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Cardiac Sympathetic Activity During Acute Myocardial Ischemia.

Brett Hamilton Neely
University of Alabama at Birmingham

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CARDIAC SYMPATHETIC ACTIVITY DURING ACUTE MYOCARDIAL ISCHEMIA

by

BRETT HAMILTON NEELY

A DISSERTATION

Submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Department of Physiology and Biophysics in The Graduate School, University of Alabama at Birmingham

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1989

ABSTRACT OF DISSERTATION
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Title CARDIAC SYMPATHETIC ACTIVITY DURING ACUTE MYOCARDIAL ISCHEMIA

Efferent sympathetic activity was simultaneously recorded from two thoracic cardiac nerves in each of a total of 67 chloralose anesthetized adult dogs. Prior to recording, efferent innervation patterns were determined by electrical stimulation for each nerve. In each animal one of the nerves selected for recording was shown to innervate the proposed ischemic region, while the other nerve was selected because it was shown to innervate non-ischemic regions. Left ventricular ischemia (15-30 minutes) was produced by the total occlusion of a branch of either the left anterior descending (LAD) or left circumflex (LCX) coronary arteries. Heart rate was controlled by electrical pacing.

In animals with intact sympathetic and vagal afferents (n=22), cardiac efferent sympathetic activity to ischemic regions was found to decrease in response to coronary occlusion (LAD or LCX). In contrast, in the same animals cardiac efferent sympathetic activity to non-ischemic regions was either unchanged (LAD) or increased (LCX) following coronary occlusion. In animals (n=7) subjected to partial thoracic sympathectomy (severance of the right and left anterior ansae), preganglionic sympathetic activity to ischemic regions was also found to decrease in response to coronary occlusion (LAD or

LCX), while activity to non-ischemic regions was unchanged. Bilateral cervical vagotomy (n=10) or local cardiac deafferentation with phenol (n=8) prior to coronary occlusion prevented the differential cardiac efferent sympathetic neural responses to coronary occlusion. Fourteen animals with intact sympathetic and vagal afferents were subjected to reperfusion and a second 30-minute coronary occlusion. Cardiac efferent sympathetic activity to ischemic, as well as non-ischemic, regions remained unchanged during the second LAD or LCX occlusion. Mean arterial pressure did not change significantly in response to coronary occlusion in any of the animals studied.

Taken collectively, our findings indicate that 1) regional left ventricular ischemia elicits differential efferent sympathetic neural responses which are characterized by decreased sympathetic activity to ischemic regions with either no change (LAD) or increased activity (LCX) to non-ischemic regions, 2) the differential reflex changes in cardiac efferent sympathetic activities are dependent upon both the location of the ischemic insult and the efferent destination of the recorded nerves, 3) the ischemia-induced reflex changes in cardiac efferent sympathetic activities are due to afferent signals originating from the ischemic region, 4) both cardiac sympathetic and vagal afferent fibers participate in the ischemia induced cardio-cardiac reflex, and 5) the reflex sympathetic response to regional left ventricular ischemia is attenuated by a previous ischemic insult.

Abstract Approved by: **Committee Chairman** G R Hageman

Program Director G R Hageman

Date _____ **Dean of Graduate School** Jerry L Duckett

TO MY MOTHER AND FATHER
Gloria and James

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CHAPTER I

INTRODUCTION

An understanding of the neural events associated with myocardial ischemia is of great importance, since many if not all of the current therapeutic interventions exert effects upon the autonomic nervous system. It is well known that myocardial ischemia alters the function of cardiac myocytes (2, 15, 20, 44, 80, 130, 167), but, in addition, the same ischemic insult may exert some of its effects through alterations in the function of efferent cardiac nerves.

Since the initial observations were made concerning the genesis of reflexes from the mammalian ventricle over 100 years ago (39, 177), many new techniques have been applied to study cardiac reflexes. Most notable was the development of the technique which allowed direct recording of the activity in autonomic nerves. Current technology has advanced the technique to the extent that now not only can one observe and record neural activity, but also precisely quantitate the level of activity.

From observations made in both man (53, 139, 180, 185) and experimental animals (52, 104, 141, 152, 159, 185), several generalized conclusions have been reached concerning the status of the autonomic nervous system during myocardial ischemia. It was found that soon after the occurrence of a confirmed ischemic event plasma catecholamines became elevated (53, 139, 152, 180, 183, 185). The magnitude of the changes in plasma catecholamines were found to differ depending on the

region of myocardium subjected to ischemia (183). It was concluded that, in man, the predominant response to anterior myocardial ischemia was increased sympathetic nervous system activity, while posterior ischemia elicited increased parasympathetic nervous system activity. In none of these studies (52, 53, 104, 139, 141, 152, 159, 180, 183) was neural activity recorded from either sympathetic or parasympathetic nerves.

More recently, activities in mostly sympathetic (26, 37, 47, 49, 50, 57, 98, 99, 101, 110, 116, 169, 179, 185) and some parasympathetic (37, 57) nerves have been recorded during acute myocardial ischemia. In many of the studies (49, 57, 98, 101, 110), sympathetic activity was recorded from only one nerve (not necessarily cardiac) and was found to increase in the presence of myocardial ischemia, although several studies (37, 47, 50, 98, 101, 116, 179) did report decreases. The duration of the ischemic event was limited to less than 5 minutes in almost all of these studies. In addition, the ischemic event was usually global, being produced by occlusion of the left main coronary artery or one of its major branches. Furthermore, no significant differences in neural responses were found between anterior or posterior myocardial ischemia. Thus, to date, little direct evidence exists supporting the conclusions concerning the status of the cardiac autonomic nerves during acute myocardial ischemia (53, 139, 180).

The purposes of this investigation were to a) produce small localized anterior or posterior ischemic insults via occlusion of either a diagonal branch of the left anterior descending or marginal branch of the left circumflex coronary arteries, b) simultaneously record changes

in two cardiac sympathetic nerves for a period of up to 30 minutes of myocardial ischemia, c) compare and contrast the systemic hemodynamic and cardiac neural reflex responses to anterior and posterior ischemic events, d) determine the influence(s) of multiple coronary artery occlusions upon hemodynamic and neural responses, and e) determine the influence(s) of a cardiac-selective versus cardiac-non-selective deafferentation upon the ischemia induced changes in hemodynamic and neural responses to coronary occlusion.

CHAPTER II

LITERATURE REVIEW

Cardiac Innervation

The anatomy of the cardiac nerves has been extensively studied in the dog (5, 6, 93, 105, 123). The greatest limitation of these studies is the lack of functional correlates during the anatomical identification. Studies performed by Mizeres (124-127) were the first attempts at describing structure-function relationships for the cardiac nerves. More recently, Randall and others (6, 7, 54, 60, 62, 144-146, 165) have rigorously examined structure-function relationships of the cardiac nerves in the dog. Detailed descriptions of the intrinsic innervation of the heart have previously been thoroughly reviewed (5, 6, 93, 105). Therefore, discussion of the innervation of the heart will be limited to points relevant to this study.

In the right thorax of the dog, arising from the cranial pole of the right stellate ganglion are the ventral (anterior) and dorsal (posterior) ansae subclavia (143, 144, 146). The ventral ansa courses ventral to the subclavian artery and connects with the thoracic vagus at the level of the stellate ganglion. The dorsal ansa courses dorsal to the subclavian artery and interconnects with the (right) middle cervical ganglion. Both the ventral and dorsal ansae subclavia are considered sympathetic nerves and act to convey information between the stellate and cervical ganglia. Arising from the dorsal ansa are

several small neural branches which are known collectively as the right stellate cardiac nerve. The right stellate cardiac nerve has been shown to be the major if not exclusive route of efferent sympathetic activity to the region of the sinoatrial node (144). Electrical stimulation of the right stellate cardiac nerve produces dramatic positive chronotropic responses and positive inotropic responses from the atria (144). Stimulation of the right stellate cardiac nerve produces profound responses in the atria; however, similar responses are not elicited from either the atrioventricular node or ventricles (144). In the anesthetized dog, transection of either the right stellate ganglion, the ansae subclavia or the right stellate cardiac nerve exerts a profound negative chronotropic effect (144, 176). Armour (3) has shown the right stellate cardiac nerve to contain few afferent fibers.

Also in the right thorax of the dog arising from the right thoracic vagus inferior to the ventral ansa subclavia are several major cardiac branches which have been denoted as the craniovagal and caudovagal nerves by Mizeres (124). Histological studies (5, 144) employing the horseradish peroxidase (HRP) technique have demonstrated the presence of both sympathetic and parasympathetic fibers in both the craniovagal and caudovagal nerves. Electrical stimulation of either the craniovagal or caudovagal nerves has been shown to produce mixed efferent sympathetic and parasympathetic effects (7). The majority of the efferent parasympathetic effects are limited to the right side of the heart, while the efferent sympathetic effects have been demonstrated to influence both right and left sides (3, 7). The mixed sympathetic-parasympathetic efferent effects are not exclusively limited to either

the atria or ventricles and in fact have been shown to effect both regions of the heart (144). Both the craniovagal and caudovagal nerves have also been shown to contain few afferent fibers. When present, these fibers usually arise from the regions of the atrial-caval junctions, both atria and the right ventricle (3, 4).

The remainder of the nerves normally present in the right thorax of the dog include the right recurrent cardiac nerve and the thoracic vagosympathetic trunk (125). The right recurrent cardiac nerve has been shown to possess a relatively complex arrangement in relation to its anatomy. The caudal pole arises from the middle cervical ganglion (125) and ramifies with branches from the recurrent laryngeal and vagus nerves. HRP staining (7, 144) has demonstrated both sympathetic and parasympathetic neurons in the right recurrent cardiac nerve. Neurophysiological studies (3) have demonstrated that the recurrent cardiac conveys afferent information from the regions of the great vessels, atria, right ventricle, ventricular septum and occasionally the left ventricle. At the mid-thoracic level the right vagosympathetic trunk contains both afferent and efferent fibers from cardiac and non-cardiac structures.

In the left thorax of the dog several anatomical similarities exist with right sided nerves. Many of the left sided cardiac nerves originate from the middle cervical ganglion (126). Distal to the middle cervical ganglion, the anatomical arrangement of the (left) thoracic nerves becomes more variable due to interconnections between adjacent nerves (5). Arising from the cranial pole of the left stellate ganglion are the ventral (anterior) and dorsal (posterior) ansae subclavia. Both the ventral and dorsal ansae interconnect with the (left) middle

cervical ganglion. The ventrolateral cardiac nerve (VLCN) originates from the caudal pole of the middle cervical ganglion and courses laterally over the aorta and pulmonary arteries entering the pericardium near the region of the left atrium (124). As denoted by its name, the ventrolateral cardiac nerve is ventral and lateral to the left thoracic vagus. Additionally, the VLCN may have numerous interconnections with the thoracic vagus and occasionally with the left anterior ansa. The VLCN has been shown (144) to contain few if any parasympathetic neurons and as such is considered to be primarily a sympathetic nerve. The VLCN normally innervates a large portion of the left ventricle, most often the posterior side, as well as the region of the atrioventricular node (144). Studies (6, 7) have shown the VLCN to contain few afferent fibers. Afferent fibers in the VLCN, when present, have been found to arise from the region of the left atrium (3, 4).

Originating from the medial portion of the left thoracic vagus and receiving numerous connections from the middle cervical ganglion is the ventromedial cardiac nerve (VMCN). The VMCN courses ventral and medial to the left thoracic vagus giving off branches near the aorta and pulmonary bifurcation. The VMCN enters the pericardium near the region of the left atrium (144). The VMCN has been shown to variably contain both sympathetic and parasympathetic neurons, although its most prominent effects are sympathetic in nature (144). Electrical stimulation of the VMCN elicits positive inotropic responses from the anterior regions of both ventricles, most notably the left (144). The VMCN has been shown to contain few afferent fibers (3, 4).

The remainder of the nerves normally present in the left thorax of the dog include the innominate, the dorsal cardiac, the left recurrent

cardiac and the thoracic vagosympathetic trunk. The innominate nerve originates from the middle cervical ganglion and receives connections from the thoracic vagus and occasionally from the left anterior ansa and/or stellate ganglion (144). Mizeres (124) was unable to trace the innominate nerve all the way to the heart due to the diffuse anatomy of the nerve. It has subsequently been shown (5, 6) that the innominate nerve courses medially along the border of the innominate artery and enters the pericardium from the left dorsal side. Electrical stimulation of the innominate nerve elicits responses which appear to consist of both sympathetic and parasympathetic components (144). The innominate has been shown (3, 6) to contain numerous afferent neurons arising from the regions of the great vessels, both atria and both ventricles. Nonidez (134), in his study of the afferent innervation of the canine heart, proposed that the innominate nerve was the route for the baroreceptor information signalled in the aortic depressor nerves. The dorsal cardiac nerve arises from the medial portion of the middle cervical ganglion and courses medial to the left thoracic vagosympathetic trunk (126). Much like the innominate, electrical stimulation of the dorsal cardiac nerve can result in mixed responses consisting of both sympathetic and parasympathetic components (6). Neural recordings from the dorsal cardiac nerve have demonstrated pulse synchronous afferent activity (3). The small branches of the left thoracic vagus originating at the level of the heart are known collectively as the left recurrent cardiac nerve (62). These nerves have been demonstrated to contain a variable number of both sympathetic and parasympathetic neurons (62). Electrical stimulation of the left recurrent cardiac nerve frequently results in moderate negative chronotropic responses from the sinus node, with concomitantly strong

negative dromotropic responses from the atrioventricular node (62). It appears that these nerves may also contain afferent fibers, although due to their relatively greater variability this question remains unresolved. As in the right thorax, the left thoracic vagosympathetic trunk possesses many diffuse properties. At the level of the esophageal plexus practically no myelinated afferent fibers are found (69). Thus, it has been concluded that the majority of the medullated afferent fibers contained in the vagosympathetic trunk in the high thoracic or cervical regions are of either cardiac or pulmonary origin (6, 134).

Cardiac Reflexes

Since the initial observations (39, 177) concerning the genesis of reflexes from the mammalian ventricle, a great deal of information has accumulated concerning cardiac reflexes. It is now well known that cardiac reflexes may not only affect the performance of the heart, but also that of other organ systems (8, 25, 32). Therefore, the nomenclature used to describe the various cardiac reflexes is highly dependent upon the organ system under study.

To date, over 25 different studies have been published which examined reflexes originating from atrial receptors. The anatomical arrangement of the sensory fibers and their end-organ receptors is quite complex, which is reflected in their description by Linden (106-108) as being that of a net or web. Presently, the function of this sensory system still remains in part a mystery due to methodological limitations. The endocardium of both atria contains the greatest number of afferent fibers (131-134). Histological studies have reported the greatest number of unencapsulated and free nerve endings to be located ventricular refractory in the regions of the junctions of the superior

and inferior vena cava and the right atrium and the pulmonary veins and the left atrium (20, 90, 106-108).

The physiological functions of the atrial receptors have been debated since the early 1900's when Bainbridge (9) made his initial observations. Bainbridge concluded that the reflex increase in heart rate observed with rapid intravenous infusions was due to stimulation of receptors located in the region of the right atrial-caval junction (9). More recently, the work of Linden and others (59, 90, 106-108) has provided an accurate description of atrial reflexes. Linden's first observations (106) demonstrated that stretch of the region of the left atrial-pulmonary vein junction resulted in increases in heart rate with little or no change in arterial pressure. Further work on the atrial mechanoreceptor reflex revealed that the afferent signals travel primarily in the vagus (25, 107). Linden also concluded that the efferent response for the mechanoreceptor reflex is conveyed solely through the sympathetic nerves (106, 107). Whether the same reflex exists in the right atrium as proposed by Bainbridge is still debated, although Kappagoda and others (59, 87) have demonstrated similar increases in heart rate elicited by stretch of the right atrial-caval junction. Similar to the left atrial reflex, the right atrial mechanoreceptor reflex can be demonstrated within physiological levels (8-12 cm H_2O) of right atrial pressure.

Reflexes originating in the atria are primarily mechanoreceptive (107, 137, 138). Several chemical compounds can elicit reflex changes in heart rate similar to those observed with atrial distention, although it now appears that these agents exert their effects through nonspecific activation of sensory fibers (25, 32, 88, 107, 115, 137). The atrial mechanoreceptor reflex is influenced by changes in pH (108). The

greatest responses are observed at or slightly above physiological levels, while responses are attenuated during acidoses (108).

Reflex changes due to atrial mechanoreceptor activation are highly localized within the cardiovascular system. Mechanoreceptor activation causes neither changes in ventricular contractile performance nor changes in total peripheral resistance (107). Direct recordings of sympathetic efferent activity from nerves originating distal to the renal artery revealed no changes in activity in response to atrial distension (99). Simultaneous recordings of efferent sympathetic activities in the ansa subclavia and renal nerves demonstrated increased activity in the ansa and decreases in activity in the renal nerves during atrial distension (106). It has been proposed (107), that the function of the reflex tachycardia in response to atrial distention is to maintain the heart at a relatively constant size during periods of increased venous return by decreasing diastolic filling time.

While atrial reflexes elicit increases in heart rate and/or blood pressure, the direction and magnitude of changes due to ventricular reflexes depend on the type of sensory fibers which are activated and the type of the afferent stimulus. Similar to the atria, the architecture of the ventricular sensory fibers and their end organs has yet to be fully defined. A variety of unencapsulated endings have been demonstrated in the epicardium (138-160). Whether these same structures also exist in the endocardium or within the myocardium is unknown.

Since studying ventricular sensory receptors via histological techniques is complex (13), information concerning these receptors has been derived primarily from studies recording afferent neural activity in cardiac sensory fibers (4, 10, 23, 31, 33, 109, 114, 133, 172, 173).

Thus, the recorded activity cannot supply information concerning detection at the receptive site; instead, activity reflects both the detection and transduction of the afferent stimulus. This point is of critical importance since some of the cardiac sensory fibers (172, 173) show characteristics of accommodation upon prolonged exposure to certain stimuli. Therefore, one cannot separate the role of the receptive site versus that of the afferent neuron in the accommodation process.

In contrast to atrial reflexes, ventricular reflexes with vagal afferents generally elicit decreases in heart rate and/or blood pressure (40, 79, 94, 160, 169, 178). Ventricular mechanoreceptor reflexes were first described by Daly and Verney (40), who reported that occlusion of the aortic root elicited bradycardia and hypotension. Since distension of the pulmonary veins or the left atrium had no effect, it was concluded that the reflex must be of left ventricular origin. Electrical stimulation of vagal afferent fibers also results in hypotension and bradycardia (3, 31, 46, 138, 168). Measurement of conduction velocities and threshold stimulation voltages of vagal afferents eliciting depressor responses suggests that these fibers are nonmyelinated (3). Direct recording of afferent vagal activity in cardiac nonmyelinated vagal fibers has demonstrated activity unrelated to the cardiac cycle, as well as pulse synchronous activity (3, 4, 33).

Ventricular mechanoreceptor reflexes involving myelinated vagal afferent fibers have also been reported (4, 33). Afferent fibers characteristically exhibit pulse synchronous activity related to ventricular systole (31, 33). Electrical stimulation of ventricular myelinated vagal afferent fibers generally elicits increased heart rate and/or blood pressure (4, 31, 138). Brown (21, 22) has reported a

subpopulation of ventricular mechanoreceptors located either in or very near the coronary arteries which are responsive to intracoronary pressure. These coronary mechanoreceptors relay afferent impulses via both myelinated and unmyelinated vagal afferents (21, 22). The overall importance of myelinated vagal afferents has been questioned since they comprise only 20% of cardiac vagal afferents, and less than 10% of these fibers originate in the ventricles (4, 138).

Ventricular mechanoreceptors with sympathetic afferent fibers were first reported in 1936 by Nettleship (131) and later confirmed by Nonidez (134). Nerve endings of this class of mechanoreceptors are found throughout all chambers of the heart, appearing as free unmyelinated terminals located most extensively in extracellular spaces (23, 45). The free nerve endings are connected to the central nervous system by both myelinated (23, 45) and unmyelinated (115, 140) sympathetic afferent fibers. The myelinated fibers have been reported to be mainly of the A-delta class (4). Both myelinated and unmyelinated afferent fibers have their cell bodies in the dorsal root ganglion of the upper five thoracic (T1-T5) segments of the spinal cord (23, 115). Brown and Malliani (26) have also reported the existence of coronary mechanoreceptors with myelinated sympathetic afferent fibers. Similar to those with vagal afferents, these coronary mechanoreceptors are responsive to intracoronary pressure (26).

Electroneurographic studies (18, 31) of myelinated sympathetic afferent fibers of ventricular origin have reported pulse synchronous activity. Recordings from unmyelinated sympathetic afferents (4) have revealed tonic activity apparently unrelated to the cardiac cycle. Activation of ventricular mechanoreceptors with sympathetic afferents

requires stimuli of greater magnitude than those which excite mechanoreceptors with vagal afferent fibers (25). In addition, ventricular mechanoreceptors with sympathetic afferents have been shown to adapt to prolonged stimulation (23, 26, 70). Electrical stimulation of sympathetic afferent fibers elicits reflex increases in heart and blood pressure (113). Stimulation of ventricular sympathetic afferent fibers has also been demonstrated to result in decreased activity in efferent parasympathetic neurons (156, 157).

Ventricular chemoreflexes involving vagal afferent fibers were reported as early as 1867 when von Bezold and Hirt (177) used extracts of veratrum alkaloids to elicit a depressor reflex. Subsequently, Jarish and Zotterman (79) confirmed and extended the findings of von Bezold and Hirt (177). In 1954 Dawes and Comroe (41) examined the data concerning cardiovascular chemoreflexes and developed nomenclature which more accurately described each reflex. Thus, the chemoreflex observed by von Bezold and others became known as the coronary chemoreflex (41, 119), or what now is also commonly known as the Bezold-Jarish reflex. Studies by Coleridge and associates (32, 33) determined that the agents employed to elicit the coronary chemoreflex activated unmyelinated vagal afferent fibers. It is presently believed that unmyelinated vagal afferents are responsible for the majority of the depressor chemoreflexes originating in the ventricles (32).

Ventricular chemoreflexes with sympathetic afferent fibers have also been reported (10, 114). Activation of this type of sympathetic afferent fiber results in increased heart rate and/or blood pressure (10, 115, 136, 140). The neural structure responsible for the detection of chemical stimuli has not been determined, but it appears that the

free nerve endings of the sympathetic afferent fibers are the most likely candidates (4, 25, 32). Much of the pioneering work concerning sympathetic chemoreflexes in the ventricles was performed by Coleridge and associates (10, 31-33). Chemical agents such as bradykinin, veratridine, cyanide, serotonin and histamine have all been demonstrated to excite sympathetic afferent fibers (10, 133). Brown and Malliani (26) have demonstrated that sympathetic afferents responsive to changes in intracoronary pressures are also sensitive to chemical agents, most notably the veratrum alkaloids. The existence of ventricular sympathetic chemoreflexes has previously been debated (25), since intracoronary injection of the above chemical agents elicits primarily decreases in heart rate and blood pressure, while epicardial application elicits increases in rate and pressure (13, 163). This seeming disparity has been demonstrated to be due to the relative potency of depressor reflexes (163). In vagotomized animals, intracoronary bradykinin causes reflex increases in heart rate and blood pressure (10, 13, 163). Conversely, intracoronary bradykinin in sympathectomized animals with intact vagi causes reflex decreases in heart rate and blood pressure (10, 13). Thus, it is believed that vagal depressor influences on the cardiovascular system predominate when both vagal and sympathetic afferent fibers are stimulated by blood borne agents. This conclusion is supported by the observation that the pressor effects of agents like bradykinin are amplified after bilateral vagotomy (163).

Acute Myocardial Ischemia

Ischemia is a condition which occurs when the delivery of oxygen and nutrients and removal of metabolites by the coronary circulation is inadequate (2, 15, 19, 28, 80, 81, 96, 130). The cellular effects of

ischemia upon the myocardium can be divided into two categories: the direct effects on the myocyte and the indirect effects due to alterations in cardiac regulatory processes (i.e., reflexes). Attempts have been made to study the direct effects of ischemia upon the ventricular myocyte, but have been fraught with the persistent problem of whether or not the experimental conditions accurately mimic those in the intact ischemic heart. Hypoxia has been employed to mimic conditions during ischemia, although it is known that ischemia differs from hypoxia in that a small but variable amount of oxygen delivery is still maintained to the myocytes (2, 20). Furthermore, during ischemia but not hypoxia, the removal of metabolites such as potassium ions, hydrogen ions and lactic acid is severely impaired because of the reduced coronary blood flow (28, 58, 80, 96).

It has been shown that during anoxia, and presumably also during ischemia, there are changes in membrane transport of substrates and ions. Metabolic processes within the myocyte are also altered during ischemia, resulting in increased glycolysis and inhibition of pyruvate and fatty acid oxidation (132, 148, 149). The result of these changes is the depletion of ATP and creatine phosphate and increases in the concentrations of ADP, AMP, phosphate, lactate and the NADH:NAD ratio (128, 132, 148, 149). Many of the these changes are due directly attributable to the shift from aerobic to anaerobic metabolism during ischemia (132).

During acute ischemia the myocardium becomes depleted of energy stores; therefore, processes such as protein turnover become deranged (17, 30, 89, 128). Incorporation of tritiated amino acids into membrane proteins has been reported to be greatly slowed during acute ischemia

(17, 30, 89, 128). Protein synthesis during acute ischemia has been reported to be greater than during anoxia, but still significantly less than during aerobic conditions (30, 89). Thus, without the synthesis of the necessary membrane proteins the ischemic process can progress to the point of irreversibility.

Over 40 years ago Tennant and Wiggers (167) reported that following total occlusion of a coronary artery the ischemic myocardium lost much, if not all, of its contractile function. Recent studies (20, 73, 120, 170) have confirmed these findings, demonstrating that the ischemic myocardium becomes partially to totally asynergic and/or akinetic during even brief ischemic episodes. During prolonged bouts of ischemia (> 45 minutes), severely compromised myocardium may exhibit paradoxical movement (170). Should the ischemic region be sufficiently large, left ventricular function can become compromised. Early phases of ventricular failure are characterized by increases in end diastolic pressure and end diastolic volume with concomitant decreases in stroke volume, stroke work and ejection fraction (20). These changes will result in decreased cardiac output, which if sustained, results in heart failure. Left ventricular failure shows hemodynamic manifestations when 20 to 25% of the left ventricle becomes akinetic (2).

Alterations in heart rate and blood pressure associated with the presence of myocardial ischemia have been reported in man (119, 180) and experimental animals (152, 185). Pantridge and associates (139, 180) reported the occurrence of autonomic disturbance at the onset of myocardial ischemia in man. Evidence of sympathetic hyperactivity was indicated by tachycardia (> 100 beats/minute) and/or hypertension (> 160/100 mm Hg). Parasympathetic hyperactivity was indicated by

bradycardia (< 60 beats/minute) and/or atrioventricular block with or without hypotension (< 100 mm Hg systolic pressure). In patients admitted within 30 minutes of the onset of symptoms, only 17% had heart rates or blood pressures within normal limits (119). More than 30% of the patients exhibited signs of increased sympathetic activity, while 50% had signs of increased parasympathetic activity. Eight percent of all patients had complete atrioventricular block. In contrast, in a subgroup of patients admitted 30 minutes or more after the onset of symptoms, only 56% exhibited signs of autonomic disturbance (119). The incidence of pressor or depressor responses was found to be correlated with the location of the ischemic myocardium. Pressor responses were more frequent with anterior ischemia, while depressor responses were more frequent with posterior ischemia.

Alterations in cardiac regulatory processes due to myocardial ischemia have been shown to involve both neural and humoral elements (142). In man (53, 139, 180, 185) and experimental animals (152, 183, 185) the observed increases in heart rate and/or blood pressure can be correlated with increases in plasma catecholamines. Increases in both norepinephrine and epinephrine were found (152); therefore, it is likely that both the sympathetic nerves and the adrenal glands contributed to the observed changes. It is also likely that the sympathetic reflex response to myocardial ischemia progresses through stages involving different mechanisms, since the increases in plasma catecholamines (152, 183, 185) occur several minutes after the onset of ischemia but at a time when blood pressure is already elevated.

The candidate realizes that the term myocardial ischemia covers a broad topic. Therefore, in all of the subsequent discussions either of the terms acute coronary artery occlusion or myocardial ischemia may be

used interchangeably. It is also recognized that the degree of myocardial ischemia will change considerably throughout the course of a coronary occlusion. However, since the major thrust of this proposal was to determine changes in cardiac efferent sympathetic activities, no attempt has been made to quantitate the degree of ischemia at any one measurement point. The amount of ischemic myocardium was instead determined upon completion of the occlusion protocol. All occlusions employed in the study were single stage complete (100%) occlusions. It should be noted however, that these coronary occlusions can be considered as acute and not chronic. Finally, the candidate also recognizes that the possibility exists that factors other than ischemia (e.g. low coronary flow, stretch etc.) may play a role in determining the sympathetic reflex response to acute coronary occlusion. However, since all animals were subjected to similar protocols, I have chosen to use the term ischemia in its broadest sense.

Sympathetic Influences During Myocardial Ischemia

A great deal of information has appeared in the literature concerning the role of the autonomic nervous system in the initiation and maintenance of arrhythmias (34, 56, 60, 71, 102, 111, 117, 118, 145, 147, 154, 175). Han and associates (64-67) demonstrated the potentially deleterious effects of activation of sympathetic nerves upon the recovery of excitability (i.e., repolarization) in the ventricles. Intravenous infusions of catecholamines produced uniform shortening of ventricular refractory periods, while stimulation of sympathetic nerves resulted in nonuniform shortening of refractory periods (64-67). These investigators concluded that the greater temporal dispersion of

periods due to stimulation of sympathetic nerves could play a critical role in the genesis of arrhythmias (64-67). Studies examining ventricular vulnerability (75, 122) and ventricular fibrillation threshold (76, 95) have reported significant alterations of both parameters in the presence of catecholamines. Stimulation of the stellate ganglia (75, 76, 95, 122) increased ventricular vulnerability to electrical extrastimuli and lowered ventricular fibrillation thresholds in otherwise normal myocardium. Myocardial ischemia has also been reported to increase ventricular vulnerability to extrastimuli as well as decreased ventricular fibrillation threshold (27, 43). The combination of both ischemia and increased catecholamines results in synergistic changes in ventricular vulnerability to extrastimuli and ventricular fibrillation threshold (27, 43).

From the available data (118), it now appears that activation of individual sympathetic or parasympathetic nerves may not by itself be sufficient to result in arrhythmias. In fact, it has been demonstrated (118) that arrhythmogenesis is greatest upon simultaneous activation of both sympathetic and parasympathetic nerves. During myocardial ischemia, the influences of the sympathetic and parasympathetic nervous systems alone or in combination become critical factors when considering the electrical and mechanical stability of the heart. Therefore, the benefits and drawbacks of autonomic support or autonomic blockade (14, 35, 55, 74, 100, 142, 150, 153) during an ischemic event has become a topic of great controversy. Further complicating the task of assessing the roles of the sympathetic and parasympathetic nervous systems during ischemia are Pantridge and associates findings (139, 180) that demonstrated that the reflex responses to ventricular ischemia could not be

characterized solely as pressor or depressor. In man (139), the presence of depressor reflexes were unmasked upon blockade of adrenergic receptors during anterior ischemia, while pressor responses were unmasked during posterior ischemia upon blockade of muscarinic receptors. Thus, simultaneous activation of both limbs of the autonomic nervous system during myocardial ischemia seems likely based upon the available information in man.

Another line of evidence supporting the involvement of the autonomic nervous system in the electrical and mechanical instability of the heart during acute ischemia are the reported beneficial effects of cardiac denervation (51, 68, 82-85, 171). Both acute (85, 171) and chronic (51, 82-84, 171) total cardiac denervation have been reported to decrease the incidence of severe cardiac arrhythmias associated with ischemia. Sectioning of the sympathetic nerves has been demonstrated to lower the frequency of premature ventricular beats (71). Chronic sympathetic denervation has been demonstrated to be more effective than acute denervation (171). Differences between chronic and acute sympathectomy were reported (92, 171) to be due to the depletion of tissue catecholamine stores in chronic, but not acute denervations. This point is of critical importance, since changes in cardiac tissue catecholamines have been observed during ischemia (72, 184). Recent studies (155, 158, 159) have demonstrated several complex interactions resulting from partial cardiac sympathectomy. Sectioning of the left stellate ganglion alone has been reported (158, 159) to decrease the incidence of ventricular dysrhythmias during and following an ischemic event, whereas removal of the right stellate ganglion has been reported to be even more arrhythmogenic than leaving all sympathetic nerves intact (154).

Although the early data derived from humans and experimental animals supported the conclusion that the cardiovascular changes associated with myocardial ischemia involved reflex mechanisms, direct evidence of an ischemia-induced cardio-cardiac reflex was not available prior to 1963. Costantin (37) was the first to record and report changes in efferent sympathetic activity during myocardial ischemia. Unfortunately, no method existed to quantitate these changes; therefore, only qualitative descriptions were published. Malliani and associates (116) subsequently confirmed Costantin's findings and were the first to quantitate sympathetic preganglionic efferent activity during acute myocardial ischemia. Of utmost importance to Malliani's study (116) was the fact that the animals had been vagotomized prior to occlusion so that only the sympathetic nerves were left intact. Thus, it was demonstrated that both the afferent and efferent pathways of the reflex involved the sympathetic nerves (116).

Although many studies which recorded cardiac sympathetic efferent activity during acute myocardial ischemia (49, 57, 98, 101, 110) support Malliani's findings (116), several reports (37, 47, 50, 98, 101, 116, 179) have demonstrated decreased sympathetic activity during acute ischemia. In studies which reported increased cardiac sympathetic activity (49, 57, 110), it was not uncommon to find that the ischemic insult involved the entire left ventricle or that the vagi had been transected prior to the coronary occlusion. In contrast, in the studies reporting decreased sympathetic activity during acute ischemia (37, 47, 50, 98, 101), the location of the ischemic myocardium was usually localized and all nerves other than those employed for recording remained intact. Thus, similar to man (119, 139, 180), in the neurally

intact animal preparation, depressor responses may outweigh pressor responses depending on the extent and location of the ischemic region.

Changes in sympathetic efferent activities to non-cardiac organs during myocardial ischemia have been reported (87, 98, 99, 164, 169). Simultaneous recordings of efferent sympathetic activities in the periarterial ear nerve and in the splanchnic nerve of the rabbit (98) have demonstrated differential changes in activity upon coronary artery occlusion. Shortly after the onset of the coronary occlusion, activity in the splanchnic nerve was reported to increase, while activity in the ear nerve was reported to decrease (98). Following coronary occlusion, efferent sympathetic activity to the kidney has been reported to decrease at a time when blood pressure is reduced (169). This finding is particularly important, since at comparable levels of hypotension produced by hemorrhage, sympathetic activity to the kidney has been shown to increase (25, 169).

Recently, attempts have been made to assess the level of involvement of the central nervous system in the reflex alterations in autonomic activity in response to acute myocardial ischemia (47, 166, 169, 179). The available data (114, 116) suggest that the sympathetic responses to coronary occlusion do not require higher integrative centers for their manifestation. Thus, the sympathetic reflexes elicited by coronary occlusion were concluded to be of spinal origin (114, 116). However, the depressor response to coronary occlusion appears to involve central integration as well as significant interactions with areas controlling sympathetic outflow as suggested by the effects of vagotomy (116). Both the pressor and depressor sympathetic and reflex responses to acute myocardial ischemia have been shown to be modulated by descending information from the brain (47, 166,

169, 179). For example, baroreceptor activation alters the magnitude but not the direction of the changes in the efferent sympathetic activities during acute myocardial ischemia (116, 166).

Summary

It is currently not known whether acute coronary occlusion elicits reflex changes in all of the cardiac efferent nerves equally or whether there are only changes in certain discrete populations of nerves. It is also not known whether or not the location of the occluded coronary artery plays a role in determining the direction and magnitude of the ischemia-induced reflex changes in cardiac efferent sympathetic activities. Differences in the reflex response to acute coronary occlusion which may be correlated to the location of the ischemic myocardium may be due to differences in cardiac vagal and/or cardiac sympathetic afferent signalling. Furthermore, it is not known whether or not the effects of coronary occlusion are cumulative. Thus, it is not known whether changes in cardiac sympathetic activities will be sustained throughout coronary occlusions longer than 5 minutes or whether a second coronary occlusion will elicit similar changes in cardiac sympathetic activities. Finally, no information exists concerning changes in cardiac efferent sympathetic activities during the initial reperfusion of the ischemic myocardium.

CHAPTER III

SPECIFIC AIMS

The objective of this research was to determine whether or not during acute myocardial ischemia there exists a cardio-cardiac reflex which can result in an reflex imbalance of sympathetic efferent activities to the heart. The hypothesis considers that the location of the ischemic ventricular myocardium, duration of the coronary occlusion, the number of ischemic insults and the type of afferent innervation each plays a role in determining the direction and magnitude of changes in sympathetic activities to the heart. It is further hypothesized that each of the above factors will differentially affect cardiac sympathetic activity during the ischemic period versus the period immediately following the release of the coronary occlusion (i.e. reperfusion). Six specific aims were designed to test the hypothesis.

Specific Aim 1. To determine the effects of atrial pacing upon cardiac efferent sympathetic activities.

Specific Aim 2. To determine the effects of the location of the ischemic ventricular myocardium upon reflex changes in cardiac efferent sympathetic activities.

Specific Aim 3. To determine the effects of the duration of the coronary artery occlusion upon the magnitude of reflex changes in cardiac efferent sympathetic activities.

Specific Aim 4. To determine the effects of a second coronary artery occlusion upon reflex changes in cardiac efferent sympathetic activities.

Specific Aim 5. To determine the effects of selective deafferentation performed prior to coronary occlusion upon ischemia-induced changes in cardiac efferent sympathetic activities.

Specific Aim 6. To determine the effects of an initial 5 - minute reperfusion period upon cardiac efferent sympathetic activities.

CHAPTER IV

METHODS

General Preparations

Sixty-seven adult mongrel dogs of either sex weighing 16-27 kg were sedated with ketamine hydrochloride 100 mg I.M. (Ketalar (R), Parke-Davis) and anesthetized with alpha-chloralose (Sigma Chemical, St. Louis, Mo.) 100 mg/kg I.V. (38, 59, 63).

Ventilation was maintained through a cuffed endotracheal tube attached to an intermittent positive-pressure Harvard pump supplying room air. Central aortic pressure (catheter inserted and advanced into the aorta from a femoral artery) and a limb lead electrocardiogram (II, III or aVR) were recorded in every animal. Mean arterial pressure was electronically derived from the aortic pressure signal. Heart rate was derived by an analog tachograph triggered by the R wave of the electrocardiogram. Core temperature was continuously monitored by a rectal probe and maintained at 37 ± 1°C. Animals were paralyzed with succinylcholine chloride (Sigma Chemical) 0.5 mg/ml I.V. drip. Anesthesia was supplemented (with alpha-chloralose) at the rate of 10 mg/kg/hr.

A bilateral thoracotomy was performed through the fourth intercostal space. To facilitate access to the small thoracic cardiac nerves, we removed the second and third ribs. The pericardium was incised, reflected and attached to form a cradle for the heart. A bipolar pacing electrode was sutured on the left atrial appendage. A

16-18 French balloon catheter was inserted into the remaining femoral artery to be used to elicit changes in aortic pressure during testing of nerve preparations (see below).

Determination of Innervation Patterns

Regional myocardial contractile force was measured with Walton-Brodie strain gauge arches (12 mm; 120 Ohm) sutured to the epicardium of the anterior and posterior left ventricle. At least one gauge was placed in the region distal to the coronary artery undergoing occlusion while the remaining gauge was located on the opposite surface of the left ventricle (Figure 1). References to ventricular contractile performance within the text refer to ischemic regions only.

In the right thorax, we identified and carefully isolated six cardiac nerves (stellate cardiac, recurrent cardiac, craniovagal, caudovagal, anterior and posterior ansa subclavia) (5, 6). In the left thorax, we identified and carefully isolated seven additional cardiac nerves (ventrolateral cardiac, ventromedial cardial, dorsal, recurrent cardiac, innominate, anterior and posterior ansa subclavia) (5, 6). Each intact nerve was electrically stimulated for 10 seconds with a square wave stimulator (Grass S-44) and stimulus isolation unit (Grass SIU5) at 4-20 Hz, 2 msec. pulse duration and 10-15 volts. Heart rate, blood pressure, electrocardiographic and strain gauge responses to neural stimulation were recorded on an eight-channel polygraph (Hewlett-Packard 7888-A) and were used to assess the innervation pattern of the nerve under scrutiny (Figure 2).

Once innervation patterns had been determined for all of the thoracic cardiac nerves, two nerves were then selected for recording of efferent sympathetic activity. One nerve was selected such that it was

Figure 1

Schematic representation showing the anatomy of the left anterior descending (LAD) and left circumflex (LCX) coronary arteries. In this example, an occlusion would be performed at the level of the third marginal branch (M3) of the LCX. Placements of Walton-Brodie strain gauge arches for purposes of assessing ventricular contractile performance are also illustrated. The region which would be painted with phenol (in animals subjected to local cardiac deafferentation prior to the coronary artery occlusion) is denoted by the shaded area (see text for details). RA = right atrium; LA = left atrium; RV = right ventricle; LV = left ventricle.

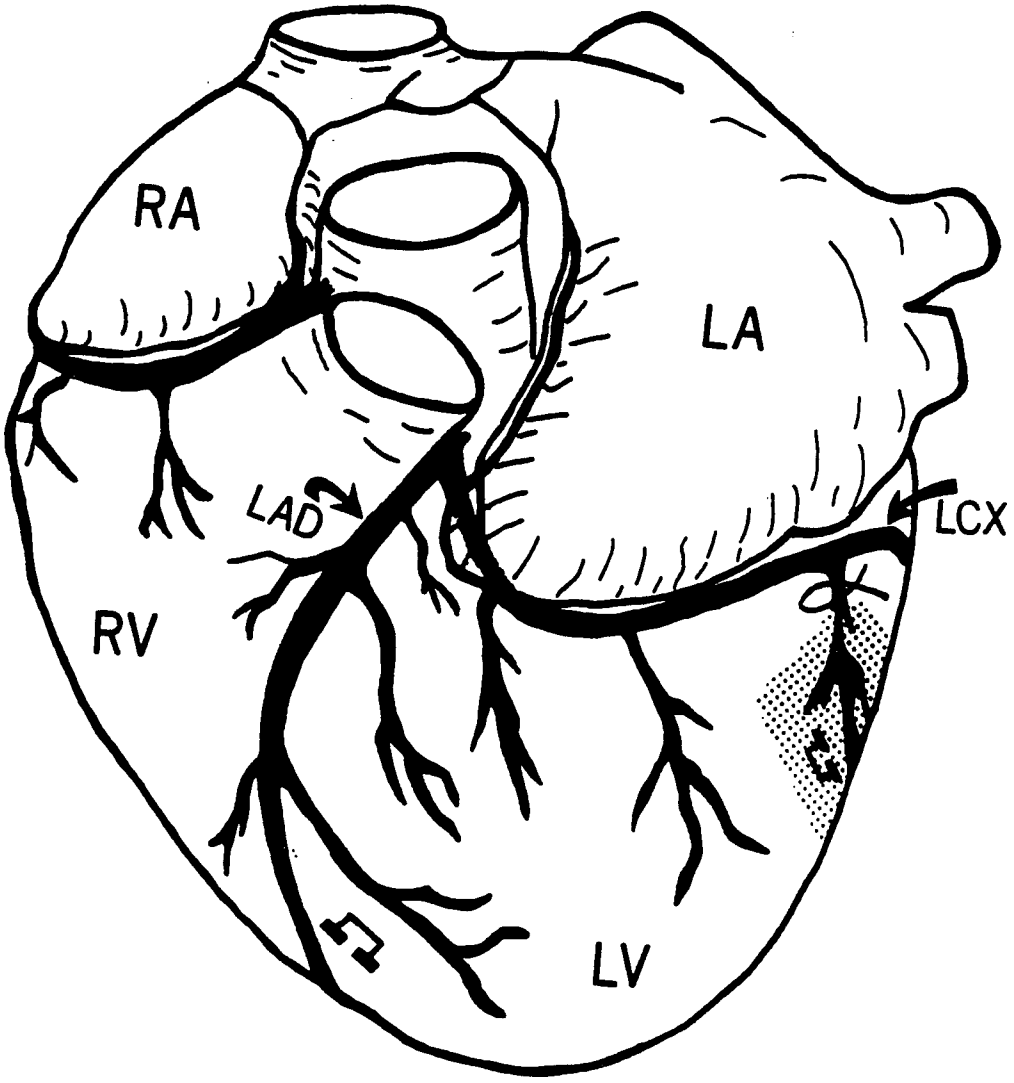
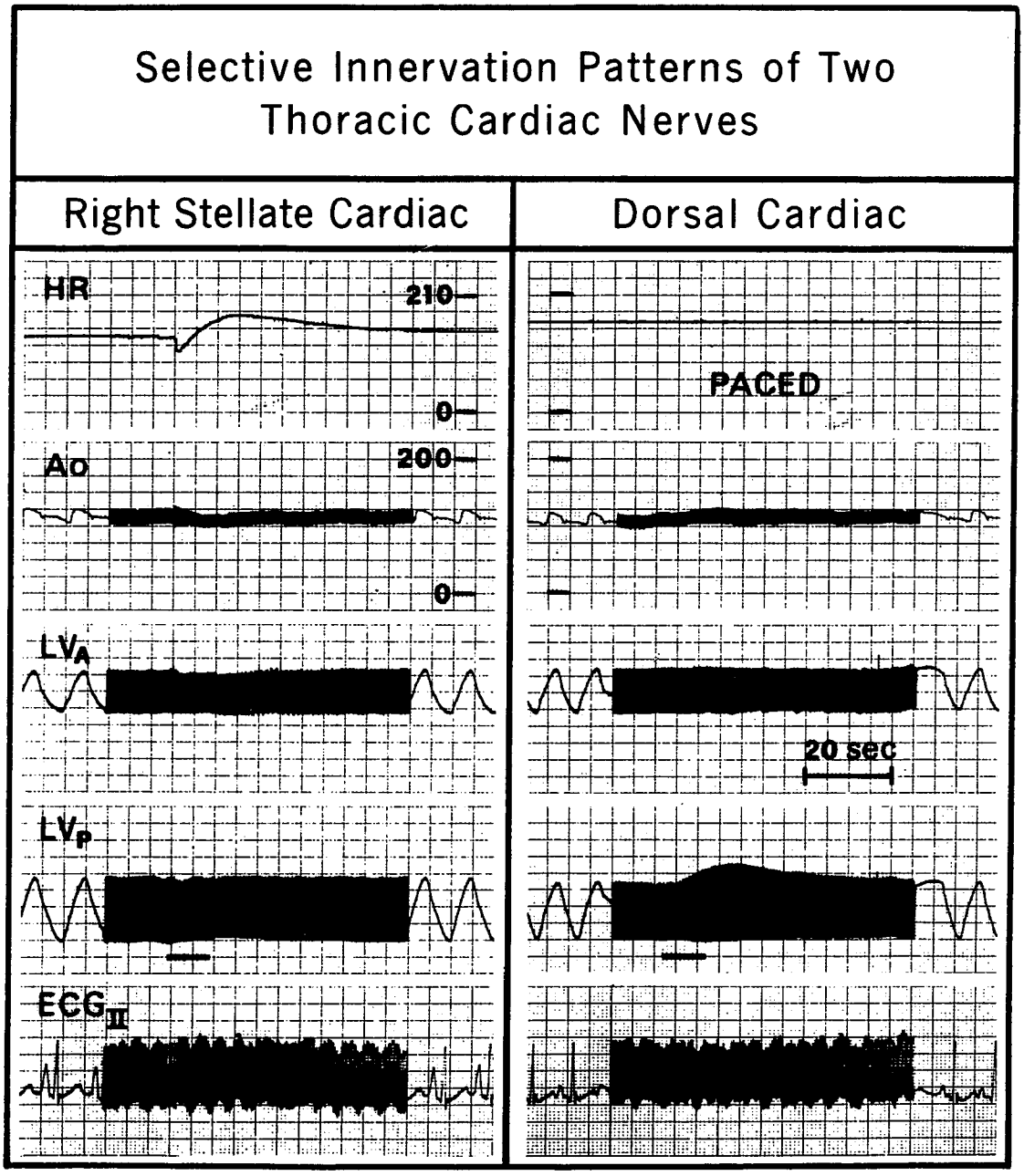

RA
LA
LAD
LCX
RV
LV

Figure 2

Representative example of the determination of efferent innervation patterns of two thoracic sympathetic cardiac nerves. HR = heart rate calibrated in beats/minute; Ao = aortic pressure calibrated in mmHg; LVa = left ventricular contractile performance - anterior; LVp = left ventricle contractile performance - posterior; ECG II = limb lead II electrocardiogram. Each nerve was stimulated for 10 seconds (2 msec pulse duration, 10-15 volts) during the interval indicated by the bar in the LVp channel. To eliminate the possible interference from the emergence of an AV junctional rhythm during stimulation of the dorsal cardiac nerve, heart rate was maintained constant by atrial pacing (PACED).


Selective Innervation Patterns of Two Thoracic Cardiac Nerves
Right Stellate Cardiac
Dorsal Cardiac
HR
210
0
PACED
Ao
200
0
LVA
20 sec
LVP
ECGII

known to innervate regions which would undergo ischemia upon occlusion of the coronary artery, while the remaining nerve was selected such that it was known not to innervate ischemic regions.

Coronary Artery Preparation

Only animals possessing normal distribution of the left anterior descending (LAD) coronary artery or left circumflex (LCX) coronary artery were used in this study. Criteria used to delineate the nature of the arterial distribution were modified from those of Jones and associates (82-85). Normal distribution was considered present when one or more branches of the respective coronary artery originated proximal to the terminal apical portion of the artery. When the preparation had been accepted as possessing a normal distribution, either a diagonal branch of the LAD or a marginal branch of the LCX was selected. The branch of the artery was carefully dissected from the surrounding fat and fascia just beyond its bifurcation with the parent coronary artery. Extreme care was taken to assure that the pericoronary nerves were not damaged during the dissection. A silk ligature was then placed beneath the artery and left loose until the time of occlusion. Table 1 summarizes the coronary arteries selected for each animal.

Once the coronary artery had been isolated and prepared, the two previously selected cardiac nerves were again stimulated (Figure 3). Heart rate, blood pressure, electrocardiogram and strain gauge responses were monitored and used to assess what damage if any had occurred to the efferent innervation during isolation and preparation of the the coronary artery. Preparation of the coronary artery in the above manner resulted in less than a 5% reduction of the heart rate, blood pressure,

Table 1

Summary of Animals. ARTERY OCCLUDED: LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery. NERVES RECORDED: RAA = right anterior ansa; RSC = right stellate cardiac; CrV = cranial vagal; RecC = recurrent cardiac; LAA = left anterior ansa; VMCN = ventromedial cardiac; VLCN = ventrolateral cardiac; DCN = dorsal cardiac. N = nerves innervating non-ischemic regions; I - nerves innervating ischemic regions. PREPARATION TYPE: 0 = neurally intact; 1 = sham; 2 = bilateral vagotomy; 3 = epicardial phenolization.

TABLE 1

SUMMARY OF ANIMALS

	ARTERY OCCLUDED		NERVES RECORDED									PREPARATION TYPE
			RIGHT				LEFT					
DOG #	LAD	LCX	RAA	RSC	CrV	RecC	LAA	VMCN	VLCN	DCN	RecC	
383	X		N				I					0
283	X		N				I					0
183	X		N				I					0
483		X	N				I					0
182		X	N				I					0
482		X	N				I					0
1983	X		N				I					0
2083	X				N			I				0
2183		X									I	1
184		X		N				I				0
284		X				N		I				0
384		X		N					I			0
484		X		N					I			0
583	X				N			I				0
683	X							I		N		0
783	X							I			N	0
883	X			N				I				0
983		X						N	I			0
1083	X							I	N			1
1183	X							I		N		0
1283	X							I		N		0
1383	X								I		N	0
1483	X							I				0
1683		X		N					I			0
1783		X		N					I			0
1883		X		N					I			0
316		X		N					I			0
320		X			N				I			0
321		X		N					I			0
329	X			N					I			1
330	X			N					I			0
402	X			N				I				0
411		X		N					I			0
412	X			N				I				1
413	X			N				I				0
419		X		N					I			1
420		X		N					I			0
423	X				N				I			1
424	X				N				I			0
426		X		N					I			2
9849	X			N	I							2
9874	X			N							I	2
9914	X			N				I				2
9876		X		N					I			2
9902		X		N					I			2
9921		X		N						I		2
9979	X			N				I				2
9996		X		N					I			3
9993		X		N					I			3
9995		X		N					I			3
1022	X			N	I							3
1023	X			N				I				1
1024	X			N				I				3
1041		X		N					I			2
1051	X			N				I				1
1052	X			N				I				3
1054	X			N				I				1
1055	X			N				I				2
1071		X		N					I			1
1072		X		N					I			3
1632	X			N	I							1
1633	X			N	I							3

electrocardiogram and strain gauge responses to repeated nerve stimulation.

Recording of Efferent Neurograms

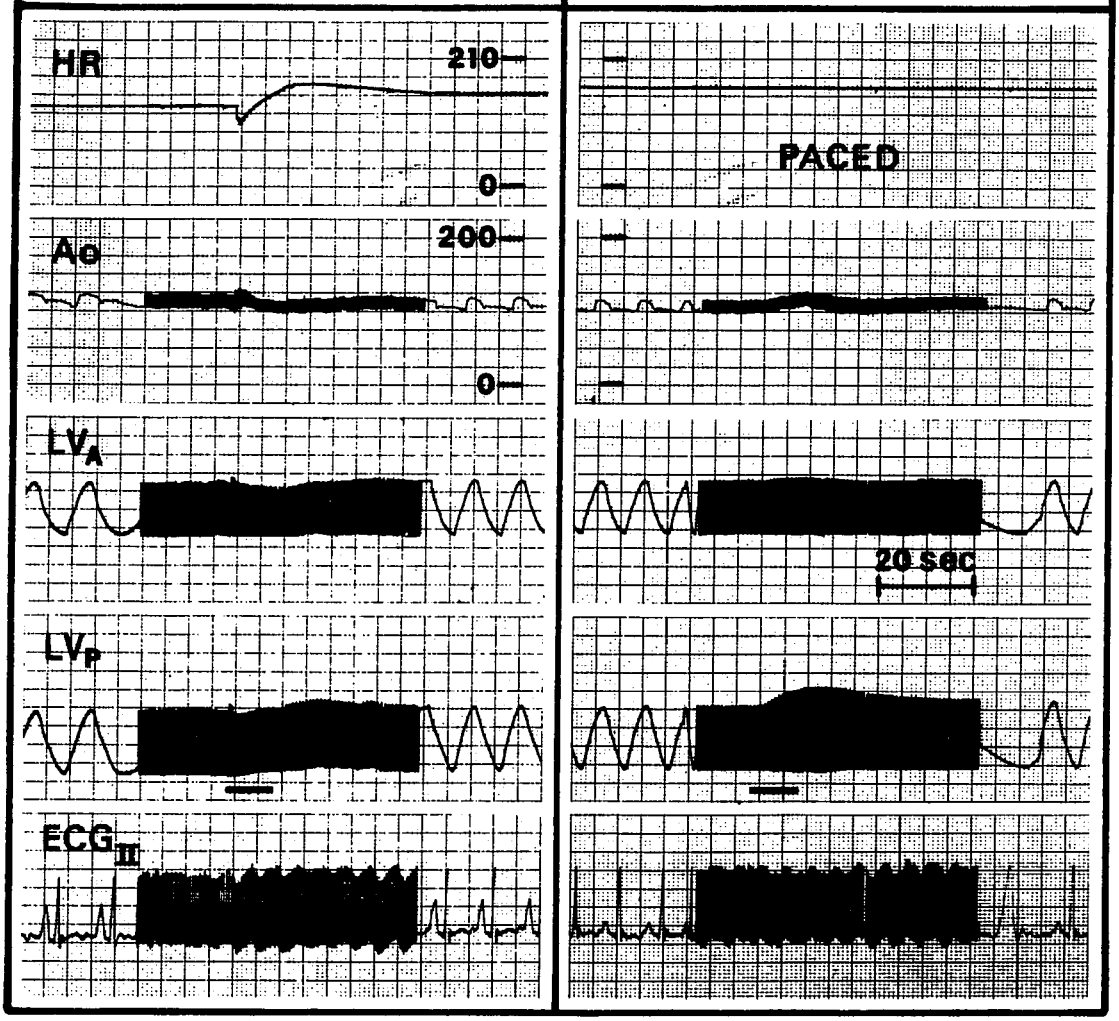
Efferent neurograms were recorded by tying and severing the nerve and placing the central end into a small nerve bath filled with mineral oil. Under direct vision with a binocular microscope (Zeiss Op-1) the nerve was carefully stripped of its major sheath and then progressively split into small strands. The small multifiber strands were then placed across two silver (0.25 mm diameter) contiguous electrodes. Prior to recording or testing of neural preparations, a 30-minute stabilization period was allowed. To varify that the recorded nerve filaments were responsive to cardiovascular interventions, we tested each filament of the split nerve. The balloon catheter placed in the aorta (via the femoral artery) was distended by filling it with saline which caused a marked (20-40 mm Hg) increase in mean aortic pressure proximal to the catheter. This elicited a baroreflex which characteristically resulted in withdrawal of sympathetic activity (42, 61). Upon release of the balloon, aortic pressure transiently decreased, characteristrically resulting in increased sympathetic activity (42, 61). Each filament was tested at least three times and was rejected for study if the changes in efferent activities were not reproducible. Table 1 summarizes the nerves recorded in each animal.

It is important to note that the isolation and preparation of sympathetic efferent fibers in the above manner should not significantly interfere with afferent signals from the regions of the heart under study. Armour and Randall (4, 6) have shown that, in the dog, the major afferent route from the anterior left ventricle is the right recurrent

Figure 3

Confirmation of efferent innervation patterns of two sympathetic cardiac nerves after the isolation of the third marginal branch (M3) of the left circumflex coronary artery. Same animal as illustrated in Figure 2. HR = heart rate calibrated in beats/minute; Ao = aortic pressure calibrated in mmHg; LVa = left ventricular contractile performance - anterior; LVp = left ventricular contractile force - posterior; ECG II = limb lead II electrocardiogram. Each nerve was stimulated for 10 seconds during the interval indicated by the bar in the LVp channel. To eliminate the possible interference from the emergence of an AV junctional rhythm, heart rate was maintained constant by atrial pacing (PACED) when stimulating the dorsal cardiac nerve.

Selective Innervation Patterns of Two Thoracic Cardiac Nerves Following LCA Isolation



cardiac nerve while the corresponding afferent pathway for the posterior left ventricle is the left innominate nerve.

Atrial Pacing

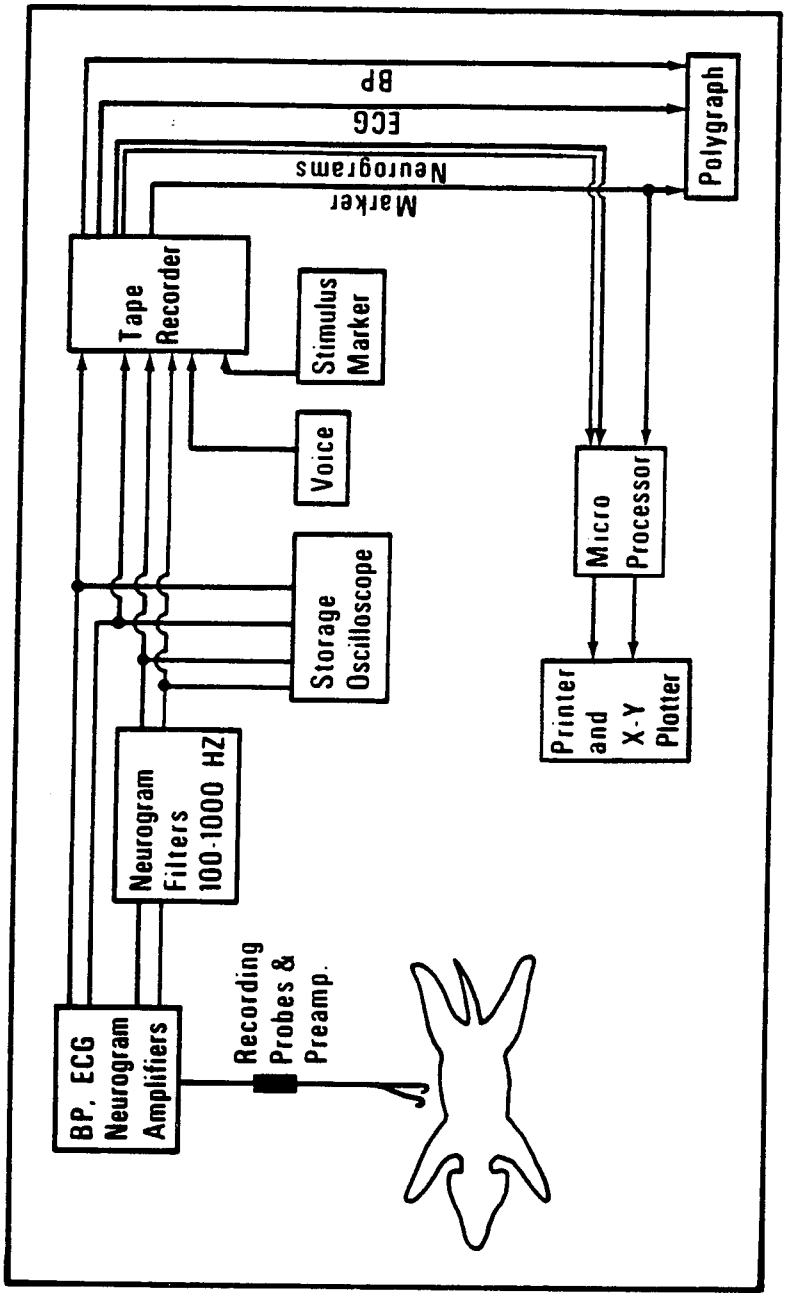
Transection of the nerves which provide efferent sympathetic activity to the sinus node results in a progressive decrease in sinus rate over the following two to three hours (144, 176). Since many of the nerves which were to be recorded from the sinus node, and transection of these nerves could result in rate changes independent of the reflex under scrutiny, heart rate was maintained constant during the coronary occlusion protocols by atrial pacing. Pacing was initiated from the tip of the left atrial appendage with a square wave stimulator (Grass S-44) and stimulus isolation unit (Grass SIU5). Each animal underwent incremental pacing in 5-10 beat changes until a rate was achieved at which mean arterial pressure declined from control levels. The experimental pacing rate was then selected to be 65% of that rate.

Neurogram Analysis

A customized microprocessor system was used to quantitate neural activities. The system is built around a S100 bus with an 8-bit microprocessor and 64 kilobytes of random access memory (Figure 4). The design of the system allows simultaneous monitoring, recording and analysis of on-line data. Neural activities, blood pressure and the electrocardiogram can be monitored and stored on a 4-channel storage oscilloscope (Hewlett-Packard 181A) equipped with a Polaroid camera (HP 197A). In addition, each of the above variables as well as a voice channel and event marker are also stored on a seven channel magnetic tape recording system (Hewlett-Packard 3413A). The microprocessor system makes use of a voltage sensitive Schmitt trigger which provides

Figure 4

Schematic diagram of instrumentation used for recording and analysis of efferent sympathetic activity. On-line data can be simultaneously stored on magnetic tape and analyzed by microprocessor based system. BP = blood pressure; ECG = electrocardiogram.


BP
ECG
Neurograms
Marker
Polygraph
Tape Recorder
Stimulus Marker
Voice
Micro Processor
Storage Oscilloscope
Printer and X-Y Plotter
Neurogram Filters 100-1000 HZ
Recording Probes & Preamp.
BP, ECG Neurogram Amplifiers

minimum threshold voltage levels. The Schmitt trigger setting and tape recorder channel output are adjusted at the beginning of the analysis so that little or no neural traffic remains undetected while only a small fraction of the noise of the system is counted (59, 61, 63). Histograms of the multifiber preparations are plotted from 15 seconds of data following a stimulus signal (recorded on the marker channel of the tape). The neural activity during resting (i.e., control) conditions is normalized at 100%. All subsequent neural activities are expressed as a percentage of control. This normalization of the data permits statistical evaluation within individual dogs and between preparations with differing numbers of active fibers. Comparisons between experiments are made by analyzing percent changes in neural activities.

The software used to run the microprocessor system was designed and written by Andrew F. Spear of the Cardiovascular Electronics Laboratory of the University of Alabama at Birmingham. The main nerve traffic analysis program NERAN and its subroutines, as well as a schematic illustrating the interactions between the main program and subroutines, can be found in the Appendix.

Removal of Afferent Pathways

The effects of deafferentation performed prior to coronary occlusion upon ischemia-induced changes in cardiac sympathetic efferent activities were examined. A cardiac non-selective vagal deafferentation was produced by transection of both vagosympathetic trunks at the midcervical level. The second procedure was a selective cardiac deafferentation. Animals were initially tested for their responsiveness to a stimulus of known cardiac origin. A small (5 mm diameter) filter paper disk saturated with a 10 µg/ml solution of nicotine (Sigma Chemical) was placed on the epicardium distal to the coronary snare.

Sixty seconds of heart rate, blood pressure, electrocardiographic and neural responses to the nicotine stimulus were recorded and repeated in triplicate allowing 10 minutes to elapse between trials. Phenol (75% solution), a sclerosing agent (13, 91), was then used to circumscribe and paint a 1 to 1.5 cm region surrounding the area supplied by the artery undergoing occlusion (Figure 1). Responsiveness to the nicotine stimulus was retested after phenolization. The preparation was considered to be locally deafferentated when the nicotinic stimulus did not elicit changes in heart rate, blood pressure, the electrocardiogram or neural activities. Table 1 summarizes the types of preparations (according to afferent innervation).

Arterial Blood Gas Measurements

Arterial blood samples were withdrawn from a catheter inserted into the subclavian artery and advanced to the root of the aorta. Blood samples were taken during the control period and during the ischemic period 1 minute prior to the release of the coronary occlusion. Animals which were subjected to more than one coronary artery occlusion had blood gas determinations made for each control and occlusion period. Analysis of arterial pH and blood gases were graciously performed by the respiratory physiology laboratory of Dr. S. M. Cain at the University of Alabama at Birmingham.

Determination of Ischemic Myocardium

The method of Kloner et al. (96) was used to assess the amount of myocardium made ischemic by the coronary artery occlusion. Myocardium metabolism is normally aerobic (128, 148), but quickly switches to anaerobic metabolism if arterial flow is insufficient to provide enough oxygen. The transition from aerobic to anaerobic metabolism in ischemia can be directly observed if methylene blue is present in the tissue.

This deep blue dye becomes colorless when it is reduced to leukomethylene blue by the excess hydrogen ions present in ischemic tissue (96). Therefore, oxygenated myocardium containing methylene blue remains a deep blue, while ischemic myocardium is similar in color to cyanotic tissue (i.e., burgandy).

To avoid any possible interactions involving changes in cardiac sympathetic activity and the methylene blue, intravenous infusions of a 1.0% solution of methylene blue (Sigma Chemical) were begun after the last occlusion protocol. The solution was prepared fresh in saline and equilibrated to 37°C prior to infusion. The infusion proceeded over 5-10 minutes with a pump set at a rate sufficient to raise the total dose to 3-4 mg/kg of body weight and to turn the entire heart a deep blue. At this point the coronary artery was re-occluded for the same time interval as performed in the occlusion protocol. At the end of this period the heart was rapidly excised and placed into 4°C phosphate buffered saline. The atria were removed and the ventricular myocardium was sliced in a breadloaf fashion. The left ventricle was defined as the tissue remaining after the atria were removed and the right ventricle was cut away from the interventricular septum. Table 2 summarizes the amount of myocardium made ischemic by coronary artery occlusion in the animals subjected to the methylene blue determination.

Statistical Analysis

Data for individual animals were coded for each of the following categories: location of the occluded coronary artery, efferent destination of the recorded nerves, duration of the coronary occlusion, occlusion number, heart rate, mean arterial pressure, ventricular contractile force and amount of ischemic myocardium. Group data were analyzed by the Statistical Analysis System (SAS) Program at the Rust

Computer Center, University of Alabama at Birmingham. Statistical consultation was provided by Dr. Edwin Bradley from the Department of Biostatistics and Biomathematics, University of Alabama at Birmingham.

A preliminary analysis of variance (161, 162) incorporating all of the above listed categories and each of their first order interactions was performed on the group data. A more detailed analysis was then performed using a repeated measures analysis of variance (161, 162) to test the effect of the time of measurement for each type of preparation and all type-level combinations. Furthermore, one-way and two-way factor analysis of variance were run at each measurement time to determine the effects of the type of preparation. Pairwise comparisons between means utilized Fisher's protected least significant differences test and paired t tests (161, 162).

TABLE 2

SUMMARY OF CORONARY ARTERY OCCLUSIONS AND EXTENT OF ISCHEMIA†

DOG #	ARTERY OCCLUDED	NUMBER OF OCCLUSIONS	DURATION(S)	PERCENT ISCHEMIC*
320	LCX	1	30	12.9
321	LCX	2	30/15	17.8
330	LAD	2	15/15	20.9
402	LAD	1	30	13.2
411	LCX	2	30/30	22.9
413	LAD	2	30/15	33.4
420	LCX	2	30/15	18.3
424	LAD	2	30/15	14.0
426	LCX	1	30	23.7
9849	LAD	2	30/15	17.3
9874	LAD	2	30/30	25.2
9914	LAD	2	30/30	10.7
9876	LCX	2	15/30	21.8
9902	LCX	2	30/30	21.2
9921	LCX	2	30/15	11.7
9979	LAD	2	30/30	10.1
9993	LCX	2	30/30	15.5
9995	LCX	2	30/30	17.8
9996	LCX	2	30/30	11.1
1022	LAD	2	30/30	12.5
1024	LAD	1	30	12.9
1041	LCX	2	30/30	8.4
1052	LAD	2	30/30	13.0
1055	LAD	2	30/30	9.9
1072	LCX	2	30/30	17.0
1633	LAD	2	30/30	15.1

† As assessed by the methylene blue technique
* Expressed as a percentage of left ventricular mass
LCX = left circumflex; LAD = left anterior descending

CHAPTER V

RESULTS

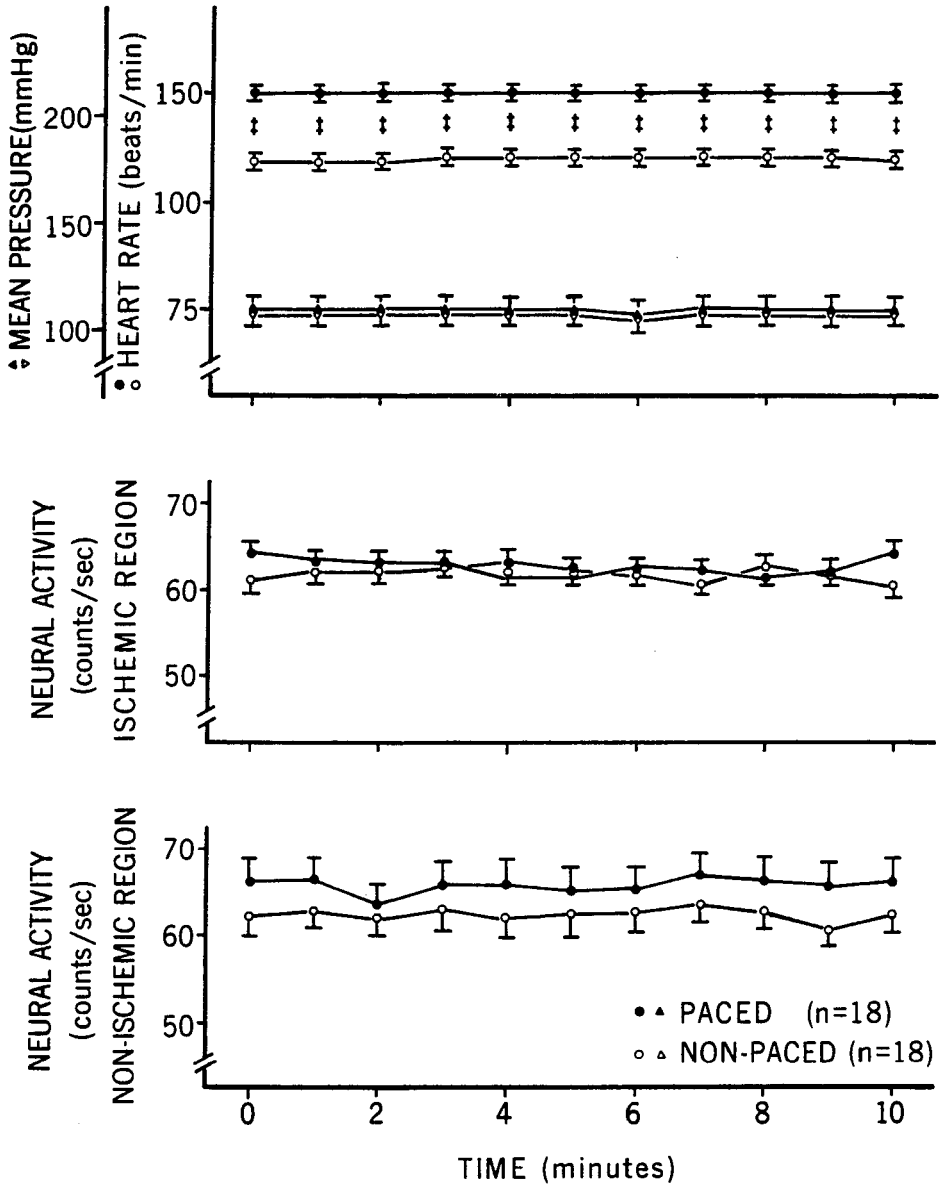
Effects of Cardiac Pacing

The effects of left atrial pacing upon mean arterial pressure, ventricular contractility and efferent cardiac sympathetic activity were examined in 18 animals. All variables were recorded for two 10-minute periods during both sinus rhythm and left atrial pacing. The order in which the recordings were performed (i.e., sinus rhythm and atrial pacing or atrial pacing and sinus rhythm) was randomized and a 10-minute stabilization period allowed between recordings. Heart rate during sinus rhythm averaged 124 ± 5 beats/minute (mean ± SE), while heart rate during left atrial pacing averaged 151 ± 2 beats/minute ($p < 0.0001$) (Figure 5). Mean arterial pressure was not significantly affected by atrial pacing, averaging 113 ± 5 mm Hg during sinus rhythm and 112 ± 5 mm Hg during pacing. Ventricular contractility (expressed as a percentage of sinus rhythm) was not significantly different during atrial pacing (98 ± 3%). Efferent cardiac sympathetic activities were recorded in a total of 36 preparations. Since these animals were also used for occlusion protocols, data for neural activities are presented as a function of the region of innervation as it would apply to an occlusion protocol (Figure 5). The "ischemic regions" included the anterior and posterior left ventricle while the "non-ischemic regions"

Figure 5

Effects of left atrial pacing upon mean arterial pressure and cardiac efferent sympathetic activities. Ischemic and non-ischemic regions refer to the regions according to their condition during an occlusion protocol (see text). Values have been adjusted for dog variability and are illustrated as mean ± SE.

† $p < 0.0001$ paced versus non-paced comparison.


MEAN PRESSURE(mmHg)
HEART RATE (beats/min)
200
150
100
150
100
75
NEURAL ACTIVITY
(counts/sec)
ISCHEMIC REGION
70
60
50
NEURAL ACTIVITY
(counts/sec)
NON-ISCHEMIC REGION
70
60
50
PACED (n=18)
NON-PACED (n=18)
0
2
4
6
8
10
TIME (minutes)

included the sinus node and right atrium. Since direct comparisons of neural activities within animals were necessitated by the experimental design, all values for neural activities are expressed as counts/second. Cardiac sympathetic activities were not significantly different during sinus rhythm (ventricle 62.1 ± 2.1; right atrium 62.4 ± 4.4 counts/second) or left atrial pacing (ventricle 62.5 ± 2.0; right atrium 65.8 ± 5.0 counts/second).

Time-Related Changes in Sham Animals

The occurrence of unpredictable changes in mean arterial pressure, left ventricular contractility or cardiac efferent sympathetic activity in the absence of myocardial ischemia was examined in 11 animals hereafter referred to as sham animals. Animals were fully instrumented including the isolation and preparation of a coronary artery and were paced from the left atrial appendage. Five minutes of spontaneous activity were recorded as controls and ventricular contractility and sympathetic neural activities normalized at 100%. Immediately following the control period, a 30-minute test period was recorded during which no coronary artery was occluded. Efferent cardiac sympathetic actvity was broken down into right and left sided nerves. In general, the nerves of the right side predominantly innervated the atria, while the nerves of the left side innervated the ventricles.

The group data were analyzed by an analysis of variance and are summarized in Table 3. As indicated in the analysis (Table 3 part II), neither mean arterial pressure, ventricular contractility nor efferent sympathetic activities changed significantly from control levels during the 30-minute test period.

TABLE 3

EFFECTS OF TIME ON CARDIAC SYMPATHETIC ACTIVITY, MEAN ARTERIAL PRESSURE AND LEFT VENTRICULAR CONTRACTILE PERFORMANCE

I. MEAN ± SE

	Mean Pressure (mmHg)	Contractile Performance	Symp Activity (Right)	Symp Activity (Left)
Control Period	117±4	100%	100%	100%
Test Period	118±2	98±1%	98.7±2.3%	99.2±4.4%

II. ANALYSIS OF VARIANCE

Dependent Variable	Source	D.F.	Sums of Squares	F	P
Mean Arterial Pressure					
	Dog	10	932.419	1.78	0.0701
	Time	24	1129.233	0.90	0.6064
	Error	140	7347.740		
Contractile Performance					
	Dog	10	786.635	1.14	0.3356
	Time	24	1096.685	0.66	0.8794
	Error	140	9643.464		
Sympathetic Activity (R)					
	Dog	10	92709.175	560.24	0.0001
	Time	24	329.040	0.83	0.6958
	Error	140	95354.937		
Sympathetic Activity (L)					
	Dog	10	6091.215	21480.16	0.0001
	Time	24	0.563	0.83	0.6969
	Error	140	3.970		

Ventricular contractile performance and sympathetic activities are normalized at 100% for control and during the test period are expressed as a percentage of control. Means have been adjusted for dog variability. Cardiac sympathetic activities have been divided into right side and left side nerves.

Effects of Anterior Versus Posterior Myocardial Ischemia Upon Preganglionic Sympathetic Activity

The effects of anterior versus posterior myocardial ischemia upon preganglionic sympathetic efferent activity were compared in 7 animals. Anterior ischemia was produced by occlusion of a diagonal branch (D2 or D3) of the left anterior descending (LAD) coronary artery (n=4). Posterior ischemia was produced by occlusion of a marginal branch (M3 or M4) of the left circumflex (LCX) coronary artery (n=3). The neural preparations employed for recording consisted of the right and left anterior ansae. Efferent innervation patterns were obtained in each animal. In general, stimulation of the right anterior ansa resulted in pronounced changes in heart rate with little if any change in left ventricular contractility. Stimulation of the left anterior ansa resulted in dramatic increases in left ventricular contractility with little if any change in heart rate. When stimuluation of the left ansa elicited heart rate changes, in every case the cardiac rhythm was determined to be of AV junctional origin.

Since these 7 animals comprised the preliminary studies examining the hypothesis, only mean arterial pressure and efferent preganglionic sympathetic activity were monitored continuously throughout the experiment. In addition, the duration of each ischemic insult was limited to 15 minutes. All preparations were paced. Group data for changes in mean arterial pressure and preganglionic sympathetic activities (ischemic and non-ischemic regions) were assessed by an analysis of variance (Table 4).

Absolute differences (i.e., different levels between dogs) in either heart rate (between dogs) or mean arterial pressure did not significantly influence the preganglionic response to coronary occlusion in nerves to ischemic or non-ischemic regions. The location of the

TABLE 4

CHANGES IN PREGANGLIONIC SYMPATHETIC ACTIVITY DURING MYOCARDIAL ISCHEMIA

ANALYSIS OF VARIANCE

Dependent Variable: Sympathetic Activity Ischemic Regions

Source	D.F.	Sum of Squares	F	P
Dog	6	323819.537	27.57	0.0001
Heart Rate	1	264.652	0.14	0.4192
Mean Arterial Pressure	1	24.183	0.01	0.8070
Artery Location	1	548.699	0.28	0.2448
Time	16	71712.462	2.29	0.0001
Error	69	135071.624		

Dependent Variable: Sympathetic Activity Non-Ischemic Regions

Source	D.F.	Sum of Squares	F	P
Dog	6	272255.051	44.86	0.0001
Heart Rate	1	51.964	0.05	0.7354
Mean Arterial Pressure	1	285.890	0.28	0.4281
Artery Location	1	1646.097	1.63	0.2990
Time	16	1243.729	1.23	0.2461
Error	69	69793.426		

occluded coronary artery did not significantly influence the sympathetic preganglionic response. However, the duration of the coronary occlusion did significantly affect preganglionic sympathetic activity to ischemic regions (Table 4). In contrast, preganglionic sympathetic activity to non-ischemic regions did not manifest any significant changes (increases or decreases) during the 15-minute coronary occlusion.

Since the initial analysis determined that one could not ascribe a significant role for the location of the occluded artery and due to the lack of selective innervation patterns of the neural preparations (i.e. preganglionic nerves), I pooled the data. The emphasis for the final analysis (Table 5) was to determine the effects of the duration (time dependency of the ischemic insult upon changes in preganglionic sympathetic activities to ischemic and non-ischemic regions. Preganglionic sympathetic activity to non-ischemic regions did not exhibit time-dependent trends during the 15-minute coronary artery occlusions. In contrast, preganglionic sympathetic activity to ischemic regions became significantly depressed (compared to control activity) 5 minutes after coronary occlusion and remained depressed throughout the remainder of the 15-minute occlusion.

Effects of Anterior Versus Posterior Myocardial Ischemia Upon Cardiac Postganglionic Sympathetic Activity

The effects of anterior versus posterior myocardial ischemia upon cardiac postganglionic sympathetic activity were compared in 22 animals. Anterior ischemia was produced by occlusion of a diagonal branch (D2 or D3) of the left anterior descending (LAD) coronary artery (n=10). Posterior ischemia was produced by occlusion of a marginal branch (M3 or M4) of the left circumflex (LCX) coronary artery (n=12). Simultaneous recordings were obtained from two thoracic cardiac postganglionic

TABLE 5

EFFECTS OF LEFT VENTRICULAR ISCHEMIA UPON MEAN ARTERIAL PRESSURE AND CARDIAC PREGANGLIONIC SYMPATHETIC ACTIVITY

I. MEAN ± SE

Time (min)	Mean Arterial Pressure (mmHg)	Symp. Activity Ischemic Regions	Symp. Activity Non-Ischemic Regions
Control	103 ± 1	100%	100%
Occlusion	103 ± 2	92.9 ± 2.1	98.9 ± 4.2
0.25	103 ± 2	96.4 ± 2.3	97.2 ± 4.4
1	104 ± 1	98.7 ± 2.3	96.5 ± 4.3
2	104 ± 1	95.4 ± 2.3	98.5 ± 4.3
3	101 ± 2	96.1 ± 2.3	97.0 ± 4.3
4	101 ± 1	94.9 ± 2.3	97.6 ± 4.7
5	101 ± 2	90.1 ± 2.4 *	92.4 ± 5.7
6	103 ± 1	87.4 ± 4.5 *	95.1 ± 5.8
7	102 ± 1	90.1 ± 4.4 *	89.3 ± 5.8
8	102 ± 2	94.8 ± 4.4	91.5 ± 5.7
9	102 ± 2	86.6 ± 4.4 *	90.1 ± 5.7
10	102 ± 1	94.1 ± 4.3	91.8 ± 5.7
11	102 ± 1	88.8 ± 4.4 *	89.8 ± 5.7
12	102 ± 1	89.1 ± 4.4 *	91.7 ± 5.6
13	101 ± 2	85.4 ± 4.3 *	94.7 ± 5.6
14	101 ± 1	86.8 ± 4.5 *	88.7 ± 5.6
15	101 ± 2	85.9 ± 4.4 *	102.6 ± 5.6

II. ANALYSIS OF VARIANCE

Dependent Variable	Source	D.F.	Sum of Squares	F	P
Mean Arterial Pressure					
	Dog	6	38272.057	437.27	0.0001
	Time	16	125.557	0.54	0.9220
	Error	72	1050.303		
Sympathetic Activity (Ischemic)					
	Dog	6	3715.693	15.57	0.0001
	Time	16	1279.802	2.01	0.0262
	Error	72	2863.932		
Sympathetic Activity (Non-Ischemic)					
	Dog	6	5291.842	22.43	0.0001
	Time	16	932.386	1.48	0.2550
	Error	72	2830.837		

Sympathetic activities are normalized at 100% for control conditions and are expressed as a percentage of control during the ischemic period. Means have been adjusted for dog variability.

* $p < 0.05$ compared to control

sympathetic nerves. Innervation patterns for each nerve were localized to discrete regions of the atria or ventricles. Since there were at least 20 possible combinations of thoracic postganglionic nerves to record from (see Methods), the decision was made to compare ischemia-induced changes in activity in nerves to ischemic regions to those occurring in nerves to primarily only one other region of the heart. Therefore, whenever possible, changes in postganglionic sympathetic activity to ischemic regions were compared to changes in activity to the region of the sinus node.

In animals subjected to LAD occlusion, postganglionic sympathetic activity to ischemic regions was recorded from the ventromedial cardiac nerve in 7 animals and from the ventrolateral cardiac nerve in 3 animals. Postganglionic sympathetic activity to non-ischemic regions was recorded from the right stellate cardiac nerve in 4 animals, from the recurrent cardiac nerve in 2 animals, from the craniovagal nerve in 2 animals and from the dorsal cardiac nerve in 2 animals. Innervation patterns for the right stellate cardiac, recurrent cardiac and craniovagal nerves confirmed that these nerves supplied sympathetic efferent innervation to the sinus node. In the 2 preparations in which postganglionic sympathetic activity was recorded from the dorsal cardiac nerve, efferent innervation went to the posterior left ventricle.

In animals subjected to LCX occlusion, postganglionic sympathetic activity to ischemic regions was recorded from the ventrolateral cardiac nerve in 10 animals and from the ventromedial cardiac nerve in 2 animals. Sympathetic activity to non-ischemic regions was recorded from the right stellate cardiac nerve in 10 animals, from the recurrent cardiac nerve in 1 animal and from the craniovagal nerve in 1 animal.

TABLE 6

CHANGES IN CARDIAC POSTGANGLIONIC SYMPATHETIC ACTIVITY
DURING MYOCARDIAL ISCHEMIA

ANALYSIS OF VARIANCE

Dependent Variable: Sympathetic Activity Ischemic Regions

Source	D.F.	Sum of Squares	F	P
Artery	1	18166.072	44.86	0.0001
Dog * Run No (Location)	29	322085.009	27.42	0.0001
Heart Rate	1	264.652	0.65	0.4192
Mean Arterial Pressure	1	24.183	0.06	0.8070
Location * Time	31	19024.673	1.52	0.0375
Error	702	284275.135		

Dependent Variable: Sympathetic Activity Non-Ischemic Regions

Source	D.F.	Sum of Squares	F	P
Artery	1	62092.783	136.58	0.0001
Dog * Run No (Location)	29	272255.051	20.65	0.0001
Heart Rate	1	51.964	0.11	0.7354
Mean Arterial Pressure	1	285.890	0.63	0.4281
Location * Time	31	46802.245	3.32	0.0001
Error	702	319147.267		

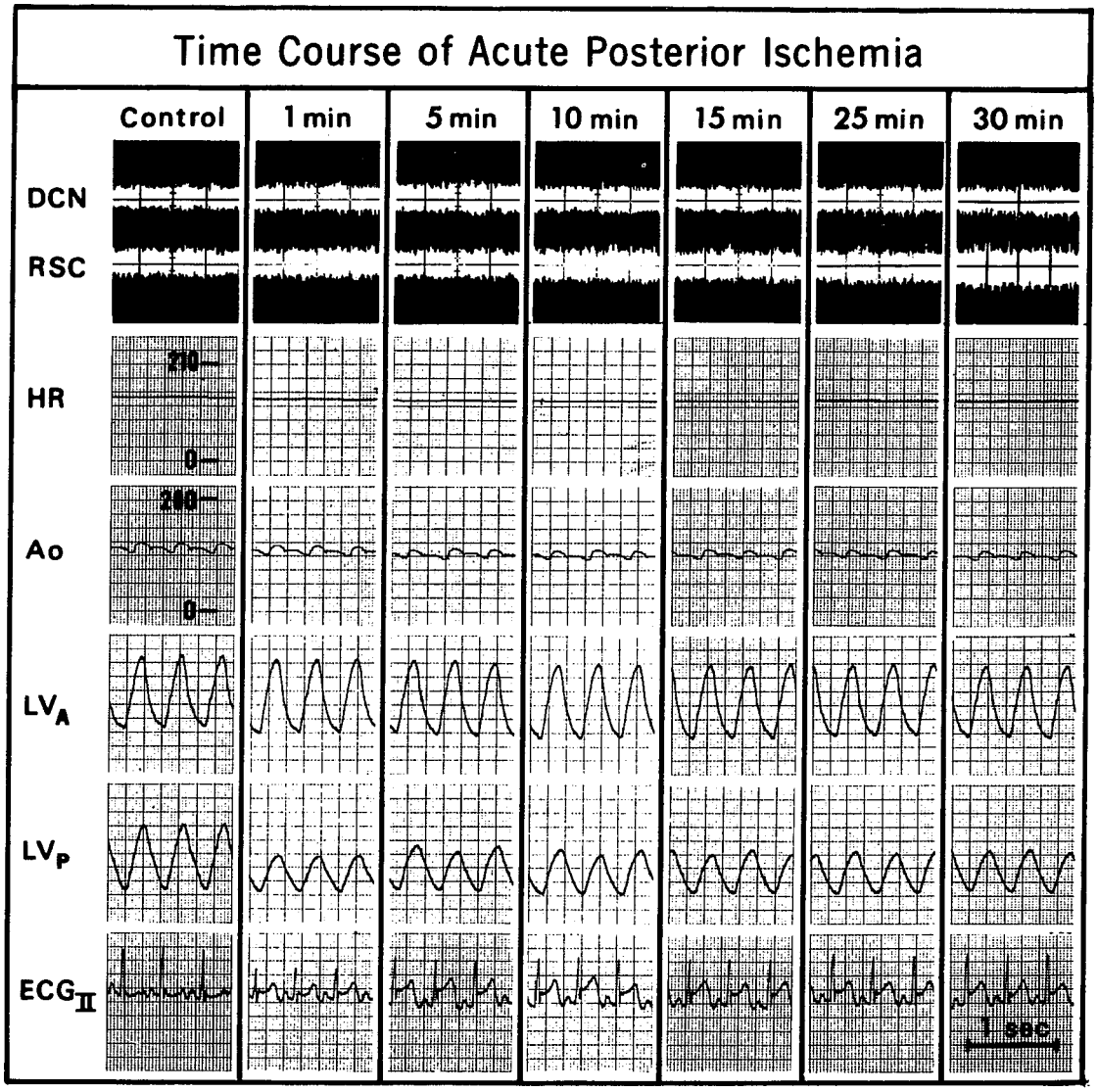
Innervation patterns for the right stellate cardiac, recurrent cardiac and craniovagal confirmed that these nerves supplied efferents to the sinus node. A representative example of the time course of a 30-minute left circumflex coronary artery occlusion is depicted in Figure 6. Note in the example the characteristic depression in contractility (from the ischemic region) and S-T segment elevation in the electrocardiogram.

The group data were analyzed by an analysis of variance (Table 6). Differences in heart rate or mean arterial pressure between animals did not significantly influence the postganglionic sympathetic response to coronary occlusion. However, both the location of the occluded coronary artery and the duration of the occlusion significantly influenced neural responses. The total duration of ischemia is the product of the number of occlusions times the duration of each individual occlusion. The analysis indicated that, both the duration (Location*Time) and number of occlusions (Dog*RunNo[Location]) significantly influenced the neural response to left ventricular ischemia (Table 6). The influence(s) of repetitive occlusions upon cardiac efferent sympathetic activities are dealt with in a later section.

The group data for changes in postganglionic sympathetic activities, mean arterial pressure and left ventricular contractility are illustrated in Figure 7. In animals subjected to LAD occlusion postganglionic sympathetic activity to ischemic regions became significantly less than control after 2 minutes of ischemia. In animals subjected to LCX occlusion postganglionic sympathetic activity to ischemic regions became significantly less than control after 4 minutes. In contrast, postganglionic sympathetic activity to non-ischemic regions exhibited vastly different responses depending upon the location of the

Figure 6

Representative example illustrating the time course of acute posterior ischemia. The top two channels are efferent neurograms recorded from the dorsal cardiac nerve (DCN) and the right stellate cardiac nerve (RSC). HR = heart rate calibrated in beats/minute; Ao = aortic pressure calibrated in mmHg; LVa = left ventricular contractile performance - anterior; LVp = left ventricular contractile performance - posterior; ECG II = limb lead electrocardiogram. Heart rate was maintained constant with atrial pacing.


Time Course of Acute Posterior Ischemia
Control
1 min
5 min
10 min
15 min
25 min
30 min
DCN
RSC
HR
210
0
Ao
200
0
LV_A
LV_P
ECG_II
1 sec

occluded coronary artery. Postganglionic sympathetic activity to non-ischemic regions became significantly greater than control 5 minutes after LCX occlusion and reached levels which were approximately 160% of control after 30 minutes (Figure 7). In contrast, postganglionic sympathetic activity to non-ischemic regions did not change significantly from control levels during LAD occlusion.

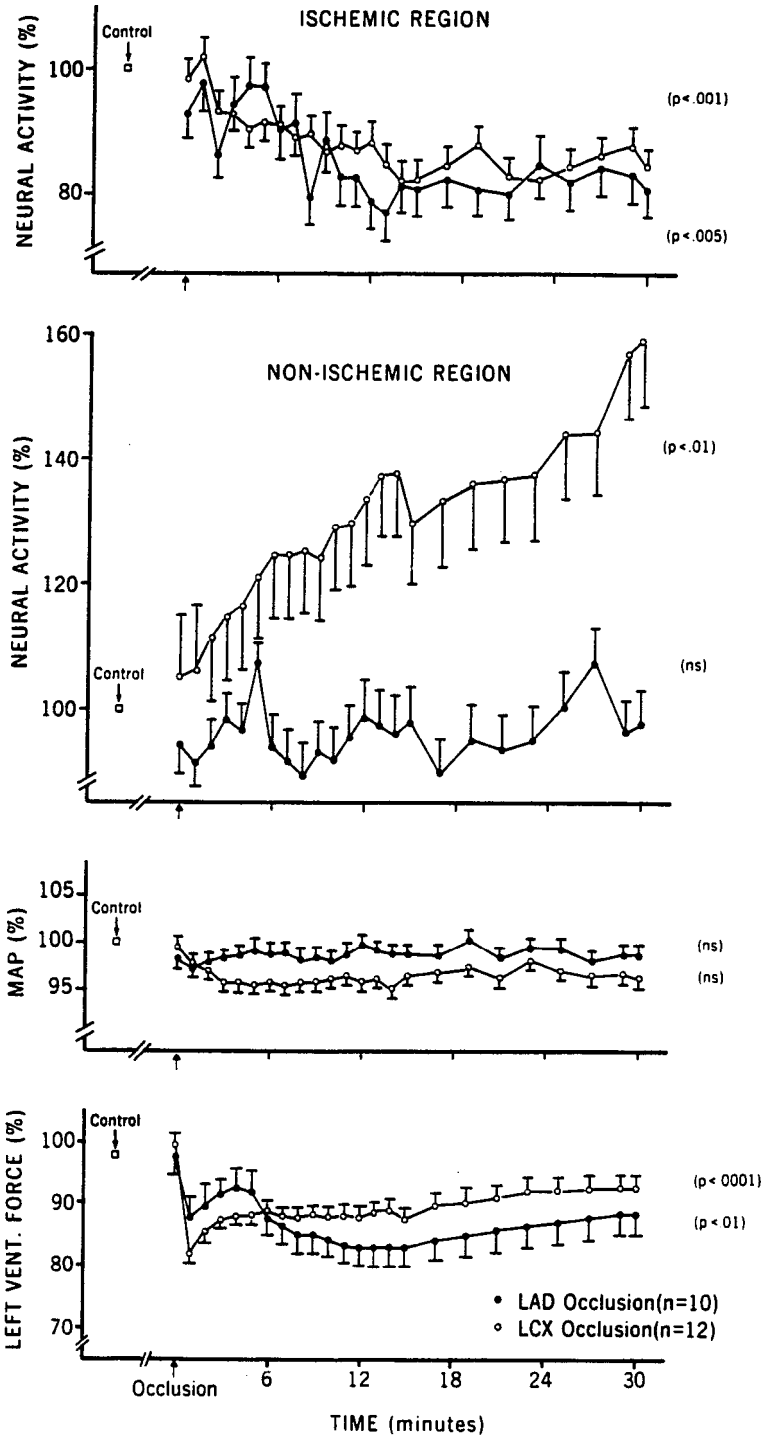
Mean arterial pressure did not exhibit any time-dependent changes during either LAD ($p = 0.89$) or LCX ($p = 0.44$) occlusions. Mean pressure varied less than ± 3% from control (114 ± 1 mmHg) during LAD occlusion and less than 5% from control (107 ± 2 mmHg) during LCX occlusion (Figure 7).

Ischemia-induced changes in left ventricular contractility in affected regions were similar during LAD and LCX occlusions (Figure 7). Ventricular contractility was significantly depressed from control immediately (15 seconds) following occlusion of either coronary artery. During both LAD and LCX occlusion ventricular contractility performance exhibited a transient return towards control levels during the first 5 to 8 minutes. However, ventricular contractility still remained depressed from control throughout the occlusion.

Possible differences in the amount of ischemic myocardium were examined, since this is one factor which might influence the reflex neural response. Only a branch of the parent coronary artery was occluded; therefore, ischemia was limited solely to the left ventricle. In animals subjected to LAD occlusion, the mass of the ischemic tissue averaged 16.9 ± 1.3% of left ventricular wet weight. In the animals subjected to LCX occlusion, the mass of ischemic tissue averaged 16.0 ± 1.9% of the left ventricular wet weight.

Figure 7

Group data illustrating changes in postganglionic sympathetic activities (ISCHEMIC AND NON-ISCHEMIC REGIONS), mean arterial pressure (MAP) and left ventricular contractility (ischemic region) as a function of the occluded coronary artery and duration of the occlusion. Sympathetic activities, mean arterial pressure and ventricular contractility were normalized at 100% during control and expressed as a percentage of control during the coronary occlusion. LAD = left anterior descending; LCX = left circumflex. P values are from the analysis of variance for time dependent changes.


ISCHEMIC REGION
Control
NEURAL ACTIVITY (%)
100
80
(p<.001)
(p<.005)
NON-ISCHEMIC REGION
160
140
120
100
(p<.01)
(ns)
MAP (%)
105
100
95
(ns)
(ns)
LEFT VENT. FORCE (%)
100
90
80
70
(p< 0001)
(p< 01)
• LAD Occlusion(n=10)
◦ LCX Occlusion(n=12)
Occlusion
6
12
18
24
30
TIME (minutes)

Arterial pH and blood gases were assessed just prior to the beginning of the coronary artery occlusions and again during the last minute of the occlusions. Neither arterial pH (before 7.29 ± 0.03; during 7.28 ± 0.04) nor blood gases (before pO_2 87.6 ± 3.4 mmHg, pCO_2 28.9 ± 2.2 mmHg; during pO_2 86.9 ± 3.0 mmHg, pCO_2 27.0 ± 1.7 mmHg) exhibited significant changes.

Changes in Postganglionic Sympathetic Activity in Animals Exhibiting Ventricular Fibrillation During Lad Occlusion

Ventricular fibrillation was observed in 3 animals during LAD occlusion. Since these animals did not complete the first occlusion protocol, the data were analyzed separately in an attempt to determine whether a correlation could be established between the incidence of ventricular fibrillation and changes in cardiac sympathetic activity. Of the three animals, 1 exhibited ventricular fibrillation 3 minutes after occlusion, and the other 2 exhibited ventricular fibrillation 7 minutes after occlusion (Table 7). Postganglionic sympathetic activity to either ischemic or non-ischemic regions did not exhibit significant changes prior to the onset of ventricular fibrillation. It is noteworthy that sympathetic activity to ischemic regions initially decreased, but this depression was short lived.

Changes in Postganglionic Sympathetic Activity in an Animal Exhibiting Ventricular Fibrillation During LCX Occlusion

During LCX occlusion ventricular fibrillation was observed in only 1 animal out of a total of 13 animals (Table 7, part II). Prior to the occurrence of ventricular fibrillation cardiac postganglionic sympathetic activity to ischemic or non-ischemic regions was unchanged by LCX occlusion. Mean arterial pressure was initially unchanged by LCX occlusion. However, 2 minutes after initiating the occlusion and just

TABLE 7

CHANGES IN CARDIAC POSTGANGLIONIC SYMPATHETIC ACTIVITY IN ANIMALS EXHIBITING VENTRICULAR FIBRILLATION

I. LEFT ANTERIOR DESCENDING OCCLUSIONS

Time (min)	n	Mean Arterial Pressure (mmHg)	Symp. Activity Ischemic	Symp. Activity Non-Ischemic
Control	3	86 ± 9	100%	100%
Occlude	3	82 ± 11	108.2 ± 11.8	109.7 ± 8.8
0.25	3	84 ± 9	98.7 ± 4.3	102.6 ± 2.3
1	3	84 ± 11	93.8 ± 0.9	102.5 ± 2.1
2	3	80 ± 10	96.7 ± 0.4	101.5 ± 7.4
3	3	82 ± 11	99.7 ± 3.7	107.3 ± 8.8
4	2	89 ± 18	96.2 ± 1.6	96.1 ± 7.9
5	2	89 ± 19	102.7 ± 0.7	100.8 ± 5.4
6	2	87 ± 21	129.8 ± 32.2	135.1 ± 29.3
7	2	88 ± 18	151.4 ± 43.1	119.4 ± 20.5

II. LEFT CIRCUMFLEX OCCLUSION

Time (min)	n	Mean Arterial Pressure (mmHg)	Symp. Activity Ischemic	Symp. Activity Non-Ischemic
Control	1	90	100%	100%
Occlude	1	90	86.3	91.9
0.25	1	90	92.4	86.1
1	1	90	100.2	88.3
2	1	83	95.2	92.9

Sympathetic activities are normalized at 100% during control and are expressed as a percentage of control during the ischemic period. Values are mean ± SE

prior to the occurrence of ventricular fibrillation, mean pressure was observed to decrease.

Effects of Repeated Episodes of Myocardial Ischemia Upon Cardiac Preganglionic Sympathetic Neural Activity

Whenever possible, the effects of a second coronary artery occlusion were examined in each animal. At least 60 minutes were allowed to elapse from the time of the release of the first occlusion to the beginning of the second recording period. The same nerves were employed for recording and the same coronary artery was occluded during the second ischemic episode.

Preganglionic sympathetic activities were recorded in 7 animals during a second coronary occlusion. The group data were tested with an analysis of variance where emphasis was placed on the determination of differences between the first and second occlusions. The analysis is summarized in Table 8. As indicated in part II of the table, a significant interaction was found between the occlusion number (RUN) and the sympathetic neural response in an individual animal. During the first occlusion sympathetic activity to ischemic regions decreased. In contrast, during the second occlusion activity remained unchanged. Thus, the response to left ventricular ischemia differed depending on whether or not the animal had been exposed to a previous ischemic insult.

Mean arterial pressure exhibited similar responses during both the first and second coronary artery occlusions. Mean arterial pressure did not change significantly from control (Tables 5 and 8 part I). Coronary occlusion mean arterial pressure did not differ from the respective control, however, during the second occlusion protocol the control level of mean pressure was significantly ($p < 0.01$) higher and remained so during the occlusion (Table 8 part I).

TABLE 8

EFFECTS OF REPETITIVE CORONARY ARTERY OCCLUSIONS UPON SYMPATHETIC PREGANGLIONIC ACTIVITY

I. MEAN ± SE - Second Occlusion

Time (min)	Mean Arterial Pressure (mmHg)	Symp. Activity Ischemic Regions	Symp. Activity Non-Ischemic Regions
Control	111 ± 2	100%	100%
Occlusion	112 ± 3	99.9 ± 10.4	110.3 ± 11.1
0.25	112 ± 2	97.8 ± 6.3	91.1 ± 11.6
1	113 ± 4	102.2 ± 8.4	93.8 ± 9.2
2	113 ± 2	98.5 ± 8.4	100.0 ± 11.1
3	113 ± 4	102.1 ± 9.2	98.1 ± 4.6
4	113 ± 3	98.2 ± 9.2	96.7 ± 6.3
5	113 ± 1	93.5 ± 9.8	90.1 ± 10.4
6	120 ± 4	92.6 ± 17.5	84.4 ± 15.2
7	121 ± 2	82.4 ± 19.9	81.4 ± 9.8
8	121 ± 2	82.7 ± 19.9	81.1 ± 11.4
9	121 ± 3	87.5 ± 19.9	78.0 ± 19.8
10	120 ± 1	96.1 ± 17.5	80.9 ± 17.7
11	121 ± 4	87.5 ± 19.9	80.1 ± 12.3
12	119 ± 2	89.2 ± 15.3	76.5 ± 18.9
13	117 ± 1	87.6 ± 14.5	77.5 ± 19.5
14	117 ± 2	88.3 ± 11.5	74.4 ± 16.9
15	117 ± 3	88.8 ± 11.5	82.5 ± 17.3

II. ANALYSIS OF VARIANCE

Dependent Variable	Source	D.F.	Sum of Squares	F	P
Sympathetic Activity (Ischemic)					
	Run * Dog	9	4438.432	10.23	0.0001
	Time	16	1445.723	1.87	0.02
	Error	71	3420.995		
Sympathetic Activity (Non-Ischemic)					
	Run * Dog	9	5938.557	21.39	0.0001
	Time	16	925.836	1.87	0.025
	Error	71	2190.671		

Sympathetic activities are normalized at 100% for controls and expressed as a percentage of control during the ischemic period. Means adjusted for dog variability.

Effects of Repeated Episodes of Myocardial Ischemia Upon Cardiac Postganglionic Sympathetic Activity

Only 14 of the original 22 animals were subjected to a second coronary artery occlusion. Animals which developed severe arrhythmias (ventricular tachycardia or fibrillation) at the release of the first occlusion or during the 60-minute recovery period were not subjected to a second occlusion. In a given animal, the same nerves were employed for recording and the same coronary artery occluded during the second occlusion. Since the previous analyses (Table 6) had indicated that there was a significant difference in the sympathetic postganglionic neural response to LAD versus LCX occlusion, the group data were analyzed as a function of the location of the occluded artery (Table 9).

In 8 of the original 10 animals which were subjected to LAD occlusion, a significant interaction was found for the occlusion number and the neural response of an individual animal (i.e., Run*Dog). Significant duration of occlusion dependent influences were also found (Table 9 part I). Thus, changes in postganglionic sympathetic neural activity in response to LAD occlusion differed depending on whether or not the animal had been previously subjected to a coronary occlusion. The group data for the first and second LAD occlusions are illustrated in Figure 8. As illustrated, postganglionic sympathetic activities to ischemic regions differed during the first and second LAD occlusion. During the first LAD occlusion postganglionic sympathetic activity to ischemic regions became depressed. In contrast, during the second LAD occlusion postganglionic sympathetic activity to ischemic regions initially remained at or near control and then gradually increased. Activity to ischemic regions increased to levels which were in excess of

TABLE 9

EFFECTS OF REPETITIVE CORONARY ARTERY OCCLUSIONS UPON CARDIAC POSTGANGLIONIC SYMPATHETIC ACTIVITY

ANALYSIS OF VARIANCE

I. LEFT ANTERIOR DESCENDING - Second Occlusion

Dependent Variable: Sympathetic Activity Ischemic Regions

Source	D.F.	Sum of Squares	F	P
Run * Dog	17	140700.935	18.44	0.0001
Time	24	50585.004	4.70	0.005
Error	338	151694.243		

Dependent Variable: Sympathetic Activity Non-Ischemic Regions

Source	D.F.	Sum of Squares	F	P
Run * Dog	17	20991.088	8.44	0.0001
Time	24	6759.912	1.93	0.005
Error	338	44173.698		

II. LEFT CIRCUMFLEX - Second Occlusion

Dependent Variable: Sympathetic Activity Ischemic Regsions

Source	D.F.	Sum of Squares	F	P
Run * Dog	17	1379590.084	88.05	0.0001
Time	24	49014.050	2.22	0.001
Error	350	322590.940		

Dependent Variable: Sympathetic Activity Non-Ischemic Regions

Source	D.F.	Sum of Squares	F	P
Run * Dog	17	9156.563	21.52	0.0001
Time	24	475.707	0.61	0.9277
Error	350	7657.702		

120% of control at 30 minutes. Neither during the first nor the second LAD occlusion did postganglionic sympathetic activity to the non-ischemic regions change significantly from control (Figure 8).

Changes in ventricular contractility (ischemic regions) differed between the first and second LAD occlusions. During the second LAD occlusion no duration dependent trends ($p = 0.6$) were found. Neither during the first nor second LAD occlusion did mean pressure change significantly from the respective control (1st 114 ± 1 mmHg; 2nd 124 ± 5 mmHg).

In 6 of the original 12 animals subjected to a second LCX occlusion, a significant interaction was found for the occlusion number and the neural response of an individual animal (i.e., Run*Dog; Table 9). The group data for changes in postganglionic sympathetic activities and mean arterial pressure during the first and second LCX occlusions are illustrated in Figure 9. Comparison of the levels of postganglionic sympathetic activities to ischemic regions during the first and second LCX occlusions revealed significant differences in the pattern of response. Postganglionic sympathetic activity to ischemic regions became depressed during the first occlusion, whereas activity remained at control levels throughout the second occlusion. Similarly, postganglionic sympathetic activity to non-ischemic regions was unchanged during the second LCX occlusion (Figure 9).

Changes in mean arterial pressure were also found to differ between the first and second LCX occlusions (Figure 9). During the first LCX occlusion mean arterial pressure changed approximately ±5% from the control (107 ± 2 mmHg). During the second LCX occlusion mean arterial pressure remained near control levels (107 ± 6 mmHg) until approximately

Figure 8

Group data illustrating the responses of postganglionic sympathetic activities (ISCHEMIC AND NON-ISCHEMIC) and mean arterial pressure (MAP) as a function of the occlusion number and the duration of occlusion in animals subjected to left anterior descending coronary artery occlusions. Sympathetic activities and mean arterial pressure were normalized at 100% during control and expressed as a percentage of control during the coronary occlusion. P values are from the analysis of variance for time dependent changes.

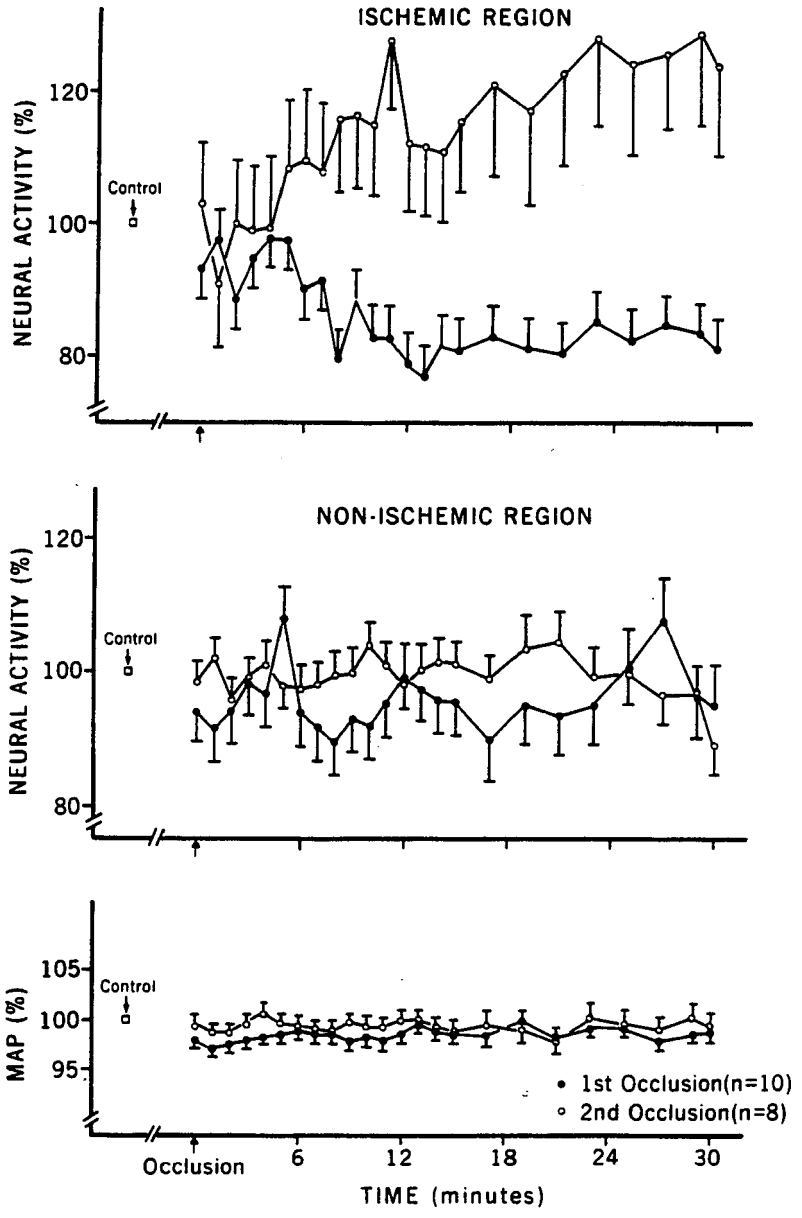
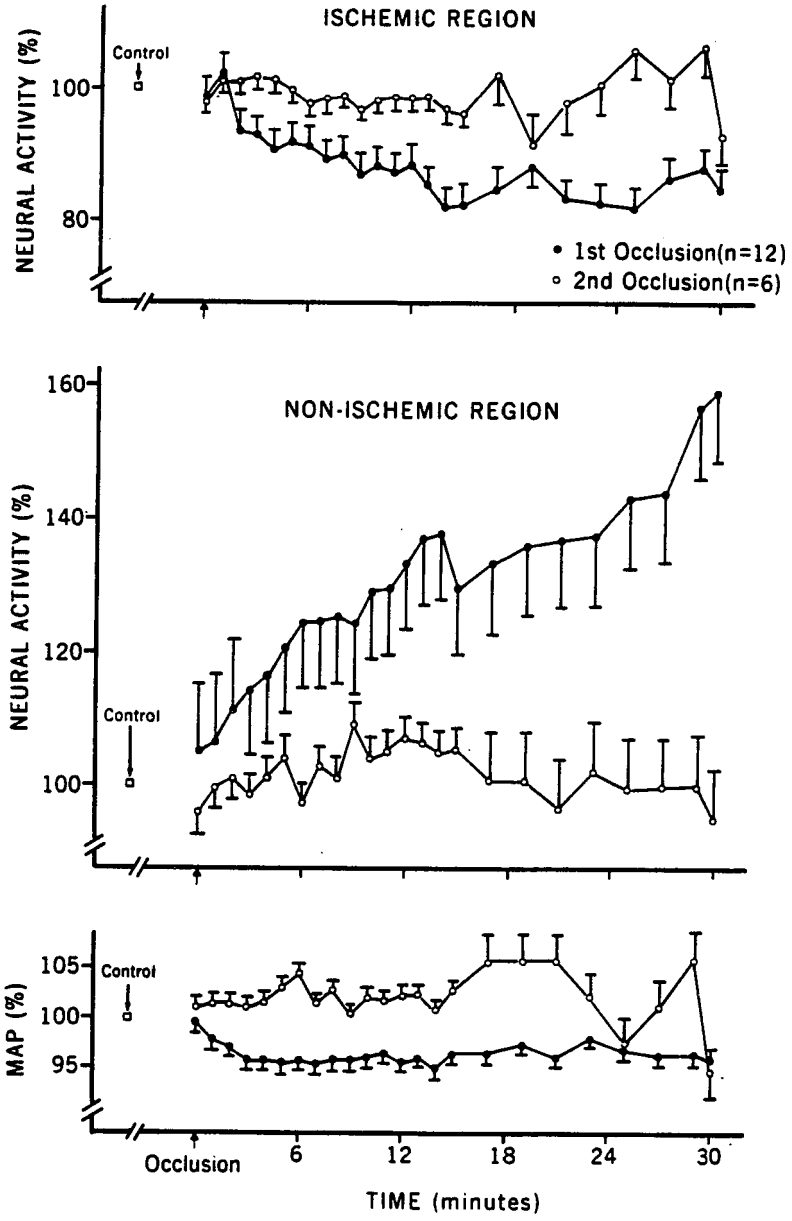

ISCHEMIC REGION
NEURAL ACTIVITY (%)
120
100
80
Control
NON-ISCHEMIC REGION
NEURAL ACTIVITY (%)
120
100
80
Control
MAP (%)
105
100
95
Control
• 1st Occlusion(n=10)
◦ 2nd Occlusion(n=8)
Occlusion
6
12
18
24
30
TIME (minutes)

Figure 9

Group data illustrating postganglionic sympathetic activities (ISCHEMIC AND NON-ISCHEMIC REGIONS) and mean arterial pressure (MAP) as a function of the occlusion number and the duration of occlusion in animals subjected to left circumflex coronary artery occlusions. Sympathetic activities and mean arterial pressure were normalized at 100% during control and expressed as a percentage during the coronary occlusion. P values are from the analysis of variance.


ISCHEMIC REGION
Control
NEURAL ACTIVITY (%)
100
80
• 1st Occlusion(n=12)
◦ 2nd Occlusion(n=6)
NON-ISCHEMIC REGION
160
140
120
100
Control
NEURAL ACTIVITY (%)
105
100
95
Control
MAP (%)
Occlusion
6
12
18
24
30
TIME (minutes)

15 minutes had elapsed from the beginning of the occlusion. At this time mean arterial pressure became more labile, as evidenced by the swings in pressure during the final 15 minutes of the occlusion (Figure 9). Changes in ventricular contractility (ischemic regions) also differed between the first and second LCX occlusions. During the second LCX occlusion no duration dependent trends ($p = 0.1$) were found.

Arterial pH and blood gases were assessed just prior to the beginning of the second coronary artery occlusion as well as during the last minute of the occlusion. Neither arterial pH (before 7.30 ± 0.01; during 7.30 ± 0.02) nor blood gases (before pO_2 81.5 ± 4.8 mmHg, pCO_2 30.7 ± 4.6 mmHg; during pO_2 84.1 ± 5.0 mmHg, pCO_2 30.6 ± 1.2 mmHg) exhibited significant changes.

Effects of Deafferentation Upon Cardiac Postganglionic Sympathetic Activities During Myocardial Ischemia

The contributions of afferent nerves in the initiation and maintenance of the ischemia-induced changes in cardiac postganglionic sympathetic activity were examined in 18 animals. Prior to LAD occlusion 5 animals were subjected to bilateral cervical vagotomy while 4 animals were subjected to epicardial phenol painting. Prior to LXC occlusion 5 animals were subjected to bilateral cervical vagotomy while 4 animals were subjected to epicardial phenol painting. Group data for the deafferentated animals were compared to the data from the corresponding group of animals with intact afferent nerves.

Bilateral cervical vagotomy prevented the ischemia-induced changes in cardiac postganglionic sympathetic activity independent of the location of the occluded coronary artery (Table 10). However, during LAD occlusion the effects of vagotomy appeared to be limited to nerves innervating ischemic regions (Table 11). In contrast, in animals subjected to LCX occlusion vagotomy not only prevented the reflex

decrease in activity to ischemic regions, but also the reflex increase to non-ischemic regions (Table 12).

In animals subjected to epicardial phenol painting (phenol deafferentation) prior to occlusion, no significant duration dependent changes were observed to non-ischemic regions upon LAD occlusion (Table 10). However, a significant duration dependent change in sympathetic activity to ischemic regions was observed. Sympathetic activity to ischemic regions remained at or above control levels throughout the LAD occlusion (Table 11). In contrast, phenol deafferentation significantly altered neural responses to both ischemic and non-ischemic regions during LCX occlusion (Table 12).

LAD occlusion following vagotomy did not mean arterial pressure from control levels (117 ± 8 mm Hg). In contrast, LAD occlusion following phenol deafferentation caused mean arterial pressure to steadily increase to levels 114 ± 4 % of control (103 ± 12 mmHg; $P < 0.05$). In animals subjected to LCX occlusion following either vagotomy or phenol deafferentation, no significant changes in mean arterial pressure were found (Table 10). Control pressurea in vagotomized (103 ± 12 mm Hg) or phenol animals (109 ± 13 mm Hg) were not significantly different than in animals with intact afferents.

Ischemia-induced changes in ventricular contractility with occlusion also differed depending upon the type of deafferentation. Changes in ventricular contractility in vagotomized animals subjected to LAD occlusion were biphasic. Contractility transiently increased (+16 %) during the first 6 minutes of the occlusion, then exhibited only negative inotropic responses (74.7 ± 9% of control; $p < 0.05$). LAD occlusion following phenol deafferentation severely depressed (63 ± 10% of control; $p < 0.01$) contractility. Similarly, LCX occlusion after

vagotomy also severely depressed contractility (60 ± 3% of control; $p < 0.01$). In contrast, LCX occlusion after phenol deafferentation only minimally depressed contractility (93 ± 3% of control); followed by a steady increase (119 ± 7% of control; $p < 0.05$) throughout the remainder of the occlusion.

Arterial pH and blood gases were assessed in 11 of the 18 animals subjected to deafferentation. Arterial blood samples were taken just prior to the beginning of the coronary occlusion and again during the last minute of each occlusion. Neither arterial pH (before 7.30 ± 0.09; during 7.25 ± 0.13) nor blood gases (before pO_2 90.9 ± 12.9 mmHg, pCO_2 26.9 ± 3.81 mmHg; during pO_2 91.23 ± 10.5 mmHg, pCO_2 24.97 ± 3.39 mmHg) exhibited significant changes.

Effects of Reperfusion Upon Preganglionic Sympathetic Activity

The effects of an initial period (5 minutes) of reperfusion upon preganglionic sympathetic activities were examined in 7 animals. The analysis of the group data is summarized in Table 13. The analysis indicated that there were no time-dependent changes in sympathetic activity to ischemic or non-ischemic regions during the first 5 minutes after reperfusion (Table 13, part II). During the last minute of the occlusion, preganglionic sympathetic activity to ischemic regions averaged 85.9 ± 4.4%; ($p < 0.05$) of control. Upon release, activity to ischemic regions immediately returned to control levels.

As previously indicated, preganglionic sympathetic activity to non-ischemic regions did not exhibit significant duration dependent changes during the 15-minute coronary occlusions. During the last minute of the ischemic period activity to non-ischemic regions averaged 102.6 ± 5.6% ($p = 0.9$) of control. No change in activity was observed upon reperfusion.

TABLE 10

COMPARISION OF THE EFFECTS OF DEAFFERENTATION ON CHANGES IN CARDIAC POSTGANGLIONIC SYMPATHETIC ACTIVITY DURING ACUTE MYOCARDIAL ISCHEMIA

ANALYSIS OF VARIANCE

I. VAGAL TRANSECTION

LAD OCCLUSION P Value*	Variable	LCX OCCLUSION P Value*
0.7341	Mean Arterial Pressure	0.3637
0.7981	Contractile Performance	0.0001
0.9532	Symp. Activity Ischemic	0.9945
0.9435	Symp. Activity Non-ischemic	0.0878

II. PHENOL

LAD OCCLUSION P Value*	Variable	LCX OCCLUSION P Value*
0.0196	Mean Arterial Pressure	0.9808
0.0682	Contractile Performance	0.7531
0.0061	Symp. Activity Ischemic	0.6198
0.3503	Symp. Activity Non-Ischemic	0.0260

* P values are from ANOVA for changes in the variable associated with the passage of time. The model for all analyses included the terms dogs (variability) and time (duration).

TABLE 11

EFFECTS OF DEAFFERENTATION ON CARDIAC POSTGANGLIONIC SYMPATHETIC ACTIVITY DURING LEFT ANTERIOR DESCENDING CORONARY ARTERY OCCLUSION

I. MEAN ± SE

Time (min)	VAGOTOMY Sympathetic Activity†		PHENOL Sympathetic Activity†	
	Ischemic	Non-Ischemic	Ischemic	Non-Ischemic
Control	100%	100%	100%	100%
Occlusion	107.6 ± 8.0	101.5 ± 3.1	93.0 ± 3.2	99.4 ± 1.8
0.25	106.7 ± 8.0	104.5 ± 3.1	100.9 ± 3.2	102.4 ± 1.8
1	107.5 ± 8.0	100.7 ± 3.1	98.6 ± 3.2	102.3 ± 1.8
2	109.2 ± 8.0	102.4 ± 3.1	100.0 ± 3.2	101.6 ± 1.8
3	105.1 ± 8.0	104.7 ± 3.1	97.8 ± 3.2	102.3 ± 1.8
4	94.4 ± 9.1	104.2 ± 3.6	99.3 ± 3.2	97.2 ± 1.8
5	96.8 ± 9.1	104.8 ± 3.6	102.4 ± 3.2	101.2 ± 1.8
6	96.7 ± 9.1	100.3 ± 3.6	101.4 ± 3.2	103.7 ± 1.8
7	95.5 ± 9.1	101.5 ± 3.6	99.8 ± 3.2	103.4 ± 1.8
8	95.6 ± 9.1	102.6 ± 3.6	98.5 ± 3.2	103.0 ± 1.8
9	95.6 ± 9.1	104.5 ± 3.6	100.4 ± 3.2	100.4 ± 1.8
10	92.5 ± 9.1	100.6 ± 3.6	106.7 ± 3.2	98.9 ± 1.8
11	101.1 ± 9.1	102.7 ± 3.6	105.0 ± 3.2	102.1 ± 1.8
12	111.8 ± 9.1	105.4 ± 3.6	102.3 ± 3.2	103.0 ± 1.8
13	101.9 ± 9.1	103.4 ± 3.6	103.8 ± 3.2	100.8 ± 1.8
14	102.6 ± 9.1	101.6 ± 3.6	106.7 ± 3.2	99.7 ± 1.8
15	105.0 ± 9.1	102.4 ± 3.6	103.8 ± 3.2	100.2 ± 1.8
17	102.1 ± 9.1	99.4 ± 3.6	109.2 ± 3.2	101.8 ± 1.8
19	99.6 ± 9.1	97.8 ± 3.6	110.1 ± 3.2	101.2 ± 1.8
21	95.3 ± 9.1	99.0 ± 3.6	110.0 ± 3.2	102.6 ± 1.8
23	97.2 ± 9.1	100.6 ± 3.6	110.0 ± 3.2	105.5 ± 1.8
25	110.1 ± 9.1	104.4 ± 3.6	112.1 ± 3.2	99.4 ± 1.8
27	113.8 ± 9.1	107.4 ± 3.6	101.4 ± 3.2	102.1 ± 1.8
29	107.9 ± 9.1	104.1 ± 3.6	106.2 ± 3.2	104.4 ± 1.8
30	89.8 ± 9.1	96.0 ± 3.6	102.8 ± 3.2	98.6 ± 1.8

† Sympathetic activities are normalized at 100% for control and are expressed as a percentage of control during the ischemic period. Means have been adjusted for dog variability.

TABLE 12

EFFECTS OF DEAFFERENTATION ON CARDIAC POSTGANGLIONIC SYMPATHETIC ACTIVITY DURING LEFT CIRCUMFLEX CORONARY ARTERY OCCLUSION

I. MEAN ± SE

Time (min)	VAGOTOMY Sympathetic Activity†		PHENOL Sympathetic Activity†	
	Ischemic	Non-Ischemic	Ischemic	Non-Ischemic
Control	100%	100%	100%	100%
Occlusion	98.9 ± 4.5	97.7 ± 3.0	100.3 ± 2.6	101.9 ± 3.7
0.25	94.4 ± 4.5	96.4 ± 3.0	100.0 ± 2.6	101.7 ± 3.7
1	100.8 ± 4.5	99.3 ± 3.0	100.3 ± 2.6	101.9 ± 3.7
2	99.0 ± 4.5	96.9 ± 3.0	99.1 ± 2.6	100.1 ± 3.7
3	96.5 ± 5.2	97.2 ± 3.4	97.3 ± 2.6	94.0 ± 3.7
4	98.8 ± 5.2	95.7 ± 3.4	103.5 ± 2.6	97.0 ± 3.7
5	99.9 ± 5.2	102.0 ± 3.4	101.5 ± 2.6	103.1 ± 3.7
6	102.8 ± 5.2	104.8 ± 3.4	100.4 ± 2.6	98.2 ± 3.7
7	99.6 ± 5.2	106.8 ± 3.4	105.7 ± 2.6	98.5 ± 3.7
8	97.7 ± 5.2	107.0 ± 3.4	102.6 ± 2.6	98.8 ± 3.7
9	94.0 ± 5.2	100.6 ± 3.4	101.4 ± 2.6	101.4 ± 3.7
10	100.6 ± 5.2	100.9 ± 3.4	104.7 ± 2.6	88.8 ± 3.7
11	100.7 ± 5.2	99.2 ± 3.4	103.1 ± 2.6	98.7 ± 3.7
12	101.5 ± 5.2	104.8 ± 3.4	102.7 ± 2.6	90.0 ± 3.7
13	99.6 ± 5.2	97.3 ± 3.4	107.7 ± 2.6	97.0 ± 3.7
14	101.8 ± 5.2	100.2 ± 3.4	102.8 ± 2.6	96.3 ± 3.7
15	98.9 ± 5.2	106.2 ± 3.4	105.6 ± 2.6	94.6 ± 3.7
17	96.5 ± 5.2	99.7 ± 3.4	105.6 ± 2.6	96.3 ± 3.7
19	97.6 ± 5.2	102.2 ± 3.4	106.0 ± 2.6	94.7 ± 3.7
21	93.7 ± 5.2	105.0 ± 3.4	103.0 ± 2.6	94.5 ± 3.7
23	95.8 ± 5.2	101.8 ± 3.4	110.1 ± 2.6	98.2 ± 3.7
25	99.5 ± 5.2	103.1 ± 3.4	107.1 ± 2.6	94.8 ± 3.7
27	101.3 ± 5.2	110.4 ± 3.4	107.3 ± 2.6	99.5 ± 3.7
29	91.3 ± 5.2	101.1 ± 3.4	109.4 ± 2.6	99.4 ± 3.7
30	92.3 ± 5.2	109.1 ± 3.4	108.9 ± 2.6	96.6 ± 3.7

† Sympathetic activities are normalized at 100% for control and are expressed as a percentage of control during the ischemic period. Means have been adjusted for dog variability.

Effects of Reperfusion Upon Cardiac Postganglionic Sympathetic Activity

The effects of an initial period (5 minutes) of reperfusion upon cardiac postganglionic activities were examined in 20 animals. Animals which developed ventricular fibrillation at the release of the coronary occlusion or during the 5 minutes of recording during the reperfusion period were analyzed separately; therefore, only 9 of the original 10 animals subjected to LAD occlusion were entered into the analysis. Since previous analyses (Table 6) had indicated that the direction and magnitude of reflex changes in postganglionic sympathetic activities were dependent upon the location of the ischemic myocardium, the data were subdivided accordingly.

No time-dependent changes in cardiac postganglionic sympathetic activities were found to be ischemic ($p = 0.5$) or non-ischemic ($p = 0.3$) regions during the first 5 minutes after reperfusion. However, closer examination of the data revealed phasic changes in activity to both ischemic and non-ischemic regions during this period (Table 14). Critical to the description of changes in neural activities coinciding with reperfusion is how (relatively) the data are expressed. When postganglionic sympathetic activity was expressed as a function of the pre-occlusion control, it was found that following release of an LAD occlusion sympathetic activity to ischemic regions rapidly increased to 128% of pre-occlusion levels following 3 minutes of reperfusion. When the same data are expressed as a function of the neural activity directly preceding (2 minutes) release of the occlusion, a similar pattern of response is observed although the magnitude of the changes are significantly greater (Table 14).

TABLE 13

EFFECTS OF REPERFUSION OF ISCHEMIC LEFT VENTRICULAR MYOCARDIUM UPON MEAN ARTERIAL PRESSURE AND PREGANGLIONIC SYMPATHETIC ACTIVITIES

I. MEAN ± SE

Time (min)	Mean Arterial Pressure (mmHg)	Symp. Activity Ischemic Regions	Symp. Activity Non-Ischemic Regions
Control	103 ± 1	100%	100%
Pre-Release	101 ± 2	85.9 ± 4.4 *	102.6 ± 5.6
Release	106 ± 3	100.3 ± 3.2	89.7 ± 2.9
0.25	108 ± 4	96.5 ± 3.1	92.6 ± 2.2
1	107 ± 3	96.4 ± 2.7	89.9 ± 2.0
2	109 ± 3	97.3 ± 2.8	92.1 ± 2.0
3	108 ± 4	97.1 ± 2.5	90.6 ± 2.0
4	109 ± 3	95.8 ± 2.5	91.2 ± 2.0
5	109 ± 4	99.2 ± 2.6	96.9 ± 2.0

II. ANALYSIS OF VARIANCE

Dependent Variable	Source	D.F.	Sum of Squares	F	P
Mean Arterial Pressure					
	Dog	6	3276.483	7.31	0.0001
	Time	6	64.651	0.15	0.8968
	Error	53	3959.244		
Sympathetic Activity (Ischemic)					
	Dog	6	6639.359	19.13	0.0001
	Time	6	131.654	0.38	0.9098
	Error	53	3066.389		
Sympathetic Activity (Non-Ischemic)					
	Dog	6	3563.584	8.71	0.0001
	Time	6	536.274	1.31	0.2650
	Error	53	3614.265		

Sympathetic activities were normalized at 100% for control and are expressed as a percentage of control for all other measurements. Means adjusted for dog varability.
Pre-release = Average activity during the 2 minutes immediately preceeding release of the coronary occlusion.

* $p < 0.05$ compared to control

Postganglionic sympathetic activity to non-ischemic regions increased after release of the LAD occlusion and peak activity also occurred following 3 minutes of reperfusion. Neither mean arterial pressure nor ventricular contractility exhibited significant changes during the initial 5 minutes of reperfusion.

It was also of interest to know whether or not the neural responses to reperfusion change with sequential coronary occlusions. Of the original 9 animals subjected to LAD occlusion and reperfusion, only 7 survived second occlusion and reperfusion. When the data for the second reperfusion are expressed as a function of the activity preceding the second occlusion, sustained increases in activity to ischemic regions were found during the entire 5 minutes of recording during the reperfusion period (Table 14). If the data are expressed as a function of the activity directly preceding the second release, the relationship described above is not observed. In fact, when the data are expressed as a function of pre-release activity, no significant changes were observed during the second reperfusion period. Postganglionic sympathetic activity to non-ischemic regions did not exhibit significant changes during the second reperfusion period (Table 14). This finding was independent of manner in which the data were expressed (i.e., pre-occlusion versus pre-release). Neither mean arterial pressure nor ventricular contractility exhibited significant changes during the second reperfusion period.

Only 11 of the original 12 animals subjected to LCX occlusion were analyzed. No time-dependent or phasic changes in postganglionic sympathetic activities were found ischemic ($p = 0.7$) or non-ischemic ($p = 0.2$) regions during the first 5-minute period of reperfusion. As previously indicated, postganglionic sympathetic activity to ischemic

regions was significantly depressed (-21%) at the end of the first LCX occlusion. Following release of the occlusion no recovery was observed. However, there was a further depression of sympathetic activity to ischemic regions. Postganglionic sympathetic activity to non-ischemic regions was significantly elevated by the end of the 30-minute LCX occlusion. No significant changes in activity occurred upon release of the occlusion (Table 15).

Mean arterial pressure exhibited a time dependent decrease ($p < 0.0001$) during the first reperfusion period following LCX occlusion. Upon release, mean pressure immediately became depressed (91 ± 1% of control; $p < 0.01$). Ventricular contractility was significantly depressed from control at the end of the first occlusion (Figure 8). Upon release of the LCX occlusion, contractile performance transiently improved, but then became depressed (85 ± 6% of control; $p < 0.05$).

Of the original 11 animals subjected to an initial LCX occlusion and reperfusion, only 5 survived a second occlusion and reperfusion. During the second reperfusion period no time-dependent trends were found for changes in postganglionic sympathetic activities to ischemic ($p = 0.9$) or non-ischemic ($p = 0.7$) regions. As previously described, during the second LCX occlusion activity to ischemic or non-ischemic regions was not significantly different from control levels. Activity to ischemic or non-ischemic regions remained unchanged upon release of the second LCX occlusion (Table 15).

Mean arterial pressure did not change significantly during the second LCX occlusion. Mean pressure decreased (93 ± 2% of control, $p < 0.05$) upon release, but had returned to control levels (106 ± 2% of control; $p = 0.3$) following 5 minutes of reperfusion. Ventricular contractility was minimally depressed (90 ± 6% of control; $p = 0.1$) by

TABLE 14

CARDIAC POSTGANGLIONIC SYMPATHETIC ACTIVITY DURING REPERFUSION OF ISCHEMIC ANTERIOR MYOCARDIUM

	Sympathetic Activity Ischemic Regions		Sympathetic Activity Non-Ischemic Regions	
Time (min)	%Pre-Occlusion	%Pre-Release	%Pre-Occlusion	%Pre-Release
I. FIRST OCCLUSION (n=9)				
Release	98.4 ± 7.7	115.6 ± 13.2	96.8 ± 3.4	103.5 ± 3.9
0.25	97.2 ± 7.7	115.2 ± 13.2	95.2 ± 3.4	101.7 ± 3.9
1	104.1 ± 10.1	120.0 ± 17.4	103.4 ± 4.3	110.8 ± 5.0
2	107.1 ± 10.1	123.4 ± 17.4	107.4 ± 4.3*	115.6 ± 5.0*
3	127.7 ± 10.1*	171.2 ± 17.4*	110.8 ± 4.3*	119.3 ± 5.0*
4	106.2 ± 10.1	125.1 ± 17.4	95.9 ± 4.3	102.2 ± 5.0
5	97.1 ± 10.1	110.5 ± 17.4	103.7 ± 4.3	111.2 ± 5.0
II. SECOND OCCLUSION (n=7)				
Release	126.9 ± 11.9	102.6 ± 6.4	96.0 ± 2.9	103.0 ± 3.3
0.25	131.9 ± 11.9*	105.6 ± 6.4	101.0 ± 2.9	108.9 ± 3.2
1	152.2 ± 15.1*	111.9 ± 8.1	96.6 ± 3.6	104.4 ± 4.1
2	125.4 ± 15.1*	101.0 ± 8.1	100.3 ± 3.6	108.3 ± 4.1
3	132.1 ± 15.1*	106.3 ± 8.1	97.6 ± 3.6	105.7 ± 4.1
4	112.1 ± 15.1	91.6 ± 8.1	96.1 ± 3.6	103.7 ± 4.1
5	112.4 ± 15.1	93.3 ± 8.1	96.4 ± 3.6	103.8 ± 4.1

Values are mean ± SE.
† Pre-occlusion values are expressed as a percentage of control.
†† Pre-release values are expressed as a percentage of 2 minutes of neural activity directly preceeding coronary artery release.
* $p < 0.05$ compared to (respective) control

TABLE 15

CARDIAC POSTGANGLIONIC SYMPATHETIC ACTIVITY DURING
REPERFUSION OF ISCHEMIC POSTERIOR MYOCARDIUM

	Sympathetic Activity Ischemic Regions		Sympathetic Activity Non-Ischemic Regions	
Time (min)	%Pre-Occlusion	%Pre-Release	%Pre-Occlusion	%Pre-Release
I. FIRST OCCLUSION (n=11)				
Release	79.4 ± 2.0*	98.7 ± 3.0	132.4 ± 4.3*	95.4 ± 3.5
0.25	81.4 ± 2.2*	100.7 ± 3.3	129.5 ± 4.7*	93.3 ± 3.9
1	77.6 ± 3.1*	96.9 ± 4.6	130.9 ± 6.5*	94.3 ± 5.3
2	78.9 ± 3.1*	97.0 ± 4.6	132.8 ± 6.5*	95.9 ± 5.3
3	76.6 ± 3.1*	93.0 ± 4.6	132.6 ± 6.5*	95.7 ± 5.3
4	73.3 ± 3.1*	88.4 ± 4.6*	134.5 ± 6.5*	97.5 ± 5.3
5	76.5 ± 3.1*	92.2 ± 4.6	129.5 ± 6.5*	93.3 ± 5.3
II. SECOND OCCLUSION (n=5)				
Release	99.0 ± 3.9	104.1 ± 4.1	100.6 ± 2.4	100.2 ± 2.4
0.25	96.7 ± 3.9	101.6 ± 4.1	101.6 ± 2.4	101.6 ± 2.4
1	97.5 ± 7.0	102.5 ± 7.5	106.3 ± 4.3	106.0 ± 4.4
2	95.2 ± 7.0	100.2 ± 7.5	103.4 ± 4.3	102.9 ± 4.4
3	95.8 ± 7.0	100.8 ± 7.5	106.3 ± 4.3	106.0 ± 4.4
4	100.4 ± 7.0	105.5 ± 7.5	102.7 ± 4.3	102.7 ± 4.4
5	97.6 ± 7.0	102.7 ± 7.5	111.8 ± 4.3*	111.2 ± 4.4*

Values are mean ± SE.
† Pre-occlusion values are expressed as a percentage of control.
†† Pre-release values are expressed as a percentage of 2 minutes of neural activity directly preceding coronary artery release.
* $p < 0.05$ compared to (respective) control.

the end of the second LCX occlusion. Reperfusion resulted in a further reduction in contractility during the first minute, followed by a return to control after 5 minutes (101 ± 4%; p = 0.9) .

Changes in Cardiac Postganglionic Sympathetic Efferent Activities In Animals Exhibiting Ventricular Fibrillation During Reperfusion

Only 1 animal out of a total of 13 animals subjected to an initial LCX occlusion exhibited ventricular fibrillation during the first 5 minutes of reperfusion (Table 16). In this animal, postganglionic sympathetic activity to ischemic regions was not significantly different from control following 30 minutes of ischemia. In contrast, sympathetic activity to non-ischemic regions significantly increased (+336%) during LCX occlusion. Upon reperfusion, sympathetic activity to ischemic or non-ischemic regions remained virtually unchanged for pre-release levels (Table 16). Mean arterial pressure was significantly depressed (-19% from control levels) by LCX occlusion. Upon reperfusion, mean pressure decreased further, but following 1 minute of reperfusion and prior to the occurrence of ventricular fibrillation had returned to control levels.

Effects of Deafferentation Upon Changes in Cardiac Postganglionic Sympathetic Efferent Activities During Reperfusion

The effects of deafferentation upon changes in cardiac postganglionic sympathetic efferent activities during reperfusion were examined in 16 animals. Of the 9 animals which were subjected to deafferentation prior to LAD occlusion, only 8 survived occlusion and reperfusion. of these 8 animals, 4 were subjected to bilateral vagotomy and 4 to epicardial phenol painting. Similarly, of the 9 animals subjected to deafferentation prior to LCX occlusion only 8

TABLE 16

CHANGES IN CARDIAC POSTGANGLIONIC SYMPATHETIC ACTIVITY IN AN ANIMAL EXHIBITING VENTRICULAR FIBRILLATION DURING POSTERIOR REPERFUSION

Time (min)	Mean Arterial Pressure (mmHg)	Symp. Activity Ischemic	Symp. Activity Non-Ischemic
Control	120	100%	100%
Pre-Release	97	105.3	336.4
Release	96	103.9	319.3
0.25	95	105.6	337.2
1	115	110.3	338.1

Sympathetic activities are normalized at 100% during control and are expressed as a percentage of control during reperfusion.

Pre-release refers to average activity during the 2 minutes just prior to release of LCX occlusion and is expressed as a percentage of the pre-occlusion control.

survived occlusion and reperfusion. Of these 8 animals 4 were subjected to bilateral vagal transection and 4 to epicardial phenol painting.

In deafferentiated animals (vagotomy or phenol) no significant changes in postganglionic activities were observed to either ischemic or non-ischemic regions during the initial 5 minutes of reperfusion (Table 17). Comparision of the levels of sympathetic activities during reperfusion revealed a significant difference between animals with intact vagal afferents (Table 14 & 25) and deafferentiated animals (Table 18 & 19).

Mean arterial pressure did not exhibit any significant changes during the initial 5 minutes of reperfusion in the deafferentated animals (Table 17). Mean pressure remained within 2 ± 1% of control (vagotomy: 116 ± 2 mmHg; phenol: 120 ± 3 mmHg) in both groups during reperfusion.

TABLE 17

COMPARISION OF THE EFFECTS OF DEAFFERENTATION ON CHANGES IN CARDIAC POSTGANGLIONIC SYMPATHETIC ACTIVITY DURING REPERFUSION OF ISCHEMIC VENTRICULAR MYOCARDIUM

ANALYSIS OF VARIANCE

I. VAGAL TRANSECTION

ANTERIOR ISCHEMIA P Value*	Variable	POSTERIOR ISCHEMIA P Value*
0.6390	Mean Arterial Pressure	0.2732
0.9327	Contractile Performance	0.9758
0.3248	Symp. Activity Ischemic	0.1154
0.1255	Symp. Activity Non-ischemic	0.3417

II. PHENOL

ANTERIOR ISCHEMIA P Value*	Variable	POSTERIOR ISCHEMIA P Value*
0.9424	Mean Arterial Pressure	0.5625
0.0027	Contractile Performance	0.8047
0.2454	Symp. Activity Ischemic	0.2296
0.6216	Symp. Activity Non-Ischemic	0.0768

* P values are from ANOVA for changes in the variable associated with the passage of time. The model for all analyses included the terms dog (variability) and time (duration).

TABLE 18

EFFECTS OF DEAFFERENTATION ON CARDIAC POSTGANGLIONIC SYMPATHETIC ACTIVITY DURING REPERFUSION OF ISCHEMIC ANTERIOR MYOCARDIUM

Time (min)	VAGOTOMY (n=4)		PHENOL (n=4)	
	Sympathetic Activity		Sympathetic Activity	
	Ischemic	Non-Ischemic	Ischemic	Non-Ischemic
Release	80.8 ± 2.6 (91.6 ± 4.2)	101.6 ± 2.2 (108.8 ± 2.5)	103.5 ± 4.2 (100.8 ± 4.2)	99.2 ± 1.6 (100.7 ± 1.6)
0.25	82.1 ± 2.6 (93.7 ± 4.2)	95.6 ± 2.2 (101.9 ± 2.5)	101.9 ± 4.2 (99.3 ± 4.2)	100.0 ± 1.6 (101.4 ± 1.6)
1	84.0 ± 2.6 (96.9 ± 4.2)	99.0 ± 2.2 (105.7 ± 2.5)	110.5 ± 5.2 (107.9 ± 5.1)	102.5 ± 2.0 (103.8 ± 2.0)
2	74.6 ± 2.6 (83.0 ± 4.2)	94.4 ± 2.2 (100.8 ± 2.5)	114.5 ± 5.2*(111.8 ± 5.1)	97.8 ± 2.0 (99.2 ± 2.0)
3	80.9 ± 2.6 (90.6 ± 4.2)	97.0 ± 2.2 (103.6 ± 2.5)	116.3 ± 5.2*(113.7 ± 5.1)	98.2 ± 2.0 (99.6 ± 2.0)
4	79.6 ± 2.6 (88.8 ± 4.2)	102.7 ± 2.2 (109.7 ± 2.5)	117.0 ± 5.2*(114.3 ± 5.1)	100.9 ± 2.0 (102.3 ± 2.0)
5	79.0 ± 2.6 (89.8 ± 4.2)	100.7 ± 2.2 (107.9 ± 2.5)	112.0 ± 5.2 (109.5 ± 5.1)	99.2 ± 2.0 (100.5 ± 2.0)

Values are least squares mean ± SE.
Sympathetic activities are normalized at 100% for control and expressed as a percentage of control. Values appearing in parentheses are expressed as a percentage of the average neural activity 2 minutes preceeding the release of the occlusion (pre-release).

TABLE 19

EFFECTS OF DEAFFERENTATION ON CARDIAC POSTGANGLIONIC SYMPATHETIC ACTIVITY DURING REPERFUSION OF ISCHEMIC POSTERIOR MYOCARDIUM

Time (min)	VAGOTOMY (n=4)		PHENOL (n=4)	
	Sympathetic Activity		Sympathetic Activity	
	Ischemic	Non-Ischemic	Ischemic	Non-Ischemic
Release	91.2 ± 3.3 (99.1 ± 3.5)	106.1 ± 2.4 (97.8 ± 2.3)	97.0 ± 6.1 (100.8 ± 6.1)	108.2 ± 2.0 (100.1 ± 1.9)
0.25	96.3 ± 3.3 (104.4 ± 3.5)	106.0 ± 2.4 (97.8 ± 2.3)	100.0 ± 6.1 (103.7 ± 6.1)	109.2 ± 2.0 (101.0 ± 1.9)
1	94.5 ± 3.3 (102.4 ± 3.5)	103.3 ± 2.4 (95.2 ± 2.3)	104.8 ± 6.1 (109.1 ± 6.1)	106.7 ± 2.0 (98.7 ± 1.9)
2	105.8 ± 3.3 (114.7 ± 3.5)*	100.6 ± 2.4 (92.6 ± 2.3)	91.5 ± 6.1 (95.0 ± 6.1)	115.7 ± 2.0 (107.3 ± 1.9)
3	98.1 ± 3.3 (108.4 ± 3.5)	100.6 ± 2.4 (92.7 ± 2.3)	97.2 ± 6.1 (101.2 ± 6.1)	110.6 ± 2.0 (102.2 ± 1.9)
4	94.0 ± 3.3 (101.7 ± 3.5)	105.0 ± 2.4 (96.8 ± 2.3)	108.1 ± 6.1 (111.5 ± 6.1)	113.1 ± 2.0 (104.5 ± 1.9)
5	98.8 ± 3.3 (108.2 ± 3.5)	99.9 ± 2.4 (92.1 ± 2.3)	86.1 ± 6.1 (89.9 ± 6.1)	108.7 ± 2.0 (100.4 ± 1.9)

Values are least squares mean ± SE.
Sympathetic activities are normalized at 100% for control and expressed as a percentage of control. Values appearing in parentheses are expressed as a percentage of the average neural activity 2 minutes preceeding the release of the occlusion (pre-release).

* $p < 0.05$ compared to (respective) control

CHAPTER VI

DISCUSSION

The occurrence of autonomic disturbances early after the onset of myocardial ischemia has been documented in both man (53, 119, 139, 180, 185) and experimental animals (1, 37, 44, 49, 50, 57, 86, 98, 110, 116, 164, 185). However, the role of the autonomic nervous system in the genesis and/or maintenance of arrhythmias occurring during myocardial ischemia is still unclear. Reflex changes in the autonomic control of the heart which occur during ischemia are the net result of changes in afferent signalling from the heart and other cardiovascular areas. Of critical importance in the discussion of whether or not the autonomic nervous systems plays an active role in arrhythmogenesis are the temporal relationships between the onset of the ischemia, the occurrence of changes in autonomic activity and the appearance of arrhythmias. The task of delineating these relationships is further complicated by the presence of other cardiovascular homeostatic mechanisms which can act to modify the level of activity in the cardiac autonomic nerves (25, 42).

Early investigators (15, 52, 104) attempted to determine whether or not alterations in the level of autonomic activity occurred during myocardial ischemia by employing measurements of peripheral resistance as an index of autonomic activity. Soon after the onset of ischemia, peripheral resistance was reported to increase (15, 52, 104). Thus, the authors speculated that a generalized increase in the level of sympathetic activity was responsible for the observed changes. In

addition, heart rate and blood pressure data from both clinical and experimental studies also fostered speculation that myocardial ischemic elicited reflex activation of the autonomic nervous system (53, 139, 180). Pantridge and associates (139, 180) reported that within 1 hour of the onset of symptoms of myocardial ischemia, the location of the ischemic myocardium could be predicted from the reflex changes in heart rate and blood pressure. The use of anti-adrenergic or anticholinergic agents either alone or in combination allowed these authors to conclude that, in man, the predominant response to anterior ischemia was enhanced sympathetic nervous system activity, while the predominant response to posterior ischemia was enhanced parasympathetic nervous system activity (139). Furthermore, additional support for an ischemia-induced sympathetic reflex has been provided by studies which demonstrated a lower incidence of arrhythmias following the administration of anti-adrenergic agents (14, 55, 71, 74, 100, 139, 150). Although each of these studies concluded that reflex alterations in sympathetic activity occurred in response to myocardial ischemia, none actually recorded activity from sympathetic nerves.

Prior to 1963, no reports had been published concerning changes in the level of activities in sympathetic nerves in response to myocardial ischemia. Costantin (37) was the first to record and report changes in activity in sympathetic nerves during acute myocardial ischemia. Coronary artery occlusion was reported to result in a transient decrease in sympathetic activity that subsided prior to release of the occlusion. However, at that time no method was available to quantitate the reported changes. Therefore, it was impossible to correlate the magnitude of the neural changes with either the duration of the ischemia or any other hemodynamic parameters.

Malliani and associates (116) were the first to record and quantitate changes in sympathetic activity during acute myocardial ischemia. They reported that, in the cat, coronary occlusion elicited an increase in sympathetic preganglionic activity. The present study is the first to record and quanitate activities in both right and left sided preganglionic sympathetic nerves during acute myocardial ischemia. Coronary occlusion elicited reflex decreases in preganglionic sympathetic activity in nerves innervating ischemic regions, while activity to non-ischemic regions was unchanged. Several differences exist between the present study and that by Malliani et al. (116) which could account for the apparent disparity. In the present study, preganglionic sympathetic activity was simultaneously recorded from both the right and left anterior ansae. The ansae are located distal to the stellate ganglia (5, 6, 124). Malliani et al. (116) recorded from the left third ramus communicans, which is located proximal to the left stellate ganglion (6, 124). The autonomic ganglia have been demonstrated to possess an integrative function (174). In addition, in the canine, a small percentage of sympathetic nerves have been shown to synapse within the stellate ganglia before exiting to either the caudal cervical ganglia or to the heart (24, 135, 181). Therefore, sympathetic activity recorded from the anterior ansae may differ significantly from that recorded from nerves located proximal to the stellate ganglia.

Efferent innervation patterns of the preganglionic sympathetic nerves are more difficult to localize to discrete regions of the heart due to the greater heterogeneity of the efferent nerves and the high degree of divergence that occurs at the autonomic ganglia (5, 124, 135, 181). Similar difficulties were encountered when we attempted to

confirm efferent innervation patterns of the anterior ansae. Although efferent innervation patterns could not be localized to discrete regions of the heart, we catagorized each neural preparation employed for recording as innervating either predominately ischemic or non-ischemic regions. The study by Malliani et al. (116) did not attempt to determine efferent innervation patterns. Therefore, no inferences can be made concerning changes in activity to ischemic versus non-ischemic myocardium in Malliani's study (116). Furthermore, in the present study, sympathetic preganglionic activity was recorded from multifiber preparations, while the majority of the preparations in the study by Malliani et al. (116) were single fiber. Although the predominant response to coronary occlusion (in the cat) was reported (116) to be increased preganglionic sympathetic activity, it should be noted that almost one third of all fibers recorded did not change their activity and a smaller population of fibers even exhibited decreased activity. The possibility exists that the single fibers recorded by Malliani et al. (116) were not representative of the population of efferent fibers innervating the ischemic region. This possibility seems even more likely upon consideration of the fact that, in the present study, no significant differences in the nature of the reflex changes were found during either left anterior descending or left circumflex occlusions (Table 4). Thus, differences in the sympathetic preganglionic response to coronary occlusion could be due to differences in the type(s) of neural preparations employed for recording. Furthermore, differences in the anatomy of the coronary circulation (78), as well as other factors (151) in the different animal models, may also contribute to the observed differences.

More recently, sympathetic efferent activity has been recorded during acute myocardial ischemia and has been reported both to increase (49, 57, 98, 101, 110) and decrease (47, 50, 98, 101, 179). Within individual studies (37, 101, 116, 179), differences in both the direction and extent of changes in sympathetic activities have been reported. Thus, disparities within, as well as between, studies preclude any conclusions concerning the status of the cardiac sympathetic nerves during myocardial ischemia. Our observations in animals in which we recorded preganglionic activities, as well as those from earlier studies, (116) would suggest that changes in preganglionic sympathetic activity are not an adequate index of changes in local autonomic control and, therefore, cannot provide the data necessary to reach specific conclusions concerning the behavior of individual cardiac sympathetic nerves. In addition, subtle changes in sympathetic activity could have been concealed when recording from preganglionic nerves due to the greater heterogeneity of the neural preparations. For instance, upon coronary occlusion one population of neurons could have increased their activity while another population decreased their activity. Should the reflex sympathetic neural response to coronary occlusion result in heterogeneous responses from the neurons within the preganglionic multifiber preparation (as is suggested by the results in the series of experiments employing postganglionic nerves), it is likely that such subtle changes would not be detected.

Additional experiments were designed and carried out which examined reflex changes in sympathetic activity in the canine thoracic cardiac postganglionic nerves during 30 minutes of acute myocardial ischemia. Unlike preganglionic nerves, the efferent innervation patterns of the

cardiac postganglionic nerves could be localized to discrete regions of the heart. This finding is in agreement with the work of Randall and colleagues (6, 7, 54, 60, 62, 144-146, 165). In the present study, electrical stimulation of cardiac postganglionic sympathetic nerves elicited discrete changes in either rate, rhythm or contractility. Therefore, it is likely that reflex changes in efferent sympathetic activities observed in different animals are more homogeneous, since the postganglionic nerves are less variable in their efferent destinations (144-146).

Two major weaknesses of previous studies which recorded sympathetic activity during acute myocardial ischemia have been the lack of information concerning concomitant changes in other nerves and the failure to determine efferent innervation patterns of the recorded nerves. To the best of our knowledge, only two studies (98, 101) exist where sympathetic activity was simultaneously recorded from more than one nerve. Furthermore, only two studies (57, 101) exist where neural activity was recorded from more than one cardiac nerve. The present study is the first to record and quantify sympathetic postganglionic efferent activities in two nerves with known cardiac innervation patterns. In contrast, in order to make similar comparisons previous studies have had to rely upon sequential measurements of sympathetic activity which were made during repeated coronary artery occlusions. Due to the cumulative effects of repetitive coronary occlusions (see discussion below), the simultaneous measurement of activities in at least two nerves is an absolute prerequisite for meaningful comparisons. Although the ability to record neural activities from a greater number of nerves theoretically increases possible comparisons, increasing the number of decentralized nerves could also produce problems. Should the

reflex changes in efferent activities be dependent upon afferent signals transmitted via any of the additional nerves severed for recording, the result could be the partial or total loss of the reflex. In contrast, recording from only two nerves provides us with the necessary information to make meaningful comparisons under identical circumstances and with the least possible interruption of afferent pathways.

Most studies (37, 47, 49, 50, 57, 98, 101, 110, 116) which recorded neural activity during acute myocardial ischemia never confirmed the efferent destination(s) or the cardiovascular nature of their neural preparations. The importance of confirming the efferent destinations of the recorded nerves, particularly the cardiac nerves, is exemplified in the present study. In 7 of the 10 animals subjected to occlusion of the left anterior descending coronary artery, the region of the anterior left ventricle was innervated by the ventromedial cardiac nerve. This finding is in agreement with the studies performed by Randall and associates (6, 144-146). However, in the remaining animals, the ventrolateral cardiac nerve was found to innervate the region of the anterior left ventricle. Thus, efferent innervation patterns in almost one third of the animals subjected to left anterior descending coronary artery occlusion differed from that predicted from the literature. The selection of nerves employed for recording becomes imprecise and could lead to erroneous conclusions concerning reflex changes in cardiac sympathetic activity during myocardial ischemia without confirmation of the efferent destinations. Furthermore, it is equally important to assess whether the recorded nerve responds to cardiovascular stimuli. In the present study each neural preparation was tested several times for its baroreceptor responsiveness. All nerves from which we recorded

responded appropriately to increases or decreases in arterial pressure (42) prior to initiation of the coronary occlusion.

Thirty minutes of left ventricular ischemia was found to elicit differential reflex changes in cardiac postganglionic sympathetic nerves. Neither the time course nor the magnitude of the reflex inhibition of sympathetic activity to ischemic regions were significantly different during left anterior descending or left circumflex coronary artery occlusions (Figure 7). The data clearly indicated that the predominant reflex response to acute left ventricular ischemia was a reduction of sympathetic activity to ischemic regions. However, both the magnitude and direction of reflex changes in activity to non-ischemic regions were correlated with the location of the occluded coronary artery (Table 6). Occlusion of a branch of the left anterior descending coronary artery did not elicit significant changes in sympathetic activity to non-ischemic regions. In contrast, occlusion of a branch of the left circumflex coronary artery elicited significant increases in sympathetic activity to non-ischemic regions. Thus, in response to acute coronary occlusion, reflex changes in cardiac postganglionic sympathetic activity to non-ischemic regions are characterized by increased activity (relative increase during left anterior descending coronary artery occlusion). The efferent destination(s) of the recorded nerves are critical in any discussion concerned with changes in neural activity during ischemia. In the majority of the animals subjected to left circumflex occlusion (10 of 12 animals), sympathetic activity was recorded from nerves shown to innervate the region of the sinus node. In humans, posterior ischemia is frequently associated with bradycardia (139). Similar observations have not been made in the anesthetized canine model without the

selective ablation of cardiac sympathetic afferent fibers (47, 49, 168). In contrast, posterior ischemia (left circumflex occlusion) in the conscious canine has been reported to elicit reflex increases in heart rate (16, 87, 141). Administration of atropine, a muscarinic receptor antagonist, did not alter the heart rate responses to circumflex occlusion in the conscious canine (141). Thus, the investigators (141) proposed a reflex increase in sympathetic activity to the sinus node. Our finding that in response to circumflex occlusion cardiac postganglionic sympathetic activity increases by approximately 60% in nerves which innervate the sinus node supports the conclusions drawn from observations in the conscious canine (16, 87, 141).

Lathers et al. (101) reported that occlusion of the left anterior descending coronary artery resulted in either no change or increased activity in the right side sympathetic nerves of the cat. Although each of the nerves recorded by Lathers et al. (101) was tested for its baroreceptor responsiveness, efferent innervation patterns were never assessed. Armour and Hopkins (5) have demonstrated that the nerves employed for recording in Lathers et al.'s study (101) innervate the atria. Thus, one can speculate that the reported changes in sympathetic activity were not only to non-ischemic regions, but were even more likely to the region of the sinus node. Therefore, our data suggest that studies which reported decreased cardiac sympathetic activity during ischemia (47, 50, 98, 101, 179) were likely to be recording from nerves innervating ischemic regions. Furthermore, our study also suggests that studies which reported increased activity in response to coronary occlusion (49, 57, 98, 101, 110, 116) were likely to be recording from nerves innervating non-ischemic regions of the heart.

To the best of our knowledge only one other study (98) has simultaneously recorded from two postganglionic sympathetic nerves during acute myocardial ischemia. Kullmann and Jink (98), recorded efferent activities from the periarterial (accompanies the retroauricular artery) and splanchnic nerves in the rabbit during coronary occlusion. Upon occlusion, activity in the periarterial nerve was reported to increase, while activity in the splanchnic nerve decreased. Thus, in the rabbit and similar to the findings of the present study, coronary artery occlusion elicited differential changes in postganglionic efferent sympathetic activities. The finding that coronary occlusion elicits differential changes in efferent sympathetic activities in both cardiac and non-cardiac nerves may help to unravel the controversy (48, 112) concerning the existence of an ischemia-induced sympathetic cardio-cardiac reflex.

The mass of myocardium rendered ischemic during a coronary artery occlusion has been reported to influence both the direction and extent of the reflex response to ischemia (86, 110, 120). Most studies which reported changes in sympathetic activity during ischemia chose to occlude either the entire left anterior descending or left circumflex coronary arteries or the left main coronary artery. These occlusions usually severely compromised the left ventricle. Cardiac output and arterial pressure would characteristically decrease, which acted to further decrease perfusion pressures and exacerbate the ischemic insult. In these preparations severe ventricular arrhythmias occurred within minutes of the initiation of the coronary occlusion. Thus, interpretation of the data is quite complicated since ventricular arhythmias causes changes in arterial pressure which also elicit reflex alterations in sympathetic activity (25, 42, 116, 166). In an attempt

to avoid these very problems, we produced only small localized ischemic insults to elicit reflex changes in cardiac efferent sympathetic activities. Similar sized ischemic insults during either left anterior descending or left circumflex coronary artery occlusions (16-17% of LV wet weight) resulted in identical reductions in sympathetic activity to ischemic regions (Figure 7). In a comparable study, Feola et al. (50) recorded efferent sympathetic activity (presumably to ischemic regions) during left circumflex occlusion in the dog. Sympathetic activity was recorded from only one (cardiac) nerve, but was reported to decrease upon circumflex occlusion. The magnitude of the reflex depression in cardiac sympathetic activity reported by Feola et al. (50) was greater than that which we report; however, in that study the entire left circumflex had been occluded.

A major element of controversy in studies which have examined changes in sympathetic activity during ischemia has been the profound decrease in arterial blood pressure which usually accompanies the coronary occlusion. The appearance of dramatic changes in arterial pressure following coronary occlusion makes it impossible to determine whether changes in cardiac sympathetic activity were due to reflexes originating from within the ischemic myocardium or to systemic baroreceptors. The extent to which baroreflexes can modify the reflex sympathetic response to acute myocardial ischemia has been examined (116,166). Animals with intact aortic and carotid baroreceptors exhibited increased sympathetic activity following left anterior descending or left circumflex occlusion (116). In sinoaortic denervated animals sympathetic activity was unchanged during occlusion of the left anterior descending coronary artery, but was depressed during left circumflex occlusion (116). As might be expected, the changes in

arterial pressure following coronary occlusion were of lesser magnitude in animals with intact baroreceptors (116, 166). Felder and Thames (47) also compared the changes in efferent sympathetic activity during coronary occlusion to those observed following hemorrhage. The authors concluded that the depressor reflex initiated by myocardial ischemia could be blunted or even completely obliterated by baroreflexes, since sympathetic activity increased more in response to decreases in arterial pressure (i.e., hemorrhage) in the absence of ischemia.

In the present series of experiments it is unlikely that the differential changes in sympathetic activities observed upon coronary artery occlusion were due to activation of systemic baroreceptors. Ischemia induced alterations in heart rate which could cause changes in arterial pressure and elicit baroreflexes were prevented by maintaining heart rate constant via atrial pacing. Atrial pacing per se was without effect on ventricular contractility, mean arterial pressure or cardiac efferent sympathetic activity (Figure 5). In addition, in none of the animals employed in this study did mean arterial pressure change significantly upon coronary occlusion. The observed small changes (±5%) in mean arterial pressure are not likely to be the afferent stimulus responsible for the reflex changes in cardiac efferent sympathetic activity. Our laboratory has previously demonstrated (61) that comparable changes in arterial pressure in the absence of myocardial ischemia do not elicit comparable changes in cardiac efferent sympathetic activity. Furthermore, the only group of animals which exhibited moderate changes in arterial pressure were those subjected to reperfusion following coronary occlusion. It is equally unlikely that these changes were the afferent stimulus responsible for the changes in

cardiac efferent sympathetic activity because such changes, when present, always followed and never preceded the neural changes. Thus, one cannot determine from the present study what role, if any, the cardiac nerves play in previously observed (182) reflex responses during reperfusion.

Several series of experiments were carried out to determine the source of the afferent signalling responsible for the differential changes in cardiac sympathetic efferent activities observed during coronary occlusion. A cardiac-selective local deafferentation was produced by the application of phenol, a sclerosing agent (13, 91), to the region of the left ventricle supplied by the coronary artery to be occluded. The application of phenol to the canine ventricle differentially affects cardiac sympathetic and vagal afferents (13). Barber et al. (13) reported that epicardial phenol painting interrupted cardiac sympathetic afferents. However, phenol was not as effective in interrupting cardiac vagal afferents. In our study, local cardiac deafferentation with phenol prevented the differential changes in cardiac efferent sympathetic activities during coronary occlusion (Table 10). If one considers the changes in cardiac efferent sympathetic activity in light of the available literature concerning cardiac vagal afferent signalling (3, 4, 16, 25, 94, 97, 169, 172, 179), the changes in sympathetic activity to ischemic regions could be considered as a vagally mediated depressor response. Similarly, if one considers the changes in activity in nerves innervating non-ischemic regions during left circumflex occlusion (3, 4, 18, 23, 25, 70, 155, 179), the response could be considered as sympathetic pressor response.

The data from the phenol deafferentated animals suggest, although do not prove, that epicardial phenol painting interrupted both cardiac

sympathetic and vagal afferent signalling. Several differences exist between the present study and that of Barber et al. (13) which could account for the apparent disparity. In our experiments, phenol was applied over the entire region of the left ventricle which could be visually identified as being supplied by the coronary artery to be occluded. In contrast, Barber et al. (13) only circumscribed limited regions of tissue with thin lines. Thus, it is likely that more afferent fibers were affected by the phenol pre-treatment in the present study. In addition, there is a significant difference in the nature of the afferent stimulus in our study and the study of Barber et al. (13). It is likely that increased afferent signalling occurs from mechanoreceptive, chemoreceptive and possibly even nociceptive afferents during the acute coronary occlusions employed in the present study. In contrast, Barber et al. (13) used epicardial bradykinin to excite cardiac sympathetic afferents and nicotine to excite cardiac vagal afferents. However, bradykinin and nicotine have been demonstrated to excite both cardiac vagal and sympathetic afferents in a variety of experimental preparations (25, 32, 33, 88, 109, 115, 140, 160, 163, 178). Therefore, the delineation of the effects of phenol as reported by Barber et al. (13) may not be as clear as originally proposed.

The individual contributions of sympathetic and vagal afferents were assessed in animals which were subjected to either a selective thoracic afferent sympathectomy or bilateral cervical vagotomy. In animals in which preganglionic sympathetic efferent activity was recorded during coronary occlusion, both the right and left anterior ansae were transected. Thus, prior to the coronary occlusion two major sympathetic afferent routes were eliminated. In these animals coronary

occlusion (left anterior descending or left circumflex) still elicited the characteristic reflex depression of sympathetic efferent activity to ischemic regions. However, the reflex increase in sympathetic activity to non-ischemic regions was not observed. The data would suggest that the anterior ansae are major sympathetic routes used to relay the afferent signals from the ischemic region to the central nervous system.

As previously discussed, the finding that preganglionic sympathetic efferent activity decreases following coronary occlusion is in opposition to the findings of Malliani et al. (116). In addition to the afore mentioned differences, at least one other explanation exists which could account for differences in the nature of the reflex response to acute coronary occlusion. Malliani et al. (116) only recorded from the left third ramus communicans; therefore, the remainder of the sympathetic afferents (right and left side) were intact. In the present series of experiments both the right and left anterior ansae were transected. Thus, animals in which we recorded preganglionic sympathetic activity during acute ischemia had a greater degree of sympathetic deafferentation than those in the study by Malliani et al. (116). Another reservation must be raised about the study by Malliani et al. (116) which is now routinely quoted in the literature as the "characteristic" response to coronary occlusion. In a later study (115) Malliani et al. report that, in the cat, the major neural pathway for the sympathetic afferent signaling during acute left ventricular ischemia is the left third ramus communicans. If this is truly the case, then one must have reservations concerning the conclusions of the earlier study (116) which reported increased preganglionic activity in the left third ramus communicans during acute ischemia. Furthermore, some animals employed in the earlier study by Malliani et al. (116)

exhibited massive increases in sympathetic preganglionic activity (>700% change from control), while others exhibited only minimal changes (<30% change from control). The data in Malliani's study (116) were not subjected to statistical analysis, and one wonders whether or not the reported changes would be significant given both the method employed to count neural activity (frequencies calculated from only several seconds of data) and the high degree of variability between preparations. In the present study we did not encounter such a problem. Not only were the sympathetic neural responses within a treatment group qualitatively similar but they were also quantitatively similar as evidenced by the relatively small standard errors of the means.

In our hands there was little difference between deafferentation produced by vagotomy or phenol. Bilateral cervical vagotomy was effective in preventing the reflex depression of cardiac sympathetic efferent activity in nerves innervating ischemic regions. This finding is in support of the proposition (25) that cardiac vagal afferents are responsible for initiating depressor reflexes. It is unclear as to why we did not observe a reflex increase in sympathetic activity in nerves innervating non-ischemic regions in the vagotomized animals. However, observations made in other studies (25, 179) suggest that cardiac vagal afferents can modulate excitatory reflexes. Whether or not this occurred in the present series of experiments is unknown.

After an initial 30-minute coronary occlusion and 60 minutes of reperfusion, occlusion of the same coronary artery for a second 30-minute period failed to elicit similar reflex changes in cardiac sympathetic activities (Figures 8 and 9). The greatest difference in the nature of the efferent sympathetic response to myocardial ischemia

was between the first and second left anterior coronary occlusions. Sympathetic activity recorded from nerves innervating non-ischemic regions remained unchanged during either the first or second left anterior descending coronary artery occlusion. However, sympathetic activity recorded from nerves innervating ischemic regions decreased during the first occlusion and second increased during the second occlusion. The mechanisms responsible for the differences observed between the first and second occlusions are unclear. Such mechanisms could include adaptations in the afferent receptors, defects in the transmission of the afferent signals to the central nervous system and/or central nervous system adaptations. This observation has important implications concerning previous studies (101, 116, 179) which relied upon sequential measurements of sympathetic activity made during repetitive coronary occlusions in order to make comparisons between different nerves.

Changes in cardiac sympathetic efferent activity in animals subjected to a second 30-minute coronary artery occlusion were compared to those recorded in animals subjected to deafferentation prior to an initial coronary occlusion. No significant differences were found between the reflex responses in the two groups. The data suggest, although do not prove, that the initial coronary occlusion or the subsequent reperfusion period altered the afferent signalling from the ischemic myocardium. It does not appear that the central mechanism governing the discharge of peripheral sympathetic neurons underwent some type of adaptation, since sympathetic activity was observed to change during the initial reperfusion period (Tables 14 and 15). Similar findings have been reported for experiments employing a single coronary

occlusion of sufficient duration as to produce myocardial infarction (12). In the present study, repetitive coronary occlusions produced transmural ischemia as assessed by methylene blue. In several of the animals subjected to repetitive coronary occlusion we also employed tetrazolium dyes (11, 12) in an attempt to determine whether or not the occlusions had infarcted any tissue. In none of the animals tested was there any evidence of infarction as assessed by the tetrazolium dye.

Previous studies recording cardiac afferent activity during myocardial ischemia have reported both phasic and sustained increases in activity (172, 173) during brief (< 5 minutes) coronary occlusions. One cannot determine from the present series of experiments the critical factors which determine at what point during the coronary occlusion cardiac afferent signalling from the ischemic region becomes deranged. However, the data would suggest that the integrity of the afferent receptors, the neurons responsible for conveying the information to the central nervous system and the central integration of the reflex are all still functional up to a period of 30 minutes of myocardial ischemia, since efferent responses are maintained within this time frame.

Due to limitations of the methodologies employed to record neural activity from the peripheral sympathetic nerves, it was necessary to sever the nerves distally in order to ensure that only efferent activity was recorded. Therefore, no direct conclusions can be made concerning the role of the observed changes in the initiation and/or maintenance of arrhythmias, since these changes were not translated into neurotransmitter release. It is tempting to speculate however, that the observed changes in sympathetic activity could play a role in arrhythmogenesis. The data from the animals which exhibited ventricular

fibrillation during left anterior descending coronary artery occlusion (Table 7) would certainly suggest that changes in neural activity in the remaining intact sympathetic nerves could have contributed to the arrhythmia. Unlike animals with intact afferents, sympathetic activity to ischemic and non-ischemic regions increased during coronary occlusion in animals which exhibited ventricular fibrillation. It is interesting to note however, that the majority of the animals employed in the present study did not develop severe ventricular arrhythmias during the coronary occlusion or the initial five minutes of reperfusion. Thus, by transection of the nerve for recording eliminated the role the observed changes would have had in the initiation and/or maintenance of arrhythmias. In the one animal exhibiting ventricular fibrillation during the initial reperfusion period, the data suggest that a generalized increase in sympathetic activity may have influenced arrhythmogenesis. However, the bulk of the data of the present study would suggest that changes in cardiac sympathetic efferent activity are not likely to be the overwhelming factor responsible for initiating arrhythmias during reperfusion (182). Whether cardiac sympathetic activity changes following 5 minutes of reperfusion and whether such changes are associated with arrhythmias cannot be answered.

Previous studies (27, 60, 64, 71) would suggest that discrete differential changes in cardiac sympathetic efferent activity analogous to those which we report within the present study would be more likely to have arhythmogenic influences than a generalized increase as was previously proposed (116). The study of Han et al. (64) provided convincing evidence that discrete sympathetic stimulation creates greater temporal dispersion of ventricular refractory periods than does a generalized increase in sympathetic influences. Ischemia alone has

been shown to shorten ventricular refractory periods and decrease the ventricular fibrillation threshold (75, 76, 95, 122). Therefore, it would appear that the reciprocal changes in sympathetic activity to ischemic and non-ischemic regions might act to create more homogeneous conditions during acute ischemia. Whether or not this actually occurs cannot be answered by the present study. The discovery of a cardio-cardiac reflex characterized by the inhibition of sympathetic activity to ischemic regions with relative or absolute increases in activity to non-ischemic regions raises many questions concerning the development of future therapeutic treatments, since many of the current pharmacological agents (14, 35, 55, 74, 100, 142, 150, 153) employed during myocardial ischemia also affect the function of the neurons of the autonomic nervous system.

Summary

In the anesthetized dog model we have demonstrated that acute coronary occlusion elicits differential changes in efferent activities in the small thoracic cardiac nerves. The direction and magnitude of these changes are dependent upon 1) the efferent destination of the nerve, 2) the duration of the coronary occlusion, 3) the location of the occluded coronary artery, and 4) the integrity of cardiac afferent signalling.

Our data supports the following conclusions: 1) acute coronary occlusion induces afferent signals from the ischemic sympathetic efferent activities; 2) the ischemia-induced cardio-cardiac reflex is characterized by decreased activity to ischemic regions with either no change or increased activity to non-ischemic regions; 3) the inhibition of sympathetic activity to ischemic regions is elicted via cardiac vagal afferents, while cardiac sympathetic afferents are responsible for the

increased activity to non-ischemic regions; and 4) previous coronary artery occlusion disrupt cardiac afferent signalling from the ischemic region.

To the best of our knowledge this study is the first to provide evidence of an ischemia-induced reflex which differentially alters cardiac efferent sympathetic activities. The discovery of such a reflex should be of significance to those in the clinical setting who must routinely treat individuals presenting with the symptoms of myocardial ischemia.

Our findings indicate several future studies which are necessary to determine the role of the sympathetic nervous system in arrhythmogenesis during acute myocardial ischemia. Future research should concentrate upon the development of techniques which will allow the simultaneous recording of afferent and efferent activities from the same intact cardiac nerve. Such a technique would allow changes in neural activities to be expressed as neurotransmitter release. This ability will allow the effects of changes in activity upon cardiac electrical stability to be assessed. In addition, it would also alleviate the possibility of disrupting afferent pathways. Additional future studies involving electrical stimulation of the cardiac nerves in a pattern analogous to those observed during the present study should also be performed. These studies would provide information which would allow conclusions concerning the arrhythmogenic role of differential changes in sympathetic activities during acute coronary occlusion.

CHAPTER VII

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APPENDIX

NERVE TRAFFIC ANALYSIS PROGRAM AND SUBROUTINES

NERAN.FOR

```
NERANA ORIG 5-14-81 AFS
A PROGRAM TO COUNT NERVE TRAFFIC FIRINGS
FOR A SUCCESSION OF TIME INTERVALS. EACH TIME
INTERVAL IS A BIN. THE DURATION OF EACH IMTERVAL IS THE
BIN WIDTH. THE TOTAL TIME IS THE RUN TIME. AFTER RUN,
MAY BE SCALED BY FACTOR OF 2**N WHEN N IS - OR +.
THE DATA MAY CONVERTED INTO HISTOGRAM FORM BY
TAKING AS THE VALUE OF EACH BIN, THE AVERAGE OF THE
2,4,8 ETC BINS OF WHICH IT IS GROUPED. THE CONVERTED
DATA MAY BE DRAWN BY AN X-Y PLOTTER AND MAY BE
STORED ON DISK.
     PROGRAM NERAN
      XXXXXXXXXXXXXXX
     DIMENSION SCAFAC(2)
     INTEGER DATE (4),RUN,TSTART,TSTOP,SET,CLKCNT,FTG1(3),FTG2(3),
    1FN(6),D1,D2,D3
     LOGICAL A,B,Z,F,S,U,D,P,T,X,LET,CHAN(2)
     LOGICAL BIN(200,3,2),SBIN(200,3,2),DBIN(200,3,2),C
     COMMON /COM1/DATE,RUNDUR,RUN,M,SCAFAC,CLKCNT,JMAX,KMAX,
    1FTG1,FTG2,BIN
     DATA Z,F,S,U,D,P,T,C/'Z','F','S','U','D','P','T','C'/
     DATA A,B,X/'A','B','X'/,CHAN(1),CHAN(2)/'A','B'/
      XXXXXXXXXXXXXXX
   1 FORMAT(14)
   2 FORMAT(A1)
     WRITE(3,20)
  20 FORMAT(1X,'START NERVE TRAFFIC ANALYSIS PGM')
     WRITE(3,21)
  21 FORMAT(1X,'MOUNT DATA DISK AND TYPE CR    ')
     READ(3,1)IND
IND IS ONLY OBJECT OF READ ST.
     CALL LOGDIS
     WRITE(3,26)
  26 FORMAT('+','TYPE DATE,6 DIGITS,E.G. 092181    ')
     READ(3,31)D1,D2,D3
  31 FORMAT(3I2)
CHANGE DATE INTO ASCII FORMAT
      ENCODE(DATE,32)D1,D2,D3
```

```
  32   FORMAT(I2,'-',I2,'-',I2)
       WRITE(3,33)(DATE(I),I=1,4)
  33   FORMAT('+',4A2)
 LOAD INTERUPT ROUTINES
      CALL POK
 DETERMINE BIN WIDTH
  36 FORMAT(1X,'TYPE RUN DURATION IN SECS.WITH DECIMAL    ')
     WRITE(3,46)
  46 FORMAT (1X,'TYPE NO.CHANNELS 1 OR 2    ')
     READ(3,1)KMAX
     WRITE(3,47)
  47 FORMAT('+','TYPE NEXT RUN NO. ')
     READ(3,1)RUN
     RUN=RUN-1
                                  NEW RUN
  50 RUN=RUN+1
     JMAX=0
     IND=1
     CALL CLEARA
     CALL CLEARB
     WRITE(3,51)RUN
  51 FORMAT(1X,'NEW RUN NO.=',I2)
     WRITE(3,36)
     READ(3,56)RUNDUR
  56 FORMAT(F5,1)
     CLKCNT=RUNDUR*1000./200.
     WRITE(3,58)CLKCNT
  58 FORMAT('+','BIN WIDTH=',I4,'MS.')
  60 WRITE(3,61)
  61 FORMAT(1X,'TYPE SET:1,2,OR 3;CR IF NONE   ')
 1 TONE;2 INTERVEN;3 POST TONE
     READ (3,1)SET
  70 IF(SET.EQ.0)GOTO 108
       IF(SET.EQ.2)IND=2
     J=SET
 ZERO BINS
     DO 80 K=1,KMAX
     DO 80 I=1,200
       BIN(I,J,K)=0
   80 CONTINUE
 IND=1 IF THERE IS AN INTERVENTION, (SET 2)
 100 WRITE(3,101)
 101 FORMAT('+','TYPE START FOOTAGE AND (CR) TO START    ')
     READ(3,10FTG1(J)
                              COUNT PULSES
     CALL COUNT(BIN(1,J,1),BIN(1,J,2),CLKCNT)
     WRITE(3,105)
 105 FORMAT('+','TYPE STOP FOOTAGE    ')
     READ(3,1)FTG2(J)
     GO TO 60
```

```
                        SCALE AND COMBINE ROUTINES
108 JMAX=J
NOW INITIALIZE SBIN ARRAYS TO EQUAL BIN ARRAYS
    DO 109K=1,KMAX
      DO 109 J=1,JMAX
      DO 109 I=1,200
      SBIN(I,J,K)=BIN(I,J,K)
109 CONTINUE
    IND2=0
110 DO 170 K=1,KMAX
    M=1
    ACCSF=1.0
    CALL CLEARA
    CALL CLEARB
     WRITE(3,116)CHAN(K),IND
116 FORMAT(1X,'SEE SET ',A1,I1, )
    CALL OUT(32,0)
    CALL NERCNT(SBIN(1, INF,K))
    IF(INF2,EQ.1)GOTO 128
                        SUBTRACT TONE ROUTINE
200 WRITE(3,201)
201 FORMAT('+','TYPE S TO SUBTRACT TONE FROM INTERVENTION  ')
    READ(3,2)LET
    IF(LET.NE.S)GOTO 219
205 DO 210 K=1,KMAX
    DO 210 I=1,200
      DBIN(I,K)=SBIN(I,2,K)-SBIN(I,1,K)+(K*25)
210 CONTINUE
    CALL OUT(32,0)
    CALL NERCNT(DBIN(1,1))
    IF(KMAX-2)219,215,219
215 CALL OUT(33,0)
    CALL NERCRT(DBIN(1,2))
219 GO TO 260
                              PLOT
260 WRITE(3,261)
261 FORMAT('+','FOR PLOT TYPE P OR X IF NOT   ')
    READ(3,2)LET
      IF(LET.EQ.P)GOTO 270
      IF(LET.EQ.X)GOTO 300
      GOTO 260
270 CALL AXES(RUNDUR)
280 WRITE(3,281)
281 FORMAT(1X,'TO PLOT A SET TYPE A OR B FOR CHAN,AND 1,2,OR 3 FOR
   1SET,X OUT  ')
    READ(3,283)LET,NJK
283 FORMAT(A1,I1)
      IF(LET.EQ.A)K=1
      IF(LET.EQ.B)K=2
    IF(LET.EQ.D)GOTO 295
    IF(LET.EQ.Z)GOTO 270
    IF (LET.EQ.X)GOTO 300
    J=NJK
```

```
  290 CALL PLOT(SBIN(1,J,K),M)
      GO TO 280
  295 K=NJK
      CALL PLOT(DBIN(1,K),M)
      GO TO 280
                              PRINT
  300 WRITE(3,301)
  301 FORMAT('+','TYPE T TO TYPE DATA FOR RUN OR X OUT  ')
      READ(3,2)LET
      IF(LET.NE.T)GOTO 350
      CALL NERLST
  350 CONTINUE
  360 WRITE(3,361)
  361 FORMAT(1X,'TYPE D FOR DATA DUMP')
      READ(3,2)LET
      IF(LET.NE.D)GOTO 370
      CALL NDUMP(BIN,JMAX,KMAX)
  370 CONTINUE
  400 WRITE(3,401)
  401 FORMAT(1X,'TYPE D FOR DISK STORE,OR CR')
      READ(3,2)LET
      IF(LET.NE.D)GOTO 50
                            STORE ON DISK
      ENCODE(FN,404)D1,D2,D3,RUN
  404 FORMAT('A',3I2,'R',I2,1X)
      CALL OPEN(6,FN,1)
      WRITE(6)(DATE(I),I=1,4),RUN,RUNDUR,CLKCNT,JMAX,KMAX,
     1(FTG1(I),I=1,JMAX),(FTG2(I),I=1,JMAX),
     2(((BIN(I,J,K),I=1,200),J=1,JMAX),K=1,KMAX)
      ENDFILE 6
        WRITE(3,420)
        WRITE(3,420)
  420   Format('0')
      GO TO 50
      END
```

SUBROUTINE NERCRT.MAC

```
 NERCRT.MAC WAS LD1RAM.MAC AFS 7-16-81
 LOGDIS ADDED 9-18-81
 LD1RAM.MAC AFS S6-22-81
 CALL NERCRT(ARG1)
 A SUBROUTINE FOR LOADING AN ARRAY INTO GRAPHICS BOARD.
 ASSUMES CHANNEL HAS BEEN SELECTED
 BY AN OUT TO 20H FFOR CHANNEL A
 OR BY AN OUT TO 21H FOR CHANNEL B
 ARG1 IS ARRAY OF LOGICAL NUMBERS
 BASELINES ARE INPUT TO BD BY ROUTINE EXT.TO THIS
        ENTRY NERCRT,CLEARA,CLEARB,LOGDIS
NERCRT: OUT(27H),A      ;RESET TR CNTR
        LD B,200
REPEAT: LD A,(HL)
        CPL
        OUT (22H),A     ;LATCH DATA BYTE
        LD A,25
```

```
DELAY:  DEC A
        JR NZ,DELAY
        OUT (24H),A     ;ADV RT CNTR
        INC HL
        DJNZ REPEAT
        OUT (23H),A
        RET
 CLEARA,CLEARB AFS 7-18-81
 CLEARA AND CLEARB CLEAR THE CRT WITHOUT CHANGING THE COMPUTER
 ARRAYS.CLEARA CLEARS CRT CHAN A,CLEARB CLEARS B
 CLEARA: OUT (27H),A   ;RESET RT COUNTER
         OUT (20H),A    ;SELECT CRT CHAN A
         CALL CLEAR
         RET
 CLEARB: OUT (27H),A
         OUT (21H),A    ;SELECT CRT CHAN B
        CALL CLEAR
        RET
 CLEAR:  LD B,255   ;ZERO 255 HORIZ LOCATIONS
 GO:     LD A,0
        CPL
        OUT (22H),A     ;LATCH A IN GRAPHICS BD
        LD A,20    ;CREATE DELAY HERE
 DEL:    DEC A
        JR NZ,DEL
        OUT (24H),A     ;ADV RT CNTR
        DJNZ GO
        OUT (23H),A     ;RESET CHAN SELECT
        RET
```

SUBROUTINE LOGDIS

```
LOGDIS 9-18-81 AFS
SUBR TO TO LOG ON DRIVE A AFTER CHANGING DISK
STORES 13 DEC IN C REG AND CALLS BDOS
LOGDIS: NOP
BDOS    EQU     0005H
       LD C,13
       CALL BDOS
       RET
       END
```

SUBROUTINE COUNT.MAC

```
COUNT.MAC AFS 5-29-81
CRT ADDED 7-13-81.
COUNT SUBR FOR USE WITH NERANA
CALL COUNT (ARG1,ARG2,ARG#)
ARG1 IS ADDR OF 1ST BIN ARRAY IN HL
ARG2 IS ADDR OF 2ND BIN ARRAY IN DE
ARG3 IS ADDR OF NO. OF CLK CNTS PER BIN
       ENTRY COUNT
```

```
COUNT:  LD (ADDRA),HL
        LD IX, (ADDRA)
        LD (ADDRB),DE
        LD IY,(ADDRB)A   ;SET IX POINTER TO 1ST B CHAN BIN
        LD A,(BC)        ;THIS ARG IS NO. OF CLK CNTS/BIN
        LD L,A
        INC BC
        LD A, (BC)
        LD H,A
        LD (CLKCNT),HL
        OUT (27H),A       ;RESET REAL TIME CNTR ON GRAPHICS BD
        LD B,200          ;NO. OF BINS SET AT 200
        LD DE, 1          ;USE DE TO DECR.HL
        IM 1    ;SELECT INT MODE 1
THE IN'S BELOW DO NOT INPUT DATA, BUT SEND PULSES TO INTERFACE BD
        IN A, (2AH)      ;RESET START LATCH
        IN A, (2BH)      ;RESET CLOCK LATCH
NOW WAIT FOR START PULSE.BIT 0,CHAN 28H, WILL EQUAL 1
WAIT:  IN A, (28H)
      BIT 0,A           ;Z FLAG=1 WHEN BIT 0=0
      JR Z,WAIT         ;JMPS TO WAIT WHEN BIT 0=0
NOW COUNT THE BIN. 1ST INITIALIZE
CNTBIN: LD HL,(CLKCNT)  ;LD HL WITH NO.OF CLK CNTS PER BIN
        LD A,0          ;USE LOGIC OUTPUT 14H TO RESET CLK
        OUT (14H),A     ;TO START TIMING NOW
        LD A,1
        OUT (14H),A
        OUT (15H),A     ;TURN ON INTERUPTS,LOGIC OUT CHAN 15H
        EI      ;ENABLE INTS.
NOWCNT: IN A,(28H)       ;28H IS START OF CLK PORT
        BIT 1,A
        JR Z,NOWCNT     ;LOOKS FOR CLK WHILE COUNTING INTERUPTS
        IN A,(28H)      ;RESET CLK LATCH
        AND A
        SBC HL,DE       ;DECR.HL
        JR NZ,NOWCNT
        DI              ;DISABLE MASKABLE INT.
DJNZ DECS.B AND JMPS BACK UNTIL B=0
WHEN B=0 WE CHANGE BINS IF BIN CNT IS NOT 0
        IN A, (2BH)     ;RESET CLK LATCH
CHGBIN: LD A,0
       OUT (15H),A     ;TURN OFF INTERUPTS
       CALL CRT
       INC IX  ;POINT TO NEXT BIN(S)
       INC IY
       DJNZ CNTBIN     ;GO TO NEXT BIN IF BIN CNT NOT 0
OR LEAVE THIS SUBR IF IT IS 0
       OUT (23H),A
       RET
ADDRA:  DEFS 2
ADDRB:  DEFS 2
CLKCNT: DEFS 2
```

```
INTERUPT ROUTINES,USE POKE IN MAIN PGM
FOR PINT INC (IX), EI,AND RETI
FOR NMI INC(IY),EI, AND RETN
THE CRT SUBR.SENDS BINS JUST COUNTED TO GRAPH.BD
CRT:    OUT (23H),A
        OUT (20H),A     ;SELECT CHAN A
        LD A,(IX)
        CPL
        OUT (22H),A     ;SEND LAST A BIN CNT TO GRAPH.BD
        LD A,25
DELAY:  DEC A
        JR NZ,DELAY
        OUT (23H),A     ;RESET SELECT LATCH
        OUT (21H),A     ;SELECT CHANB
        LD A,(IY)
        CPL
        OUT (22H),A     ;SEND LAST B BIN CNT TO GRAPH BD
        LD A,25
DELAY2: DEC A
        JR NZ,DELAY2
        OUT (24H),A     ;ADV REAL TIME CNTR
        RET
        END
```

SUBROUTINE POK.FOR

```
 POK.FOR AFS REV 7-8-81
SUBR TO SERVICE PINT AND NMI INTERUPTS
WHICH IS POKED INTO ABS MEM.LOCS
      SUBROUTINE POK
PINT JMPS TO ML 38H=56
PINT ROUTINE:INC(IX) DDH,34H,0H=221,52,0
             EI   FBH=251
            RETI EDH,4DH=237,77
      CALL POKE (56,8)
      CALL POKE (57,221)
      CALL POKE (58,52)
      CALL POKE (59,0)
      CALL POKE (60,8)
      CALL POKE (61,251)
      CALL POKE (62,237)
      CALL POKE (63,77)
NMI JMPS TO ML 66H=102
NMI ROUTINE:EX AF,AF'8H=8
            INC(IY) FDH,34H,0H=253,52,0
            EI FBH=251
           RETN EDH,45H=237,69
```

```
      CALL POKE(102,8)
      CALL POKE(103,253)
      CALL POKE(104,52)
      CALL POKE(105,0)
      CALL POKE(106,8)
      CALL POKE(107,237)
      CALL POKE(108,69)
      RETURN
      END
```

SUBROUTINE NERLST.FOR

```
NERLST AFS 9-17-81
SUBR FOR FOR NERANA TO CALC AND PRINT OUT DATA END OF EACH RUN
                          VARIABLES:
AVCNT  REAL  MEAN BINC CNT FOR SET
BIN    LOG    ARRAY OF BIN CNTS(MAIN DATA)
CLKCNT INT   MS/BIN
FRCSIG REAL SIG CNTS AS FRAC OF SET
JMAX    INT   NO. OF SETS
KMAX    INT    NO.OF CHANS.
IBIN   INT   TEMP VAR = BIN(I)
M       INT   NO. OF BINS/BAR
MAXCNT  INT MAX CNT OF ANY BIN IN SET
NUMSIG  INT NO. OF SIG. BINS IN SET
RUNDUR  REAL RUN DURATION IN SEC.
SIGCNT  INT  CNT T'HOLD FOR SIGNIFICANCE
SIGTOT  INT  SIGNIFICANT CNTS/SETS
TOTCNT  INT  TOTAL CNTS/SETS
XMAX    REAL  TEMP VAR = MAXCNT
XSGTOT  REAL  TEMP VAR = SIGCNT
XTOTCT  REAL  = TOTCNT
    SUBROUTINE NERLST
    ********************************
    INTEGER DATE(4),RUN,CLKCNT,TOTCNT,SIGTOT,SIGCNT,
   1FTG1(3),FTG2(3),STOT(2)
    REAL LST(2)
    LOGICAL BIN(200,3,2),CHAN(2)
    DIMENSION SET(3),SCAFAC(2),FST(2),TF(2,2),TL(2,2),SF(2,2)
    COMMON /COM1/DATE,RUNDUR,RUN,M,SCAFAC,CLKCNT,JMAX,KMAX,
   1FTG1,FTG2,BIN
    DATA CHAN(1),CHAN(2),SET(1),SET(2),SET(3)/'A','B','TONE',
   1'INTV','TONE'/
     ********************************
  1 FORMAT(I6)
  3 FORMAT(F3.1)
  4 DO 5 I=1,5
      WRITE(3,7)
  5 CONTINUE
    WRITE(3,10)(DATE(I),I=1,4)
  7 FORMAT('0')
    WRITE(3,8)
  8 FORMAT(1X,"TYPE NO.OF SDS,F.P.    ')
    READ(3,3)SIGS
```

```
 10 FORMAT(1X,32X,4A2)
    WRITE(3,15)RUN
 15 FORMAT(1X,32X,'RUN ',I2)
    WRITE(3,20)RUNDUR,M,(SCAFAC(I),I=1,KMAX)
 20 FORMAT(1X,3X,'RUN TIME ',F5.1,'SEC',7X,I2,'BINS/BAR',8X,
   1'SCALE FACTORS A: X',F4.1,'B: X',F4.1)
    XCLCNT=CLKCNT
    WRITE(3,25)CLKCNT
 25 FORMAT(1X,3X,'BIN TIME ',I4,'MS ')
                    WRITE MAIN DATA HEADING
    WRITE(3,30)
 30 FORMAT(1X,'  DHAN ',3X,'SET',3X,'TOTAL',2X,'MEAN',4X,'MAX',3X,
   1'SIG',4X,'NUMBER','  SD  ','  P56  ','P5BINS ',' T')
    WRITE(3,35)
 35 FORMAT(1X,16X,'COUNT  ','COUNT  ','COUNT  ','COUNT  ',
   1"SIG.BINS')
                        COMPUTE STATS
    DO 120 K=1,KMAX
    DO 120 J=1,JMAX
      TOTCNT=0
      SIGTOT=0
      NUMSIG=0
      MAXCNT=0
      STOT(1)=0
      STOT(2)=0
      IND1=0
      IND2=0
      SS=0
    JP5BIN=0
      DO 70 I=1,200
        IBIN=BIN(I,J,K)
        TOTCNT=TOTCNT+IBIN
        BINSQ=IBIN*IBIN
        SS=SS+BINSQ
        IF(IBIN.LE.MAXCNT)GO TO 70
        MAXCNT=IBIN
 70  CONTINUE
STS.TO 73 FOR SETS 1&2
      XTOTCT=TOTCNT
      AVCNT=XTOTCT/200.
      VAR=(SS-XTOTCT*XTOTCT/200.)/199.
      SD=SQRT(VAR)
      XMAX=MAXCNT
      IF(J.EQ.2)GOTO 76
STS.TO 76 FOR SET 1 ONLY
      XM1=AVCNT
      S1=SD
      JP5=SIGS*SD+AVCNT+.5
      T=0.0
      GOTO 80
STS.TO 80 FOR SET 2 ONLY
```

```
 76    XM2=AVCNT
       S2=SD
       DM=XM2-XM1
       SDM=(S1*S1+S2*S2)/200.
       SDM=SQRT(SDM)
       T=DM/SDM
 80    CONTINUE
       SIGCNT=.333*(XMAX-XM1)+XM1+.5
       DO 90 I=1,200
         IBIN=BIN(I,J,K)
       IF(IBIN.LE.SIGCNT)GOTO 85
        NUMSIG=NUMSIG+1
        STOT(1)=STOT(1)+IBIN-SIGCNT
        LST(1)=I
        IF(IND.EQ.1)GOTO 85
        IND1=1
        FST(1)=I
 85    CONTINUE
        IF(IBIN.LE.JP5)GOTO 90
        JP5BIN=JP5BIN+1
        STOT(2)=STOT(2)+IBIN-JP5
        LST(2)=I
        IF(IND.EQ.1)GOTO 90
        IND2=1
        FST(2)=I
 90    CONTINUE
       IF(J.NE.2)GOTO 100
    DO 95 L=1,2
      TF(L,K)=XCLCNT*FST(L)/1000.
      TL(L,K)=XCLCNT*LST(L)/1000.
      SF(L,K)=STOT(L)
      SF(L,K)=SF(L,K)/XTOTCT
 95  CONTINUE
100  CONTINUE
WRITE CALCULATED DATA
    WRITE(3,100)CHAN(K),SET(J),TOTCNT,AVCNT,MAXCNT,SIGCNT,
   1NUMSIG,SD,JP5,JP5BIN,T
101 FORMAT(1X,4X,A1,4X,A4,I6,F7.1,3X,I3,2X,I5,5X,I3,6X,F4.2,
   1I4,I6,F8.3)
120 CONTINUE
      WRITE(3,7)
      WRITE(3,123)
123   FORMAT(1X,3X,'1ST SIG.BIN',4X,'SIG.CNTS',2X,'LAST SIG.BIN')
    DO 130 K=1,KMAX
    DO 130 L=1,2
      WRITE(3,124)TF(L,K),SF(L,K),TL(L,K)
124   FORMAT(1X,4X,F6.1,'S',6X,F6.3,8X,F6.1)
130 CONTINUE
    WRITE(3,136)
136 FORMAT(1X,4X,'TAPE LOCATION')
    WRITE(3,137)
137 FORMAT(1X,'SET  START STOP')
    DO 139 J=1,JMAX
      WRITE(3,138)J,FTG1(J),FTG2(J)
```

```
138 FORMAT(1X,I2,4X,I4,I5)
139 CONTINUE
      WRITE(3,7)
      WRITE(3,7)
    WRITE(3,140)
140 FORMAT(1X,'TYPE 1 TO REPEAT OR (CR)')
    READ(3,1)IND
    IF(IND.EQ.1)GOTO 4
    RETURN
    END
```

SUBROUTINE SCALE.FOR

```
SCALE.FOR 5-21-81 AFS
MOD 7-8-81
COMBIN ADDED 7-21-81
STS.14,15 ETC 7-22-81
SCALE MULTIPLIES ARRAY SBIN BY XSCALE, A REAL NO.
TO GENERATE NEW ARRAY SBIN
    SUBROUTINE SCALE(SBIN,XSCALE)
    *************************
    LOGICAL SBIN(200)
    *************************
THE IF ST.BELOW CAUSES THE CONVERSION OF LOGICAL NUMBERS
ABOVE 127, WHICH CONVERT TO NEG. REAL NOS., TO BE CHANGED
TO REAL NOS.GTR THAN 127
    DO 20 I=1,200
    TEMP=SBIN(I)
      IF(TEMP)14,15,15
 14 TEMP=TEMP+256.
 15 TEMP=XSCALE*TEMP+.5
    SBIN(I)=TEMP
 20 CONTINUE
    RETURN
    END
    SUBROUTINE COMBIN(SBIN,M)
    *************************
    LOGICAL SBIN(200)
    INTEGER BINTOT,BINAVE
    *************************
    ND=200-M+1
    DO 50 J=1,ND,M
      K=J+M-1
      BINTOT=0
        DO 20 I=J,K
        BINTOT=BINTOT+SBIN(I)
20      CONTINUE
      BINAVE=BINTOT/M
        DO 40 I=J,K
        SBIN(I)=BINAVE
40  CONTINUE
50  CONTINUE
    RETURN
    END
```

SUBROUTINE DELAY.FOR

```
DELAY AFS 6-8-81
MOD 6-9-81
SUBROUTINE TO CREATE A DELAY PROPORTIONAL TO D1*D2
USE CALL DELAY(D1,D2) WHERE D1 AND D2 ARE INTEGERS
    SUBROUTINE DELAY(D1,D2)
    INTEGER D1,D2
    DO 10 I=1,D1
      DO 10 J=1,D2
 10 CONTINUE
    RETURN
    END
```

SUBROUTINE PLOT.FOR

```
PLOT AFS 6-8-81
MOD 6-9-81
SEPERATED INTO SUBROUTINES AXES AND PLOT IN FILE NAMED PLOT
    SUBROUTINE AXEX(RUNDUR)
    ****************************
    INTEGER D,D1,D2,D5,D10,D20,D40
    DATA D1,D2,D5,D10,D20,D40/1,2,5,10,20,40/
    ****************************
    D=4000
BRING PEN TO ORIGIN(LOWER LEFT)
    IX=-400
    IY=-511
    CALL PLOTX(IX)
    CALL DELAY(D20,D)
    CALL PLOTY(IY)
    CALL DELAY(D20,D)
IND IS JUST USED HERE AS AN OBJECT OF THE READ STATEMENT
 19 FORMAT(I1)
DRAW X AXIS WITH TIC MARKS
 COMPUTE TIME BETWEEN TICS AS A FUNCTION OF RUN TIME
TT IS TIME BETWEEN TICS
    IRD=RUNDUR+.5
    TT=1.
    IF(IRD.GT.20)TT=5.
    IF(IRD.GT.50)TT=10.
    IF(IRD.GT.100)TT=20.
COMPUTE XFAC=INT UNITS PER SEC
    XFAC=800./RUNDUR
COMPUTE THE NUMBER OF TICS,NUMTIC
    NUMTIC=(RUNDUR/TT)+.9
NOW DRAW X AXIS
    DO 30 I=1,NUMTIC
      XI=I
      IX=XI*TT*XFAC-400.
      CALL PLOTX(IX)
      CALL DELAY(D5,D)
      IY=-495
```

```
      CALL PLOTY(IY)
      CALL DELAY(D1,D)
      IY=-511
      CALL PLOTY(IY)
      CALL DELAY(D1,D)
 30 CONTINUE
RETURN PEN TO ORIGIN
    IX=-400
    CALL PLOTX(IX)
    CALL DELAY(D10,D)
DRAW Y AXIS AT 5 CNTS PER TIC,20 INT.U.PER TIC
    DO 40 I=1,400,20
      IY=I-511
      CALL PLOTY(IY)
      CALL DELAY(D5,D)
      IX=-384
      CALL PLOTX(IX)
      CALL DELAY(D1,D)
      IX=-400
      CALL PLOTX(IX)
      CALL DELAY(D1,D)
 40 CONTINUE
RETURN PEN TO ORIGIN
    IY=-511
    CALL PLOTY(IY)
    CALL DELAY(D10,D)
    RETURN
    END
```

SUBROUTINE PLOT

```
AFS 6-9-81
CHANGED TO INT.NOS 8-3-81
SUBROUTINE TO OUTPUT BIN SET TO X-Y PLOTTER
          SUBROUTINE PLOT(SBIN,M,RUNDUR)
          ****************************
          INTEGER D,D1,D2,D5,D10,D20,D40
          LOGICAL SBIN(200)
          DATA D1,D2,D5,D10,D20,D40/1,2,5,10,20,40/
          ****************************
          D=1500
          DO 50 I=M,200,M
          IY=SBIN(I)
          IF(IY.GE.0)GOTO 30
CONVERTS FROM +,-128 RANGE TO 0 TO 255 RANGE
          IY=IY+256
       30 IY=4*IY-511
          CALL PLOTY(IY)
          CALL DELAY(D10,D)
          IY=4*I-401
          CALL PLOTX(IX)
          CALL DELAY(D5,D)
       50 CONTINUE
      RETURN TO ORIGIN
```

```
          IY=-511
          CALL PLOTY(IY)
          CALL DELAY(D10,D)
          IX=-400
          CALL PLOTX(IX)
          CALL DELAY(D10,D)
      GRAPH IS COMPLETE AND PEN AT ORIGIN
          RETURN
          END
```

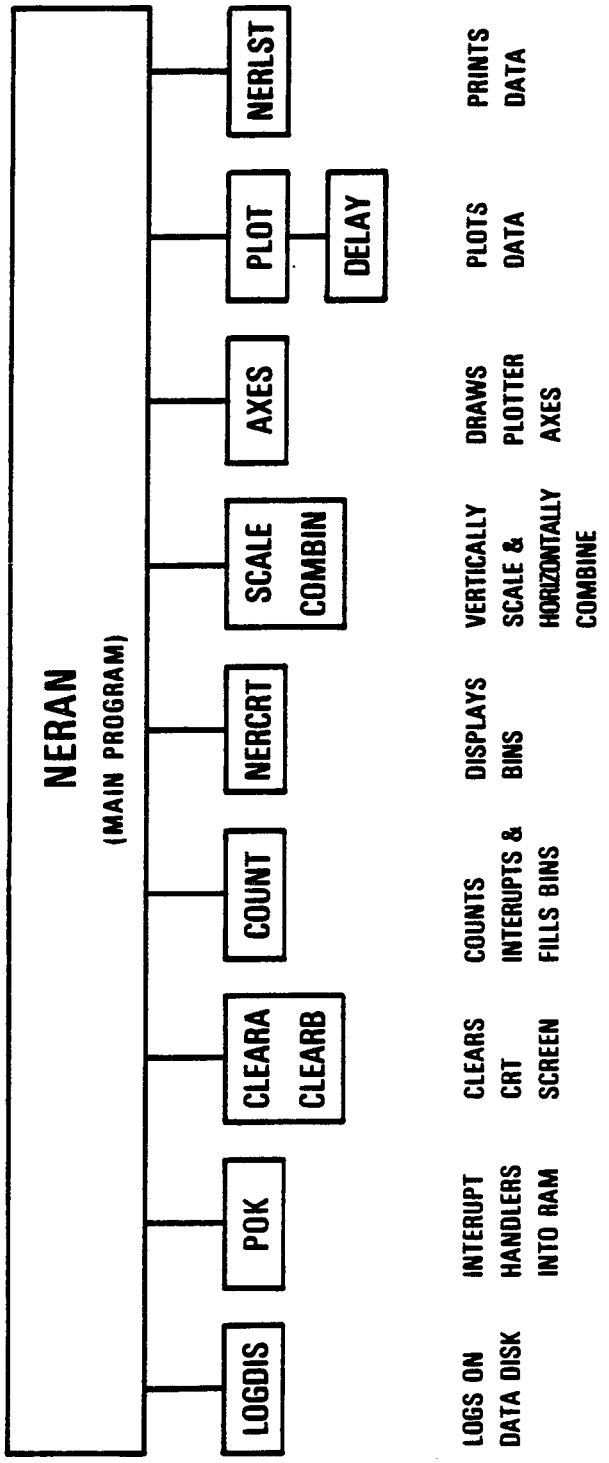
SUBROUTINE NDUMP.FOR

```
NDUMP AFS 8-10-81
SUBROUTINE FOR PRINTING DATA ARRAYS IN NERANA
   SUBROUTINE NDUMP(BIN,JMAX,KMAX)
   **********************************
   LOGICAL BIN(200,3,2)
   **********************************
   DO 50 K=1,KMAX
   DO 50 J=1,JMAX
       WRITE(3,20)K,J
20   FORMAT(1X,28X,'CHAN= ',I1,' SET= ',I1)
     ILO=-19
     DO 40 L=1,10
        ILO=ILO+20
        IHI=ILO+19
        WRITE(3,30)(BIN(I,J,K),I=ILO,IHI)
30       FORMAT(1X,2014)
40 CONTINUE
50 CONTINUE
   RETURN
   END
```

SUBROUTINE NERFIL.FOR

```
NERFIL 9-21-81 AFS
A PROGRAM TO OPEN NERANA DATA FILES AND WRITE OUT DATA
LOAD NERFIL,NERCRT,NDUMP
    ****************************
    PROGRAM NERFIL
    LOGICAL BIN(200,3,2)
    INTEGER RUN,FN(6),DATE(4),CLKCNT,FTG1(3),FTG2(3)
    ****************************
 1  FORMAT(I4)
    WRITE(3,11)
11  FORMAT(1X,'CHANGE DISK & TYPE CR')
    READ(3,1)
    CALL LOGDIS
LOGDIS SUBROUTINE IS IN NERCRT FILE
    JMAX=2
    KMAX=2
    WRITE(3,20)
```

```
20  FORMAT(1X,'TYPE FILENAME WITH LAST CHARACTER BLANK')
    READ(3,21)(FN(I),I=1,6)
21  FORMAT(6A2)
    CALL OPEN(6,FN,1)
    READ(6)(DATE(I),I=1,4),RUN,RUNDUR,CLKCNT,JMAX,KMAX,
   1(FTG1(I),I=1,JMAX),(FTG1(I),I=1,JAMX),
   2(((BIN(I,J,K),I=1,200),J=1,JMAX),K=1,KMAX)
    WRITE(3,31)(DATE(I),I=1,4),RUN,RUNDUR,CLKCNT,JMAX,KMAX,FTG1(1),
   1FTG2(1),FTG1(2),FTG2(2)
31  FORMAT(1X,4A2,I3,F6.1,I6,2I2,4I5)
    CALL NDUMP(BIN,JMAX,KMAX)
    END
```


NERAN
(MAIN PROGRAM)
LOGDIS
POK
CLEARA
CLEARB
COUNT
NERCRT
SCALE
COMBIN
AXES
PLOT
DELAY
NERLST
LOGS ON DATA DISK
INTERUPT HANDLERS INTO RAM
CLEARS CRT SCREEN
COUNTS INTERUPTS & FILLS BINS
DISPLAYS BINS
VERTICALLY SCALE & HORIZONTALLY COMBINE
DRAWS PLOTTER AXES
PLOTS DATA
PRINTS DATA

GRADUATE SCHOOL
UNIVERSITY OF ALABAMA AT BIRMINGHAM
DISSERTATION APPROVAL FORM

Name of Candidate Brett Hamilton Neely

Major Subject Physiology

Title of Dissertation Cardiac Sympathetic Activity During Acute Myocardial Ischemia

Dissertation Committee:

G R Hageman, Chairman

S B Barker

Thomas A. Jones

W A Woodside

Stephen M. Cain

Jimmy D. Neill

Director of Graduate Program G R Hageman

Dean, UAB Graduate School

Date 11/21/88

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