Effect of Brief Myocardial Ischemia on Sympathetic Coronary Vasoconstriction

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The purpose of the present study was to determine whether sympathetic coronary vasoconstrictor responses are altered after brief ischemia and reperfusion. Adult mongrel dogs were anesthetized and instrumented for measurements of heart rate, arterial pressure, left ventricular pressure, left ventricular dP/dt, anterior myocardial wall thickening, and left circumflex coronary artery (LCX) and left anterior descending coronary artery (LAD) blood flow velocities. Changes in coronary vascular resistance were recorded during intravenous bolus doses of norepinephrine and bilateral electrical stimulation of the stellate ganglia. After β-adrenergic blockade and bilateral vagotomy, electrical stimulation of the stellate ganglia increased coronary vascular resistance in the LAD and LCX beds by 38±5% and 39±5%, respectively. After a 15-minute LAD occlusion, repeat electrical stimulation produced increases in coronary resistance of 16±3% and 45±8%, respectively (p<0.05 for the LAD before versus after the occlusion). The peak increase in coronary vascular resistance to two doses of norepinephrine was unchanged. After a shorter period of myocardial ischemia (7 minutes), similar increases in coronary resistance to stellate stimulation were observed before (27±4%) and after (26±6%) myocardial ischemia. The mechanism of this impaired sympathetic coronary vasoconstriction was further tested by examining the responses to bretylium and tyramine. Brief ischemia did not alter the coronary constrictor responses to either bretylium or tyramine, suggesting that mechanisms governing prejunctional release of norepinephrine are intact in the postischemic coronary arterial bed. The postischemic myocardium was characterized by mild reductions in left ventricular dP/dt and marked reductions in transmural myocardial wall thickening, characteristic of myocardial stunning. We conclude that after brief myocardial ischemia, coronary vasoconstriction to sympathetic activation is impaired, whereas constriction to direct receptor activation (norepinephrine) and stimulated prejunctional release of a neurotransmitter (bretylium or tyramine) remain intact. These data are consistent with the interpretation that sympathetic efferent neural conduction is impaired in regions of stunned myocardium. (Circulation Research 1992;71:960–969)

KEY WORDS • myocardial ischemia • sympathetic coronary vasoconstriction • left anterior descending coronary artery • left circumflex coronary artery • myocardial stunning

Periods of coronary artery occlusion lasting less than 20 minutes are not associated with myocardial necrosis but are accompanied by marked reductions in myocardial function during reperfusion. This reversible and temporary decrease in ventricular function after brief coronary occlusion has been termed “myocardial stunning” and has been a source of recent intense investigation. Although the hemodynamic and functional myocardial changes associated with myocardial stunning have been well characterized, little is known regarding the effects of brief periods of ischemia followed by reperfusion on other cardiac structures.

Brief coronary occlusion followed by reperfusion alters coronary vascular reactivity. In epicardial arteries exposed to prolonged ischemia, coronary vasodilation to endothelium-dependent agents but not endothelium-independent vasodilators is abolished. Studies of the coronary microcirculation reveal reduced vasodilation to infused adenosine after brief myocardial ischemia; however, peak reactive hyperemic responses are unchanged. These changes in coronary vascular reactivity may be very important, since a close curvilinear relation exists between postischemic myocardial perfusion and wall thickening, indicating that in the postischemic state even small changes in coronary flow can profoundly affect mechanical function.

The effect of brief ischemia on sympathetic coronary vasoconstriction is difficult to predict. Sympathetic neurotransmission within ischemic or infarcted myocardium is reduced. Ciuffo et al demonstrated reduced cardiac inotropic responsiveness to neural sympathetic activation after a 25-minute coronary occlusion. Thus, impaired neural conduction may attenuate coronary vasoconstriction to sympathetic stimulation. Conversely, reduced mechanical function might decrease the local concentration of vasodilator metabolites produced dur-
ing contraction. This coupled with impaired endothelium-dependent coronary vasodilation would predict an augmented constriction to adrenergic stimuli. One study suggests that regulation of coronary flow may be dissociated from myocardial metabolism in the stunned state.14 Hours after reperfusion, coronary flow returns to normal but with depressed aerobic myocardial metabolism. This dissociation of flow from metabolism would be expected to enhance the constriction to adrenergic activation. Therefore, we examined whether coronary responses to sympathetic activation are altered after brief myocardial ischemia. Potential mechanisms of the altered neural responsiveness of coronary vessels were also examined.

**Materials and Methods**

**General Preparation**

In adult mongrel dogs (18–28 kg) of either sex, anesthesia was induced with intravenous thiopental sodium (25 mg/kg) and maintained with α-chloralose (45 mg/kg i.v.). Supplemental injections of α-chloralose (20 mg/kg) were given as needed to suppress corneal reflexes and reflex pressor responses to skin pinch. Arterial blood was sampled for determinations of Po2, Pco2, and pH. These parameters were maintained within physiological ranges (Po2: 80–100 mm Hg; Pco2: 35–45 mm Hg; and pH 7.35–7.45) by adjustments in the ventilatory rate and supplemental oxygen delivery and by injections of sodium bicarbonate (2 meq/ml i.v.). Rectal temperature was maintained at 36–37°C with a heating pad.

Arterial blood pressure was measured with a transducer (model CP-01) connected to a polyethylene catheter placed into the right femoral artery. The phasic arterial pressure signal was directed to two couplers for measurements of systemic and mean arterial pressure and to a cardiotachometer for recording heart rate. Another polyethylene cannula was inserted into the right femoral vein for administration of fluids and pharmacological agents: norepinephrine (5 or 10 μg), bretylium tosylate (4 mg), tyramine hydrochloride (5 μg), and α-chloralose.

Through a ventral neck incision, both cervical vagi were isolated and transected. The heart and left stellate ganglion were exposed by removing the second through fifth left ribs. In 21 dogs, the stellate ganglia were isolated, secured within a pair of bipolar microstimulating electrodes, and covered with saline-soaked gauze. Stimulation parameters were 5–10 V, 1.0-msec pulses at 15 Hz (10-second duration).

A pericardial sac was formed. In nine dogs, a micromanometer-tipped catheter (Millar Instruments, Inc., Houston, Tex.) was placed into the left ventricle via the left atrial appendage or through the left ventricular apex for continuous monitoring of left ventricular pressure and left ventricular dP/dt.

All agents were dissolved in normal saline and prepared on the day of the study. Changes in mean arterial pressure, heart rate, and left anterior descending coronary artery (LAD) and left circumflex coronary artery (LCX) blood flow velocities were recorded for each drug administration or nerve stimulation. Data reported were taken between 3 and 5 seconds after the onset of stimulation, when the peak decrease in coronary flow velocity (and increase in coronary vascular resistance) occurred. Responses to at least two repeated injections of each drug or stimulations were averaged and used for data analysis. In three preliminary experiments, the stellate ganglia were surgically decentralized proximal to the stimulus electrodes. The duration and magnitude of coronary constriction to stimulation of the stellate ganglia were similar to those observed in intact preparations. All reported data are from experiments in animals with intact cardiac sympathetic pathways.

**Sonomicrometry**

Regional wall thickening and left ventricular dP/dt were measured in nine dogs in the perfusion territory of the LAD by using a pair of 7-MHz ultrasonic transit-time dimension gauges.15 After a 15-second occlusion of the LAD, sonomicrometer crystals were implanted into the center of the distal LAD bed where cyanosis was most prominent. One crystal was sutured onto the epicardial surface, and the other was inserted at a 45° angle to a depth of 4–7 mm in the subendocardium directly below the overlying surface crystal. Output from the sonomicrometer signal processing unit was directed to an oscilloscope, and the voltage was recorded continuously on a chart recorder for visual and graphic display of anterior wall thickening. Accurate placement of sonomicrometer crystals was confirmed if Evans blue dye (4% solution, 3 ml injected in the proximally ligated LAD at the end of the study) completely stained the excised ventricular segment containing the crystals. This was the case in each animal tested. Regional wall thickening was measured as a percent change in myocardial wall thickness from end diastole (measured at the time of onset of the increase in dP/dt) to end systole (measured 50 msec before the time of peak negative dP/dt).3

**Coronary Flow Velocity**

Coronary blood flow velocity was measured in both LAD and LCX arteries by use of suction-applied epicardial Doppler flow probes designed at the University of Iowa.16,17 Briefly, a 20-MHz piezoelectric crystal is mounted at a 45° angle in a silastic-cupped housing and held to the epicardial surface using suction (4 mm Hg). This probe minimizes the risk of coronary denervation from surgical isolation of the vessels required for circumferential probes. Reproducible zero-flow velocities and reactive hyperemia responses greater than 3:1 are regularly achieved with this probe.16 Changes in flow velocity correlate well with absolute changes in flow when measured with timed venous collections, radiolabeled microspheres, and electromagnetic flow probes.3,16,18 The probe on the LAD was attached distal to the site of occlusion.

All analyses (except baseline measurements) were made by using changes in coronary flow velocity and changes in coronary vascular resistance rather than absolute values.

Phasic arterial pressure, mean arterial pressure, heart rate, left ventricular pressure, left ventricular dP/dt, left ventricular wall thickness, and LAD and LCX blood flow velocities were continuously recorded on an eight-channel Dynograph recorder. Data were tabulated from the recorded output and were analyzed by computer.
Experimental Procedure

After instrumentation, propranolol was administered in a dose (2 mg/kg i.v.) sufficient to block the tachycardia (36±7 beats per minute before propranolol, 1±0.5 beats per minute after propranolol) to isoproterenol (3.5 μg i.v.). Supplemental bolus injections were given periodically throughout the experiment. When hemodynamics were stable, intravenous injections of norepinephrine (5–10 μg) and vehicle (saline) were made in random order. Smaller doses of norepinephrine produced variable and often undetectable degrees of coronary vasoconstriction. In some experiments, either bretylium tosylate (n=6) or tyramine hydrochloride (n=5) was injected intravenously as well. In four experiments in which tyramine was given, repeated doses were administered. In four of five studies, the hemodynamic response to tyramine was taken as the average of two separate injections of the same dose. In each case, both injections produced a similar hemodynamic response. Multiple bilateral stimulations of the stellate ganglia with a 15-minute interstimulus interval were performed.

Next, lidocaine (2%, 2 ml i.v.) and heparin (1,000 units/ml, 4 ml i.v.) were administered 1 minute before a 7–15-minute LAD occlusion (distal to the first or second diagonal branch). The LAD was occluded by one of two methods, direct epicardial pressure or intraluminal obstruction. Compression was performed by gentle epicardial pressure using a cotton-tipped swab or with a 4-0 silk suture snare placed widely around the coronary vessel to include underlying myocardium. The snare was loosely tied over the epicardial surface, leaving a small gap between the snare and the epicardial vessel. An angioplasty balloon catheter (2F) was inserted between the surface of the epicardial vessel and the overlying suture. When inflated, the suture forced the balloon against the epicardial vessel, thereby occluding it. The pressure applied by both methods (manual or balloon inflation) was the minimum amount to completely abolish coronary flow as measured by a distally placed Doppler flow probe.

Intravascular obstruction was produced in six additional dogs by isolating the first diagonal branch of the LAD and retrogradely inserting a 3.0–3.5F angioplasty catheter into the lumen of the main LAD vessel. The balloon was inflated with the minimum pressure necessary to reduce coronary flow velocity (distally attached main LAD probe) to zero. The inflation was sustained for 15 minutes, after which the balloon was removed. Coronary responses to stellate stimulation were studied before balloon inflation and after balloon removal.

The LAD was chosen because of more reliable and reproducible posts ischemic responses compared with the LCX. Just before release of the LAD occlusion, lidocaine (2%, 1 ml i.v.) was again given.

Thirty minutes into the reperfusion period, when hemodynamics had reached baseline, intravenous injections of norepinephrine, bretylium, or tyramine and bilateral stellate stimulations were performed. In eight dogs, stellate stimulation was repeated at 1 and 2 hours after the onset of reperfusion.

Next, the LAD was occluded proximal to the intracoronary catheter, and Evans blue dye (3 ml) was injected distally to mark the risk area. The animal was killed (saturated KCl, 10 ml i.v.), and the heart was quickly removed and stored in 10% formalin for later sectioning to ensure proper crystal placement. In seven dogs, the myocardial area at risk was determined by a modification of a method used previously.20 Briefly, hearts were removed from formalin and sliced in six or seven transverse sections. All but the most basal section were traced on an acetate sheet, marking both stained and unstained regions of the left ventricle. The sum of the areas of the stained regions was divided by the sum of the areas of each left ventricular slice, and the risk area was expressed as a percentage of the left ventricular summed area.

Criteria for Acceptable Study

Animals included for data analysis met the following criteria: mean arterial pressure of >65 mm Hg, arterial pH of 7.35–7.45, PaCO2 of 30–40 mm Hg, PaO2 of >70 mm Hg, LAD and LCX flow signals with a signal to noise ratio of >20 and reactive hyperemia to a 15-second coronary occlusion of >3.0:1, and reproducible hemodynamic and coronary responses to pharmacological agents. Of 58 dogs studied, three were excluded because of low arterial pressure or nonreproducible flow responses to stellate stimulation or norepinephrine. Five animals were excluded because of ventricular fibrillation during the peri-ischemic period. Of 58 animals studied, data were analyzed from 50.

Statistical Analysis

Differences between baseline hemodynamics during control conditions and after myocardial stunning were detected using Student’s two-tailed t test. In cases where multiple comparisons were performed (changes in resistances between control and experimental beds before and after ischemia), an analysis of variance with repeated measures was used. Tukey’s multiple comparison test was performed for post hoc determination of significant differences. Coronary responses to bretylium demonstrated tachyphylaxis. Therefore, comparisons were made only between control and experimental beds (n=6) after ischemia. The effect of bretylium before ischemia was determined in three dogs. The response to only one injection was used for analysis. Statistical significance is defined as p<0.05. Data are presented as mean±SEM.

Results

After β-adrenergic blockade, electrical stimulation of the stellate ganglia bilaterally (10 V) produces an increase in arterial pressure, no change in heart rate, and an early and transient decrease in coronary flow velocity accompanied by an increase in coronary vascular resistance in both the LAD (peak increase, 38±5%) and LCX (peak increase, 39±5%) coronary beds (Figure 1, Table 1). Intravenous infusion of norepinephrine (10 μg) also increased coronary vascular resistance in both beds (LAD, 45±7%; LCX, 46±9%; Figure 1). Saline infusion was without effect.

Thirty minutes after release of a 15-minute occlusion of the LAD, baseline heart rate (118±3 beats per minute before and 119±3 beats per minute after occlusion) and arterial pressure (104±4 mm Hg before and
95±3 mm Hg after occlusion) remained unchanged. The pressor response to bilateral electrical stimulation of the stellate ganglia was unchanged (Table 1). Whereas coronary vasoconstriction in the unoccluded bed (47±8% increase from baseline) was similar to that observed before the occlusion, constriction in the LAD bed was markedly diminished (16±3% peak increase in resistance after brief myocardial ischemia; p<0.05 versus before occlusion and versus LCX bed). Coronary constrictor responses in both vascular beds to two doses of intravenous norepinephrine were not altered by occluding the LAD for 15 minutes (Figures 1 and 2, Table 2). These data indicate that coronary reactivity to α-adrenergic stimulation remains intact after brief periods of ischemia, while responses to peripheral neural activation are reduced.

**TABLE 1.** Baseline Hemodynamic Values and Peak Changes in Flow Velocity and Resistance to Stellate Stimulation in Left Anterior Descending Coronary Artery and Left Circumflex Coronary Artery Beds

<table>
<thead>
<tr>
<th>Stellate stimulation</th>
<th>Baseline</th>
<th>Change from baseline (%)</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>HR (bpm)</td>
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<tr>
<td><strong>Epicardial compression studies</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>20</td>
<td>118±3</td>
</tr>
<tr>
<td>15 Minutes, 10 V</td>
<td>7</td>
<td>121±4</td>
</tr>
<tr>
<td>7 Minutes, 10 V</td>
<td>10</td>
<td>120±5</td>
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<tr>
<td><strong>After LAD occlusion</strong></td>
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<tr>
<td>15 Minutes, 10 V</td>
<td>20</td>
<td>119±3</td>
</tr>
<tr>
<td>15 Minutes, 5 V</td>
<td>7</td>
<td>122±4</td>
</tr>
<tr>
<td>7 Minutes, 10 V</td>
<td>10</td>
<td>126±5</td>
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<tr>
<td><strong>Intracoronary balloon studies</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>6</td>
<td>136±12</td>
</tr>
<tr>
<td><strong>After LAD occlusion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 Minutes, 5 V</td>
<td>6</td>
<td>127±3</td>
</tr>
</tbody>
</table>

HR, heart rate; bpm, beats per minute; AP, arterial pressure; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; CBFV, coronary blood flow velocity; CVRI, coronary vascular resistance index. Values are mean±SEM.

For stellate stimulation, the duration of coronary occlusion is given in minutes, and the intensity of electrical stimulation is given in volts.

*p<0.05 vs. control; †p<0.05 vs. LCX.
Coronary Vascular Responses to Neuronally Released Norepinephrine

To examine whether reduced prejunctional stores or attenuated release of norepinephrine is responsible for the diminished coronary constrictor to stellate stimulation, we examined responses to intracoronary infusions of tyramine and bretylium (Figure 3 and Table 2).

Tyramine produced an increase in coronary vascular resistance in the LAD (30±10%) and LCX (20±6%) beds. After a 15-minute coronary artery occlusion, the constriction to tyramine was unchanged (34±11% in the LCX, 32±8% in the LAD). This suggests that the mechanisms regulating release of cytosolic norepinephrine from the adrenergic nerve terminals are not impaired after brief ischemia.

Coronary vasoconstriction to bretylium was similar in LAD and LCX beds after a 15-minute LAD occlusion (Figure 3, Table 2). Since bretylium primarily releases vesicular norepinephrine from adrenergic nerve terminals, it is unlikely that reduced vesicular release of the neurotransmitter is responsible for the impaired coronary constriction in the LAD bed. These data are consistent with normal release of norepinephrine from sympathetic nerve terminals in regions of postischemic myocardium.

Intravascular Balloon Occlusion

In six dogs, an intracoronary angioplasty balloon catheter was used to occlude the LAD. This protocol tested whether postischemic reductions in sympathetic coronary constriction were due to direct effects of epicardial compression. In these experiments, an intracoronary balloon catheter was used to occlude the LAD. Stellate stimulation (5 Hz, 10 V) produced similar increases in LAD (24±2%) and LCX (20±4%) vascular resistance. After 15 minutes of ischemia and 30 minutes of reperfusion, constriction in the LAD bed to sympathetic stimulation (12±2%) was reduced compared with both the LCX bed (21±3%, p<0.05) and the LAD bed before ischemia (24±2%, p<0.05) (Figure 4). Baseline and stimulated changes in heart rate and arterial pressure were not altered by ischemia and reperfusion (Table 1).

Effect of Brief Coronary Occlusion on Neural Responses

In 10 animals, coronary responses to sympathetic nerve stimulation were tested before and after occlusion.
sion of the LAD for 7 minutes. The early reduction in flow velocity and increase in coronary resistance to sympathetic nerve stimulation were not altered by 7 minutes of LAD occlusion (27±4% increase in LAD bed resistance before and 26±5% increase after occlusion; Figure 5, Table 1). Responses in the LCX bed were also unaffected (Figure 5). Therefore, shorter periods of coronary artery occlusion are not associated with attenuated constrictor responses to sympathetic nerve stimulation.

Responses in Coronary Vessels Marginal to the Risk Region

In three animals, multiple Doppler flow probes were used to monitor coronary flow velocity in central and marginal vessels within the risk region. Stellate stimulation increased coronary vascular resistance similarly in both regions (Figure 6). However, after a 15-minute LAD occlusion, coronary constriction to stellate stimulation appeared reduced in the centrally located vessel but not in the marginal one in each of these three dogs. This observation suggests that heterogeneous regional vascular responses to sympathetic nerve stimulation occur after brief periods of ischemia.

Left Ventricular Function

In nine animals, left ventricular dP/dt was measured during control conditions and during reperfusion. Peak baseline dP/dt was decreased during reperfusion (1,800±140 mm Hg/sec) compared with the control value (2,268±184 mm Hg/sec) (p<0.05).

Myocardial transmural wall thickening was measured in nine animals. Systolic thickening during control conditions was 18±6%. During LAD occlusion, no change in thickness occurred during systole (−0.5±4%). Approximately 30 minutes after a 15-minute coronary occlusion, coronary flow velocity returned to normal in the previously ischemic bed (2.2±0.1 kHz shift before versus 1.9±0.1 kHz shift after ischemia; p=NS); however, regional wall thickening was still reduced (3.7±1%). This reduction was maintained for the remainder of the experiment (1–2 hours). In two of these animals, no decrease in systolic wall thickening was observed, although sonomicrometer placement was verified post mortem (by Evans blue dye staining) as completely within the risk region.

Myocardial Risk Region

The area of the myocardial region at risk was planimetered in seven dogs and expressed as a percent of the summed areas of the left ventricular and septal slices. The LAD perfusion field, defined by injection of Evans blue dye into the LAD at the site of coronary occlusion, was 13±3% of the left ventricle.

Discussion

After brief myocardial ischemia, left ventricular function remains depressed despite the return of normal myocardial perfusion. This reversible impairment of resting function, myocardial stunning, persists for several hours to days. The new finding of this study is that, in addition to effects on myocardial contractility, brief
myocardial ischemia attenuates coronary vasomotor responses to neural activation. This is the first demonstration that coronary vasoconstriction to sympathetic stimulation is impaired after brief myocardial ischemia. The altered reactivity is not due to impaired vascular responsiveness but likely reflects abnormal conduction of sympathetic impulses to the coronary vasculature.

A similar reduction in responsiveness by brief ischemia has been demonstrated to the inotropic effect of sympathetic nerve stimulation by Ciuffo et al.\textsuperscript{15} Inotropic responses to infusing norepinephrine were not affected by ischemia; thus, postjunctional receptor mechanisms were intact.

In the present study, coronary responses to sympathetic activation were examined. It was hypothesized that, since vasodilator mechanisms are impaired in stunned myocardium,\textsuperscript{8,10,21,22} neurogenic influences may play a more prominent role. However, in accordance with Ciuffo et al,\textsuperscript{15} we observed a reduced response to activation of the sympathetic innervation of the coronary vasculature. Coronary α-adrenergic receptor responses were intact, and prejunctional release of norepinephrine appeared unaltered after ischemia.

Other investigators report no reduction in cardiac neural conduction after brief myocardial ischemia.\textsuperscript{23,24} Johnstone et al\textsuperscript{23} performed multiple short (5-minute) coronary artery occlusions separated by 10 minutes of reperfusion. They measured no impairment of regional epicardial segment shortening after 12 cycles of occlusion/reperfusion. It is possible that these short periods of coronary occlusion produce an ischemic insult that is not sufficient to alter neural responsiveness. Our findings were consistent with this interpretation; we demonstrated intact coronary responses to neural activation after 7-minute periods of occlusion followed by reperfusion. The effect of multiple brief periods of myocardial ischemia may be different on myocardial function than on cardiac neural innervation.

Heusch et al\textsuperscript{24} measured changes in regional wall thickness to cardiac nerve stimulation before and after a 15-minute LCX occlusion. They recorded reduced thickening to both nerve stimulation and norepinephrine during reperfusion. These attenuated responses recovered in parallel over the 24 hours following coronary occlusion. Reductions in baseline and stimulated contractile function to both stellate stimulation and norepinephrine suggest altered end-organ responsiveness, which could mask underlying changes in cardiac neurotransmission. It is likely that the duration and magnitude of ischemia as well as the method of evoking ischemia (with or without intermittent reperfusion) determine whether cardiac neural responsiveness is altered after ischemia. In the present study, 15 minutes of LAD occlusion impaired coronary vasoconstriction to stellate stimulation but not to adrenergic activation with norepinephrine. This interpretation depends on several methodological considerations.

**Methodological Considerations**

Myocardial ischemia was produced by gentle occlusion of the epicardial coronary artery with a cotton-tipped swab for 15 minutes. This method of occlusion was chosen because it is relatively atraumatic and it obviates the need for surgical dissection of the epicardial artery, with attendant risk for mechanical denervation.\textsuperscript{25,26}

With this method, malalignment or rotation of the swab could produce partial release of the occlusion, introducing periods of reperfusion and interrupting the duration of the ischemic stimulus. This did not occur in the present study, since a Doppler flow velocity probe positioned distal to the occlusion site ensured zero flow during the entire 15-minute occlusion.

Sympathetic denervation is a potential result of coronary artery occlusion by epicardial compression. However, for several reasons, we believe that this method of coronary occlusion does not produce traumatic sympathetic denervation.

First, we examined the effect of intraluminal balloon occlusion of the LAD in six dogs. This method of coronary occlusion circumvents the possibility of direct epicardial compressive damage to the sympathetic nerves. A similar reduction in sympathetic coronary constriction was observed with both epicardial and intravascular methods of coronary occlusion. This is consistent with findings of previous studies that also used extravascular and intravascular occlusion techniques.\textsuperscript{27,28}

Second, we observed that sympathetic coronary constriction in the peripheral regions of the risk zone in three animals was not affected by brief coronary occlusion. Since the majority of the innervation to the heart and coronary vessels runs along the adventitia of the large epicardial arteries and their branches,\textsuperscript{25,29,30} this observation suggests that innervation to this border zone region remained intact after 15–20 minutes of coronary artery occlusion. The preserved innervation of these peripheral segments likely reflects a smaller ischemic insult, possibly from collateral flow.

Third, we observed no alteration in sympathetic coronary vasoconstriction after a 7-minute coronary artery occlusion produced by epicardial compression. If this technique resulted in traumatic damage to the perivascular innervation, one might expect to observe
reduced coronary constriction to stellate stimulation after a 7-minute occlusion.

Finally, methods of epicardial coronary manipulation that are more invasive than those used in the present study (e.g., placement of circumferential flow probes and/or snare) preserve coronary innervation distal to the instrumentation.31,32

In addition to neural influences, several other factors important in coronary flow regulation could have contributed to the responses observed in this study.

Changes in arterial pressure can alter coronary blood flow directly by changes in perfusion pressure and indirectly through changes in myocardial oxygen consumption. Similarly, adrenergic-induced changes in left ventricular end-diastolic pressure and left ventricular dP/dt may alter coronary blood flow independent of direct vascular actions. If the effect of sympathetic nerve stimulation on these parameters was different in the control condition compared with the reperfused state, this could have contributed to observed changes in coronary flow. It is unlikely, however, that evoked changes in global myocardial metabolism, preload, or afterload account for the observations in this study, since comparisons were made between experimental and control beds in the same animals over time. Changes in arterial pressure and left ventricular end-diastolic pressure should exert similar effects on both control and experimental beds. Brief myocardial ischemia altered neural conduction only in the experimental bed.

In this study, we did not measure regional myocardial oxygen consumption, and it is possible that the differences between experimental and control perfusion territories may have contributed to the different responses observed to nerve stimulation after ischemia. For three reasons, however, this is unlikely. First, if underlying changes in regional oxygen consumption were primarily responsible for differential responses to nerve stimulation, the same differences should have been evident during intravenous bolus doses of norepinephrine. This was not the case. Second, similar baseline coronary flow velocities before and after stunning argues against large changes in myocardial oxygen consumption after ischemia. Third, brief periods of myocardial ischemia have

![Diagram showing sites of occlusion and CBFV measured for this experiment.](http://circres.ahajournals.org/doi/abs/10.1161/01.CIR.0000170995.65434.64)
not uniformly been shown to alter regional myocardial oxygen consumption, although regional function may be depressed. Laxson et al. demonstrated no differences in regional myocardial oxygen consumption in stunned compared with control regions of the canine heart. Thus, it is unlikely that myocardial metabolic changes related to ischemia contributed to the differential responses observed in control and experimental vascular regions.

In two dogs, myocardial function returned to normal by 30 minutes of reperfusion despite akinesis during ischemia. The reason for this is unclear, but marked variability in recovery of postischemic function has previously been described. Possible differences in collateral flow, degree of ischemic dysfunction, or preocclusive metabolic rate may contribute to different time courses of recovery.

One limitation of this study is the use of direct stellate activation rather than a more physiological stimulus for coronary constriction (e.g., baroreflex or exercise). Stellate stimulation was used for three reasons. First, we have observed that coronary constriction to direct sympathetic activation is reproducible over time and is therefore a reliable indicator of the integrity of the neurocoronary axis. Second, coronary constriction to direct sympathetic activation, as opposed to most reflex physiological coronary constrictor responses, occurs early during the stimulus, at a time when there are minimal changes in heart rate and arterial pressure. Secondary alterations in coronary flow due to changes in pressure and myocardial metabolism would confound the interpretation of results. Third, direct sympathetic activation produces coronary vasoconstriction that is relatively unaffected by central neural inhibitory influences, vagal and ganglionic modulation of neural impulses, and competition from other reflexes that might be activated during more physiological forms of coronary vasoconstriction. Thus, for the purposes of consistency and selectivity, we chose direct activation of sympathetic nerves. Future studies should address the extent to which reduced sympathetic efferent responses during reperfusion interact with other neural, humoral, and hemodynamic factors to regulate coronary flow during physiological stimuli that produce coronary constriction.

**Mechanism of Altered Coronary Vasoconstriction**

Although this study demonstrates that brief coronary occlusion attenuates sympathetic coronary vasoconstriction during reperfusion, it is unclear where along the neuroeffector axis this alteration occurs. Sympathetic coronary vasoconstriction was impaired in the center of the risk region but not in the control beds or in more peripheral myocardial regions supplied by branches of the occluded vessel. This implies that ischemia is acting locally to alter coronary responsiveness to nerve stimuli in the postischemic myocardium. In this study, postfunctional adrenergic coronary constriction is not altered after brief ischemia, consistent with previous observations. Stimulated prejunctional release of cytosolic (tyramine) or vesicular (bretylium) norepinephrine produced similar degrees of coronary vasoconstriction in postischemic and control regions. Although depletion of norepinephrine from nerve terminals has been demonstrated after prolonged periods of ischemia, this factor likely does not contribute to postischemic attenuation of coronary vasoconstriction. The most plausible hypothesis, consistent with previous studies, is an altered conduction through epicardial sympathetic efferent fibers. This conclusion is supported indirectly by the present study. Future investigations measuring efferent sympathetic cardiac nerve activity could directly test efferent neural conduction in postischemic myocardium.

Postischemic impairment of autonomic cardiac neurotransmission is independent of the method of coronary artery occlusion and has been demonstrated in both efferent and afferent limbs of the sympathetic innervation. Although not addressed in the present study, it has been suggested that the mechanism of this postischemic attenuation in neurotransmission may be the result of alterations in local release of prostaglandins or changes in regional Po2, K+, or pH.

**Implications of the Present Study**

Cardiac sympathetic tone is increased during myocardial ischemia. It has been suggested that attenuation of cardiac sympathetic influences may be beneficial in the postischemic myocardium by preventing the associated increase in metabolism, which would further dissociate oxygen supply and demand and result in further mechanical dysfunction in postischemic tissue. The present study suggests an additional benefit. Mechanical function in the postischemic or stunned myocardium is critically dependent on myocardial perfusion. Thus, inhibition of sympathetic mediated coronary vasoconstriction might act to maintain perfusion to the region of stunned myocardium by attenuating the effects of reflex and centrally evoked increases in cardiac sympathetic tone. This is of particular importance in the presence of a coronary stenosis, where neurogenic coronary vasoconstriction is augmented.

In summary, neurogenic coronary vasoconstriction is impaired after brief periods of myocardial ischemia. This impairment does not appear to involve altered coronary responsiveness to norepinephrine or depletion of prejunctional catecholamine stores.
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