Distribution of Left Ventricular Sympathetic Afferents Demonstrated by Reflex Responses to Transmural Myocardial Ischemia and to Intracoronary and Epicardial Bradykinin

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Background. Stimulation of left ventricular (LV) receptors with sympathetic afferents generally results in reflex sympathoexcitatory responses. Stimulation of LV receptors with vagal afferents results in reflex sympathoinhibitory responses. Vagal afferents are known to be preferentially distributed to the inferoposterior (IP) wall of the LV. We tested the hypothesis that there is also a preferential distribution of LV sympathetic afferents.

Methods and Results. We measured reflex responses to stimulation of sympathetic afferents located in the anterior and IP LV. We used myocardial ischemia and chemical stimuli to increase the activity of the sensory endings in 15 chloralose-anesthetized, mechanically ventilated dogs with sinoaortic denervation and vagotomy. Reflex responses were assessed by direct recordings of efferent renal sympathetic nerve activity (RSNA). In nine dogs, maximal RSNA changes elicited by transmural anterior myocardial ischemia (22.6±3.9% increase from baseline nerve traffic) were not significantly different from maximal RSNA changes observed during transmural IP ischemia (27.1±4.4%). Similar changes in mean arterial and left atrial pressures were noted during transmural anterior and IP ischemia. In eight dogs, maximal changes of RSNA elicited by epicardial or intracoronary bradykinin to the anterior LV were not significantly different from those observed during bradykinin to the IP LV (anterior epicardial bradykinin, 76.7±11.7%; IP epicardial bradykinin, 72.2±10.0%; anterior intracoronary bradykinin, 84.6±21.0%; IP intracoronary bradykinin, 88.8±17.3%).

Conclusions. We conclude that cardiac receptors with sympathetic afferents are distributed equally to the IP and anterior regions of the LV. (Circulation 1993;87:240–246)

Key Words • afferents, sympathetic • afferents, vagal • reflexes, cardiovascular • sympathetic activity

Many of the cardiovascular responses to myocardial ischemia are reflexive in nature and are the consequence of activation of sensory endings located in the left ventricular myocardium. The left ventricle contains two distinct populations of sensory receptors. The first group comprises receptors subserved by afferent fibers that travel to the central nervous system in the vagus nerves (vagal afferents). Activation of these receptors elicits cardioinhibitory, vasodepressor, and sympathoinhibitory responses. A second group of ventricular receptors is subserved by afferent fibers that course to the spinal cord and central nervous system via the sympathetic nerves (sympathetic afferents). Stimulation of these receptors generally results in reflex vasopressor and sympathoexcitatory responses. In addition to being activated by myocardial ischemia, ventricular receptors are also chemosensitive and can be activated by chemicals such as nicotine (vagal afferents) and bradykinin (sympathetic afferents).

Experimental data from animals and clinical observations in humans indicate that vagal afferents are preferentially distributed to the inferoposterior wall of the left ventricle. As a result, reflex inhibitory responses are most apparent when stimuli are applied to the inferoposterior left ventricle. Furthermore, experiments performed by Barber and colleagues suggest that vagal afferent fibers travel mainly in the deeper endocardial layers of the left ventricle.

In contrast, the study by Barber et al indicates that the sympathetic afferent fibers are located mainly in the superficial epicardial layers of the left ventricle. Recent data from our laboratory support this observation. We found that excitatory reflexes mediated by cardiac sympathetic afferents in response to coronary occlusion are apparent only when myocardial ischemia is transmural and involves the superficial epicardial layers. Although clinical observations in humans suggest that excitatory responses occur more frequently during ischemia of the
anterior left ventricle, it has not been determined systematically whether there is a preferential distribution of sympathetic afferents to a particular region of the left ventricle. In our initial experiments, reflex responses were measured only during anterior myocardial ischemia. In the present experiments, we compared reflex responses with regional myocardial ischemia and with chemical stimuli applied to sympathetic afferents in both the anterior and inferoposterior left ventricle to determine whether these receptors are preferentially distributed to a specific region.

Methods

Experiments were performed in 15 anesthetized, mechanically ventilated dogs with cervical vagotomy and sinoaortic denervation. The animals were anesthetized with thiamylal sodium (15–25 mg/kg) followed by α-chloralose (80 mg/kg i.v.). Additional doses of chloralose (10 mg/kg i.v.) were administered hourly. The animals were ventilated with a combination of oxygen and room air. Arterial blood gases were determined at intervals, and either the respiratory settings were adjusted or sodium bicarbonate was given to maintain pH between 7.35 and 7.45. Cannulas were placed in the femoral artery and vein, and arterial pressure was monitored continuously. In 12 dogs, a cannula was also placed in the left atrium for continuous measurement of left atrial pressures. Body temperature was maintained at 37°C by external warming. During 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 both cervical vagi. The carotid baroreceptors were denervated by ligation and sectioning of all structures that coursed between the internal and external carotid arteries. Abolition of the reflex increases in arterial pressure and sympathetic nerve activity during transient bilateral carotid occlusion were considered indicative of adequate carotid sinus denervation in each experiment. Previous studies indicate that the technique of cervical vagotomy abolishes reflex responses mediated by the cardiopulmonary vagal afferents and the aortic arch baroreceptors.11-13

The heart was exposed by an incision made in the fifth left intercostal space, and an opening was made in the pericardium. In nine animals, the proximal segments of both the left anterior descending (LAD) and left circumflex (LCx) coronary arteries were carefully isolated. Each vessel was instrumented with a snare occluder, a hydraulic occluder, and a Doppler velocity transducer. In eight animals, a small cannula (PE-50) was passed into the LAD and LCx via a distal branch of these arteries for injection of bradykinin. In all experiments, the epicardial surface was kept 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

Recordings were made from efferent renal sympathetic nerves. A retroperitoneal 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 surrounding connective tissue. The nerve was sectioned distally, and the nerve sheath was removed. The nerve was immersed in mineral oil and placed on bipolar platinum–iridium electrodes for recording of action potentials. The method of recording and quantifying nerve activity has been described previously in detail. Briefly, the signal was amplified by a Grass bandpass amplifier (P511, Grass Instruments Co., Quincy, Mass.) with high- and low-frequency filters set at 1,000–3,000 Hz and 30–100 Hz, respectively. The output of this amplifier was fed into an audio amplifier and a spike counter (model 706C Nerve Traffic Analysis System, University of Iowa, Iowa City) that counted and integrated all nerve spike activity whose amplitude exceeded a preselected voltage level (just above noise). At the conclusion of the experiment, the nerve was crushed to eliminate all spike activity and to ensure that noise was not included in the spike quantification.

Experimental Protocol

In nine experiments, measurements of arterial and left atrial pressures and renal sympathetic nerve activity were made during 2-minute periods of transmural myocardial ischemia. Responses both to anterior and to inferoposterior ischemia were recorded in each animal. Transmural anterior myocardial ischemia was elicited by total occlusion of the LAD after a flow-limiting stenosis was placed on the LCx. Conversely, transmural inferoposterior myocardial ischemia was elicited by total occlusion of the LCx after a flow-limiting stenosis was placed on the LAD. The purpose of the coronary stenosis was to limit collateral flow to the region normally perfused by the completely occluded coronary artery and to create greater transmural ischemia in the region supplied by the occluded artery. The stenosis was adjusted to abolish coronary reactive hyperemia without reducing basal levels of coronary flow. Coronary reactive hyperemia was assessed by observing the flow velocity responses that were elicited by a transient (5-second) complete occlusion of the stenosed artery. The stenosis was tightened until there was elimination of the reactive hyperemia after release of the transient complete occlusion. Previous experiments from our laboratory have demonstrated that this model results in myocardial ischemia that is more transmural and involves the epicardial layers to a greater extent than does simple coronary occlusion in which there is no limitation of collateral flow.10,15 The order in which coronary occlusions were performed was randomized. Adequate time (30–45 minutes) was allowed between each occlusion for stabilization.

In eight experiments, measurements of arterial and left atrial pressures and renal sympathetic nerve activity were made during epicardial and intracoronary administration of bradykinin. Epicardial bradykinin (mean dose, 143±27 μg; range, 100–300 μg) was applied to 1.5×1.5-cm gauze sponges placed on either the anterior or inferoposterior surfaces of the left ventricle. Intracoronary bradykinin (mean dose, 59±9 μg; range, 25–
100 μg) was injected into either the LAD or LCx through the coronary cannulas. The order in which these maneuvers were performed was randomized. In each experiment, we chose a dose of bradykinin that resulted in a large reflex increase in renal nerve activity. The same dose of bradykinin was administered to the anterior and inferoposterior left ventricle in each experiment. In all cases, adequate time (15–20 minutes) was allowed between each maneuver for stabilization of parameters.

At the completion of six of these experiments, vital stains (6 cm² of either 1% aniline blue or Ponceau fuchsins solution) were infused through each coronary cannula to demarcate the perfusion bed of each artery. The animal then was killed, and the heart was removed and fixed in 10% formalin. After fixation, the heart was divided into slices 0.5 cm thick, and the stained regions of the left ventricle were separated and weighed to estimate the amount of myocardium exposed to intracoronary bradykinin.

Data Analysis

Arterial pressure, left atrial pressure, electrophysograms, and integrated renal nerve activity were recorded continuously on an electrostatic recorder (Gould ES 1000, Gould, Inc., Greenbelt, Md.). Heart rate was measured from the arterial pressure waveforms. In the coronary occlusion experiments, baseline measurements of heart rate, mean arterial and left atrial pressures, and sympathetic nerve activity (impulses per second) were made after the coronary stenosis was adjusted and during the 30-second period that preceded the coronary occlusion. Repeat measurements were made for each 30-second period of the 2-minute coronary occlusion. A final recovery measurement was made 5 minutes after release of the occlusion. In the bradykinin experiments, heart rate, pressure, and nerve activity measurements were made immediately before bradykinin administration and at the time of maximal reflex changes. Since multiunit nerve preparations were used, nerve activity changes were expressed as percent changes from control values. With multiunit preparations, the absolute value of nerve activity is dependent on the number of active fibers placed on the recording electrodes. The number of active fibers may vary widely from one experiment to another. Statistical comparisons between interventions require that nerve activity be normalized to basal values.

Measurements made in each animal were combined, and means±SEM were calculated. Baseline values of mean arterial and left atrial pressures were compared by paired t tests. Analysis of heart rate measurements indicated that the values were not normally distributed. Therefore, baseline heart rate values and maximal changes in heart rate were analyzed by the Mann-Whitney ranked-sum test. A repeated-measures ANOVA was used to determine whether there were any significant differences in the hemodynamic and nerve activity responses to anterior or inferoposterior myocardial ischemia. In addition, the maximal nerve traffic changes observed during each coronary occlusion were compared by a paired t test. In the bradykinin experiments, the maximal nerve traffic and arterial/left atrial pressure changes observed after intracoronary or epicardial bradykinin administration to the anterior or inferoposterior left ventricle were compared by paired t tests. Paired t tests also were used to compare the weights of myocardium perfused by the coronary canulas placed into the LAD and LCx. In all cases, a value of p<0.05 was considered statistically significant.

Results

Reflex Responses to Myocardial Ischemia

Baseline values of mean arterial pressure, left atrial pressure, and heart rate measured immediately before each coronary occlusion are shown in Table 1. There were no significant differences in any of these basal measurements. The changes in mean arterial and left atrial pressures observed during the two coronary occlusions are shown in Figures 1 and 2, respectively. Mean arterial pressure decreased during both anterior and inferoposterior transmural myocardial ischemia. Conversely, left atrial pressure increased during both anterior and inferoposterior transmural ischemia. The

| TABLE 1. Baseline Heart Rate and Hemodynamic Values Measured Before Each Experimental Maneuver |
|------------------|------------------|------------------|
|                  | Baseline measurements |                |
|                  | HR (bpm) | MAP (mm Hg) | LAP (mm Hg) |
| Myocardial ischemia | 131±9     | 110±5      | 4.2±1.6    |
| Anterior         | 130±8     | 116±10     | 5.0±2.1    |
| Posterior        | 144±7     | 117±4      | 6.1±2.0    |
| Epicardial bradykinin | 144±6    | 114±7      | 6.7±1.3    |
| Anterior         | 147±6     | 121±7      | 8.4±2.4    |
| LCx              | 150±6     | 119±9      | 8.5±2.8    |

HR, heart rate; bpm, beats per minute; MAP, mean arterial pressure; LAP, left atrial pressure; LAD, left anterior descending coronary artery; LCx, left circumflex coronary artery.

![Graph showing responses of mean arterial pressure (MAP) during occlusion of the left anterior descending coronary artery (LAD) with a left circumflex coronary artery (LCx) stenosis (solid symbols) and during LCx coronary artery occlusion with an LAD stenosis (open symbols). Measurements were made at baseline (B) immediately before coronary occlusion and at each 30-second interval during the 2-minute coronary occlusion. A recovery measurement (R) was made 5 minutes after release of the coronary occlusion. Values shown represent mean±SEM.](http://circ.ahajournals.org/)

FIGURE 1. Graph showing responses of mean arterial pressure (MAP) during occlusion of the left anterior descending coronary artery (LAD) with a left circumflex coronary artery (LCx) stenosis (solid symbols) and during LCx coronary artery occlusion with an LAD stenosis (open symbols). Measurements were made at baseline (B) immediately before coronary occlusion and at each 30-second interval during the 2-minute coronary occlusion. A recovery measurement (R) was made 5 minutes after release of the coronary occlusion. Values shown represent mean±SEM.
changes in mean arterial and left atrial pressures noted during transmural anterior myocardial ischemia were not significantly different from those observed during transmural inferoposterior myocardial ischemia. There were no significant changes in heart rate during either anterior (3±1 beats per minute change) or inferoposterior (2±1 beats per minute change) ischemia.

The reflex changes in efferent renal sympathetic nerve activity noted during the two coronary occlusions are shown in Figure 3. Reflex increases in sympathetic outflow to the kidney were observed both during LAD occlusion with a LCx stenosis and during LCx occlusion with an LAD stenosis. There were no significant differences in the degree of reflex sympathoexcitation elicited by either transmural anterior or transmural inferoposterior myocardial ischemia.

The maximal changes in renal nerve activity that occurred during coronary occlusion are shown in Figure 4. The maximal increase in nerve activity during transmural anterior ischemia (22.6±3.9%) was not significantly different from the maximal nerve traffic increase during transmural inferoposterior ischemia (27.1±4.4%).

**Reflex Responses to Epicardial Bradykinin**

The maximal changes of renal nerve traffic observed in response to bradykinin applied to the anterior and inferoposterior epicardium are illustrated in Figure 5. In all cases, epicardial bradykinin elicited large increases in renal nerve activity. Application of bradykinin to the anterior left ventricle resulted in reflex increases in nerve activity that were not significantly different from those observed during application of bradykinin to the inferoposterior wall.

There were no significant differences in baseline pressures or heart rate measured before epicardial bradykinin (Table 1). Epicardial bradykinin was associated with significant increases in mean arterial pressure. During anterior bradykinin application, mean arterial pressure increased from 117±4.3 to 131±3.5 mm Hg.
Figure 6. Bar graph showing mean±SEM maximal percent changes in renal sympathetic nerve activity (RSNA) observed during injection of bradykinin into the left anterior descending coronary artery (LAD, solid bar) and into the left circumflex coronary artery (LCx, open bar).

(p=0.006). During inferoposterior bradykinin application, mean arterial pressure increased from 114±7.1 to 126±8.0 mm Hg (p=0.003). There were no significant changes in left atrial pressure or heart rate during epicardial bradykinin application.

**Reflex Responses to Intracoronary Bradykinin**

Intracoronary bradykinin also elicited large reflex increases in renal sympathetic nerve activity (Figure 6). Injection of bradykinin into the LAD resulted in maximal reflex nerve traffic increases that were not significantly different from the responses observed during injection of bradykinin into the LCx.

In contrast to the effects of bradykinin applied topically, intracoronary injection was associated with significant decreases in mean arterial pressure. During LAD injection, mean arterial pressure decreased from 121±6.6 to 62±4.7 mm Hg (p<0.0001). During LCx injection, mean arterial pressure decreased from 119±9.2 to 66±7.6 mm Hg (p<0.0001). There were no significant differences in baseline left atrial pressure or heart rate values measured before each injection (Table 1). Although small decreases were observed in left atrial pressure after bradykinin injection, these changes were not statistically significant (LAD, -2.2±1.0 mm Hg change; LCx, -2.0±1.0 mm Hg change). There were no significant changes in heart rate associated with intracoronary bradykinin (LAD, 9±4 beats per minute change; LCx, 9±5 beats per minute change).

Estimation of the amount of myocardium exposed to intracoronary bradykinin in six experiments indicated that there was no significant difference in the size of the perfusion beds supplied by either the LAD or LCx coronary cannula (LAD, 40.3±5.7 g; LCx, 35.2±3.3 g).

**Discussion**

The major new finding of our study is that left ventricular sympathetic afferents are distributed uniformly in the anterior and inferoposterior walls. This conclusion is based on measurement of reflex responses to receptor activation by three distinct methods—regional transmural myocardial ischemia, regional intracoronary bradykinin injection, and regional epicardial bradykinin application. Reflex excitatory responses mediated by sympathetic afferents were elicited equally by stimuli applied to either the anterior or inferoposterior walls of the left ventricle.

Ventricular receptors with vagal afferents are known to be preferentially distributed to the inferoposterior wall of the left ventricle. This observation is based on experimental data in animals and on clinical observations in humans that indicate that stimuli applied to the inferoposterior left ventricle elicit reflex responses of greater magnitude than do stimuli applied to the anterior left ventricle. For instance, chemical activation of ventricular vagal afferents elicits greater reflex inhibitory responses both in animals and humans when the chemicals are injected into the coronary artery that supplies the inferoposterior wall than when they are injected into the artery that supplies the anterior wall.\textsuperscript{6,16} In addition, reflex inhibitory responses associated with myocardial ischemia are larger and are observed more frequently during ischemia involving the inferoposterior wall than during ischemia of the anterior wall.\textsuperscript{5,7,8,17}

Although it has not been known previously whether sympathetic afferents are preferentially distributed to a particular region of the left ventricle, some data indicate that sympathetic afferents are located primarily in the superficial epicardial layers.\textsuperscript{9} The results of recent experiments from our laboratory support this concept. We demonstrated that reflex excitatory responses could be elicited by myocardial ischemia in dogs if the ischemia was transmural in nature and involved the superficial epicardial layers.\textsuperscript{10} Ischemia that was limited mainly to the endocardial layers did not evoke excitatory reflexes. We used our model of transmural myocardial ischemia to test the hypothesis that sympathetic afferents are preferentially distributed in the left ventricle, as are the vagal afferents. We further investigated this possibility by measuring reflex responses to regional epicardial and intracoronary administration of bradykinin. The rationale for our current experiments is similar to the rationale for the experiments that demonstrated preferential distribution of the vagal afferents. Specifically, if ventricular sympathetic afferents are preferentially distributed to the anterior or inferoposterior wall, we would hypothesize that significantly greater reflex excitatory responses would be elicited by transmural ischemia of that region or by intracoronary or epicardial administration of bradykinin to that region.

Our experimental results indicate that the magnitudes of reflex excitatory responses observed during transmural anterior myocardial ischemia are comparable to those observed during transmural inferoposterior ischemia. Furthermore, the reflex responses elicited by intracoronary or epicardial bradykinin to the anterior wall are similar to the reflex changes observed when bradykinin was administered to the inferoposterior wall. The similarity of these reflex responses to both ischemic and chemical stimulation does not support the concept that sympathetic afferents are clustered in one particular area of the left ventricle.

Although we did not quantify myocardial blood flow during these coronary occlusion experiments, we previously have validated our technique for producing transmural myocardial ischemia.\textsuperscript{10,15} In our previous experiments, measurement of myocardial blood flow with radiolabeled microspheres confirmed that occlusion of the LAD while a stenosis was in place on the LCx
resulted in significantly greater flow reductions involving a greater amount of epicardium in the ischemic risk region than did LAD occlusion alone. On the basis of these results, we feel that the combined coronary occlusion/coronary stenosis technique represents a simple and reliable method of creating transmural myocardial ischemia in dogs. Although it is possible that the degree of anterior transmural ischemia may have been different from the degree of inferoposterior ischemia in the present studies, we observed changes in mean arterial and left atrial pressures during each coronary occlusion that were similar. Since the hemodynamic responses to the two experimental maneuvers were not significantly different, it is unlikely that there were major differences in the degree of myocardial ischemia and mechanical ventricular dysfunction created by each coronary occlusion. Furthermore, it should be emphasized that our conclusions regarding the uniform distribution of left ventricular sympathetic afferents are based on the reflex responses to three different stimuli and not just on the response to regional ischemia.

Similarly, although we did not quantify coronary blood flow during the intracoronary bradykinin experiments, efforts were made to ensure that baseline arterial pressures (and thus coronary perfusion pressures) were similar before each coronary injection. In addition, we quantified the amount of myocardium supplied by the LAD and LCx arterial cannuulas; this analysis confirmed that the sizes of the perfusion beds were similar. As a result, it is unlikely that our experimental findings are affected by significant differences in the concentration of bradykinin being injected into each coronary artery or by significant differences in the amount of myocardium being exposed to intracoronary bradykinin.

Although there were differences in the magnitudes of reflex sympathoexcitation elicited by myocardial ischemia and bradykinin administration, we feel that these differences have little physiological significance. The reflex responses to intracoronary and epicardial bradykinin are purely dose dependent. If smaller doses of bradykinin had been administered, then smaller reflex responses would have been elicited, and the responses observed during ischemia and bradykinin would have been of similar magnitude. We used bradykinin as an experimental tool. As such, we administered a dose that would result in a strong stimulus to cardiac sympathetic afferents so that there would be a clearly perceptible change in renal nerve activity.

We observed major differences in the responses of arterial pressure to epicardial and intracoronary bradykinin. Epicardial bradykinin was associated with increases in arterial pressure, whereas intracoronary bradykinin elicited decreases in arterial pressure. These differences probably are related to the route of administration. Both epicardial and intracoronary bradykinin activate ventricular sympathetic afferents and elicit reflex increases in efferent sympathetic nerve activity. Normally, this reflex sympathoexcitation should increase vascular resistance and elevate arterial pressure, as was observed with epicardial bradykinin. However, bradykinin is a potent vasodilator. We have observed decreases in arterial pressure after intravenous and intra-arterial bradykinin that are similar to those observed after intracoronary bradykinin (unpublished observations). Bradykinin administered by the intracorony route probably enters the systemic circulation and lowers arterial pressure by a systemic vasodilatory action. In our experiments, this direct vascular effect of bradykinin completely negated the expected effects of augmented sympathetic outflow on vascular resistance and arterial pressure.

In summary, we have demonstrated that there are no significant differences in the excitatory reflexes mediated by ventricular sympathetic afferents in response to ischemic or chemical stimuli applied to either the anterior or inferoposterior left ventricle. This finding indicates that the sympathetic afferents are not preferentially distributed to the anterior or inferoposterior left ventricle. This differs from the vagal afferents, which are located mainly in the inferoposterior wall. Based on this observation, we conclude that there is uniform and equal distribution of sympathetic afferents in the anterior and inferoposterior left ventricle. As a result, we hypothesize that reflex excitatory responses mediated by ventricular sympathetic afferents could occur during clinical ischemic events that involve either the anterior or inferoposterior regions. This is in contrast to the depressor reflexes mediated by the vagal afferents, which are more likely to occur during ischemia of the inferoposterior region.

Acknowledgments

We wish to acknowledge the technical contributions of Teresa L. Cersley and David B. Brands to these experiments.

References


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*Circulation*. 1993;87:240-246
doi: 10.1161/01.CIR.87.1.240
*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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