger than were the changes in renal sympathetic nerve activity. This difference may reflect the additional

contribution of the spinal cardiocardiac reflex to reflex effects mediated through higher central nervous system centers.<sup>19</sup>

Our findings also may provide insight into the mechanism of sympathetic afferent activation during myocardial ischemia. Cardiac receptors with sympathetic afferents are polymodal and often have both mechanosensitive and chemosensitive properties, in that they are stimulated both by mechanical events in the contracting ventricle and by exposure to a variety of endogenous and exogenous irritant substances.<sup>26,27</sup> During myocardial ischemia, there are both alterations in the mechanical function of the ventricle and release of irritant substances such as potassium, hydrogen ions, and bradykinin. Potentially, reflex responses to myocardial ischemia could be mediated by mechanosensitive stimuli, chemosensitive stimuli, or both. In our study, increases in left atrial pressure were significantly greater during LAD+CIRC than during LAD alone. This larger increase in left atrial pressure would provide a more powerful mechanical stimulus to sympathetic afferents and would be expected to elicit a greater reflex response. However, there was no significant correlation between the changes in left atrial pressure and the reflex changes in nerve activity that occurred during coronary occlusion (Figure 4). This lack of correlation suggests that the reflex neural changes were related to factors other than (or in addition to) changes in cardiac filling pressures. On the basis of this observation, we speculate that chemical activation of sympathetic afferent receptors was largely responsible for the reflex excitatory responses that occurred during transmural myocardial ischemia.

We conclude that myocardial ischemia can elicit reflex sympathoexcitatory responses mediated by cardiac sympathetic afferents in dogs with an intact spinal cord. These excitatory responses are apparent mainly during ischemia that is transmural and involves the epicardial layers. These data are consistent with the results of other studies that indicate that ventricular sympathetic afferent fibers are located mainly in superficial epicardial layers. In addition, we speculate that our results can be taken to suggest a potential mechanism for silent myocardial ischemia in humans. Cardiac nociceptors responsible for the perception of angina pectoris are subserved by sympathetic afferent fibers. If these nociceptors are also preferentially distributed to or near the epicardial layers as were the fibers responsible for the reflex excitatory responses that we observed, then nociceptor activation and perception of cardiac pain may be more likely to occur when ischemia is transmural and involves the epicardium. Ischemia that is primarily subendocardial may not activate epicardial receptors with sympathetic afferents and may not be associated with cardiac pain. Although it is likely that there are multiple mechanisms by which ischemia can occur in the absence of anginal pain, we speculate that the preferential distribution of sympathetic afferent fi-

bers to the superficial epicardial layers of the left ventricle may contribute to its occurrence.

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**KEY WORDS** • autonomic nervous system • sympathetic activity • silent ischemia • myocardial blood flow

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