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Excitation of Afferent Cardiac Sympathetic Nerves is Modulated by Nitroglycerin (41081)

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Abstract. We examined the effect of nitroglycerin on stimulation of sympathetic nerve fibers arising from receptor sites in the left ventricular wall during acute coronary artery occlusion. Sympathetic afferent nerve activity was recorded in single and multifiber nerve preparations dissected from slips of T_3 white rami communicantes. Acute left anterior descending or left circumflex coronary artery occlusion produced an increase in segmental myocardial length and excitation of afferent cardiac sympathetic nerve fibers in the region of the myocardium supplied by the occluded artery. Intravenous administration of nitroglycerin in doses up to 100 μg/kg and sodium nitroprusside (50 μg/min) reduced systolic bulge during coronary occlusions and suppressed excitation of these myelinated and unmyelinated afferent fibers. Local intracoronary injection of nitroglycerin (100 μg) produced neither a change in systemic blood pressure nor a change in excitation of the afferent nerve fibers during coronary occlusion. Systemic administration of nitroglycerin sufficient to lower blood pressure and reduce epicardial systolic lengthening in the ischemic zone is associated with inhibition of firing of cardiac afferent sympathetic nerves.

Occlusion of the anterior descending or circumflex branch of the left coronary artery results in the development of a systolic bulge of the ventricular wall in the ischemic area as well as excitation of cardiac sympathetic afferent myelinated (1) and unmyelinated fibers (2) originating from receptors located within the ischemic myocardium or within the coronary arteries. Excitation of some of these fibers is thought to initiate angina pectoris which is considered to be due to failure of the coronary arteries to supply a sufficient quantity of oxygen to the heart. Cardiac ischemia (3), unphysiological motion of the ventricular wall (2), and an increase in lactic acid formation (4) have been implicated as some of the causes of anginal pain. For nearly a century, nitroglycerin has been used as an effective drug for the relief of angina pectoris. The precise mechanism by which nitroglycerin modifies ischemia is not clear. Although nitroglycerin may cause coronary vasodilatation, some previous investigations have indicated that peripheral actions of nitroglycerin are responsible for this agent's ability to relieve angina pectoris (5–7). Since nitroglycerin has multiple cardiovascular actions, it is not clear whether the relief of anginal pain is also due to direct inhibition of afferent cardiac sympathetic nerve endings in the heart.

We designed the present study in such a way that the effect of direct action of nitroglycerin on the coronary bed and cardiac receptors could be investigated during myocardial ischemia in the absence of changes in the systemic circulation. This was achieved by direct intracoronary injection of nitroglycerin in doses, which were large enough to markedly increase local coronary blood flow as measured by the electromagnetic flow probe. These doses were too small to cause a change in systemic blood pressure. Our study also examined the effect of systemic administration of nitroglycerin and sodium nitroprusside, in doses sufficient to lower arterial blood pressure, during excitation of cardiac afferent sympathetic (Aδ and C) nerve fibers produced by myocardial ischemia.

Material and Methods. Seventeen mongrel dogs of either sex weighing 20–30 kg were anesthetized with sodium pentobarbital (30–40 mg/kg). Respiration was controlled via an endotracheal tube and Air Shields Company ventimeter ventilator.

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The upper six or seven ribs from the left side were removed from the costovertebral joint to the costochondral junction. The sympathetic chain on the left side was dissected from the surrounding connective tissue, and the third or second thoracic white ramus communicans was isolated, sectioned, and afferent nerve activity was recorded from the distal end of the nerve. In all studies, the sympathetic trunk was cut below T_3 in order to eliminate afferents traveling up the sympathetic chain. The heart was exposed by pericardiotomy, and left ventricular pressure was recorded using a pressure transducer (Statham Instruments, P23D), and short polyethylene cannula was inserted into the left ventricle through the apex. Aortic blood pressure was recorded using a catheter advanced through the femoral artery. A polyethylene cannula was introduced through the femoral vein for intravenous infusions. To evaluate regional epicardial length changes, 1-in.-long Mercury-in-silastic length gauges (Parks Electronics) were sutured to the epicardium in the areas of the ventricle supplied by the left anterior descending (LAD) and left circumflex (LC) coronary arteries. Regional length changes were expressed as percentage changes from the control values (8). Percentage shortening of the myocardial segment was also analyzed as the difference between the end-diastolic and end-systolic dimensions divided by the end-diastolic dimensions multiplied by 100, as used by some investigators (9). Segmental diastolic length and nerve activity were analyzed by paired *t* test. A local ventricular electrocardiogram from the ischemic zone was recorded from silver pin electrodes inserted into the epicardial surface of the heart. The LAD or the LC coronary artery was cannulated with small diameter tubing (0.7 mm OD) for injection of nitroglycerin. In our preliminary studies using electromagnetic flow probes, intracoronary injection of nitroglycerin (100 μg), produced a striking coronary vasodilatation. After 1 min the coronary blood flow was 50% above that of the control. Snares were placed at the origins of the LAD and LC coronary arteries, and measurements of afferent sympathetic nerve activity coming from the left ventricle were made before, during, and after short-term coronary occlusion of 30 sec. The occlusions were carried out before and after injection of nitroglycerin (100 μg) directly into the coronary vessels, before and after systemic administration of nitroglycerin (100 μg/kg) and before and after infusion of sodium nitroprusside (50 μg/min). All parameters were recorded with a Grass Model 7 polygraph, and four of these parameters plus voice were recorded on a tape recorder (Tandberg series 100 FM) for later analysis.

Nerve recording. The second or third white ramus communicans was divided into fine filaments under a Zeiss dissecting microscope and filaments were laid across bipolar metal electrodes under oil warmed to 37°. The electrodes were connected to a high-input impedance preamplifier and amplifier equipped with high and low-pass filters (1600-100 Hz). The signal was displayed on a Tektronix type 564B storage oscilloscope and monitored with a loudspeaker. A total of 17 nerve fiber preparations of left ventricular origin in the ischemic areas were studied (11 myelinated and 6 unmyelinated afferent fibers). During the experiment, the approximate location of each receptor was confirmed by mechanical probing of the epicardial surfaces of the left ventricle with a blunt probe having a tip diameter of 1 mm. Animals were exsanguinated after every experiment and nerve activity disappeared from the arrested heart. Nerve activity in the same fiber, however, could be produced by mechanical probing of the receptor site in the arrested heart, and a more accurate receptor location was obtained. Using this technique, the receptive fields could be localized within 3–5 mm for a single fiber preparation and varied up to 3 cm^2 for the small multifiber preparations studied in these experiments. When averaged nerve activity was used, it was obtained from a precision half-wave rectifier and low-pass filter with a half amplitude point at 1 Hz. Conduction velocities were measured by stimulating one of the close cardiac nerves or the ventral ansa subclavia with a strength sufficient to evoke

an action potential from the fiber which was studied during spontaneous activity. Stimulation characteristics of the generator were a square-wave monophasic pulse, 0.1-msec pulse duration, and a constant current of 1–2 mA. In some instances, conduction velocity was measured by placing the stimulating electrodes over the receptive field of the left ventricular receptor. Usually, higher stimulus strengths were required to evoke the nerve fiber action potential using the latter technique. The conduction velocity was calculated from the time of the stimulation artifact to the beginning of each evoked action potential and the measured distance from the stimulating to the recording electrodes.

The nerve activity, epicardial length, and aortic blood pressure were examined by analyzing the tapes on an Ortec averaging computer using the R wave of the ECG as a trigger. Nerve activity during 32 consecutive heart beats was accumulated at 5-msec time increments by the computer and was printed out with the left ventricular pressure or length changes for the same heart beats using an X-Y plotter and digital printer. Changes in afferent nerve activity were considered significant if they exceeded control nerve activity by 20% or more.

Results. In our preparation, the total range of nerve activity in impulses per heart beat is dependent on the number of active afferent fibers in the nerve preparation and the absolute changes in the segmental length. It is possible to calculate the averaged increase in segmental length and averaged increase in nerve activity during a 30-sec coronary occlusion using the data accumulated by the computer. These values are expressed as a percentage change from the control values.

In the present study, conduction velocities for Aδ fibers which responded to increased myocardial length ranged from 3–27 m/sec and averaged 9.7 m/sec, and conduction velocities for C fibers ranged from 0.5 to 1.1 m/sec and averaged 0.8 m/sec. Systemic intravenous administration of nitroglycerin in a dose of 100 μg/kg or sodium nitroprusside (50 μg/min) reduced mean arterial blood pressure by 52 ± 11% from a control of 100 ± 8 mm Hg. When arterial blood pressure was low, coronary occlusion did not elicit systolic bulge, and excitation of the afferent nerve fibers was absent.

Figure 1 compares control changes in left ventricular pressure, systemic blood pressure, averaged nerve activity and myocardial segment length during left circumflex coronary occlusion (A) and occlusions after injection of 100 μg of nitroglycerin into the left circumflex coronary artery (B). Increases in the segmental diastolic length and nerve activity are similar in the top two panels, suggesting no direct effect of nitroglycerin on these receptors. The effect of intravenous injection of nitroglycerin (100 μg/kg) is seen in panel C and intravenous infusion of sodium nitroprusside 50 μg/min in panel D. Here, the increase in the left circumflex segment length is diminished during coronary occlusion due to lowered arterial and left ventricular blood pressure. Nerve activity from the same segment did not change significantly.

Faster tracings of the left circumflex segment length, blood pressures, and raw nerve activity are shown in Fig. 2. In the top three tracings, during left circumflex occlusion, the increases in segmental length and nerve activity from the same segment are similar. After injection of nitroglycerin (100 μg/kg bottom right tracings) coronary artery occlusion produced neither an increase in segment length nor an increase in nerve activity during coronary occlusion.

Figure 3 shows typical recordings obtained during an experiment at a rapid speed. The segmental length, left ventricular pressure, and nerve activity are compared during control, LC occlusion, LC occlusion after intracoronary injection of nitroglycerin, and LC occlusion after systemic injection of nitroglycerin. During the control segment length is maximal in the early part of the ejection period and substantial shortening occurs during the systolic ejection period. During the LC occlusion, diminished shortening and late systolic lengthening appear in the ischemic region. The increase in afferent nerve ac-

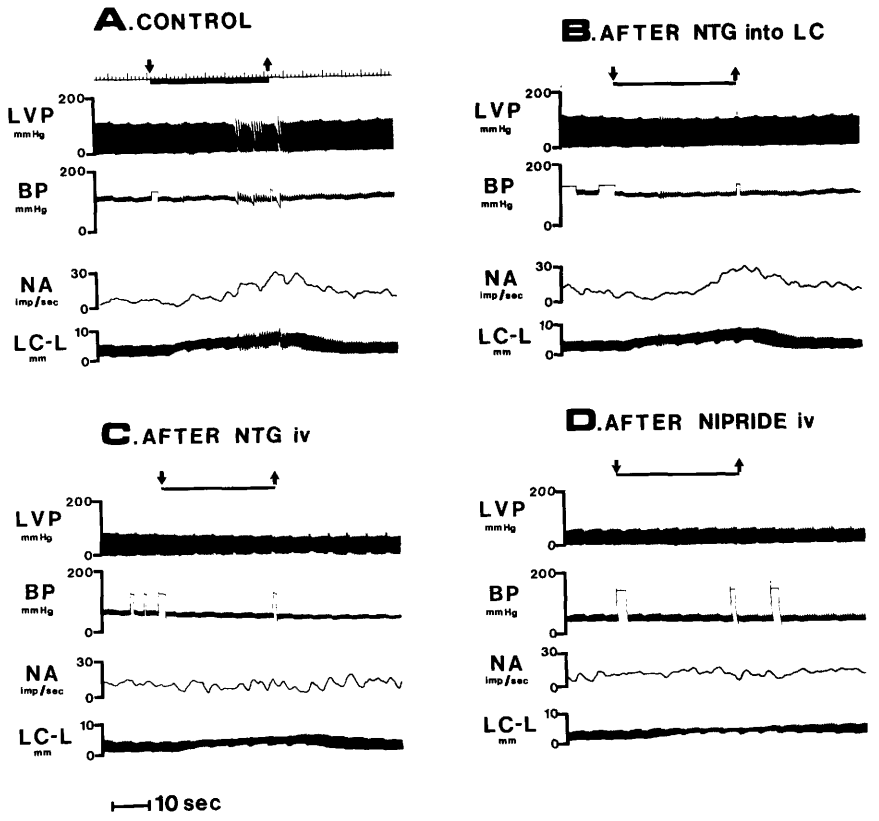

FIG. 1. Changes in left ventricular pressure (LVP), peripheral blood pressure (BP), averaged nerve activity (NA) from a small multifiber preparation and left circumflex length (LC-L) are compared during 30 sec coronary artery occlusion: (A) control occlusion (B) occlusion after injection of 100 μg of nitroglycerin into left circumflex coronary vessel, (C) occlusion after injection of 100 μg/kg of nitroglycerin intravenously, and (D) occlusion after intravenous infusion of nitroprusside (50 μg/min). For details, see text.

tivity is directly related to an increase in left ventricular segmental length produced by acute coronary artery occlusion.

Mean changes in myocardial segmental length and nerve activity from 17 cardiac receptors during control occlusions of 30 sec and occlusions following intracoronary injection of nitroglycerin or iv injection of 100 μg/kg of nitroglycerin are presented in Fig. 4. During control occlusions, the averaged increase in segmental diastolic length was 90% above the control length, and the average increase in afferent nerve activity was 170% above the control. If the percentage segment shortening is used, the averaged increase in segmental diastolic length was 23%. Changes in myocardial length and nerve activity during control occlusion and occlusion after intracoronary injection of nitroglycerin differ significantly from control but not from each other. After injection of 100 μg/kg of nitroglycerin into the systemic circulation, changes in myocardial length and nerve activity during occlusions were not significant from control.

Discussion. In animals, brief periods of experimental coronary artery occlusion cause a significant increase in sympathetic cardiac afferent nerve activity (1, 2). Brown has demonstrated that transient occlusion of the main left coronary artery in cats provokes a painlike response associated with increased neural discharge in afferent fibers of selected cardiac nerves (10). It was

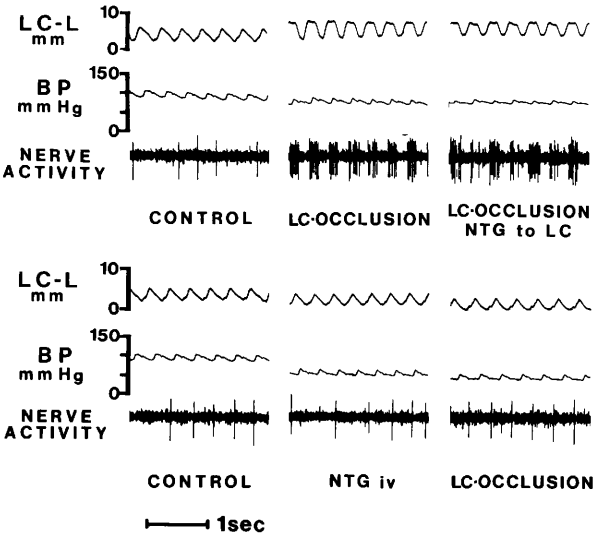

FIG. 2. Changes in the left circumflex segment length (LC-L), peripheral blood pressure (BP) and nerve activity are shown during control, LC occlusion before and after intracoronary injection of nitroglycerin (NTG 100 μg), top three tracings. Increases in segmental length and nerve activity are similar. The effect of lower systemic blood pressure due to injection of the nitroglycerin (100 μg/kg) is shown in the bottom right tracings. Lower arterial blood pressure prevented the increase in the segmental length during coronary occlusion and increase in nerve activity.

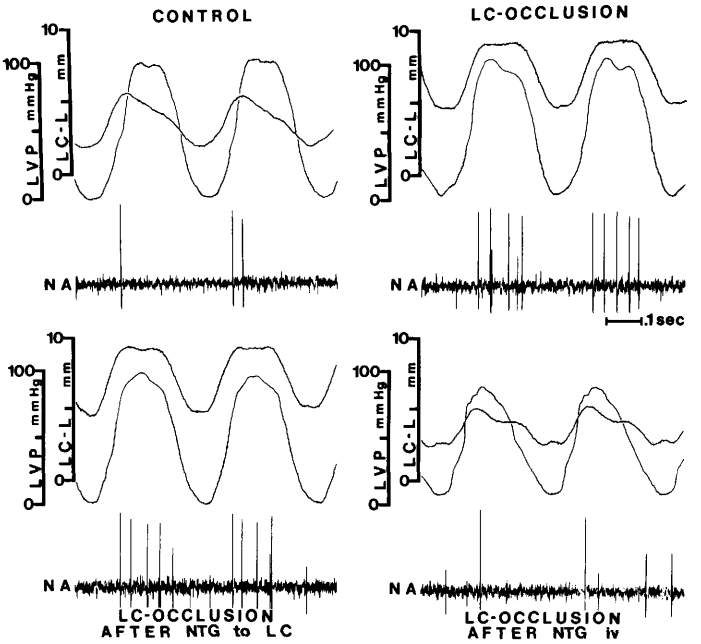

FIG. 3. Alterations of regional segment length (LC-L), left ventricular pressure (LVP) and nerve activity (NA) in a typical experiment. The LC occlusion was repeated after injection of 100 μg of nitroglycerin (NTG) into the LC coronary artery and after injection of NTG into the peripheral circulation (100 μg/kg).

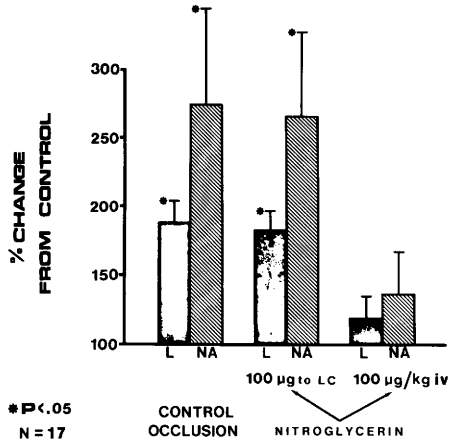

FIG. 4. Myocardial length changes and sympathetic afferent nerve activity are expressed as percentage change from control values. Mean values with 1 SEM are presented from 17 cardiac receptors and 17 experimental animals. Percentage change from control values is significant for both length and nerve activity during the 30-sec control coronary artery occlusion and after injection of 100 μg of nitroglycerin into the coronary artery. Changes in myocardial length (L) and nerve activity (NA) during control occlusion and after intracoronary injection of nitroglycerin are similar. After injection of 100 μg/kg of nitroglycerin into systemic circulation, increases in myocardial length, and nerve activity during coronary occlusion were not significant from the control.

suggested that systolic bulge during coronary occlusion was responsible for activation of the endings, since their discharge occurred with development of systolic bulge and was synchronized with each cardiac cycle (1, 11). It has been observed that accumulation of lactic acid (4), release of potassium ions from the myocardium (12), and formation of bradykinin (13) may be present during myocardial ischemia. Excitation of afferent sympathetic nerve fibers has been observed following application of these chemical agents to the left ventricular surface and injection into the coronary arteries (14). Although there is a relative degree of specificity with respect to stimuli, it is generally believed that both unmyelinated and slowly conducting myelinated sympathetic cardiac afferent nerves can conduct impulses when peripheral receptors are activated by painful stimuli (14, 15).

In man, myocardial ischemia produces pain which is abolished or greatly attenuated by stellate ganglion blockade or excision of the upper thoracic sympathetic ganglia (3). Although nitroglycerin causes coronary vasodilatation, it is unlikely that its major action in relieving ischemia is due to coronary vasodilatation. First, in the presence of a critical or subcritical coronary artery lesion, the reserve vasodilator capacity of the coronary bed is already almost completely exhaused in maintaining resting blood flow. Direct infusion of nitroglycerin into the coronary arteries in a concentration sufficient to cause an increase in coronary flow, did not relieve angina pectoris (16) produced by atrial pacing in man, whether the nitroglycerin was injected into the right or left coronary artery, into the obstructed coronary artery, or into the artery supplying collaterals to the obstructed coronary artery (16). Their observations suggest that the increase in coronary blood flow did not occur in the ischemic areas, but occurred in nonischemic areas where the arterioles were not maximally dilated. When the nitroglycerin was administered intravenously, angina was relieved, when associated with a fall in arterial pressure and in coronary sinus blood blow.

Nitroglycerin causes a reduction in myocardial oxygen demand by reducing wall stress (17). Our work confirms these findings since systemic nitroglycerin sufficient to reduce peripheral blood pressure results in a decreased frequency of cardiac afferent nerve discharge during myocardial ischemia. Both systolic pressure and ventricular volume are reduced by nitroglycerin, due to three major vascular sites of action; (i) coronary arteries, (ii) peripheral arteries, and (iii) peripheral veins. These effects differ depending not only upon the dose and route of administration, i.e., intracoronary, intravenous, or sublingual, but also the state of the experimental preparation, i.e., conscious or anesthetized subject, and on the presence or absence of coronary artery obstruction or disease. In addition to the reduction in preload, afterload, and cardiac work, nitroglycerin induces substantial and sustained reductions

in left ventricular dimensions (18). In infusion studies of nitroglycerin reported by Vatner and co-workers (18), left ventricular end-diastolic size fell and remained depressed for up to 30 min, and mean arterial pressure returned to control levels earlier. Thus, while nitroglycerin reduces both preload and afterload, the effect of decreasing preload, most likely due to venous pooling, is much more prominant and more sustained than the effect of nitroglycerin on afterload and may be its most important action related to relief of angina.

In a previous study (1) myelinated sympathetic fibers with conduction velocities in the Aδ range arising from receptors located within the left ventricle, responded to acute coronary artery occlusion with an increase in activity. The average increase in segmental length during occlusion was 80% above control length, and the average increase in afferent nerve activity was 115% above control nerve activity. The increase in afferent nerve activity was directly related to an increase in left ventricular segmental length produced by acute coronary artery occlusion. Coronary occlusion for 30 sec provided the most reproducible increase in segmental myocardial length and afferent nerve activity from the same segment.

In this study, systemic administration of nitroglycerin caused a fall in systemic blood pressure, eliminated systolic bulging during coronary occlusion, and suppressed excitation of sympathetic afferent cardiac fibers (both Aδ and C-fibers). Nitroglycerin induced inhibition of cardiac afferents may have been due to reduction of several substances which are released during myocardial ischemia, redistribution of epicardial to endocardial blood flow ratios, and/or elimination of the systolic bulge. The inhibitory action of the agent on these fibers was not observed with a dose which did not reduce systemic blood pressure. The effects of nitroglycerin on the coronary bed may play little, if any, role in the relief of angina pectoris (16, 19). It is, however, possible that in angina pectoris resulting from a primary reduction of myocardial oxygen supply by coronary vasoconstriction or spasm of a large coronary artery, the coronary dilating effect of nitroglycerin plays a decisive role in its relief (7).

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