1986

The Effects of Cardiac Sympathetic Nerve Stimulation on Coronary Blood Flow During Myocardial Ischemia

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THE EFFECTS OF CARDIAC SYMPATHETIC NERVE STIMULATION ON CORONARY BLOOD FLOW DURING MYOCARDIAL ISCHEMIA

by

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A Dissertation Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

July

1986

ACKNOWLEDGEMENTS

I wish to thank the Faculty of the Department of Physiology for the excellent training I have received from them. Each member has shown an interest in my education as a physiologist, and I hope that in the future I will be able to return to them some of the time and commitment they have given to me.

I would like to thank all of my committee members for their helpful and timely suggestions as I completed my research. In particular I wish to thank my advisor Dr. John X. Thomas, Jr., not only for the guidance but especially for the friendship he has given me over the last four years, both of which have been greatly appreciated.

In addition, I would like to thank David Defily whose expertise in animal surgery, and patient explanations of the ins and outs of the laboratory, allowed my first two years to pass by without any major crises. Thanks must also be extended to Mrs. Jeannie Thomas for all of the advice on statistical analysis and programming.

I would also like to thank my family for their understanding and leniency during the years of my training. Finally, I wish to acknowledge that without Chris, who lent a sympathetic and moderating ear for the last four years, the rough times would not have been so easily forgotten, nor would the small successes be so easily remembered.

VITA

The author, Kathleen (Flatley) Joyce, is the daughter of Thomas J. Flatley and Neva (Murphy) Flatley. She was born on September 24, 1959 in Minneapolis, Minnesota.

The author attended elementary school in the public school system of Whitefish Bay, Wisconsin. She graduated from Whitefish Bay High School in June of 1977.

In August of 1977, she entered the University of Wisconsin-Milwaukee. In May of 1981, she received a Bachelor of Arts degree in History. In, 1980, while attending the University of Wisconsin-Milwaukee, Kathleen was elected a member of Phi Beta Kappa, and in 1981, was elected a member of the academic honor society of Phi Kappa Phi.

In August of 1982, the author entered graduate school at Loyola University in the Department of Physiology. Her dissertation work was completed under the direction of Dr. John X. Thomas, Jr. The author is a student member of the American Physiological Society.

Kathleen will be continuing her research as a post-doctoral fellow with Dr. Anthony Cutilletta, a Professor in the Department of Pediatrics at Loyola University.

PUBLICATIONS

1. FLATLEY, K.A., D. V. Defily, and J.X. Thomas, Jr. Effects of cardiac sympathetic nerve stimulation on infarct size following beta blockade in anesthetized dogs. Fed. Proc. 43: 1021, 1984.

2. FLATLEY, K.A., D.V. Defily, and J.X. Thomas, Jr. Effects of cardiac sympathetic nerve stimulation during adrenergic blockade on infarct size in anesthetized dogs. J Cardiovasc Pharm 7(4): 673-79, 1985.

3. FLATLEY, K.A., and J.X. Thomas, Jr. Effect of combined alpha and beta receptor blockade on infarct size in anesthetized dogs. Fed Proc 44: 1019, 1985.

4. Thomas, J.X., Jr., and K.A. FLATLEY. Adrenergic influences on regional myocardial flow following coronary occlusion. Fed Proc 44: 1019, 1985.

5. FLATLEY, K.A., and J.X. Thomas, Jr. Effect of combined alpha-1 and beta receptor blockade on myocardial blood flow in anesthetized dogs. Circulation 72: III-62, 1985.

6. FLATLEY K.A., and J.X. Thomas, Jr. Effects of regional denervation on myocardial blood flow during coronary artery occlusion. Submitted: Circulation.

7. FLATLEY K.A., and J.X. Thomas, Jr. Influence of the cardiac nerves on blood flow to the circumflex bed. Submitted: Circulation.

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CHAPTER I

INTRODUCTION

Alpha adrenergic receptors have been reported to limit coronary blood flow under conditions of augmented oxygen demands when production of vasodilator metabolites might be expected to overwhelm the influence of alpha mediated vasoconstriction. Even when blood flow is reduced, alpha receptors have been shown to limit flow to the ischemic region. The blood supply to the heart is closely tied to myocardial metabolism. When the balance is upset under conditions of ischemia such as occur following a coronary artery occlusion, oxygen demand outstrips the oxygen supply and an infarction may result. Early investigations into prevention of myocardial infarction focused on reducing the oxygen demand by blocking myocardial beta-1 receptors which mediate increases in myocardial oxygen consumption when stimulated. Studies indicated that elevated levels of sympathetic input in the presence of beta-blockade might alter the amount of blood flow to the ischemic myocardium by limiting the flow into the region via alpha-mediated vasoconstriction.

The first study in this dissertation proposed to examine the effect of sympathetic nerve stimulation in the presence of beta-blockade on infarct size. In this preparation, the infarct size was assessed with tri-phenyltetrazolium chloride and normalized to the area at risk.

The second study was done to determine what effect sympathetic

nerve stimulation had in the presence of beta-blockade on regional myocardial blood flow following coronary artery occlusion. The hypothesis was that alpha-mediated vasoconstriction limited blood flow to the ischemic zone by reducing the collateral blood flow to the region or by restricting flow in the ischemic zone.

While alpha receptor vasoconstriction can be demonstrated under conditions of beta-blockade and elevated sympathetic input to the heart, the hypothesis that alpha receptors exhibit a tonic restraint on coronary flow, while accepted by some investigators, has been refuted as untenable by others. There were several hypotheses examined in the third study. The first was to demonstrate whether there was significant alpha-mediated tone under resting conditions following circumflex coronary occlusion, second, whether this tone limits flow to the ischemic zone and third, whether regional denervation of the ischemic zone provides any beneficial effects by removing the tonic alpha restraint. The technique used for regional denervation was developed in this laboratory and included cutting of the ventrolateral cardiac nerve where it courses over the pulmonary veins, and painting a thin strip of phenol around the perimeter of the circumflex artery perfusion zone. Topical application of phenol has been shown to penetrate approximately 0.25 cm into the epicardium causing coagulation necrosis and destruction of sympathetic nerves which run in the upper 0.5 mm of the epicardium.

The last study was done to determine whether alpha mediated vasoconstriction in the circumflex bed could be elicited by specific cardiac thoracic nerves, and whether the vasoconstriction was predomi-

nantly alpha-1 or alpha-2 mediated. Recent evidence suggests that alpha-2 receptors although originally believed to be presynaptic in origin are also located on post-synaptic effector organs.

It is hoped that the work presented in this dissertation will provide additional information regarding the influence of sympathetic nerves on coronary blood flow under pathophysiologic conditions of coronary artery occlusion and myocardial ischemia as well as on blood flow during physiologic conditions.

CHAPTER II

GENERAL LITERATURE REVIEW

Under normal physiologic conditions, the autonomic nervous system is the major external influence on coronary vascular resistance. The research presented in this dissertation is concerned primarily with determining the extent of control of coronary blood flow exerted by the sympathetic branch of the autonomic nervous system, specifically in the pathophysiologic setting of myocardial ischemia.

A. SYMPATHETIC INNERVATION OF THE HEART

The sympathetic supply to the heart is via the first through the fourth thoracic spinal sections (138). The cell bodies of the preganglionic fibers are located in the intermediolateral cell column. The axons leave the spinal nerve and synapse in the paravertebral ganglion via the white rami communicantes. Unmyelinated post-ganglionic fibers leave via the gray rami communicantes to end on effector cells within the heart. A detailed description of the innervation of the heart was provided by Woollard in 1926 (136). Forty five days after removal of the stellate ganglia, Woollard used methylene blue staining to study the innervation of the heart. The nerves supplying the heart course along the coronary arteries, forming spirals around the vessels. The coronary circulation was described as receiving the richest innervation of all arterial beds (137). Woollard concluded

from his studies that the large coronary arteries are innervated by both the parasympathetic and sympathetic systems, and that the smaller arteries are innervated primarily by vagal fibers. Penetration of the myocardium by these nerves gave rise to a plexus of fibers underlying the epicardium as well as the endocardium. More recently, confirmation of Woollard's studies has been provided by investigations utilizing histo-fluorescence. The coronary arteries have been found to be densely innervated by adrenergic terminals (32), which are located primarily in the adventitia of the vessel (33).

Subsequent studies have described the specific cardiac nerves to the heart. Nonidez gave detailed anatomical descriptions of the cardiac nerves and their distribution to the heart (100). He established that many preganglionic fibers synapse in the middle cervical ganglion, and that a number of preganglionic fibers synapse above the level of the aortic arch. In addition, Nonidez determined that parasympathetic fibers supply the structures above the coronary sulcus. In his description of the cardiac sympathetic nerves, he described the superior cardiac sympathetic nerve which carries sympathetic post-ganglionic fibers. This nerve carried fibers which end as pressoreceptors on the arch of the aorta. Nonidez did not consider this nerve to be a true cardiac sympathetic nerve, as it did not distribute below the aortic arch. The major left cardiac nerve was the middle cardiosympathetic nerve which reaches the left ventricular base and joins nerve branches of the coronary arteries to form a nerve plexus on the posterolateral surface of the left ventricle.

The nomenclature devised by Mizeres in his studies is still used today (92). The three major left side cardiac nerves were the innominate (ICN) cardiac nerve, the ventromedial cervical cardiac nerve (VMCCN) and the ventrolateral cervical cardiac nerve (VLCCN). These latter two nerves are analogous to the superior cardiosympathetic nerve and the middle cardiosympathetic nerves of Nonidez, respectively. Mizeres' studies came to some slightly different conclusions from those of Nonidez. Mizeres, in describing the course of the ICN, concluded that the ICN was not a cardiac nerve since it did not reach the left ventricular wall. Furthermore, the VMCCN was found to be composed of both branches of the autonomic nervous system, having both vagal and sympathetic fibers. Although Nonidez did not consider the superior cardiosympathetic nerve (VMCCN) a true cardiac nerve, as did Mizeres, both investigators were in accord that the VMCCN sent branches to the aorta. The VLCCN was described by Mizeres as the largest of the nerves. It also had many interconnections with the vagus, and carried sympathetic postganglionic fibers as well as a few afferent fibers. Mizeres postulated that there may be ganglia in the ventral limb of the ansa subclavia.

In a later study in 1958, Mizeres determined that stimulation of the left cardiac nerves had more of an influence on the force of contraction of the heart, rather than any noticeable effects on cardio-acceleration (93). Randall expanded these original observations of Mizeres in a number of studies investigating the functional importance of cardiac sympathetic nerves (106,107). These studies will be described in more detail later.

B. ADRENERGIC INFLUENCES ON CORONARY BLOOD FLOW

One of the earliest recorded mentions of coronary vasomotor activity was described by Newell-Martin in 1880, when he noted a vasomotor response of the coronary arteries (99). Newell-Martin had undertaken the study to determine what happens to coronary pressure throughout the cardiac cycle. The debate at that time involved whether flow occurred solely during diastole due to obstruction of the Sinus of Valsalva by the aortic leaflets during systole. During the course of this study, he noted that in one of the pressure tracings, the coronary artery pressure was greater than carotid artery pressure (76 mm Hg, and 64 mm Hg, respectively). Rather incidently, Newell-Martin remarked that often, it was difficult to cannulate a coronary branch, since it appeared to be considerably smaller upon insertion of the cannula than it had at first appeared. After a few minutes, it would dilate to twice the size it was when constricted. This visual observation on coronary vasomotor tone might at the time have seemed merely anecdotal. However, several years later, Porter provided the first evidence that coronary arteries respond to external stimuli (105). By stimulating the cervical vagus, Porter was able to decrease the flow through the cut end of a coronary artery from 13 to 8 drops per 15 seconds. The rate of flow was found to return to control levels following cessation of the stimulation period. Thus, it was demonstrated by Porter that coronary arteries can constrict in response to neural stimulation, and also that the cervical parasympathetic trunk contained sympathetic fibers.

From the beginning of the century, numerous investigations addressed the topic of adrenergic influences on coronary blood flow. Wiggers contended that vasodilation was the main response of coronaries, although he observed that when the heart was not beating, coronary blood flow decreased in response to adrenalin (134). Studies undertaken by Barbour suggested that 'vasospasm' may be due to release of adrenalin and the consequent vasoconstriction of the arteries (6). Brodie and Cullis determined that the coronary vessels were supplied with both vasoconstrictor and vasodilator fibers (15). They determined that although the primary effect was constriction which could be elicited by a low dose of adrenalin, a large dose of adrenalin overwhelmed the constrictor effect causing a vasodilation. This apparently satisfactory explanation for the paradoxical actions of adrenalin was not enough to prevent a plethora of investigations concerning adrenergic influences on coronary blood flow.

A cursory examination of the literature on this subject reveals that there are a number of studies with conflicting results. Coronary arteries have been reported to constrict (6,7,121,125) dilate (26,-135), constrict and dilate (2,3,10,12,50,56,73,95), or do nothing (31), in response to adrenergic stimuli. In some instances an initial constriction preceding a dramatic vasodilation may have been recorded by an investigator but was not remarked upon (135).

A plausible explanation for these differences can be attributed to the variety of preparations used. Studies have been done on isolated hearts (57,67), isolated vessels (2,7,9,26,73,95), nonworking hearts (55), arrested hearts (52), anesthetized animals (58,121,128),

and conscious animal preparations (83,104). Furthermore, even in the vessel strip preparation, opposing responses can be obtained depending on the size of the vessel examined (2,95,139).

The ground work for clarification of the results from these studies was laid by Cannon and Rosenblueth in a series of experiments done in the early 1930's (20). Cannon was among the first investigators to describe the effects of 'sympathin' as separate from 'adrenin'. He postulated that sympathin was the chemical mediator of sympathetic nerve stimulation and that when released it combined with a substance in the effector organ to produce either sympathin E or sympathin I. Depending on which organ was stimulated the response might vary. Thus sympathin released from the heart was excitatory, but that released from the coronary arteries was inhibitory due to the variable concentration of the sympathin I or E substances in each tissue.

It was Ahlquist however, who demonstrated that the same chemical mediator could exert different effects depending on where it was released (1). Ahlquist determined that there were two types of adrenergic receptors which mediated the effects of sympathin which he believed to be identical to adrenin. These receptors could not be categorized as purely inhibitory or excitatory as Cannon had believed. To prove his hypothesis, Ahlquist selected a series of sympathomimetic amines for which the order of potency on the following functions was determined: vasoconstriction, excitation of the uterus and ureters, contraction of the nictitating membrane, dilation of the pupil, and inhibition of the gut. The order of potency was, from most active to

least active: l-epinephrine> dl-epinephrine> arterenol > methyl arterenol > methyl epinephrine > N- isopropyl arterenol. However, the order of potency for the same amines was different, (N-isopropyl arterenol > l-epinephrine > methyl epinephrine > dl-epinephrine > methyl arterenol > arterenol, from most to least active), on the following functions: vasodilation, inhibition of the gut, and myocardial stimulation. One type of receptor was associated with most of the excitatory functions and at least one inhibitory function of the intestine (the alpha receptor), and the other receptor which he arbitrarily designated the beta receptor was associated with most of the inhibitory functions and one important excitatory function (myocardium). Thus, N-isopropyl arterenol was more potent on actions associated with beta receptors, whereas arterenol was more potent on those actions determined to be mediated by alpha receptors.

Identification of the chemical mediator released by sympathetic nerves was accomplished by von Euler, who localized norepinephrine to nerve terminals and the sediment of axons in the mid 1950's (132). By the mid 1950's, the action of sympathetic nerves was known to be via release of norepinephrine which stimulated both alpha and beta receptors, thus eliciting different end organ responses depending on the concentration of receptor present.

In 1967, Lands determined that beta receptors could be divided into two subtypes, beta-1 and beta-2 (74,75). His basis for this subdivision came from experiments he had performed on the actions of several sympathomimetic amines including isoproterenol, epinephrine and norepinephrine on different physiological functions. The effects

of these different agents were assessed on fatty acid mobilization from rat adipose tissue, cardiac stimulation, bronchodilation, and vasodilation. A similarity was found for the relative potencies of each of the drugs on lipolysis/cardiac stimulation and on bronchodilation/vasodilation. There was a low correlation between the action of norepinephrine, for instance on cardiac stimulation and bronchodilation, but the correlation between its potency on lipolysis and cardiac stimulation was high. Lands designated the receptors located on the heart and adipose tissue, and small intestine as beta-1, and those found on the uterus, diaphragm, bronchioles and vasculature as beta-2. Following the development of specific beta-agonists and antagonists in the 1960's, the location and function of beta-1 and beta-2 receptors in the cardiovascular system was clarified by different investigations.

Klocke found that isoproterenol in a potassium arrested heart caused a drop in perfusion pressure of a constant flow system which was blocked by nethalide (beta-blocker) (71). Several investigations determined that coronary beta receptors were of the beta-2 type (14,52,55,86). Gross and Feigl published a study in which they concluded that the coronary vascular receptor is of the beta-2 type. In the potassium arrested heart they found that practolol (a beta-1 antagonist) blocked the dilation evoked by isoproterenol only at very high concentrations (10^{-3} M), whereas propranolol blocked the dilation at 10^{-7} M. Salbutamol, although a selective beta-2 agonist, produced a dilation only one eighth as potent as that induced by the non-selective beta-agonist isoproterenol (52). Their findings concurred

with an earlier study by Broadley, in which salbutamol was also used to evoke direct vasodilation (14).

However, in a different study, Hamilton and Feigl determined that beta-2 receptors on the arteries are not of functional importance (55). In the beating non-working heart, they blocked beta-1 receptors with practolol, and alpha receptors with dibozane, then stimulated the left stellate ganglion at 10 Hz. They found that the vasodilation which could be attributed to beta-2 receptors under these conditions was very slight. Stimulation of beta-2 receptors by isoproterenol in the presence of
beta-1 blockade did evoke a significant vasodilation. Thus, they concluded that although beta-2 receptors can cause significant vasodilation in the coronary bed when stimulated by exogenous catecholamines, they are not of physiologic importance since neural stimulation has more of an effect on alpha and beta-1 myocardial receptors. Domenech and MacLellan found similarly that beta-2 activation caused a large dilation in the subepicardium and effected a redistribution of blood flow to the subepicardium from the subendocardium (34). This preparation involved beta-1 blockade with practolol, and stimulation of beta-2 receptors with salbutamol, and isoproterenol, hence the confounding influence of alpha receptor stimulation by neurally released catecholamines was circumvented in this study.

Beta-1 receptors have also been proposed to cause direct as well as indirect vasodilation of coronary vessels. Vatner et al. demonstrated in the conscious animal, that in the presence of beta-1 blockade, isoproterenol (beta-1,beta-2 agonist) and pirbuterol (a

beta-2 agonist) no longer caused vasodilation due to beta-1 receptors (129), which seemed to confirm the results from an earlier study (8). In the study by Baron et al., canine coronary and skeletal muscle vessel strips were blocked by phenoxybenzamine (alpha blocker), and isoproterenol caused a vasodilation which was blocked by propranolol as well as by practolol a beta-1 blocker. However, isoproterenol adminstration following beta-1 blockade with practolol did not block the vasodilation of skeletal muscle arterioles, which have predominantly beta-2 receptors (8).

The concept of metabolic control of coronary blood flow was introduced more than 70 years ago when Brodie and Cullis observed that an increase in cardiac activity led to an increase in cardiac metabolism and the release of carbonic acid. They tested the effect of this metabolic substrate by putting it directly on coronary arteries and observed that it caused dilation (15).

Shipley and Gregg noted that left stellate ganglion stimulation caused parallel increases in coronary blood flow and cardiac work (116). This came about because an increase in work led to an increase in metabolism and vigor of contraction. They proposed two explanations for their findings. First they postulated that there was a local production and release of metabolites and second, that stimulation of the stellate ganglia produced a local, relative anoxia caused by a disproportionate increase in the rate of oxygen utilization and existing coronary blood flow. Subsequent studies (38,41,49) confirmed these observations that there was a link between myocardial oxygen consumption and coronary blood flow. Eckstein used a preparation in

which he could control cardiac output, and in which the coronary arteries were perfused at a constant pressure (38). A nerve branch from the left stellate ganglion was stimulated at 50 Hz. In this study, during stimulation of the cardiac nerve, cardiac work increased by 13%, whereas myocardial oxygen consumption increased 100% from control and coronary blood flow increased 37% from control. His interpretation of these results was that since the increase in myocardial oxygen consumption was much greater than the increase in cardiac work, when cardiac sympathetic nerves were stimulated, they release an adrenin-like substance which "renders the heart anoxic and inefficient". Feinberg and Katz concurred with Shipley and Gregg that catecholamines augment coronary blood flow and cardiac work (41). However, in their study, they found a decrease in the A-V O_2 difference and an increase in coronary sinus O_2 content. This apparent vasodilation, in addition to metabolic vasodilation contradicted evidence gathered by other investigators from that time. Intracoronary injection of epinephrine and norepinephrine elicited a reduction in coronary sinus blood oxygen tension concomitant with an increase in blood flow in the fibrillating heart in a study by Berne (10). His explanation for this was that myocardial oxygen consumption increased more than coronary blood flow.

The limitation in the blood flow increase during adrenergic stimulation was also noted in a study by Hashimoto (57). In an isolated Langendorff heart which was cross-perfused from a donor animal, he found that epinephrine and norepinephrine increase myocardial oxygen consumption more than isoproterenol. However, isoproter-

enol caused greater changes in blood flow that either epinephrine or norepinephrine. Utilizing two early adrenergic blockers, he separated the alpha and beta actions of these drugs. Administration of the beta blocker dichloroisoproterenol (DCI) with the subsequent stimulation by norepinephrine and epinephrine, elicited decreases in coronary blood flow although there were no longer increases in myocardial oxygen consumption. The decrease in blood flow was reversed by alpha blockade with dibenzylene. With isoproterenol there was a proportionate increase in blood flow and MVO_2, but with epinephrine and norepinephrine, MVO_2 increased more than coronary blood flow. Dibenzylene prior to administration of norepinephrine and epinephrine, eliminated the mismatch in flow and oxygen consumption previously seen with both catecholamines.

Although there is a link between myocardial oxygen consumption and coronary blood flow, blood flow did not increase as much as expected during sympathetic stimulation in the above studies, since the reduction in coronary sinus oxygen tension indicates an increased extraction of oxygen due possibly to alpha receptor limitation of flow. This might explain the conclusions of Eckstein that the heart became inefficient during sympathetic stimulation since the matching between cardiac work and increase in blood flow was not so close during sympathetic stimulation as it was at rest (38). In retrospect, it seems obvious that alpha receptor stimulation contributed to the mismatch in coronary blood flow and oxygen consumption. In Eckstein's study, coronary sinus oxygen content decreased from 8.5 to 3.5 vol%, during an increase in blood flow, indicating a limitation in flow

during metabolic vasodilation. The differences in the preparation used by Feinberg and Katz can explain their contradictory findings that oxygen consumption decreased while blood flow increased (41). In their study, the drugs were given intravenously, the hearts were not neurally decentralized, nor were blood pressure or heart rate controlled. The effects of baroreceptor reflexes on the heart superimposed on systemic injection of sympathomimetic agents caused a wide variation in the control response to the drugs. They reported that epinephrine elicited a pressor response in 4 out of 10 of the dogs, and of the remaining 6, 5 had blood pressures which returned toward control. Moreover, heart rate was elevated in six of the animals, and depressed in four of the animals. Berne, on the other hand, administered norepinephrine intracoronary, to a fibrillating heart, thus avoiding the confusing contribution of baroreceptors reflexes, and metabolic increases in blood flow (10).

The increased work of the contractile elements leads to a net production of cellular metabolites. The identity of the elusive metabolite produced by the heart was proposed by Berne in 1964 to be adenosine (11). Adenosine fulfills many criteria for metabolically-mediated vasodilation, it is present in the normal heart, it readily crosses the cell membrane, and is rapidly metabolized in the blood and surrounding tissues (13). When Lands designated the receptor mediating cardiac stimulation as beta-1 in 1967, the link between neural stimulation and coronary blood flow was made. Since that study, the identification and localization of beta receptors discussed above led to the general consensus that beta-1 mediated increases in myocardial

metabolism were the primary neural influence on coronary blood flow, and that beta-2 receptors, although located on the coronary vasculature, play a distant second in eliciting vasodilation.

C. NEURAL INFLUENCES DURING MYOCARDIAL ISCHEMIA

The coronary circulation is subject to a number of influences both extrinsic and intrinsic. The primary determinant of coronary blood flow is the metabolism of the heart. In addition, the autonomic nervous system modulates coronary blood flow. Under circumstances in which sympathetic nervous system (SNS) activity is elevated such as in exercise, beta-1 mediated metabolic vasodilation augments coronary blood flow with the favorable consequence of increased delivery of oxygen to the myocardium. However, myocardial ischemia illustrates a case in which the increase in SNS activity can become deleterious. Following a coronary artery occlusion there is an increase in the level of plasma catecholamines (66,109,110). In addition, there is an increase in sympathetic nervous system activity (85,124). The resulting stimulation of both alpha and beta receptors leads to an oxygen supply-demand imbalance since oxygen delivery is limited due to the combination of arterial obstruction and vasoconstriction. Simultaneously, the need for oxygen is increased in the non-ischemic myocardium due to the stimulation of beta-1 receptors. Consequently, standard treatment involved administration of beta-receptor blockers such as propranolol or timolol. These pharmacologic agents were thought to provide protection due to blockade of beta-1 mediated increases in oxygen consumption (87,133). Functional studies sup-

ported these conclusions. Although stimulation of beta receptors augments myocardial contractility under normal conditions, under conditions of ischemia, function has been shown to deteriorate. Davidson et al. demonstrated in the contracting Langendorff rat heart, that isoproterenol increased left ventricular pressure (LVP) from 103 to 166 mm Hg, and LVdP/dt from 1840 to 6000 mm Hg/sec under control conditions. Following the reduction in flow to the heart from 9.5 to 2.4 ml/min, LVP was only 25 mm Hg, and LVdP/dt 683, mm Hg/sec. Infusion of isoproterenol further reduced LVP to 23 mm Hg, and LVdP/dt to 601 mm Hg/sec. Although the change from control ischemic conditions was not significant it can be seen that the usual inotropic actions of isoproterenol are inconsequential, if not detrimental to the ischemic myocardium (29). These findings were extended to the conscious animal preparation by Vatner et al. (130). Vatner found that the amount of paradoxical motion (123) in the ischemic zone, was reduced by the injection of propranolol. They also described a redistribution of blood flow to the ischemic zone. Gallagher et al. reported that during coronary artery occlusion, heart rate increased 113% from resting levels during isoproterenol injection. At the same time the percent change in wall thickening (%Δ WT) and segmental shortening, decreased from resting levels, whereas during control, %Δ WT had increased to 40% during isoproterenol from 34% at rest. During a mild stenosis, at rest %Δ WT was 28% but during isoproterenol, it decreased to -.3%, indicating paradoxical motion had developed (43). Matsuzaki et al. demonstrated that administration of atenolol improved regional left ventricular function in a severely ischemic region

during exercise compared to exercise without atenolol. Regional myocardial blood flow during exercise and coronary artery occlusion in the ischemic region was also improved due to an increase in the endo/epi ratio (89).

Most of the early studies which investigated the effects of beta blockade on infarct size reduction used propranolol. Pretreatment with 5 mg/kg of propranolol injected intraarterially caused a reduction in the amount of necrotic posterior papillary muscle in a study by Rasmussen et al. The necrotic area was reduced from 76 ± 5% in the control animals to 43 ± 5% in the animals pretreated with propranolol ten minutes before occlusion. Delayed treatment with propranolol, at three hours after occlusion, also reduced the total amount of necrotic tissue to 62 ± 4% (108).

There are some disagreements in the literature as to the beneficial effects of beta blockade on infarct size. Burmeister used three different blocking agents atenolol, nadolol and propranolol. The drugs were given 5 minutes prior to coronary artery occlusion. The left anterior descending artery was occluded for 1 hour and then allowed to reperfuse. At 24 hours the hearts were excised, sliced and put in nitroblue tetrazolium. The infarct size as a percent of the left ventricle was assessed, and Burmeister found that all three drugs reduced infarct size compared to control (19). Reduction in infarct size was demonstrated in other laboratories with various beta adrenergic blockers (63,117,131). However, there were also studies which contradicted these positive results. Lange showed that pindolol and metoprolol did not reduce infarct size, although both beta blockers

were given after occlusion (76). Peter also did not show a protective effect of propranolol (103). In a recent study by Geary et al., propranolol did not reduce the infarct size in baboons. Geary attributed his negative findings to the fact that baboons do not have the extensive preformed collateral system of dogs (44). However, other studies have investigated the effects of beta-blockade on blood flow and have found that although beta blockers may reduce infarct size, the mechanism does not appear to be blood flow mediated (72,76,-103). It is generally believed that the beneficial effects are probably due to a reduction in myocardial oxygen consumption. (87,-133). Most of the early studies did not assess infarct size as accurately as the methods available in recent studies. In humans, the only way of assessing the severity of infarction was by determinations of serial CPK levels, and S-T segment elevation, methods which are notoriously prone to error. In addition, in experimental studies, the variations in the type of anesthetic used, duration of occlusion, time of treatment, background level of catecholamines and possible involvement of cardiac reflexes in all probability add to the disparity in results. Many of these studies have been applied to the human condition in which the elevation of sympathetic input is natural during exercise, or anger. The first study of this dissertation proposed to address the effect of elevated levels of sympathetic input in the presence of beta-blockade on infarct size.

D. ALPHA RECEPTOR INFLUENCES ON CORONARY BLOOD FLOW

Numerous studies throughout the 1950's and 1960's indicated that

alpha receptors indeed had a vasoconstrictor influence. Berne described an initial decrease in blood flow during administration of norepinephrine followed by an increase in blood flow of longer duration, at the time ascribing the increase in blood flow to an increase in myocardial metabolism (10). Although he considered catecholamines to be primarily vasoconstrictors, the net effect was vasodilation (12). It was generally believed that although the primary action of neural norepinephrine was to cause vasoconstriction, this was almost immediately overwhelmed by stimulation of beta receptors.

Feigl provided indirect evidence of alpha receptor mediated vasoconstriction. In chloralose anesthetized dogs pretreated with propranolol, blood flow measured with an electromagnetic flow probe, decreased 18% from control levels during stimulation of the left stellate ganglion. This vasoconstriction was blocked by phenoxybenzamine (39).

In 1967, Pitt et al. demonstrated that in conscious dogs, alpha and beta receptors have a role in modulation of coronary blood flow. In beta-blocked animals (with propranolol), intravenous injections of catecholamines caused vasoconstriction which could be eliminated by blockade of alpha receptors with dibenzylene. Beta-receptor mediated vasodilation could be elicited in the presence of alpha receptor blockade and occurred prior to any alterations in metabolic or hemodynamic indices (104).

Zuberbuhler and Bohr also demonstrated a role for alpha receptors in control of coronary blood flow. They found that isolated

strips of large coronary arteries underwent an initial contraction followed by relaxation in response to norepinephrine and epinephrine which suggested that large vessels might have equal populations of alpha and beta receptors. In contrast, the strips taken from smaller coronary vessels almost always relaxed when exposed to catecholamines, and the relaxation was reversed by the beta blocker nethalide. This was interpreted as demonstration of an exclusively beta receptor population in the smaller resistance vessels (139). Other investigations have demonstrated a differential population of adrenergic receptors (2,95).

Malindzak using a whole animal preparation and cardiac sympathetic nerve stimulation in addition to injections of catecholamines, showed that large vessels have more of a tendency to contract in response to catecholamines, as will smaller vessels although the constrictor response of smaller 'resistive' vessels is generally masked by the compensatory metabolic responses of the myocardium to sympathetic stimulation (84).

Additional evidence in support of alpha vasoconstriction came from a study by McRaven et al. They found that injections of norepinephrine or isoproterenol caused a drop in perfusion pressure in the circumflex artery which was perfused at a constant flow. The drop in pressure was reversed following blockade of beta receptors with practolol (a beta-1 blocker). Nerve stimulation (10V, 4msec, variable frequency) after practolol administration resulted in vasoconstriction which was reversed by administering phentolamine. They concluded that alpha receptors could directly constrict coronary arteries when

stimulated by neurally released catecholamines, but that this effect is minimal and that vasodilator effects both direct and indirect played a more important role, with vascular receptors having a more important influence than myocardial receptors (91).

Vatner et al. demonstrated the ability of alpha receptors to cause a potent vasoconstriction (128). Ultrasonic dimension gauges were implanted on the circumflex artery to measure the change in coronary diameter. Following administration of methoxamine (an alpha agonist, 50 g/kg/min) there was a transient increase in coronary diameter followed by a decrease in diameter (9 ± 2%) even though arterial pressure was elevated by 65 ± 5%. The calculated resistance for large arteries and total resistance used to estimate small artery resistance, increased by 108 ± 29 and 92 ± 14% respectively, indicating that alpha vasoconstriction was transmural and involved not only the large epicardial arteries but also the smaller resistance vessels.

In another study by Macho and Vatner, prazosin was found to reduce mean arterial pressure by 15 ± 4% in conscious dogs and to decrease coronary vascular resistance significantly. Although prazosin caused significant reductions in various inotropic indices (LV dP/dt, left ventricular end-diastolic diameter etc.) it did not increase coronary blood flow significantly. Hence they concluded that prazosin decreased resistance significantly mainly by reductions in afterload and preload thereby reducing oxygen consumption, but did not exert its effects on resistance by increasing blood flow (83). Vatner had shown previously that stimulation of alpha receptors with methoxamine could produce powerful vasoconstriction in the presence of

increased perfusion pressure, however in that earlier preparation, the animals' reflexes were intact, and no ganglionic blockade was administered (128).

None of these studies declared that alpha receptors had a major influence on blood flow under resting conditions. While demonstrating that alpha-receptors cause constriction, these studies did not actually provide evidence from a physiological standpoint of the importance of alpha receptors. In the aforementioned examples, alpha-receptor influences were demonstrated only in beta blocked preparations, or vessel strips in which vasomotor tone was independent of metabolic influences.

It wasn't until the mid-1970's that there was evidence that alpha receptors may modulate coronary blood flow even with concomitant activation of beta receptors. In a study by Feigl, blood oxygen tension in the coronary sinus was measured, along with circumflex artery blood flow (40). During stimulation of the left stellate ganglion for 90 seconds the heart rate, blood pressure and coronary blood flow increased from control, while coronary sinus oxygen tension (CSO_2) decreased from 19 mm Hg to 15 mm Hg. Following administration of propranolol, stimulation of the left stellate no longer produced the marked increases in inotropic and chronotropic indices, but coronary sinus oxygen tension was further reduced from 17 to 11 mm Hg. This indicated that alpha receptors were 'unmasked' since increases in metabolism were blunted by propranolol, and the decrease in CSO2 tension was interpreted as evidence of alpha receptor vasoconstriction. Following alpha blockade with dibozane, CSO_2 and coronary

vascular resistance no longer changed in response to left stellate stimulation. This study provided insight into the involvement of alpha receptors in modulating blood flow. By measuring changes in CSO_2, instead of only looking at changes in blood flow, it was determined that even with metabolic increases in flow, alpha-receptors limited flow enough to cause an increased oxygen extraction and decreased coronary sinus oxygen tension. Although these changes had been noted in previous studies (10,38), Feigl demonstrated conclusively that alpha-receptors are responsible for the seemingly paradoxical changes in flow and oxygen tension.

Mohrman and Feigl demonstrated in the closed-chest chloralose anesthetized dog that alpha receptors restrict the beta-1 mediated increase in blood flow by 30% (94). Sympathetic activation was elicited by either intracoronary injections of norepinephrine or carotid sinus hypotension. Measurements of O_2 extraction and coronary venous O_2 content were used as determinants of the ability of the heart to extract oxygen. When alpha blockade with dibozane was initiated, less oxygen was extracted and the coronary venous O_2 content increased, indicating that there had previously been a limitation of blood flow by alpha receptor stimulation.

Other investigations have shown that alpha receptors can limit blood flow increases following physiologic augmentation of sympathetic activity during exercise (60,98). Murray and Vatner found in dogs that were exercised freely, that alpha receptors limited the increase in coronary blood flow during exercise. Heyndrickx, under similar conditions, found that alpha receptors can upset the O_2 consumption/O_2

supply ratio. During control conditions, exercise decreased the ratio from 1.43 ± 0.05 at rest to 1.27 ± 0.03 during exercise. Following alpha blockade, the ratio went from 1.31 ± 0.05 at rest to 1.33 ± 0.02 during exercise. They concluded from this that oxygen delivery is better matched to myocardial oxygen consumption during alpha blockade by blunting the effects of alpha vasoconstriction on oxygen delivery.

Contrary to the findings of Heyndrickx, in which the match between oxygen supply and demand diverged during sympathetic stimulation, Buchweitz and Weiss stimulated the ansa subclavia and found that endocardial and epicardial oxygen extraction and blood flow increased proportionately (16). Buchweitz and Weiss found in open chest anesthetized dogs, that stimulation of the ansa subclavia did not alter the O_2 supply/O_2 consumption ratio. They concluded from their study that any coronary vasoconstriction which might occur with high levels of sympathetic input to the heart does not reduce the ability of coronary blood flow to meet the increased metabolic demand for O2 and that these needs were adequately met by the vasodilation produced by the increased metabolic state of the heart. In their study, the hearts were electrically fibrillated, excised, and quickly frozen during control and stimulation of the stellate. In addition, pentobarbital anesthesia was employed, which may have blunted adrenergic sensitivity to nerve stimulation.

Recently, alpha-receptors have been found to contribute to the regulation of blood flow during flow limited conditions such as stenosis or occlusion. Buffington and Feigl examined the contribution of alpha receptors to ischemia during partial occlusion of the

circumflex artery (18). Blood flow into the left coronary artery was measured, and following a 70% area reduction, intracoronary norepinephrine resulted in increased oxygen extraction, decreased coronary sinus oxygen content, and increased coronary vascular resistance. Following alpha blockade with phenoxybenzamine, the increase in myocardial oxygen consumption was prevented. They concluded that while alpha receptors may limit the increase in blood flow during coronary stenosis, it was not severe enough to cause net lactate production by the myocardium. These results concur with those of Gewirtz et al. who found that during a severe stenosis, alpha receptors may compete with but probably do not overcome metabolic vasodilation (46).

In the presence of a coronary occlusion, 'coronary steal' has been described as the effect of shunting of blood from an one area of the myocardium to another area of reduced vascular resistance via collateral vessels. When a substance is given which causes vasodilation of one region of the myocardium, while another region remains collateral dependent and maximally vasodilated, the vasodilation in the non-collateral dependent region causes an increase in blood flow velocity, and a decrease in perfusion pressure at the origin of collateral vessels. This results in an effective shunting of blood to the non-collateral dependent regions (101).

Chiariello et al. described a 'reverse coronary steal' due to alpha receptor activation in the normal zone. In that study the left anterior descending artery (LAD) was ligated and methoxamine was given 17-30 minutes after occluding the artery. The stimulation of alpha

receptors in the normal zone caused a reduction in flow to the normal zone from 90.6 ± 4.3 to 77.7 ± 3.2 ml/min/100 gm and increased the collateral flow to the ischemic zone from 21.4 ± 3.5 to 41.0 ± 4.2 ml/min/100 gm (21). Giudicelli et al. reported similar findings. In their study, branches of the LAD were ligated in a chloralose anesthetized dog. During stimulation of the left stellate ganglion (LSGS), regional flow increased to the normal area by 35% but not to the ischemic area. When atenolol was given, LSGS decreased flow to the normal zone by 24% but increased flow to the ischemic region by 64%. These effects were abolished by the administration of phenoxybenzamine (47). Buck et al. demonstrated that the unmasking of alpha receptors by beta-2 blockade with propranolol and pindolol caused a redistribution of blood flow to the subepicardium distal to a severe stenosis due to the predominance of alpha receptors in the larger more epicardially located blood vessels (17).

The role of alpha receptors appears to be elusive under certain circumstances. Gorman and Sparks reported a progressive vasoconstriction distal to a partial occlusion (48). In this preparation, hearts were paced at 180 beats per minute. Myocardial blood flow was determined at 30 and 180 minutes after occlusion. Vasodilator reserve was demonstrated with adenosine and norepinephrine. Since norepinephrine increased flow in the ischemic region, the vasoconstriction which evolved with time probably was not due to norepinephrine. It is interesting that in this preparation, the injection of phentolamine or phenoxybenzamine increased flow in the ischemic zone, but propranolol in addition to phenoxybenzamine decreased flow. Thus, norepinephrine

increased flow via beta-adrenergic stimulation but was not responsible for the vasoconstriction. The authors could not satisfactorily explain the phenomenon as due to alpha receptor vasoconstriction, but postulated that it may have been due to the decreased release of vasodilator metabolites, the increased release of an unknown vasoconstrictor substance, or increased cell-swelling due to the ischemia.

A recent study by Grover and Weiss confirms the results of Gorman and Sparks, regarding the ability of the ischemic bed to vasodilate. They showed that with a 50% reduction of LAD flow, the oxygen supply/demand ratio of the occluded region was reduced compared to control. However, the reduced oxygen supply/demand ratio did not change when heart rate was increased up to 50% above the resting level. Thus, the authors concluded that a flow reserve must exist (53).

It is possible that blood flow to the ischemic region is further compromised by alpha-mediated vasoconstriction in the ischemic zone. A recent report from our laboratory indicated that following coronary artery occlusion, simultaneous blockade of both alpha and beta receptors during sympathetic stimulation of the heart limited myocardial damage to a significantly greater extent than blockade of beta-receptors alone (42).

Investigations concerning alpha-mediated vasoconstriction are not limited to experimental studies but have been researched in the clinical setting too. Variant angina has been well-documented, and is believed by some to be alpha mediated. Levene reported in 1976, that a 41 year old women with angina demonstrated vasospasm during coronary

arteriography which coincided with episodes of angina. Relief of symptoms by administration of i.v. phentolamine, and phenoxybenzamine improved her exercise tolerance (81). The usual treatment for angina does not seem to work for variant angina. Kirshenbaum reported that while calcium channel blockers appear to alleviate the symptoms, nitrates help only in large doses, and propranolol is ineffective therapy, and may even aggravate the condition (70). These findings were substantiated by Maze et al., who concluded further that although alpha-blockers are effective in the treatment of vasospasm, in practice they are given only if nitrates and calcium channel blockers are ineffective (90). Other studies have added information on the role of alpha receptors during coronary artery disease (68,69,96,97). Kern et al. determined that beta-blockade with propranolol can potentiate vasospasm in some patients with CAD, possibly by unopposed alpha-tone (68). In another study, 18 patients with coronary artery disease underwent the cold pressor test, with paced heart rates of 95 beats per minute. During the cold pressor test, coronary vascular resistance (calculated as mean arterial pressure over blood flow measured with thermodilution) increased by 15%. Five minutes after 100 mg of the alpha-1 blocker trimazosin, the cold pressor test increased coronary vascular resistance by only 6% (69). The ability to increase coronary vascular resistance with the cold pressor test is not limited to patients with coronary artery disease, as normal patients who do not develop spasm, will have increases in coronary vascular resistance in the presence of beta-blockade (68). This confirms an earlier report by Mudge (97), in which it was reported

that patients with coronary artery disease have limited vasodilatory mechanisms, and inappropriate increases in coronary vascular resistance during cold pressor testing.

E. ALPHA-2 RECEPTOR MEDIATED CORONARY VASOCONSTRICTION

Over the past ten years, the possible involvement of alpha-2 receptors in coronary blood flow has been examined. Studies by Starke and Langer proposed that alpha receptors can inhibit the release of norepinephrine by a negative feedback mechanism (77,78,118). Langer originally proposed that alpha receptors be separated into prejunctional and postjunctional receptors calling the former alpha-2 receptors and the latter alpha-1 receptors (77). Eventually it was determined that pharmacological differentiation of the receptors would be more appropriate, since alpha-2 receptor activity could be demonstrated on the effector organ also (78). A physiologic basis for prejunctional modulation of norepinephrine release was provided by Lokhandwala (82). Since those studies were done, alpha-2 receptors have been reported to contribute to coronary artery vasoconstriction (64). It has also been proposed that post-junctional alpha-2 receptors as well as pre-junctional alpha receptors influence coronary blood flow (24,30,58,59,61,62,119).

Heusch and Deussen concluded that there was a continuous unmasking of sympathetic vasoconstriction with increasing severity of stenosis, which is mediated by post-junctional alpha-2 receptors (58). Holtz et al. also reported that norepinephrine induced vasoconstriction was attenuated predominantly by alpha-2 blockade not by alpha-1

blockade (62). This effect was examined during cardiac sympathetic nerve stimulation (10 Hz) by Saeed et al. (113). They found that during stimulation, coronary sinus oxygen saturation was decreased, and responded similarly under beta-blockade with nadolol. With alpha-2 blockade the decrease in CSO2 was attenuated, but not with alpha-1 blockade. It was not explained why the results were similar during beta-blockade, when all evidence points toward a greater role for alpha-receptors when unmasked by beta-blockade. It appears from recent investigations, that it is more likely that both alpha-1 and alpha-2 receptors mediate coronary vasoconstriction. Decker and Schwartz reported that the post-junctional receptors in the guinea pig heart are of the alpha-1 and alpha-2 type (30). Stevens and Moulds reported similar findings in isolated human blood vessels (119).

Heusch and Deussen further categorized the alpha-receptors, alpha -1 being located on large arteries, and alpha-2 on smaller arteries (59). This was based on the response to nerve stimulation of changes in internal diameter of a large vessel, and late diastolic resistance which reflects the contribution of smaller arteries. Stimulation of the left stellate ganglion (LSS) in these studies was for 90 seconds with measurements made at 1 minute. It was interesting that in this study, LSS in the presence of propranolol caused a significant elevation of arterial pressure which was blocked by prazosin, but not by rauwolscine. This latter finding, although not discussed in detail, is in accord with a study by Randall in which LSS in the presence of beta-blockade increased mean arterial pressure (106). The increase which is not beta-mediated appears to be alpha-1

receptor mediated (58,118). However, the dearth of investigations on myocardial alpha receptors does not allow a clear explanation for these findings.

The role of alpha receptors has been thoroughly demonstrated over a wide range of physiologic states, from their importance in modulation of resting blood flow in the conscious animal, to their involvement in limitation of blood flow during exercise, and finally their role in limitation of blood flow under pathophysiologic circumstances such as partial or severe stenosis. The second study of this dissertation proposes to determine whether alpha receptors decrease flow to the ischemic bed following complete coronary artery occlusion in the presence of beta-blockade.

F. EFFECTS OF CARDIAC DENERVATION ON CORONARY BLOOD FLOW AND ISCHEMIC INJURY

In the previous discussion, the concept of sympathetic modulation of blood flow under a variety of conditions was reviewed. The question then arises as to what effect denervation would have on the heart and on coronary blood flow. A classical method for establishing the importance of a chemical mediator or hormone is to determine what physiologic events occur in its absence. Denervation of the heart was employed early to trace the pathways of nerves to the heart (136). However cardiac denervation was soon used not only to further experimental knowledge of cardiac innervation. Leriche proposed that cardiac denervation provided protection against ischemia and infarction in the clinical setting (80). Contemporaries of his debated

whether this would provide protection or simply eliminate a warning signal of angina and impending myocardial infarction.

Following Leriche's report on the beneficial effects of cardiac denervation in the treatment of intractable angina, other investigators devised their own methods of cardiac denervation (25,28), and the reports of the protective influences of cardiac denervation during cardiac disease began to accumulate. The method of denervation suggested a wide range of sympathectomy from simple removal of the stellate ganglia to removal of the superior, middle and caudal cervical ganglia, in addition to stellate ganglionectomy. The reports of the different clinical settings in which denervation was useful were as numerous as the different methods employed to denervate the heart.

One widely used technique developed by Cooper et al. involved mediastinal neural ablation (25). However a later study by Peiss et al. determined that this technique was not totally effective in eliminating neural input to the heart (102). Peiss et al. found evidence for reinnervation after one year following excision and re-implantation. Neither mediastinal neural ablation nor bilateral stellate ganglionectomy proved to be totally effective in eliminating the sympathetic nerves to the heart since some of the nerves travel via the vago-sympathetic trunk. Cox et al. in 1936 found that bilateral stellate ganglionectomy in dogs resulted in protection against sudden coronary occlusion (27). Moreover, denervation was proposed to protect against malignant arrhythmias (37,114) and against myocardial infarction due to coronary artery occlusion (65). The

manner in which cardiac denervation was purported to exert its beneficial influences on cardiac viability was not determined in these studies. Indeed, the effects of cardiac denervation on the function of the normal heart was not ascertained until a number of studies undertaken by Donald et al. (35). Donald and Shepard determined that the response to exercise in dogs which had undergone cardiac denervation was similar in terms of capacity for work. Although the ability to increase cardiac work during exercise was similar, the heart rate increased more slowly in the denervated animals. Furthermore, Guyton et al. proposed that in cardiac denervated animals the functional responses to various forms of stress were similar to responses of innervated animals due to the influence of circulating catecholamines and the supersensitivity to norepinephrine of denervated hearts (54).

The beneficial influence of denervation has been challenged in a recent study by Lavallee et al. (79). Lavallee et al. showed that in conscious animals there is no protective influence of denervation on the development of a myocardial infarction in the conscious dog. The reason for the differences between this recent study and earlier ones was believed to be due to the use of conscious vs anesthetized animals.

The mechanism of denervation protective influence on myocardial infarction has been proposed to be due to alterations in myocardial blood flow following a coronary artery occlusion. Barber et al. and DuPont et al. both showed a reduction in myocardial regional blood flow following denervation as compared to control (4,36). However DuPont et al. found that denervation caused an increase in blood flow

to border zones following coronary occlusion, although the flow to the core of the ischemic region was not increased (36). Both studies attributed the reduction in blood flow in the denervated regions to a reduction in myocardial metabolism, although neither study addressed the question of oxygen consumption and metabolism directly. Some years earlier, Gregg et al. had hypothesized that removal of cardiac nerves resulted in an overall reduction in myocardial metabolism (51).

In recent years a number of investigators have employed denervation to determine whether there is a tonic restraint of coronary blood flow by a resting level of sympathetic input to the coronary arteries (22,61).

Demonstration of alpha-receptor limitation of flow under resting conditions or a 'tonic restraint' of flow has been addressed by numerous investigators. Resting coronary blood flow was considered to be under local influence and of course sympathetic influences would only be noticeable when levels of sympathetic input were high. But consideration of resting sympathetic discharge of approximately 3 Hz, would appear to indicate that the coronary bed, under resting conditions may have a small amount of sympathetic input. Katz and Jochim did show that removal of the stellate ganglia and vagi increased resting blood flow (67). About 35 years later, Schwartz and Stone also reported that left stellectomy not only increased the blood flow distribution to the endocardium, it also increased reactive hyperemia from 476 $\pm$ 71% to 622 $\pm$ 86% of control flow. Although propranolol could reduce the reactive hyperemic response, phentolamine following sympathectomy could not. However phentolamine prior to sympathectomy

did increase reactive hyperemia by 23% from the control response (115).

Following regional sympathetic denervation with 6-Hydroxy dopamine, Holtz et al. found that the blood flow measured with microspheres, in the innervated region was 66 ± 14 ml/min/100gm compared to resting blood flow in the denervated region of 108 ± 16 ml/min/100 gm. Administration of propranolol did not alter the blood flow distribution, however phentolamine abolished the regional differences by increasing the flow in the control region to levels equivalent to the sympathectomized region. They concluded that there is a substantial level of alpha vasoconstrictor tone on coronary blood flow (61).

Vatner et al. also proposed that alpha receptors exert a tonic control over coronary blood flow. Stimulation of the carotid sinus nerve in the conscious dog resulted in a decrease in aortic pressure of 28% and a decrease in coronary blood flow of 7%, resulting in a decreased resistance of 22% from control. Propranolol and dopamine did not prevent the dilation. Phenoxybenzamine caused a reduction in resistance to levels comparable to those elicited during carotid nerve stimulation with beta-blockade. There was no change in the resistance during CSNS with phenoxybenzamine. Guanethidine (ganglionic blocker) also abolished the dilation, indicating that stimulation of the carotid sinus nerve resulted in removal of sympathetic tone. This withdrawal of alpha-tone resulted in a vasodilation which could be stopped by phenoxybenzamine or guanethidine (126). In a similar set of experiments, they found that alpha tone could be elicited by an efferent pathway involving the mechanical reflex of pulmonary stretch

receptors. With an intracoronary injection of nicotine, the consequent stimulation of chemoreceptors and increase in ventilation, induced a reflex dilation of coronary arteries via a withdrawal of sympathetics. The dilation was abolished by alpha receptor blockade or carotid sinus nerve section. This dilation occured in spite of reductions in metabolism (127).

An investigation by Chilian et al. does not support the idea of a resting level of alpha vasoconstriction of coronary arteries. In this study regional denervation was achieved by topical application of phenol. Resting blood flow in the sympathectomized region was not significantly different from blood flow in the normal zone or control region. Their results were not altered by beta-adrenergic blockade or combined alpha and beta blockade. The flows in the innervated region were 0.87 $\pm$ .08 and 0.85 $\pm$ 0.07 ml/min/gm (22).

The effects of denervation have applications in tracing anatomic pathways to the heart, in the clinical setting of transplantation, and for treatment of angina. In addition, a number of studies in the past several years have investigated the possibility that ischemia itself causes a regional denervation; and that the ischemic muscle is 'stunned' and unresponsive to nerve stimulation.

Martins et al. studied the effects of coronary artery occlusion of cardiac function in both the ischemic and non-ischemic zones and the response to left stellate stimulation and exogenous noradrenalin. Dogs were anesthetized with alpha-chloralose, and either the LAD or circumflex artery isolated. LSS during occlusion produced segmental expansion whereas noradrenaline produced segmental shortening in the

ischemic area. They explained this impaired response of the ischemic myocardium as due to the impaired response to sympathetic nerve stimulation. They did not offer a reason as to why impaired neurotransmission in the ischemic zone should cause an actual decline in regional ventricular function. It was, however not a significant reduction of expansion during LSS. LSS produced a change in length of 0.1 ± 0.3 mm, and exogenous noradrenaline of 0.5 ± 0.1 which was significantly different from the change in length during occlusion. No mechanism of denervation was advanced in this study (88).

It is thought that sympathetic efferent nerves course in the upper 0.25 -0.50 mm of the epicardium, since topical application of phenol which destroys the upper 0.25 mm of epicardium, interrupts sympathetically mediated changes in ventricular contraction. Barber et al. (1982) demonstrated that not only does a transmural myocardial infarction interrupt sympathetic neurotransmission in the ischemic bed, it also impairs neurotransmission in the nonischemic regions (5). It has been demonstrated previously that sympathetic fibers run with coronary arteries and traverse the myocardium in a base to apex fashion (45). By injecting a rapidly hardening latex solution into a branch of the LAD, the authors were able to produce a transmural infarction by eliminating the contribution of collateral blood supply to the occluded region. The infarction was assessed using triphenyltetrazolium chloride. To determine the responsiveness to sympathetic nerve stimulation, the effective refractory period (ERP) was measured during right (RSS) and left (LSS) stellate stimulation. Also, in chronically infarcted dogs, regional norepinephrine content was

measured in the ischemic and normal regions. In zones apical to the infarction, ERP did not change during RSS and left stellate stimulation, while sites basal to the infarction remained responsive to both stellate stimulation and exogenous noradrenaline. The denervation was not homogenous however in the areas apical to the occluded region, as half of the sites remained responsive to stellate stimulation. The other sites which were not affected by stellate stimulation were responsive to noradrenaline. It should be noted that the possibility of chemical denervation in areas surrounding the infarcted region due to the release of alcohol during the hardening process may have affected the results. It was not well-defined in this study how distally located the sites for the effective refractory period measurements were. It appears as though the basal location was spatially well-removed, however, the apical sites were closer to the region of infarction. The consequences may be that the closer the location to the infarction, the greater the possibility that the region was actually denervated by the latex, and not due to interruption of sympathetic neurotransmission.

A more recent study on the subject of ischemia induced denervation was done by Ciuffo et al. Myocardial segmental shortening was measured before and after an occlusion of twenty five minutes. Before the occlusion, the shortening was 12% and increased with LSS to 20% and with injected norepinephrine to 20% in the zone which was to become ischemic. After the period of ischemia and reperfusion, the shortening was only 4%. In response to LSS, shortening decreased even further to 2.4%, but did increase in response to noradrenaline (13%).

The reduced responsiveness to neural stimulation persisted for two hours after reperfusion (23).

The loss of myocardial function following ischemia and reperfusion has been documented by other investigators. Heyndrickx observed that even when blood flow had returned to control values following ischemia, the function remained depressed for a longer time. However, in that study, he did not think it likely that the loss of function was due to impaired neurotransmission (60). The mechanism of the disruption of neurotransmission is not explained, and is particularly intriguing since ischemia generally develops in the endocardium first and progresses toward the epicardium. Moreover, in the canine model the possibility of a transmural infarction is not as likely an occurrence as in other species due to the greater amount of collateral blood flow. The third study included in this dissertation was done to determine whether removal of tonic alpha-vasoconstrictor tone would increase the blood flow the ischemic zone.

G. CARDIAC SYMPATHETIC NEURAL MODULATION OF CORONARY BLOOD FLOW

An early study on the functional importance of cardiac nerves to coronary blood flow and myocardial performance was reported by Szentivanyi (120). In that study, the author claimed to have been able to differentiate the fibers innervating the heart as being vasodilator and vasoconstrictor on the basis of their stimulation thresholds. Although these studies have not been duplicated, the idea that the large bundles of cardiac nerves could have differential influences on the heart was investigated extensively by Randall et al.

Using the technique of epicardial stripping or phenol application, the heart was selectively denervated, and the projections of cardiac nerves to the heart were delineated (106). To summarize his findings, the innominate caused an increased contractility in the right ventricle and left ventricular apex. The ventromedial cardiac nerve increased right ventricular conus contractility, and the VLCCN increased contractility to the posterolateral left ventricle. He also found that the more distally the sympathetic trunk was stimulated, the more localized were the areas affected by nerve stimulation (107). Studies by Geis and Kaye determined that the right stellate ganglion (RSG) and the left stellate ganglion (LSG) innervate the anterior and posterior left ventricle. The RSG was found to send projections to the posterior base, and the LSG to project to the anterior left ventricle via branches from the VLCCN (45). These studies, although providing insight into the innervation of the heart, did not specifically determine the effects of cardiac sympathetic nerve stimulation on blood flow the heart. Specific effects on the posterior left ventricle were not described in these studies.

Rinkema et al. examined the influences of left stellate stimulation and right stellate stimulation on regional myocardial blood flow in the anesthetized dog. They found that flow to the circumflex region was increased more by LSS that flow to the anterior region. However, it was determined that the LSG innervated all portions of the left ventricular myocardium. Following administration of propranolol, vasoconstriction during LSS was determined (111). These findings were in accord with results from Ross and Mulder, and Tato et al.

(112,122). Takenada et al. demonstrated that the anterior ansa subclavia and the VLCN cause vasoconstriction especially with practolol or propranolol (121).

Thus, although the left stellate ganglion can be concluded to affect regional left ventricular blood flow, separate cardiac nerve stimulation effects on blood flow have not been investigated previously. The exact location of sympathetic pathways to coronary vessels is not established. Changes in blood flow can be demonstrated with sympathetic stimulation, and vasoconstrictor effects during beta-blockade can be used to isolate the effects of nerves going to coronary arteries from their effects on myocardial function. However, the exact pathways and nerves have not been described and isolated. Thus, the fourth study in this dissertation, examined which cardiac nerves modulate circumflex coronary blood flow, and whether the vasoconstriction elicited by these nerves is alpha-1 or alpha-2 mediated.

It is hoped that these studies will add to our present knowledge of the role of the cardiac sympathetic nerves on myocardial blood flow during ischemia. A clearer definition of sympathetic influences on the ischemic heart is important in light of the widespread clinical use of adrenergic blockers in the treatment of coronary artery disease and myocardial infarction.

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CHAPTER III

GENERAL METHODS

A. SURGICAL PREPARATION

Mongrel dogs of either sex were premedicated with either morphine sulfate (2.5 mg/kg, s.c.) or xylazine (2 mg/kg, s.c.) approximately 15-20 minutes before being given alpha-chloralose (50-100 mg/kg, i.v., Sigma Chem. Co. in solution with sodium borate, ratio of 1:1.23, chloralose: borate). Alpha-chloralose was chosen as the general anesthetic, since it does not adversely affect hemodynamic variables (2,11) and does not depress cardiovascular reflexes as does pentobarbital anesthesia (6,12,44). A polyethylene catheter (PE-260 tubing) was placed in the right femoral artery and connected to a Statham P23dB pressure transducer for continuous monitoring of arterial blood pressure. The right femoral vein was catheterized with PE-260 tubing for fluid and drug administration. The trachea was cannulated with a glass Y-tube which was connected to a Harvard respirator. Each dog was ventilated with room air at a positive end-expiratory pressure of 5 cm of water. Both cervical vagi were isolated and severed. Succinylcholine (20 mg, i.v.) was administered as a muscle relaxant and the chest was opened in the 4th left intercostal space. The corneal reflex and response to pinching of the toe pad were checked every half hour when the effects of succinylcholine had worn off, and additional anesthetic was administered when needed. The heart was kept moist with sponge soaked in saline. In addition, a

layer of plastic wrap was kept over the open chest to minimize moisture and heat loss. In studies in which the heart was paced, a stainless steel bipolar electrode was sutured to the left atrial appendage, and the heart paced at 150 pulses/min from a Frederick Haer 4i stimulator. Blood gases and pH were monitored throughout the experiment. pCO2 and pH were maintained within normal limits (pCO2 30-40 mm Hg, pH 7.38-7.45), by adjusting the rate and/or volume of the Harvard respirator, with infusions of 8.4% sodium bicarbonate (1 meq/ml) when necessary. pO2 was monitored and kept within normal limits (80-100 mm Hg) by adjusting the volume of inspired air on the ventilator, with care taken that arterial pCO2 levels were not altered. Core temperature, measured in the thoracic cavity by a YSI temperature probe, was kept between 37-39° C by means of a heating pad beneath the dog, and an infrared heating lamp above the dog when necessary.

B. STIMULATION PROTOCOL

Both the right and left ansae subclaviae of the stellate ganglia were isolated. Both the anterior and posterior limbs of the ansae subclaviae, were stimulated (7-10 Hz, 5 msec, 5-7 V) using bipolar stainless steel electrodes. Characteristic changes in the lead II electrocardiogram consequent to stimulation confirmed that both limbs of the right and left stellate ganglia had been isolated. These changes included an increase in the heart rate and increased negativity of the T-wave as described by Yanowitz upon stimulation of the right stellate ganglion. Yanowitz also described the increased

positivity of the T-wave with left stellate ganglion stimulation (43). The alteration of the polarity and peaking of the T-wave are due to the altered repolarization pattern. Geesbreght and Randall reported a shortening of the P-R interval upon stimulation of the ansae subclaviae and a disappearance of the P-wave, with reappearance upon cessation of the stimulation. They attributed this movement of the P-wave into the QRS interval to a shifting location of the pacemaker (18). Figure 3-1 is a sample trace of the Lead II EKG during both right and left ansae subclaviae stimulation.

Square wave pulses were delivered by either a Grass SD9 stimulator or a Frederick Haer constant voltage output stimulator, and monitored on a calibrated differential oscilloscope. These stimulation parameters have been used frequently in other studies involving stimulation of sympathetic nerves. Although these levels were higher than the frequency of tonic sympathetic discharge (1-3 Hz) described by Folkow, they were lower than what Folkow considered to be maximal physiologic levels (20 Hz) for stimulation of sympathetic nerves (16). In comparison to more recent studies, these levels were lower than those used in a recent study by Heusch and Deussen (20 Hz) (20), and they are of similar magnitude to those used in studies involving cardiac sympathetic nerve stimulation (10-20 Hz, 5 msec, 4-6 V, Randall et al. 1968)(30), (10 Hz, 5 msec, 3-5 V, (31) or stimulation of the first five thoracic ventral roots (10 Hz, 10 msec, 8V, Norris et al.)(28). Hemodynamic responses were recorded on an 8 channel Beckman Recorder model 612 (Frequency response: 10 div @ 10 mm, DC to 80 Hz; $\pm$ 1 db) at 25 mm/sec before, during and after the stimulation

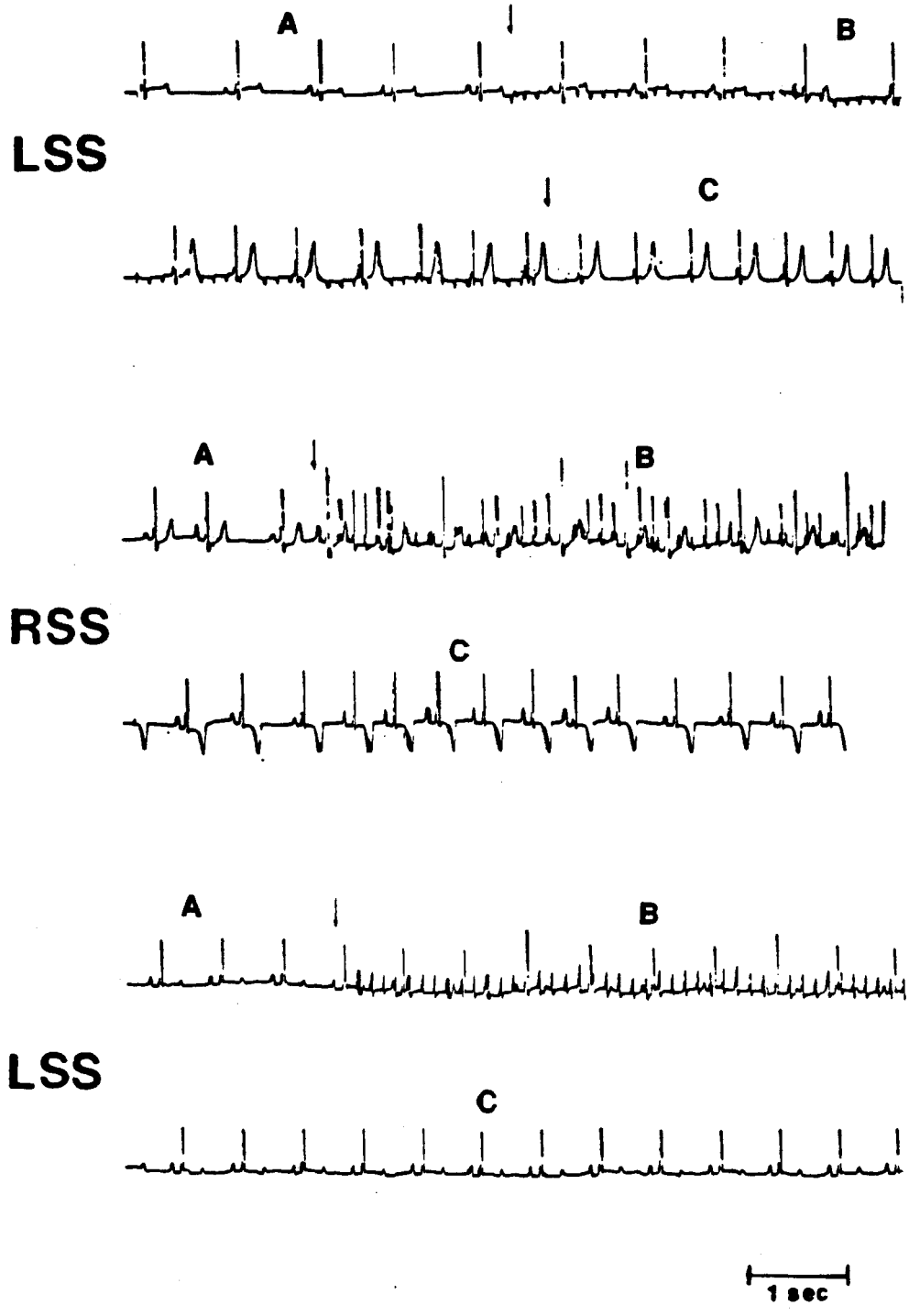
FIGURE 3-1

SAMPLE TRACING OF LEAD II EKG BEFORE, DURING AND AFTER STIMULATION OF THE LEFT AND RIGHT STELLATE ANSAE SUBCLAVIA

The upper pair of tracings shows a recording of the Lead II EKG (25mm/sec) during stimulation of the left stellate ansae subclavia (LSS, 8Hz, 5msec, 7V). The arrows indicate where ansae subclavia stimulation occurred. A represents the control EKG trace, B is the tracing during LSS, and C is the EKG following LSS. The time scale is in seconds. Note the marked increase in positivity of the T-wave during LSS, and the disappearance of the P-wave during LSS. The P-wave gradually reappeared from the QRS complex following stimulation.

The middle pair of tracings shows the Lead II EKG during stimulation of the right stellate ganglion. The arrow shows the beginning of the stimulation period. A,B,C, denote similar times as in Panel One. Right stellate stimulation was stopped immediately prior to the bottom trace. Note that prior to RSS, the T-wave was positive, and that during RSS and following RSS, the T-wave was inverted, and that the P-R interval remained constant.

The bottom pair of tracings shows the Lead II EKG during stimulation of the LSS following administration of timolol (0.2 mg/kg, i.v.). LSS was started at the arrow, and stopped immediately prior to the tracing of the bottom panel. Note that none of the changes which occurred in Panel One are present in this recording.


A
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protocol.

In each study, the ansae subclaviae of both the right and left stellate ganglia were tied with 2-0 silk and cut distal to the strands of silk. The cut distal ends were placed across the stainless steel bipolarelectrode for subsequent stimulations. Section of the right and left ansae subclaviae and the cervical vagi, effected neural decentralization of the heart. This was performed to prevent reflexes from arising following coronary artery occlusion, and to insure against afferent stimulation of the cardiac nerves. During the stimulations, the polarity of the bipolar electrode was kept the same with the negative electrode toward the heart thus avoiding the possibility of anodal nerve block.

C. ISOLATION OF THE CIRCUMFLEX ARTERY

The pericardium was incised and sutured to the walls of the chest to form a pericardial cradle. Approximately 5 mm of the circumflex artery was isolated distal to the first marginal branch in studies 1, 2, and 3. In the first study, the circumflex was ligated for 6 hours. In studies 2 and 3, the circumflex artery was occluded for a maximum of 7-8 minutes, after which the occlusion was released and the artery allowed to reperfuse for 35-40 minutes. This period of occlusion was chosen to minimize the development of ischemic damage and allows multiple assessments of blood flow during the course of several different experimental manipulations. It has been demonstrated that with periods of occlusion of up to ten minutes followed by a twenty minute period of reperfusion there is negligible ultrastructural damage (5), biochemical or metabolic alterations (32). In addition, Barber et al. have shown that a period of

occlusion of five minutes does not alter baseline blood flow when followed by a period of reperfusion of thirty minutes (4). Janes et al. reported that repeated periods of LAD occlusion of 5 minutes duration followed by a 10 minute reperfusion do not alter the responsiveness of the anterior wall to stellate ganglion stimulation as assessed by segment length and intramyocardial pressure (21). In study 4, approximately 5 mm of the circumflex artery was isolated as close as possible to its origin to allow perfusion of the majority of the circumflex bed. In the fourth study, the circumflex artery was occluded briefly while a catheter was inserted and tied in place. Normally the period of occlusion lasted no longer than 1-1.5 minutes before perfusion with blood from the carotid artery was begun. Dissection and isolation of the circumflex artery with 2-0 silk may cause interruption of the nervous supply to the posterior wall. Dolezel et al. reported that implantation of a methacrylate ring around the left coronary artery resulted in a significant decrease in the adrenergic innervation as assessed by Falck's histochemical technique (13). They hypothesized that scarring of the area of implantation consequent to the surgery resulted in compression of the pericoronary nerves and the ultimate degeneration of the adrenergic nerves supplying the area distal to the implantation. However, Knight et al. reported that even with the extensive isolation of the circumflex artery necessary for implantation of ultrasonic flow probes on the circumflex artery, the increase in posterior wall thickening during stimulation of the ansae subclaviae in the group with the flow probe (71 $\pm$ 14%) was not significantly different from the increase in posterior wall thickening of the control group (67 $\pm$ 9%). In addition posterior wall norepinephrine

levels in the flow probe group (629 ± 4 pg/mg) were not significantly different from the levels in the control group (589 ± 88 pg/mg) (24). Thus it may be that although isolation of a coronary artery partially interrupts adrenergic transmission to the region in question, norepinephrine levels do not decline significantly following instrumentation. Whether isolation of the artery interrupts nerves controlling vasomotor activity has yet to be investigated.

Lidocaine (40 mg,i.v.,bolus) was given prior to the initial coronary artery occlusion and during the course of the experiment as an additional bolus dose if there was an increased incidence of ventricular arrhythmias.

D. ADRENERGIC BLOCKING AGENTS

Table 3-1 provides a summary of the autonomic agonists and antagonists used throughout the studies. Timolol, a non-selective beta--blocker, was chosen instead of propranolol for several reasons. As described by Scriabine et al. it is 5-10 times more potent than propranolol, and exerts fewer agonist properties (35). Timolol also has fewer central effects than propranolol (38). At concentrations required to block beta-receptors, timolol does not exhibit membrane stabilizing effects as does propranolol at an equipotent dose (29,36). Blockade by timolol (0.2 mg/kg, i.v.) was considered to be adequate when stimulation of the left ansae subclavia produced no changes in the Lead II electrocardiogram (See Figure 3-1) or heart rate. The blocking dose of timolol was based on its beta-1 properties, since as was mentioned in the literature review, the beta-2 receptors do not exert an important

TABLE 3-1
AUTONOMIC AGONISTS AND ANTAGONISTS USED

SYMPATHETIC AGENT	SITE OF ACTION	DOSE USED
Adrenergic Blockers:		
Timolol	beta-1,beta-2 receptors	0.2 mg/kg,LD, 0.08 mg/kg/-hr,MD
Prazosin	alpha-1 receptors	0.5 mg/kg
Rauwolscine	alpha-2 receptors	0.3 mg/kg
Adrenergic Stimulants:		
Norepinephrine*	alpha-1&2, beta-1 and beta-2 receptors	
Phenylephrine	alpha-1 receptors	5 g/kg
BHT-920	alpha-2 receptors	0.1 mg/kg
PARASYMPATHETIC AGENT		
Parasympathetic Stimulant:		
Acetylcholine**	nicotinic and muscarinic receptors	
Parasympathetic Blockade:		
Atropine	muscarinic receptors	0.2 mg/kg

*Left stellate stimulation (8Hz, 5msec, 5-7V)
**Vagal stimulation (20Hz, 5msec, 5V)

influence on cardiac function or coronary blood flow in the dog. Maintenance doses of timolol (0.08 mg/kg/hr, i.v.) were given to insure blockade of beta-receptors throughout the experiment. This dose of timolol has been used in other studies (19) and is approximately 1/10 of the usual dose of propranolol administeredin experimental studies (20,23, 40). Alpha-1 blockade was achieved with prazosin hydrochloride (0.5 mg/kg, i.v.); a dose which is sufficient to attenuate the average 45-50 mm Hg rise in arterial pressure elicited by a 5 g/kg dose of phenylephrine (27,41). Prazosin has been demonstrated in a number of studies to exert non-significant effects on alpha-2 receptors (10,42). Phenylephrine was chosen to test the efficacy of alpha-1 blockade since it is considered to exert its agonist effects preferentially on post-junctional alpha-1 receptors. Rauwolscine (0.3 mg/kg, i.v.) is considered to be a reasonably pure alpha-2 blocking agent, since at this dose it does not block the effects of alpha-1 adrenergic agonists (26). This dose has been shown to block the increase in diastolic pressure effected by a 0.1mg/kg dose of BHT-920 (37). BHT-920 is a selective alpha-2 stimulator which is pharmacologically similar to clonidine (25,39). Atropine (0.2 mg/kg, i.v.) was given in Study 4 to eliminate the influence of the parasympathetic nerves upon stimulation of the thoracic cardiac nerves (31). Adequate blockade by atropine was tested with stimulation of the left thoracic vagus (20 Hz, 5 msec, 5V).

E. REGIONAL BLOOD FLOW DETERMINATION

In studies involving determinations of regional myocardial blood flow, radioactive labeled microspheres (14.5 $\pm$ 0.6 m) were used (3M Co,

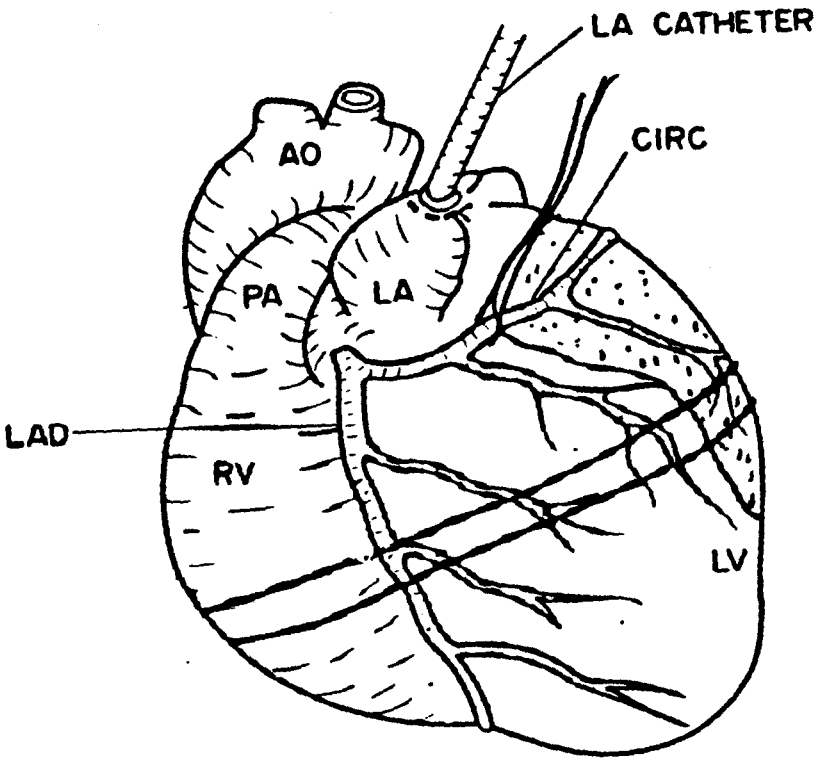
New England Nuclear). This size sphere was chosen since it causes less axial streaming than larger sized spheres (7) and less arteriovenous shunting than smaller spheres (1,7). Fortuin et al. determined that with arterial injection of microspheres of this size, less than 1% appeared in the coronary sinus (17). The microspheres were suspended in a 10% dextran solution with 0.05% polyoxyethelene-20 sorbitan mono-oleate (Tween 80) to dispel the inherent aggregation of microspheres (33). The vials containing the spheres were sonicated for at least 20 minutes prior to microsphere injection, and vigorously agitated in a vortex mixer immediately prior to use. Blood flow was calculated using the theoretical organ technique. For 30 seconds before microsphere injection, a reference blood sample was withdrawn from the femoral artery at a rate of 5-7 ml/min, and reference blood withdrawal continued for at least 90 seconds following microsphere injection. This period of withdrawal insured that all of the spheres were trapped in the animal's blood vessels or in the syringe. Also, it has been shown that 98% of the spheres are collected within the first 60 seconds (15). The withdrawal rate of the pump was calibrated at the end of each experiment to determine the exact rate of withdrawal. Microspheres were injected into the left atrium via a catheter over a period of 30-40 seconds and were flushed with 10 ml of warm saline. The electrocardiogram was monitored during the injection period, with care taken to stop injection during periods of arrhythmia. Careful handling of the left atrial catheter minimized the incidence of injection arrhythmias, as did slow injection of the microspheres. At the end of the experiment, each heart was electrically fibrillated, excised, and a segment of 5-French infant

feeding tubing was inserted into the circumflex artery distal to the site of occlusion. Microfil was injected into the circumflex catheter to delineate the perfusion area of the CX. Microfil, a liquid compound used to fill and opacify the vascular spaces of post-mortem tissues, was used in Studies 2 and 3 to define the perfusion area of the circumflex bed since it does not get filtered out of the vessels and become absorbed by neighboring vessels and tissue. Also, the stain does not dissipate when placed in 10% formalin for fixing, as Evans blue dye does. The hearts were fixed for 24-48 hours, after which samples of the myocardium were cut from both the stained and unstained regions, weighed, and placed in plastic tubes. Figure 3-2 illustrates how the ischemic area was delineated, and the method by which the heart was sliced from apex to base. A sample slice shows how the endocardial and epicardial samples were cut from the left ventricle. The reference blood withdrawal samples were also put into plastic tubes, and the syringes containing the blood samples were rinsed with saline, and the rinse put into the respective labeled counting tubes. Heart and reference organ samples were counted by a TM Analytic gamma counter (model 1185) utilizing a three inch NaI crystal sensitive to gamma emissions. The data from the gamma counter were sent directly to an APPLE computer and stored on floppy disc, for later analysis. Blood flow values were obtained by the use of a computer program which calculated blood flow in ml/min/gm using the original data stored on disc from the gamma counter. Blood flows were calculated using standard equations with the appropriate corrections for background and interference coefficients. The equation used to calculate blood flow was: MBF= (Ct/TW) x (RBW/Cb), where MBF = myocardial blood flow in ml/min

FIGURE 3-2

ILLUSTRATION OF THE METHODS USED TO OBTAIN MYOCARDIAL SAMPLES FOR REGIONAL BLOOD FLOW DETERMINATIONS

Illustration of the manner in which samples were taken for calculation of regional myocardial blood flow. AO-aorta, LA-left atrium, PA-pulmonary artery, RV-right ventricle, LAD-left anterior descending coronary artery, CIRC-circumflex coronary artery, LV-left ventricle. A sample slice in the mid wall of the left ventricle is shown in the left hand figure. The right hand figure shows how samples were cut from the left ventricle. The stippled area is the area into which Microfil had been injected, and delineates the circumflex (ischemic) region. NZ-normal zone, IZ-ischemic zone (collateral dependent zone). Sample numbers are used to define which region each sample was cut from. Sample numbers 1 and 3 are normal zone endocardium, sample numbers 2 and 4 are normal zone epicardium, sample numbers 5,7, and 9 are ischemic zone endocardium and so forth for each of the slices of left ventricle.


LA CATHETER
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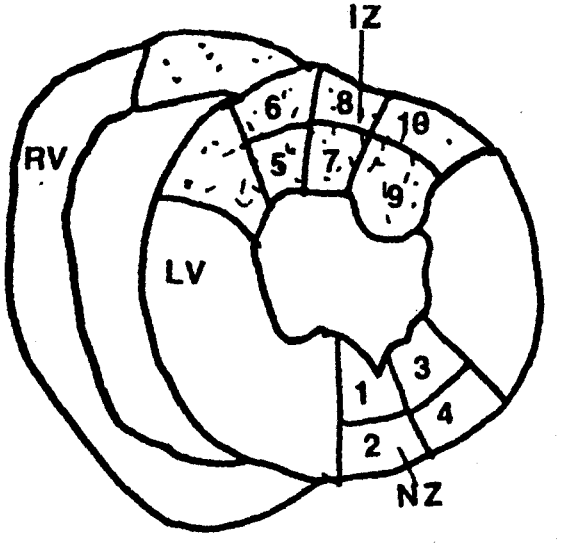
IZ
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per gm, Ct = tissue radioactivity in counts/min, TW = tissue sample weight in grams, RBW = reference blood withdrawal rate in ml/min, and Cb = total radioactivity in reference blood sample. Buckberg et al. determined by statistical analysis that a minimum of 400 microspheres per sample was necessary to obtain a statistical error of <10% in calculations of blood flows. To obtain a larger number of microspheres per sample would have necessitated the injection of such a large quantity of microspheres, that hemodynamic variables might have been changed (7). In the present studies, approximately 4.5 million microspheres per blood flow determination were injected into the left atrium. Baer et al. reported that left atrial injections of 15 m microspheres totaling 48 x 10^6 did not affect hemodynamic variables or alter regional myocardial blood flow (3). It was calculated that in order to achieve the minimum number of microspheres per sample, tissue sizes of >500 mg or tissue samples with blood flows greater than 20 ml/min would have at least 400 microspheres trapped in the micro-vasculature.

Certain basic principles as outlined by Buckberg were adhered to with the injection of microspheres (8). We used the femoral artery as the blood withdrawal organ since this has been determined to provide a more reproducible collection of microspheres than other arterial lines such as the brachial artery. Second, the left atrium was used for the injection of microspheres to provide for adequate mixing of the spheres prior to entering the left ventricle or the left ventricular outflow tract. Third, the time for withdrawal was more than adequate to collect the majority of microspheres (>95%) circulating in the blood stream, as described by others (7,15).

F. USE OF THE MICROSPHERE METHOD FOR CALCULATION OF BLOOD FLOW DURING ISCHEMIA

Utilization of the microsphere technique to determine ischemic blood flow is prone to error for several reasons. As explained above, samples containing at least 400 spheres will yield accurate blood flow measurements. However, in regions in which blood flow is compromised enough to cause ischemia, the number of microspheres lodged in the tissue might not have been sufficient. Retrospective analysis of ischemic tissues in which sample size was greater than 1 gram yielded average blood flow values for the entire heart which were not different from the blood flow values used in the actual analysis of data (see Appendix A). Second, injections of microspheres should be done under stable hemodynamic conditions. The potential influence of arrhythmias was compensated for by injecting the microspheres over 30-40 seconds, since microspheres represent mean blood flow over consecutive heart beats. In blood flow determinations following coronary artery occlusion, microspheres were injected after five minutes of occlusion, during which time hemodynamic parameters were fairly stable, with the exception of some occasional arrhythmias, thus fulfilling the requirement of a steady state for accurate measurement of blood flow. Even shorter periods of occlusion (<30 sec) have been used to measure blood flow immediately following coronary occlusion (14), thus one can be reasonably certain that a steady state was achieved by five minutes after occlusion. Third, the use of microspheres during ischemia might lead to errors in calculation of blood flow as shown by Jugdutt et al. who have found that there is a real loss

of microspheres from cardiac tissue that has become ischemic (22). However the study done by Jugdutt used a period of ischemia of several weeks in chronically instrumented dogs, and 7-10 m microspheres were used. Another reason for the errors in blood flow determination cited by Jugdutt was that there was an 'apparent' loss of microspheres from ischemic tissue due to formation of edema. This should not have been as important in an animal model in which the periods of occlusion lasted for only 5 minutes, followed by reperfusion. Basuk et al. reported that periods of 10 minutes of occlusion followed by 20 minutes of reperfusion, caused little ultrastructural damage, and only slight edema formation (5). Capurro et al. found that there was microsphere loss due to a prolonged period of ischemia prior to fixing of the samples in formalin (9). In this study the hearts were excised within 20 minutes of the last period of occlusion.

G. DATA ANALYSIS

All data were expressed as mean $\pm$ SEM. All studies involved the use of some form of analysis of variance to test for differences between groups, with a form of modified T-test to isolate the differences. All statistical tests were run on SAS (Statistical Analysis System)(34). The particular tests used, and data analysis will be discussed under each specific project.

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CHAPTER IV

EFFECTS OF CARDIAC SYMPATHETIC NERVE STIMULATION DURING ADRENERGIC BLOCKADE ON INFARCT SIZE IN ANESTHETIZED DOGS.

A. INTRODUCTION

Myocardial injury or infarction may occur as a result of either an interruption of coronary blood flow or a severe disruption in the balance between oxygen supply and demand. Augmentation of sympathetic nervous system activity following coronary occlusion is reflected by a rise in plasma catecholamine levels (33,34), and has been demonstrated directly by recordings of cardiac sympathetic nerve discharge (6,23). Activation of beta-1 receptors by cardiac sympathetic nerves or circulating catecholamines produces an increase in coronary blood flow primarily via an increase in myocardial oxygen consumption (MVO_2) and the resultant production of vasodilator metabolites (4). There is also evidence that catecholamines may effect coronary vasodilation directly by stimulation of beta-1 receptors (40) and beta-2 receptors (16) located on the coronary arteries. During myocardial ischemia, increases in MVO_2 might become crucial in determining the amount of flow available to the ischemic region. Increases in MVO2 producing vasodilation in normal tissue, may result in a "steal" of blood from the vasodilated ischemic region (10). If the oxygen demands of the myocardial cells are increased by catecholamine stimulation in the presence of limited perfusion, a vicious cycle can result, in which the blood supply to the underperfused area is compromised further due to the increasing work demands of the

heart and vasodilation of normal areas. In addition, recent investigations have demonstrated the potentially important contribution of alpha receptors in limiting coronary flow at low levels of blood pressure (7) and in the presence of a coronary stenosis (8,13). Hence, alpha-mediated vasoconstriction may not necessarily be overridden by the large metabolic demands associated with catecholamine stimulation (29) and in fact, could be deleterious by further limiting blood flow to the ischemic region. Thus, activation of both beta and alpha receptors by neurally released or circulating catecholamines might exacerbate the amount of damage initially incurred in the ischemic area.

Since a certain amount of cellular necrosis is inevitable following permanent coronary artery occlusion, there has been considerable interest in limiting the size of the infarct to prevent any further loss of functional myocardial tissue. It appears likely that factors which reduce neurally mediated increases in myocardial oxygen demands, such as beta-receptor blockade (27,42) can reverse the potentially damaging effects of catecholamines in the ischemic myocardium. Several investigations have shown that propranolol and timolol, both non-selective beta-blocking agents, can limit the size of myocardial infarcts (19,20,27,32,33,38). Moreover, propranolol has been reported to maintain perfusion of zones bordering on the infarct (32), but the extent of protection may be largely dependent on collateral flow since propranolol did not restrict infarct size in baboons, which have no preformed collaterals (12). Grong et al., reported that timolol did not increase blood flow to the ischemic area (15), however, Vik Mo et al., showed that timolol provided more protection against infarct development than

propranolol (41). The mechanisms whereby beta-blocking agents decrease ischemic damage have not been established conclusively.

Other investigations have implicated alpha receptors in the development of a myocardial infarct. Chiariello et al, showed that treatment with labetolol (a combined alpha and beta-adrenergic blocking agent) following coronary artery occlusion resulted in a smaller infarct size in rats as compared to those rats receiving a beta blocker alone (9). They attributed this to a decrease in afterload on the heart following blockade of peripheral vascular alpha receptors. The advantageous hemodynamic effects of combined alpha and beta-blockade were also reported by Nelson et al., (31).

The purpose of the present study was to assess the influence of elevated sympathetic input on the development of a myocardial infarct following circumflex artery ligation in hearts during conditions of controlled autonomic input. Specifically, we tested the hypotheses that 1) the reported beneficial effects of beta-blockade on infarct size are contingent upon a basal level of sympathetic nerve activity prior to and during coronary artery occlusion; 2) a high level of sympathetic activity following beta blockade in the neurally decentralized heart will result in an greater infarct size; and 3) the contribution of alpha receptors to the development of a myocardial infarct will be accentuated by blockade of beta-receptors with augmented sympathetic stimulation to the heart.

B. METHODS

1. General Preparation

Forty-two mongrel dogs of either sex (14-30 kg) were premedicated with morphine sulphate (2.5 mg/kg; subcutaneously) twenty minutes prior to the intravenous administration of a solution containing alpha-chloralose (100 mg/kg). A catheter was placed in the right femoral artery and connected to a Statham P23 dB pressure transducer for continuous monitoring of arterial blood pressure. The right femoral vein was cannulated for fluid and drug administration. The trachea was cannulated with a glass Y-tube, which was connected to a Harvard respirator. Each dog was ventilated with room air at a positive end-expiratory pressure of 5 centimeters of water. Both cervical vagi were isolated and severed. Through a thoracotomy in the fourth left intercostal space, both the right and left stellate ganglia were isolated and decentralized. In three groups of dogs, the anterior and posterior ansae subclavia from the left stellate ganglia were placed across a bipolar electrode for subsequent stimulation. In order to pace the heart a separate bipolar electrode was attached to the left atrial appendage at a site remote from the level of circumflex artery occlusion. The circumflex coronary artery was isolated distal to the first marginal branch with 2-0 silk for subsequent occlusion.

2. Protocol

The dogs were divided into five groups. In Groups 1, 2, 4 and 5, the hearts were neurally decentralized as described above and electrically paced at 150 beats per minute. In previous studies we had deter-

mined this heart rate to be above the average heart rate of chloralose anesthetized dogs. It was not possible to pace the hearts in Group 3, since the heart rates increased to approximately 200 beats/minute following left stellate stimulation, and the rates decreased to about 150 beats/minute over the course of two to three hours. The first group served as a control group (CTRL; n=5), receiving no beta-blocking agents or neural stimulation. In the second group (TIM; n=6), a blocking dose (0.2 mg/kg) of the non-selective beta-adrenergic blocker timolol, was administered intravenously as a bolus at least twenty minutes prior to circumflex artery occlusion. A maintenance dose of timolol (0.04 mg/kg,IV bolus) was given every hour to insure adequate beta-blockade. This dose was found to be effective in blocking both inotropic and chronotropic actions of left stellate stimulation (8 Hz, 5 msec, 5-7 volts). Group 3 (LSS; n=7) served as a control for the group of dogs receiving left stellate stimulation and timolol (Group 4). In this group the anterior and posterior ansae subclavia on the left side were stimulated electrically within five minutes of coronary artery occlusion and for the duration of the protocol (8 Hz, 5 msec, 5-7 volts) following parameters used by Barber et al.(3). No adrenergic blocking agents were administered in this group. The fourth group (LSS-TIM; n=5) also received timolol prior to and during coronary occlusion. In addition, left stellate stimulation was begun immediately following CAO. Approximately twenty minutes before coronary artery occlusion, dogs in Group 5 (LSS-TIM-PRAZ; n=6) received prazosin (0.5mg/kg, IV bolus), as well as timolol. The effectiveness of alpha receptor blockade was tested with phenylephrine (5 g/kg,IV bolus) (25). This dose of phenylephrine raised

the blood pressure an average of 64 mm Hg prior to blockade. If blood pressure did not increase more than 10 mm Hg following administration of phenylephrine, blockade by prazosin was considered to be adequate. Left stellate stimulation was also begun following CAO in this group. All dogs received 40 mg of lidocaine prior to circumflex artery ligation to reduce the incidence of lethal arrhythmias early after coronary occlusion. In each dog, the occlusion was maintained for six hours.

3. Determination of the Area at Risk

The area at risk was determined in each dog in the following manner. After six hours of occlusion, the circumflex artery was cannulated immediately distal to the ligature using 5-French infant feeding tubing. A polyvinyl catheter was placed in the left atrium for injection of Evans blue dye (1.5% solution, 1ml/kg). The dye was injected rapidly into the left atrium and allowed to circulate until the anterior free wall was stained a deep blue. The circumflex artery distal to the point of occlusion was perfused simultaneously with 50 ml of warm 0.9% NaCl using a modification of the methods described by Lange (23). Both the dye and saline were injected by hand. Immediately after injection of the dye the heart was electrically fibrillated and the left ventricle was vented with 18 gauge needles and excised. Venting was done to prevent the possible alteration of the area at risk due to acute ventricular dilation and the consequent increased wall tension. The right ventricular free wall, both atria, valvular tissue and aortic and pulmonary trunks were cut away leaving only left ventricle and septum intact. The left ventricle was sliced transversely from base to apex

into 6-8 slices, each slice approximately 1 cm in thickness, and both sides were photographed. The area at risk was defined as the unstained region of the myocardium (saline perfused), and total left ventricular area was calculated as the sum of the unstained area and the stained area following Evans blue injection. Colored pins were placed on either the basal or apical side for identification of which side was being viewed.

4. Determination of Infarct Size

Infarct size was assessed using triphenyl tetrazolium chloride solution (TTC) to stain for dehydrogenase enzymes (11,21,22). This method provides immediate quantitation of the infarct size and shows excellent correlation with the amount of necrosis determined histologically (11). The slices were rinsed in cold tap water and incubated for ten minutes in TTC maintained at 37 degrees centigrade. Each slice was again photographed to record infarct sizes. After film development the slices were projected onto a computer digitizing graphics tablet and the areas of the myocardium, area at risk, and infarct were measured by planimetry. The infarct size was normalized by estimating it as a percent of the area at risk.

Of the 42 dogs, 19 dogs fibrillated within the first 15-20 minutes post-occlusion. 13 of these dogs died before the end of the 6 hour occlusion period. The six dogs which survived the occlusion period were included in the analysis of data. Of these dogs, two were in Group 2, three were in Group 3, and one was in Group 4. All dogs which fibrillated were electrically defibrillated and given an additional dose of 40 mg lidocaine and 20 ml of an 8.4% sodium bicarbonate solution.

5. Statistical Analysis

Data were first analyzed using standard one way analysis of variance techniques. When the calculated F value was significant, differences between groups were determined using the Tukey test. Statistical significance was reached when the calculated value for p was less than 0.05. All data are presented as mean $\pm$ standard error of the mean (SEM).

C. RESULTS

In Groups 1, 2, 4 and 5, (CTRL, TIM, LSS-TIM, and LSS-TIM-PRAZ, respectively) heart rate was kept constant throughout the experiment at 150 beats per minute. The heart rate in Group 3 (LSS) increased from a control level of about 110 beats/min to 200 beats/min following left stellate stimulation. Since the heart rate reached such high levels and did not decrease to below 150 beats/min for several hours, these dogs could not be paced. Thus, the heart rates in Group 3 were higher than those in the other groups.

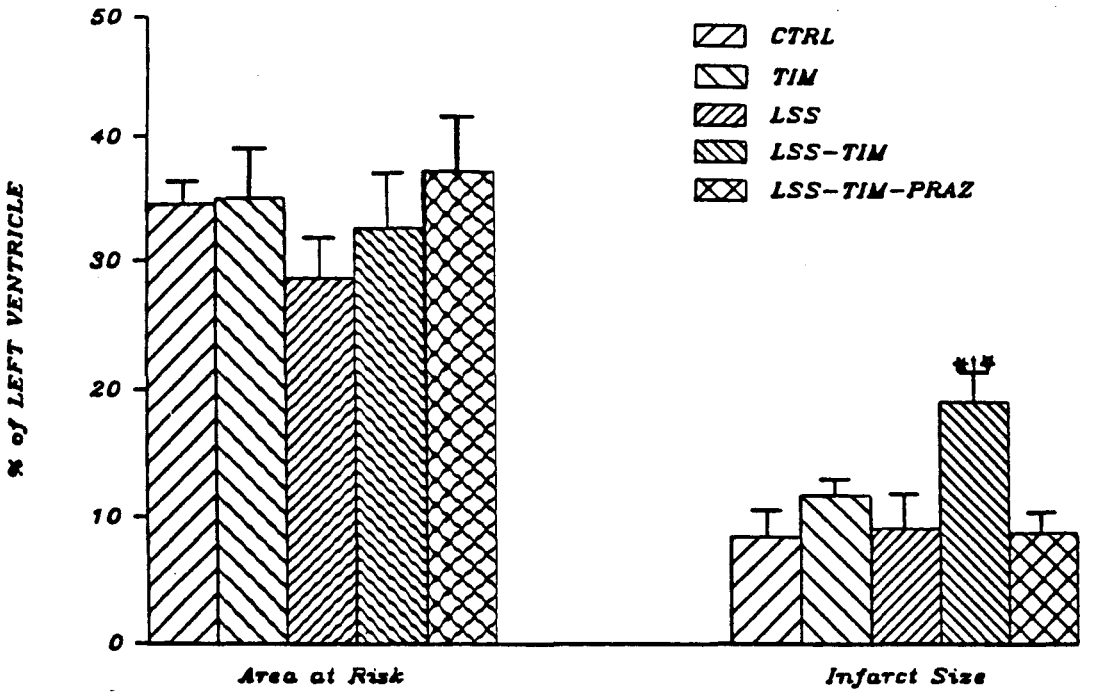
The mean arterial pressure for all dogs remained constant throughout the experiment except in dogs that fibrillated. The blood pressure in dogs that fibrillated dropped below 40 mm Hg for no more than ten minutes. If the dogs were not revived within that period of time, they were not included in the data analysis. However, all dogs which were successfully defibrillated were included in estimates of group mean arterial pressures. Mean arterial pressure was calculated as the average pressure over the six hour occlusion period. The mean arterial pressure (MAP) for the control group averaged 101.0 $\pm$ 5.5 mm Hg. For the timolol treated group, the averaged MAP was 99.5 $\pm$ 9.1 mm Hg. The group of dogs receiving left stellate stimulation had an averaged MAP of 109.2 $\pm$ 4.0 mm Hg. Group 4 MAP was 126.3 $\pm$ 9.5 and the MAP for Group 5 was 92.6 $\pm$ 5.1. The difference between groups was statistically significant (F value=2.-87, $p<.05$) using analysis of variance. However, the Tukey test could not distinguish statistical significance between the specific groups at the .05 level.

Figure 4-1 shows both the area at risk and the infarct size as a

FIGURE 4-1

INFARCT SIZE AND AREA AT RISK AS A PERCENT OF LEFT VENTRICULAR AREA

Percent of the left ventricle at risk or infarcted. On the left is the area at risk as a percent of left ventricular area. On the right is the infarct size as a percent of the left ventricular area. The data are expressed as mean values for each group ± SEM. *indicates Group 4 (LSS-TIM) was significantly different from Group 1 (CTRL) ($p<0.05$). †indicates Group 4 (LSS-TIM) was significantly different from Group 3 (LSS) ($p<0.05$). *indicates Group 4 (LSS-TIM) was significantly different from Group 5 (LSS-TIM-PRAZ) ($p<0.05$).


50
40
30
20
10
0
% of LEFT VENTRICLE
CTRL
TIM
LSS
LSS-TIM
LSS-TIM-PRAZ
*
Area at Risk
Infarct Size

percent of the left ventricular area. The mean values for the area at risk (left hand panel of Figure 1) of each group are: CTRL, 34.6 ±1.5; TIM, 35.0 ± 4.0; LSS, 28.6 ± 3.0; LSS-TIM, 32.5 ± 4.3; and, LSS-TIM-PRAZ, 37.0 ± 4.3. No significant difference was found between groups for the area at risk as a percent of the left ventricular area. Thus, coronary artery occlusion at the same approximate anatomic level in each dog resulted in no significant differences in the size of the perfusion bed of the artery between groups.

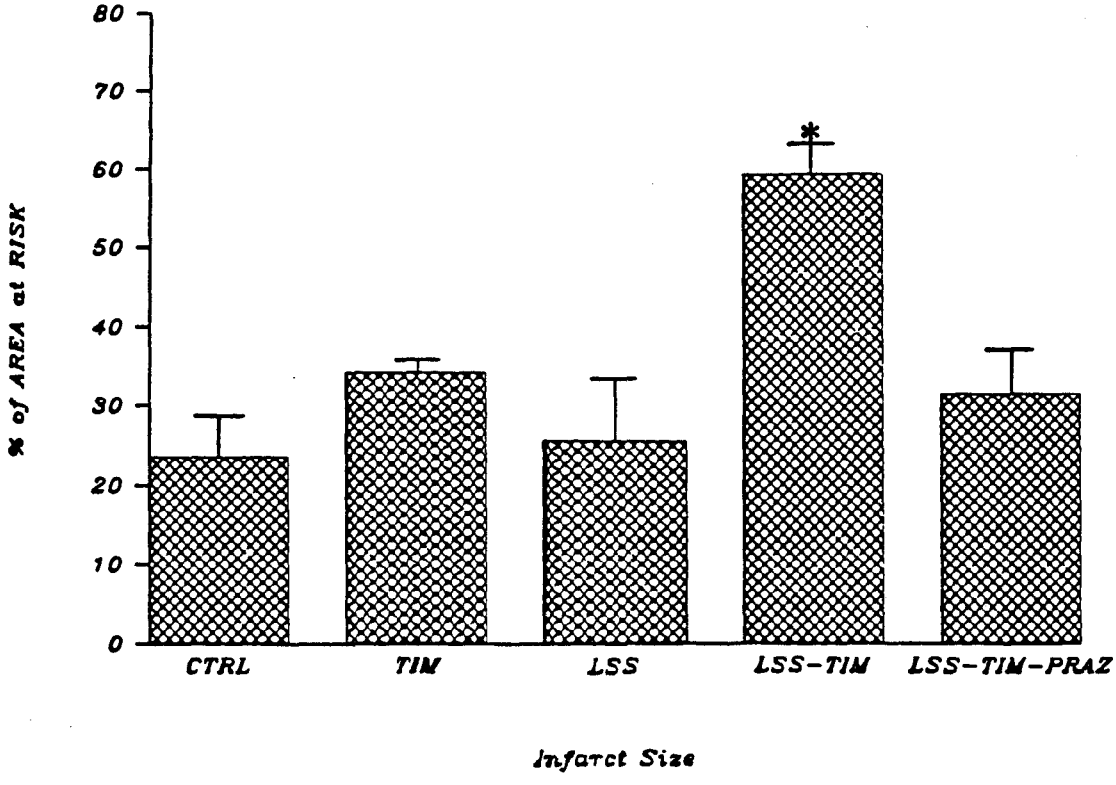
Mean values for infarct size as a percent of the left ventricular area are shown in the right hand panel of Figure 4-1 for each group are: CTRL, 8.4 ± 2.2; TIM, 11.8 ± 1.1; LSS, 9.1 ± 2.8; LSS-TIM, 19.1 ± 2.4; and, LSS-TIM-PRAZ, 8.8 ± 1.5. Significant differences were found between Group 4 (LSS-TIM) and CTRL, LSS, and LSS-TIM-PRAZ ($p<0.05$). There was no difference between LSS-TIM and TIM. The data for infarct size as a percent of the area at risk is shown in Figure 4-2. The mean value for CTRL was 23.5 ± 5.3; for TIM, 34.2 ± 1.4, for LSS, 31.4 ± 7.8; LSS-TIM 59.3 ± 3.9; for LSS-TIM-PRAZ, 25.6 ± 5.3. Significant differences were found between LSS-TIM and all other groups ($p<0.05$).

Analysis of the incidence of fibrillation, revealed that there were no significant differences in the numbers of dogs that fibrillated between the groups. Reevaluation of the data without the dogs that fibrillated did not change the significance between the groups.

FIGURE 4-2

INFARCT SIZE AS A PERCENT OF THE AREA AT RISK

The percent of the area at risk infarcted. Infarct size from each of the groups is plotted as a percent of the area at risk. Data are expressed as mean ± SEM. *indicates Group 4 (LSS-TIM) was significantly different from all other groups ($p<0.05$).


80
70
60
50
40
30
20
10
0
% of AREA at RISK
*
CTRL
TIM
LSS
LSS-TIM
LSS-TIM-PRAZ
Infarct Size

D. DISCUSSION

One of the major findings of the present study was that timolol treatment did not result in a smaller infarct size as a function of the left ventricle or as a function of the area at risk compared to control. This appears to be contrary to the results of previous studies in which beta blockade was reported to limit infarct size (19,20,27,32,33,38). The apparent discrepancy may be due to a basic difference in the experimental preparation. In those previous studies, the effect of beta-blockade was evaluated by comparing a group of animals that received the drug prior to coronary occlusion, to a group that received only the vehicle, both groups had intact cardiac innervation. Our rationale for using neural decentralization in the present study was that it would be an effective means of controlling cardiac autonomic input. In this way, we could eliminate the variability that occurs in extrinsic sympathetic activity, prevent the increase in reflex nerve activity secondary to coronary occlusion (26), and/or deliver a measurable and fixed level of selected sympathetic activation.

The increased activation of adrenergic receptors which might exacerbate ischemic damage could be due to augmented sympathetic nerve activity, increased local release (35), local reflexes originating within the myocardium (2), or come from circulating catecholamines. However, the fact that there was no significant difference between the control group and the group receiving timolol demonstrates that circulating catecholamines did not influence significantly the course of infarction. If circulating or locally released catecholamines had been a significant determinant, then the size of the infarct in the group receiving timolol

would be expected to be larger via alpha receptor stimulation than the control set of animals. Furthermore, if there were a generalized release of catecholamines in response to coronary artery occlusion, much of this increase would be in the form of epinephrine, which has a greater affinity for vascular beta-2 receptors which were blocked by timolol (1). Coronary vascular beta-2 receptors are not considered to play an important role in the control of coronary blood flow (17).

In addition the hearts were paced at 150 beats per minute to avoid the consequences that a variable heart rate would have on the development of ischemia. One of the beneficial effects of beta blockade is thought to be its ability to lower heart rate (42). By maintaining heart rate constant throughout the period of occlusion, the effects that a lower heart rate may have had on decreasing MVO2 were eliminated.

Another aspect of this study was that the infarct size as a function of the area at risk in the control group was smaller than those reported in other studies (30,36,37,43). There are several possible explanations for the observed differences. First, as explained above, in the present study all cardiac nerves were acutely sectioned, eliminating both direct and reflexly mediated nerve-stimulated catecholamine release and the concomitant increase in myocardial metabolism. Second, the specific anatomic location of the occlusion and the artery used in our study were different than those employed by others (30,36,37,43). Third, the occlusion in our study was maintained for six hours with no reperfusion. Finally, the various methods of measuring both infarct size and the area at risk of infarction, may contribute to the differences in results between separate studies. We chose to express the infarct size

as a percent of the area at risk as a means of normalizing the data. In this way we hoped to eliminate the variability in measurements between dogs that would occur if the left ventricle became the standard against which infarct size would be assessed. This method is supported by the results of our study. There was no significant difference found between TIM and LSS-TIM for the infarct size as a percent of the left ventricular area, whereas there was a difference between these two groups for the infarct size as a percent of the AAR. This can be accounted for by the inherently greater variability between left ventricular area as opposed to the circumflex artery perfusion bed.

We found it surprising that the group of dogs receiving only LSS had a similar mean infarct size to the control group. This suggests that simultaneous activation of both alpha and beta receptors may act such that their opposing influences on coronary vascular smooth muscle offset one another. When opposing beta-mediated vasodilatory effects are removed, stimulation of alpha receptors proves deleterious to the ischemic heart.

The results clearly demonstrate that efferent stimulation of the left ansae subclavia with concomitant beta-blockade produced larger infarcts than in the other groups. It is possible that the higher mean arterial pressure of this group of dogs had an adverse effect on the size of the infarct. An increase in arterial pressure represents an increase in afterload to the heart and thus, increased MVO2. Recent evidence suggests, though, that acute increases in arterial pressure do not affect the outcome of coronary artery occlusion, being neither beneficial nor detrimental to infarct development (5). It is our supposition that alpha

receptors are responsible for the large size of the infarcts in Group 4 (LSS-TIM). This is further supported by the results from Group 5 (LSS-TIM-PRAZ) in which alpha-1 receptor blockade was effected by prazosin (25). It is possible that alpha receptors are more important in myocardial ischemia with augmented levels of sympathetic input, since there was no significant difference between the control and timolol-treated groups. It might be argued that the levels of stimulation used were unphysiologic and might have overwhelmed the beta blockade. However, the level of blockade was adequate to prevent inotropic and chronotropic responses from occurring following left stellate stimulation. Thus, it is possible that the levels of cardiac sympathetic input normally present in the anesthetized dog were effectively blocked by beta-receptor blocking agents, and that these levels were not high enough to elicit significant alpha-mediated coronary vasoconstriction. However, with high levels of sympathetic input, and beta-1 adrenergic receptors blocked, alpha receptors may have a proportionally greater role in the development of a myocardial infarct. This may be the case in a conscious animal with elevated sympathetic tone during stressful situations or in exercising animals. Recently, Matsuzaki et al., have shown that myocardial function in a region of decreased flow is close to normal values at rest but deteriorates with exercise, and that atenolol (a selective beta-1-receptor blocker), although improving function in the ischemic area, does not restore it to control levels (28).

As a result of the increase in infarct size following left stellate stimulation with beta blockade (Group 4), two conclusions may be drawn. Either alpha receptor activation increases the size of the infarct by

limiting coronary blood flow to the ischemic area, or there is a localized non-beta receptor mediated increase in myocardial oxygen consumption. Alpha receptor stimulation of platelet aggregation and the resultant formation of thrombi is an alternative explanation for an alpha-mediated increase in infarct size (17). Although alpha receptors might have limited blood flow to the normal area, via unopposed vasoconstriction, no conclusions can be made regarding the actual mechanism of the increase in infarct size by alpha receptors in this study. Further investigations are needed to determine the involvement of alpha receptors in the development of ischemia, and its effects on the transmural and global distribution of blood flow.

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CHAPTER V

ADRENERGIC INFLUENCES ON CANINE MYOCARDIAL BLOOD FLOW FOLLOWING CORONARY OCCLUSION

A. INTRODUCTION

Competition of alpha-receptors with beta receptors in the regulation of coronary blood flow has been investigated for the past ten years. The premise that alpha receptors restrict coronary blood flow despite augmented oxygen needs is well-documented. Alpha-receptor vasoconstriction has been demonstrated in conscious animals (21,24,35, 43), anesthetized preparations (7,8,22,31), and isolated vessel strips (45). Alpha receptors have been postulated to exert not only a tonic influence on resting coronary blood flow (24,42,43) but also to limit the increase in blood flow consequent to exercise (23,33). Clinical evidence of intense vasospasm resulting in angina (28,34) and myocardial infarction (12,27) led other investigators to speculate on the contribution of alpha receptors to vasospasm. Alpha receptors have been implicated in the restriction of blood flow during administration of propranolol (26), and during the cold-pressor test, causing constriction severe enough to lead to angina (32).

Whether alpha receptor stimulation modulates blood flow during ischemia has not been demonstrated conclusively. Even in the case of ischemia, when dilation due to ischemic metabolites would be expected to exert an overwhelming influence on blood flow, alpha receptors have been reported to limit blood flow (7). Other investigations have suggested

that during ischemia, beta receptor blockade affords protection to the myocardium by unmasking alpha receptors in the normal region which limit flow to the normal zone, enough to cause a 'reverse coronary steal' (11,19). However, Buck et al. concluded in their study that beta-receptor blockade provided protection to the ischemic myocardium by lowering indices of oxygen demand, thus allowing the available blood supply to meet the oxygen demands of the myocardium (6). In a recent report from our laboratory, we found that combined blockade of alpha and beta receptors prior to coronary artery occlusion led to a decrease in the amount of myocardium which infarcted following coronary artery occlusion. In dogs in which beta receptor blockade was administered, infarct sizes were larger in the presence of stellate stimulation as compared to control (16). Whether this was due to an effect on myocardial blood flow remains to be determined. We postulated that the process of myocardial infarction reported in that study was exacerbated by limitation of blood flow to the ischemic region via stimulation of alpha-1 receptors. The purpose of this investigation was to elucidate the role of alpha-1 receptors on modulation of blood flow during coronary artery occlusion, with and without beta-blockade in the presence of elevated sympathetic input. In addition, we wished to determine whether prazosin would alter blood flow to the ischemic region regardless of whether it was given prior to or following coronary artery occlusion.

B. METHODS

1. General Preparation

Twenty three mongrel dogs of either sex were preanesthetized with morphine sulfate (2.5 mg/kg, s.c.) or xylazine (2 mg/kg, s.c.), 20 minutes before administration of alpha-chloralose (50-100 mg/kg, i.v.). A catheter was inserted via the right femoral artery into the thoracic aorta and connected to a Statham P23 dB pressure transducer for continuous monitoring of arterial blood pressure. A catheter was placed in the right femoral vein for intravenous administration of fluid and drugs. The left femoral artery was also cannulated for later blood withdrawal during the injection of microspheres. The trachea was cannulated with a Y-tube, the ends of which were connected to a Harvard respirator. The animals were ventilated with room air at a positive pressure of 5 cm of water. Both cervical vagi were isolated and cut. Succinylcholine was given as a muscle relaxant prior to opening the chest in the fourth left intercostal space. The dorsal and ventral limbs of both the right and left ansae subclaviae were isolated. Changes in the heart rate, P-R interval and T-wave configuration upon stimulation confirmed that both limbs had been isolated (17,44). The ansae were then cut distal to the stellate ganglia, and the cut distal ends of the left ansae subclavia were placed across a bipolar electrode. The pericardium was incised and sutured to the sides of the chest cavity to form a cradle. The circumflex artery was isolated distal to its first major branch, and a piece of 2-0 silk was placed under the artery. A catheter was inserted into the left atrial appendage for injection of microspheres. A loading dose of timolol (0.2 mg,kg, i.v.) was given prior to starting the actual

protocol, with maintenance doses of timolol administered throughout the duration of the experiment (0.08 mg/kg/hr, i.v.). Blockade of the beta-receptors was considered to be adequate when stimulation of the ansae subclavia no longer produced typical changes in either the electrocardiogram or heart rate. Blockade of alpha-1 receptors was accomplished by administration of prazosin HCL (0.5 mg/kg, i.v.). This dose of prazosin blocks the increase in blood pressure caused by a 5 g/kg,i.v. dose of phenylephrine (average increase in blood pressure 45 mm Hg) (29). The preparation was allowed to stabilize for at least 20 minutes before beginning the experimental procedures.

Blood gases were continuously monitored, and kept within normal limits (pH 7.38-7.45, pCO2 25-35 mm Hg, pO2 80-110 mm Hg) by adjusting the rate and depth of ventilation, with infusions of bicarbonate when necessary to keep pH normal. The core temperature, measured with a thermometer in the thoracic cavity was kept between 37-39° C, by means of a heating pad under the dog, and an infrared heating lamp above the dog. Plastic wrap was kept over the open chest to keep heat and moisture loss to a minimum.

2. Regional Blood Flow Determinations

In each group of animals, four blood flow determinations were made. Four of the following available species of 15 m microspheres (3-M) were used in random order: Ce-141, Cr-51, Nb-95, Sr-85 or Ru-103, and Sc-46. The microspheres were suspended in 50 ml vials of 0.5% dextran solution with 0.05% Tween 80. The vials were sonicated for 20 minutes prior to use, and agitated on a vortex mixer immediately before use. Reference

blood withdrawal from the left femoral artery was begun 30 seconds before injection of the microspheres. The microspheres were injected into the left atrium over a 30 second period and flushed with 10 ml of warm (37°C) saline. Reference blood withdrawal was continued for 90 seconds after completion of the microsphere injection. The total time for blood withdrawal averaged 2.5 minutes. Following the experimental procedures, the heart was excised, the circumflex artery ligated at the point of isolation, and a short piece of adult pediatric feeding tubing (8-French) inserted immediately distal to the ligature, and tied securely in place. Microfil was injected into the circumflex via the catheter until the epicardium of the posterior wall was visibly stained. The heart was then placed in formalin for 48 hours. After this, the heart was sliced into 6-7 slices from apex to base. Samples of the myocardium were taken from the posterior wall (stained region), and from the anterior free wall (unstained region). Each sample was cut into endocardial and epicardial halves, weighed, and placed in labeled counting tubes. Blood withdrawal samples from each of the procedures, and the heart samples were counted in a TM Analytic Gamma Counter (model 1185), and the output from the gamma counter processed directly by an APPLE II computer. Flows were calculated using standard equations with corrections for background counts and interference coefficients. The equation for blood flow determination is: $MBF = (Ct/TW) \times (RBW/Cb) \times 100$, where MBF = myocardial blood flow in ml/min/100 gm; Ct = tissue radioactivity in counts/min, TW = tissue sample weight in grams, RBW = reference blood withdrawal rate in ml/min, and Cb = total radioactivity in reference blood sample.

3. Experimental Protocol

Three groups of animals were studied. In each group four blood flow determinations were made. Figure 5-1 depicts in graph form the experimental protocols used in each group.

a. Group 1 (Prazosin;Post-CAO)

In Group 1 (n=5) the first flow determination was following beta-blockade with timolol (initial dose 0.2 mg/kg, i.v.); maintenance dose 0.08 mg/kg/hr, i.v.) and served as the control. The circumflex artery was then occluded for the remainder of the experiment. The second flow determination was made during circumflex artery occlusion, in the presence of timolol. Left stellate stimulation (LSS: 7-10 Hz, 5 msec, 7 V) was begun and the third set of microspheres were injected during LSS with the circumflex occluded. After completion of the third microsphere injection, LSS was stopped. Prazosin (0.5 mg/kg i.v.) was administered prior to the last blood flow determination, LSS was begun, and the fourth set of microspheres was injected during LSS, under conditions of alpha-1 blockade and coronary artery occlusion. The preparation was allowed to stabilize for at least 20 minutes between microsphere injections. This group will be denoted as the Prazosin;Post-CAO group since prazosin was administered after occlusion of the circumflex.

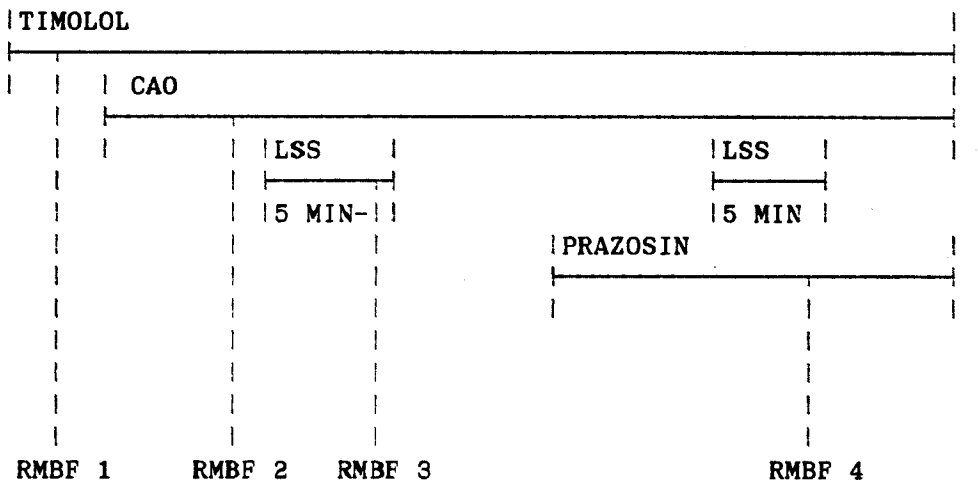
In Group 1 (Prazosin;Post-CAO), the amount of prazosin delivered to the ischemic myocardium depended on the amount of collateral blood flow to the area, since prazosin was administered following coronary occlusion. Thus, the efficacy of alpha-1 blockade was not necessarily homogeneous throughout the circumflex region. Hence, the next group of

FIGURE 5-1

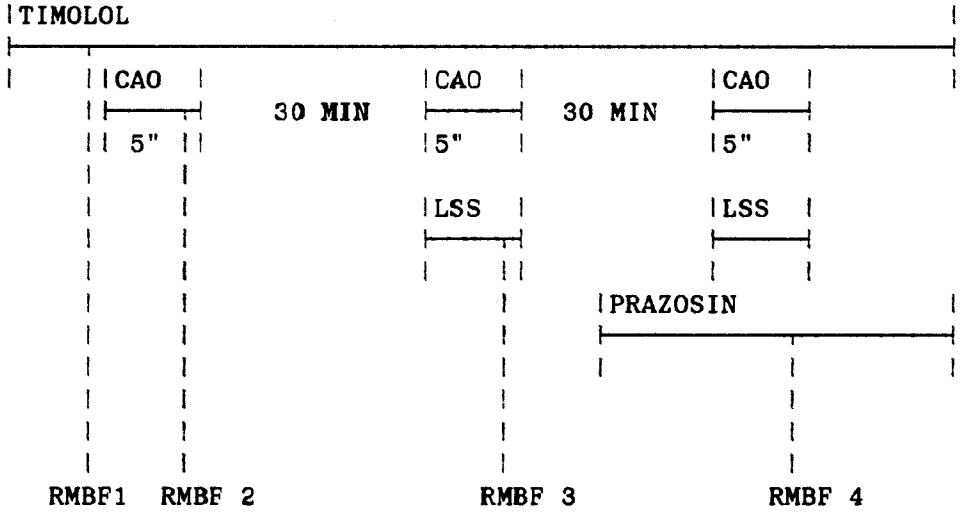
EXPERIMENTAL PROTOCOLS USED FOR GROUPS 1,2, AND 3

Abbreviations denote the following: CAO: circumflex artery occlusion; LSS: stimulation of the left ansae subclavia (8 Hz); TIMOLOL: beta-blockade with timolol; PRAZOSIN: alpha blockade; RMBF: regional blood flow determination (microsphere injection).

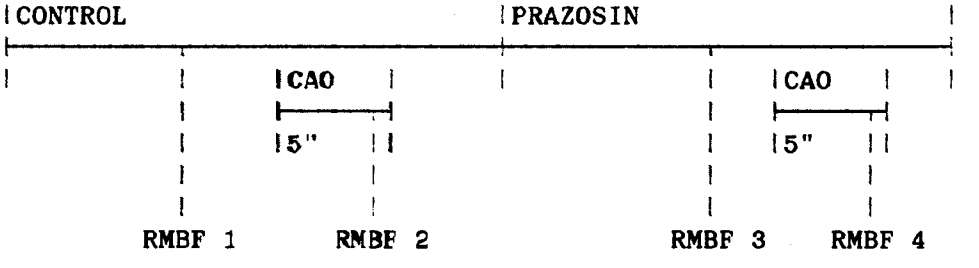
GROUP 1 (N=5): PERMANENT OCCLUSION (PRAZ-POST CAO)



GROUP 2 (N=7): REPETITIVE OCCLUSION (PRAZ-PRE CAO)



GROUP 3 (N=7): CONTROL-PRAZOSIN



animals was necessary to determine blood flow distribution during ischemia with prazosin given prior to occlusion to insure uniform blockade of the occluded region.

b. Group 2 (Prazosin;Pre-CAO)

In Group 2 (Prazosin;Pre-CAO, n=7), the occlusion periods lasted for a total of 7-8 minutes, and were followed by a thirty minute period of reperfusion. Microspheres were injected five minutes into the occlusion period, and the occlusion was released upon completion of the microsphere injection. The first flow determination was made following beta-blockade with timolol and served as the control. An atraumatic clamp was placed on the circumflex artery distal to the first marginal branch, and the second flow determination was made after 5 minutes of CAO. Upon completion of the injection, the occlusion was released and the heart allowed to reperfuse for 30-35 minutes. After this period, the CX was again clamped, stimulation of the left ansae subclaviae was begun and maintained throughout the third period of microsphere injection. At the end of the third microsphere injection, LSS was stopped, the arterial clamp was removed, and the artery allowed to reperfuse for 30-35 minutes. Prazosin (0.5 mg/kg, IV) was administered during the reperfusion period, and challenged with phenylephrine (5 g/kg, i.v.). At the end of the reperfusion period, the circumflex was again occluded, LSS was begun, and the fourth blood flow determination was made after 5 minutes of occlusion and LSS. Although total occlusion time was 7-8 minutes there is evidence that this period of occlusion does not cause ultrastructural damage (4) or alter baseline blood flow when followed by a 30 minute

period of reperfusion (2). This group will be called Prazosin;Pre-CAO since prazosin was given before occlusion of the circumflex artery.

c. Group 3 (Ctrl-Prazosin)

A third set of animals was included in this study to determine resting blood flow in the neurally decentralized preparation. In Group Three (n=7), the effects of prazosin on coronary blood flow during ischemia were tested without prior beta-blockade and with no sympathetic input. In this group of animals, the same model of circumflex occlusion was employed. The total duration of occlusion lasted from 7-8 minutes, with microsphere injections begun after five minutes of occlusion. Upon completion of the injection, the occlusion was released and the artery reperfused for thirty minutes. Four blood flow determinations were made in this group. The first microsphere injection served as a control, with no adrenergic blockers or sympathetic stimulation. The second injection was following a five minute period of coronary artery occlusion, again with no adrenergic blockers. The occlusion was released upon completion of the injection of microspheres, and allowed to reperfuse for thirty minutes. The third blood flow determination was made following a blocking dose of prazosin (0.5 mg/kg, i.v.). The last blood flow determination was made during coronary artery occlusion, in the presence of alpha-1 blockade. This group will be referred to as the Ctrl-Prazosin group to denote that prazosin was administered under control conditions, with no LSS or beta-blockade.

4. Data Analysis

Statistical tests were run on the regional myocardial blood flow values, on blood flows normalized to control flow, on resistance values, and on the endo/epi ratios. Blood flows were normalized to percent of the control blood flow. Resistance was calculated as the quotient of mean arterial blood pressure / endocardial or epicardial blood flow at the time of injection. Only left ventricular samples were analyzed. Thus anterior wall and posterior wall blood flow refer exclusively to the left ventricular blood flow. In each heart all samples from similar regions (i.e. all anterior endocardial samples) were averaged together to obtain a single blood flow value for each intervention. The averaged value was used in the analysis of data. In order to determine whether prazosin would exert a different effect on blood flow depending on the severity of the blood flow reduction following occlusion, blood flows in the posterior wall were separated in the following manner. In each dog, posterior wall blood flows were divided into two groups for analysis, Group A included those samples in which blood flow was between 20-50% of control flow following coronary artery occlusion, and Group B included those samples in which blood flow fell to less than 20% of control flow following coronary artery occlusion. Repeated measures analysis of variance was used to test for differences between experimental protocols, and Tukey's multiple range test was employed to isolate for differences. Comparisons were made between all of the groups for both the endocardium and epicardium. Statistical significance was reached at a p value of <0.05.

C. RESULTS

1. Group 1 (Prazosin;Post-CAO)

The anterior and posterior wall blood flow values for Group 1 are listed in Table 5-1. In the anterior wall there were no significant differences in blood flow between any of the groups, in either the endocardium or epicardium. In Group A of the posterior wall, the three blood flow determinations were all significantly lower in the endocardium following coronary artery occlusion compared to control ($p<0.0001$). In the epicardium, the blood flow during coronary artery occlusion (46.66 ± 4.98) was significantly lower than control (99.29 ± 11.67) ($p<.0001$). However, in the epicardium, flow increased during left stellate stimulation (LSS) and during LSS following administration of prazosin, so that the last two determinations of blood flow following coronary artery occlusion (CAO-LSS, CAO-LSS-PRAZ) were not statistically different from control. In Group B of the posterior wall, flows following CAO in both the endocardium and epicardium were all significantly lower than control ($p<0.0001$). In neither Group A nor Group B, were there were any significant differences between the interventions (CAO, CAO-LSS, CAO-LSS-PRAZ).

When the flows were normalized to the control flow value (Table 5-2), there were no significant differences between flows in the endocardium or epicardium of the anterior wall. The blood flow was significantly depressed in the endocardium and epicardium during CAO, CAO-LSS and CAO-LSS-PRAZ of Posterior Wall Groups A and B compared to control ($p<0.0001$).

There were no significant differences between groups in either the endocardial or epicardial calculated resistance in the anterior wall.

TABLE 5-1

REGIONAL BLOOD FLOW VALUES (ML/MIN/100 GM) DURING PERMANENT CORONARY OCCLUSION GROUP 1 (PRAZ;POST-CAO)

ANTERIOR LV

	ENDOCARDIUM				EPICARDIUM			
	CONTROL	CAO	CAO-LSS	CAO-LSS PRAZ	CONTROL	CAO	CAO-LSS	CAO-LSS PRAZ
MEAN	116.33	88.41	93.48	95.65	103.40	89.36	99.98	91.76
SEM	12.33	6.72	7.83	16.44	7.74	6.38	4.10	14.26

POSTERIOR LV (A)

	ENDOCARDIUM				EPICARDIUM			
	CONTROL	CAO	CAO-LSS	CAO-LSS PRAZ	CONTROL	CAO	CAO-LSS	CAO-LSS PRAZ
MEAN	114.82	38.68[1]	37.10[1]	39.61[1]	99.29	46.66[1]	56.35	57.95
SEM	16.01	6.26	14.18	16.89	11.67	4.98	11.48	17.78

POSTERIOR LV (B)

	ENDOCARDIUM				EPICARDIUM			
	CONTROL	CAO	CAO-LSS	CAO-LSS PRAZ	CONTROL	CAO	CAO-LSS	CAO-LSS PRAZ
MEAN	110.15	11.85[1]	22.49[1]	26.25[1]	93.71	40.53[1]	51.60[1]	46.98[1]
SEM	12.93	2.69	12.96	18.51	9.95	7.46	12.16	16.01

(Data are represented as mean ± SEM. Control: blood flow during beta blockade; CAO: flow during circumflex occlusion; CAO-LSS: flow during circumflex occlusion in the presence of left ansae subclavia stimulation; CAO-LSS-PRAZ: CAO-LSS following i.v. prazosin. [1]indicates significant difference from control, $p<0.0001$; $n=5$)

TABLE 5-2

NORMALIZED BLOOD FLOW VALUES (%) DURING
PERMANENT CORONARY OCCLUSION
GROUP 1 (PRAZ;POST-CAO)

ANTERIOR LV

	ENDOCARDIUM				EPICARDIUM			
	CONTROL	CAO	CAO-LSS	CAO-LSS PRAZ	CONTROL	CAO	CAO-LSS	CAO-LSS PRAZ
MEAN	100.00	86.09	82.99	82.20	100.00	93.71	100.85	88.88
SEM	0.00	8.39	9.69	7.27	0.00	4.80	8.89	7.49

POSTERIOR LV (A)

	ENDOCARDIUM				EPICARDIUM			
	CONTROL	CAO	CAO-LSS	CAO-LSS PRAZ	CONTROL	CAO	CAO-LSS	CAO-LSS PRAZ
MEAN	100.00	31.47[2]	28.30[2]	31.20[2]	100.00	48.15[2]	53.68[2]	54.69[2]
SEM	0.00	1.76	8.79	7.67	0.00	3.00	8.26	12.33

POSTERIOR LV (B)

	ENDOCARDIUM				EPICARDIUM			
	CONTROL	CAO	CAO-LSS	CAO-LSS PRAZ	CONTROL	CAO	CAO-LSS	CAO-LSS PRAZ
MEAN	100.00	10.53[2]	16.66[2]	19.37[2]	100.00	45.21[2]	54.05[2]	47.39[2]
SEM	0.00	1.67	7.93	11.49	0.00	8.86	9.90	11.28

(Data represent the mean ± SEM of percent of control flow. See Table 5-1 for definition of group names. [2]indicates significant difference from control, $p<.0001$, $n=5$)

Only the anterior wall resistances were calculated since the perfusion pressure of the posterior wall could not be assumed to be either aortic pressure, or uniform throughout the circumflex bed.

Table 5-3 lists the endo/epi ratios for the Prazosin;Post-CAO and Prazosin;Pre-CAO groups. In Group 1 (Prazosin;Post-CAO), there were no significant differences between the endo/epi ratios in the anterior wall. In the posterior wall, the endo/epi ratio was significantly lowered during left stellate stimulation (0.68 $\pm$ 0.19 mm Hg 100 gm/ml/min) as compared to control (1.16 $\pm$ 0.13) ($p<0.02$). There no other significant differences between groups.

The results from Group 1 indicate that combined alpha and beta blockade following permanent ligation of the circumflex artery does not increase flow to either the normal zone or the ischemic zone compared to the flow during left stellate stimulation without alpha blockade.

2. Group 2

Figure 5-2 depicts the regional myocardial blood flow (ml/min/100 gm) for Group 2 (Prazosin;Pre-CAO). In the top left panel of the figure are the anterior wall endocardial blood flows. Administration of prazosin significantly increased blood flow to 134.08 $\pm$ 22.21, from 81.18 $\pm$ 5.76 during control ($p<0.0001$). The middle and right hand panels of the upper graph show the blood flows for Groups A and B of the posterior wall. Endocardial blood flows shown for both groups A and B were significantly lower than control during CAO, CAO-LSS and CAO-LSS-prazosin ($p<0.0001$). There were no differences between treatment groups.

The bottom half of Figure 5-2 shows blood flow in the epicardium of

TABLE 5-3

ENDO/EPI RATIOS FOR PERMANENT AND REPETITIVE CORONARY ARTERY OCCLUSIONS

GROUP 1

	ANTERIOR				POSTERIOR(A)				POSTERIOR(B)			
	ctrl	cao	cao-lss	cao-lss-praz	ctrl	cao	cao-lss	cao-lss-praz	ctrl	cao	cao-lss	cao-lss-praz
$\bar{X}$	1.13	1.15	0.94	1.05	1.16	0.78	0.68[3]	0.79	1.18	0.40[3]	0.27[3]	0.24[3]
SEM	0.08	0.16	0.10	0.07	0.13	0.14	0.19	0.21	0.05	0.12	0.07	0.05

GROUP 2

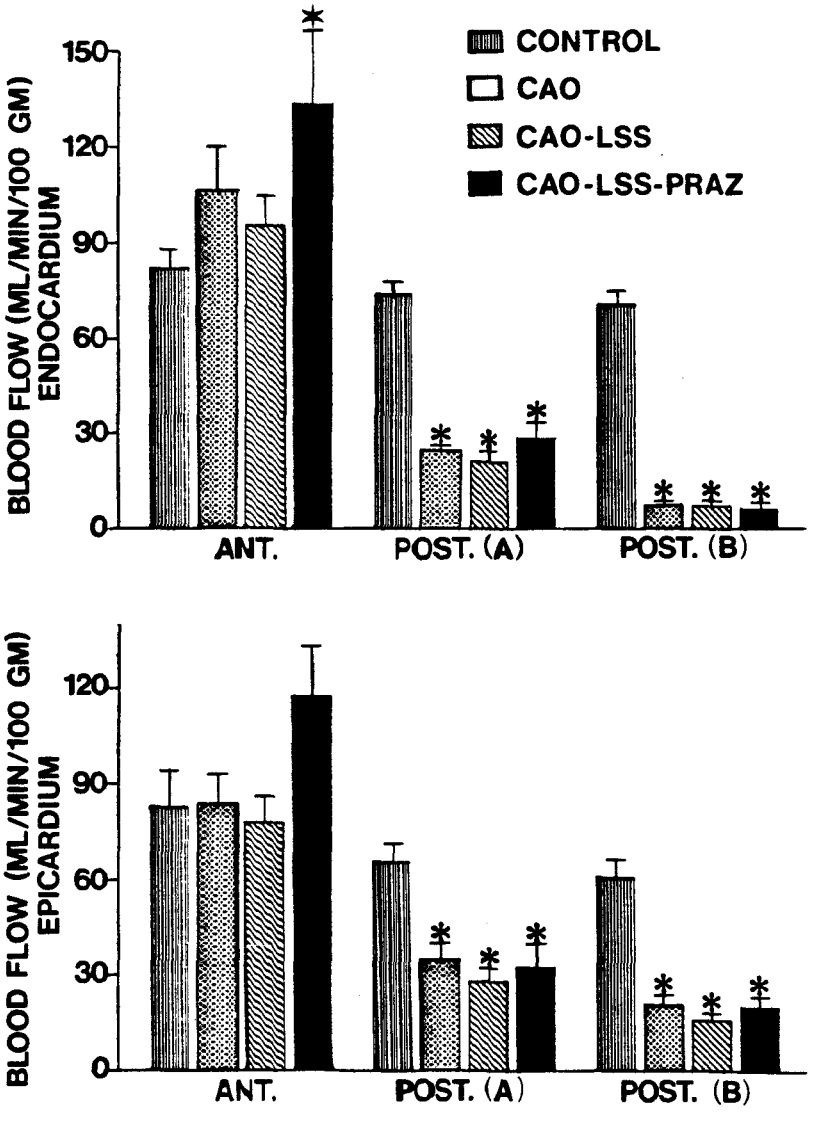
	ANTERIOR				POSTERIOR(A)				POSTERIOR(B)			
	ctrl	cao	cao-lss	cao-lss-praz	ctrl	cao	cao-lss	cao-lss-praz	ctrl	cao	cao-lss	cao-lss-praz
$\bar{X}$	1.14	1.30	1.26	1.13	1.20	0.92	0.96	1.44	1.21	0.44[4]	0.46[4]	0.30[4]
SEM	0.05	0.09	0.03	0.05	0.09	0.20	0.25	0.55	0.09	0.07	0.03	0.04

(Data are listed as mean ± SEM. Group 1 (n=5), Group 2 (n=7). [3]$p<0.02$ compared to control, [4]$p<0.0001$ compared to control. See Table 5-1 for definition of Abbreviations.)

FIGURE 5-2

REGIONAL MYOCARDIAL BLOOD FLOW VALUES (ML/MIN/100 GM)
DURING REPETITIVE CORONARY ARTERY OCCLUSION
GROUP 2 (PRAZOSIN;PRE-CAO)

Mean ± SEM values for posterior and anterior wall blood flows in the left ventricular endocardium and epicardium of Group 2. Control: blood flow during control conditions, during beta-blockade; CAO: flow during occlusion of the circumflex; CAO-LSS: flow during occlusion of the circumflex artery and simultaneous left ansa subclavia stimulation; CAO-LSS-PRAZ: flow during conditions of CAO-LSS in the presence of alpha blockade. *indicates significant difference from control ($p<0.0001$, $n=7$).


CONTROL
CAO
CAO-LSS
CAO-LSS-PRAZ
BLOOD FLOW (ML/MIN/100 GM)
ENDOCARDIUM
150
120
90
60
30
0
ANT.
POST. (A)
POST. (B)
BLOOD FLOW (ML/MIN/100 GM)
EPICARDIUM
120
90
60
30
0
ANT.
POST. (A)
POST. (B)

the anterior left ventricle (left panel) and the posterior left ventricle (middle and right panels). Prazosin did not significantly alter the flow in the epicardium (as compared to control, CAO or CAO-LSS). Flows decreased significantly from control during each of the interventions in the posterior wall ($p<0.0001$). However, there were no significant differences between any of the interventions.

Figure 5-3 shows blood flows normalized to the control blood flow value. The left hand panel of the top figure shows that blood flow following prazosin (167.03 ± 18.94) was significantly increased from the normalized control value ($p<.0001$). The middle panel and right panel of the upper figure, illustrate that blood flow was decreased significantly during all three interventions compared to control ($p<0.0001$).

The lower panel of Figure 5-3 depicts epicardial blood flows in the anterior and posterior wall. Blood flow in the anterior wall increased significantly following prazosin (168.55 ± 16.48) as compared to control (100 ± 0) and left stellate stimulation (117.34 ± 8.91) ($p<0.0001$). Flows following CAO were all significantly lower than control in both groups of posterior wall blood flows ($p<0.0001$).

Figure 5-4 shows calculated resistance values for the anterior wall. In the endocardium, resistance decreased significantly from control (1.36 ± 0.13) following prazosin administration (0.74 ± 0.13) ($p<0.0001$). Resistance subsequent to prazosin was also significantly lower than calculated resistance during left stellate stimulation without alpha-1 blockade (1.17 ± 0.11) ($p<0.0001$). In the epicardium, calculatedresistance was significantly lower following alpha-1 blockade (0.80 ± 0.11) compared to control (1.40 ± 0.17), CAO (1.29 ± 0.16) and

FIGURE 5-3

NORMALIZED REGIONAL MYOCARDIAL BLOOD FLOW VALUES (%) DURING REPETITIVE CORONARY ARTERY OCCLUSION
GROUP 2 (PRAZOSIN:PRE-CAO)

Mean ± SEM values for blood flows normalized to control (n=7). The upper half of the graph represents the anterior and posterior left ventricular endocardial blood flows. * indicates a significant difference from control ($p<0.0001$). The lower half of the graph represents the anterior and posterior left ventricular epicardial blood flows. indicates a significant difference from control ($p<0.0001$). ☆ indicates a significant difference from CAO-LSS ($p<.0001$). See Figure 5-2 for abbreviations and legend.

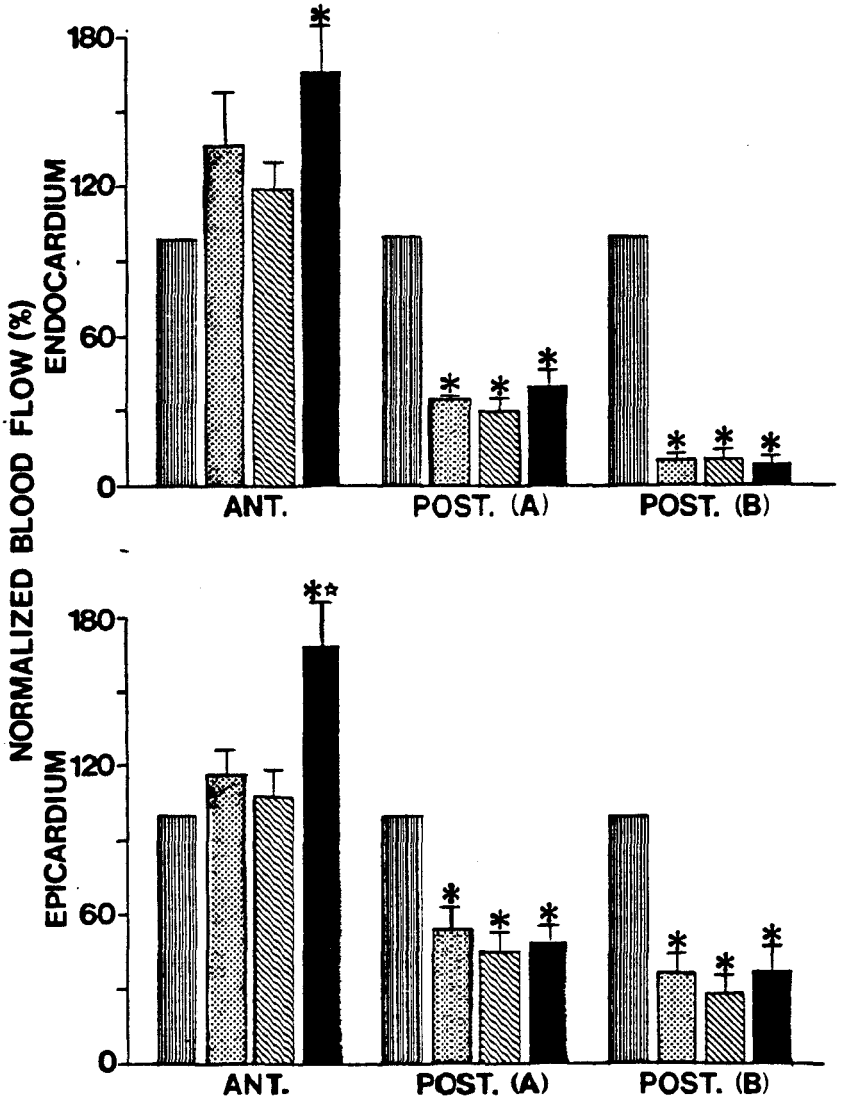
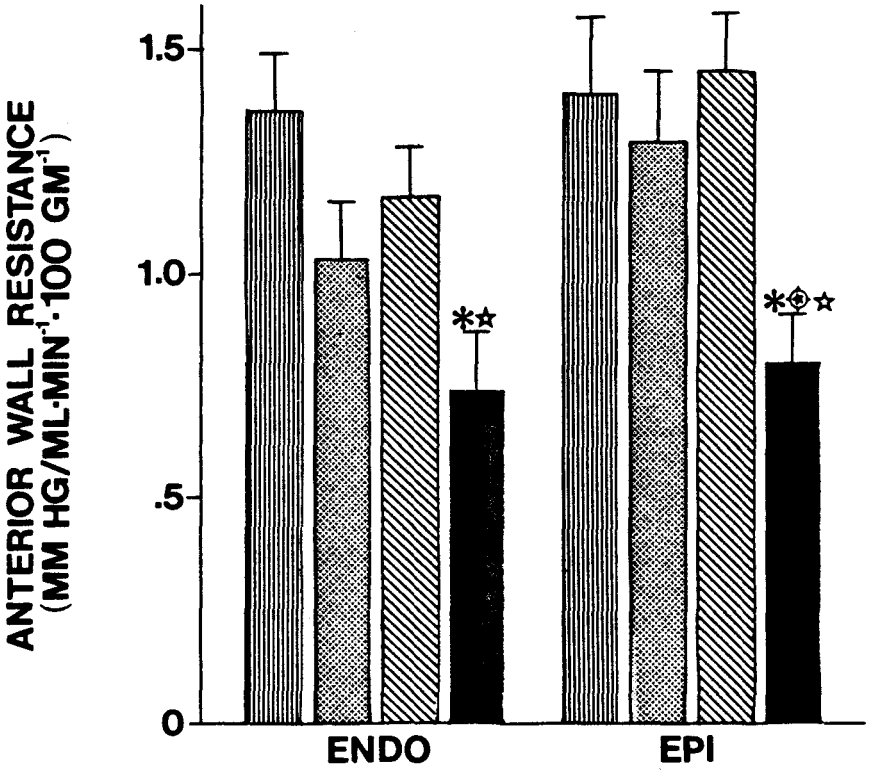

NORMALIZED BLOOD FLOW (%)
ENDOCARDIUM
180
120
60
0
ANT.
POST. (A)
POST. (B)
EPICARDIUM
180
120
60
0
ANT.
POST. (A)
POST. (B)

FIGURE 5-4

VASCULAR RESISTANCE IN THE ANTERIOR LEFT VENTRICULAR WALL
DURING REPETITIVE CORONARY OCCLUSION
GROUP 2 (PRAZOSIN:PRE-CAO)

Mean ± SEM values for the calculated left ventricular resistances, in the anterior endocardium and the epicardium (n=7). * indicates significant difference from control ($p<0.0001$). ☆indicates significant difference from CAO ($p<0.0001$). ⊛indicates significant difference from CAO-LSS ($p<0.0001$). See Figure 5-2 for legend and abbreviations.


ANTERIOR WALL RESISTANCE
(MM HG/ML·MIN⁻¹·100 GM⁻¹)
1.5
1.0
.5
0
ENDO
EPI

CAO-LSS (1.45 $\pm$ 0.13) ($p<0.0001$).

There were no significant differences in the endo/epi ratios in either the anterior wall or posterior wall Group A. In Group B of the posterior wall, the endo/epi ratios were significantly lower than control in the three interventions following CAO ($p<0.0001$) (See Table 5-3).

Thus, alpha-1 blockade with prazosin increased blood flow to the non-ischemic areas of the left ventricle, during left stellate stimulation. However, blood flow to the ischemic myocardium was not altered significantly by prazosin administration, even when given prior to coronary artery occlusion.

The third group of animals (n=7) did not show any significant changes in either blood flow or resistance in the posterior and anterior wall following administration of prazosin. Blood pressure did not change significantly following adminstration of prazosin. The absence of a drop in blood pressure following administration of prazosin in this group was most likely due to lower levels of background sympathetic activity in these dogs as opposed to the other groups. This group of dogs demonstrates that alpha-1 blockade without prior beta-blockade and in the absence of elevated sympathetic input does not increase flow or reduce resistance significantly from values prior to administration of prazosin.

3. Blood Pressure

Blood pressures during microsphere injection for Groups 1 and 2 are listed in Table 5-4. In Group 1 administration of prazosin dropped blood pressure significantly from Control, CAO, and CAO-LSS ($p<0.05$). In Group 2 prazosin decreased blood pressure significantly from control and from

TABLE 5-4

BLOOD PRESSURES (MM HG) FOR PERMANENT (GROUP1) AND REPETITIVE (GROUP 2) CORONARY ARTERY OCCLUSIONS

GROUP 1			
CONTROL	CAO	CAO-LSS	CAO-LSS-PRAZ
108.32 ± 3.63	111.54 ± 4.70	107.42 ± 6.86	88.60 ± 5.43[123]

GROUP 2			
CONTROL	CAO	CAO-LSS	CAO-LSS-PRAZ
108.43 ± 5.52	101.50 ± 5.07	106.14 ± 5.51	87.17 ± 5.13[13]

(Data are listed as mean ± SEM. Group 1 (n=5), Group 2 (n=7). [1]$p<0.05$ compared to control, [2]$p<0.05$ compared to CAO, [3]$p<0.05$ compared to CAO-LSS. See Table 5-1 for Abbrev.)

blood pressure during CAO-LSS ($p<0.05$).

The results from this study suggest that, while alpha-1 receptors exert a modulating influence on regional myocardial blood flow in normal areas, their importance can only be demonstrated in the presence of beta-blockade and elevated levels of sympathetic nerve stimulation. In addition, there does not appear to a significant influence of alpha-1 receptors on blood flow in the ischemic zone following complete coronary artery occlusion.

D. DISCUSSION

Previous investigations demonstrated that alpha receptors restrict increases in blood flow following coronary stenosis (7,8). Other investigations have demonstrated that under conditions in which beta-mediated metabolic vasodilation might be expected to overwhelm alpha receptor-mediated vasoconstriction such as severe exercise, there is still limitation of blood flow via alpha receptors (23,33).

Our goal was to examine whether sympathetic stimulation and alpha-1 blockade with prazosin would redistribute blood flow to the posterior wall following circumflex artery occlusion. Chiariello et al. reported that alpha mediated vasoconstriction in the normal zone effected a reverse 'coronary steal' into the occluded bed (11). This finding was supported by Giudicelli et al., who found that stimulation of the left stellate in the presence of beta-blockade decreased blood flow to the non-ischemic region concomitant with an increase in flow to the ischemic region (19). Contrary to those reports, the major finding of this study was that prazosin did not increase blood flow to the posterior left ventricle following circumflex coronary artery occlusion. Although prazosin induced vasodilation in the normal zone in Group 2 (in which it was given prior to occlusion), evidently this was not sufficient to precipitate 'steal' of blood from the ischemic area.

GROUP 1:

In this group (Praz:Post-CAO), there was no effect of alpha blockade on blood flow to either the anterior or posterior wall. The lack of an increase in blood flow to the posterior wall of Group 1 may be

ascribed to the post-occlusion, systemic administration of prazosin, with a resultant non-uniform distribution to the collateral dependent posterior wall. It is also possible that alpha-1 receptors do not have an important influence on blood flow following a complete coronary artery occlusion. Previous studies utilized combined alpha-1 and alpha-2 blockade to demonstrate alpha mediated vasoconstriction in their preparations. Although the role of alpha-2 receptors was not addressed in the present study, recent investigations have suggested that alpha-2 receptors, both pre- and post-junctional may influence coronary blood flow (22,24).

Insofar as flow could be delivered to the posterior wall only via collateral vessels, it is understandable that very little change in flow would be elicited in the ischemic bed as a result of anterior wall blood flow changes. Schaper reported that not only does it take several hours for nascent collateral vessels to open, the resistance of these collaterals is extremely high. Even when functional capacity has been reached after four weeks of occlusion, the resistance in collateral vessels is still 12-40 times higher than coronary vessels (40). In addition, the amount of left ventricle at risk of infarction following occlusion of the circumflex is larger (41) than the amount in previous studies (11,19) utilizing the LAD region. Schaper calculated that the relative number of collateral vessels supplying an ischemic region decreases with an increase in volume of the myocardium perfused (40). Thus, while other studies demonstrate reverse steal of blood into the ischemic region, it is unlikely that such a phenomena would exert a noticeable effect on

delivery of blood flow to, and salvage of the ischemic region in this study.

It should not be overlooked that the posterior wall might have been unable to respond to nerve stimulation and adrenergic blockade, or that the nerves themselves might have been impaired following isolation of the vessel, and occlusion of the circumflex artery (15). Several laboratories have described 'stunning' of the myocardium following coronary artery occlusion. Ciuffo et al. implanted ultrasonic dimension gauges in the midwall of the LAD region to assess myocardial function following periods of ischemia of up to two hours. They demonstrated that following a twenty-five minute period of occlusion and reperfusion, segmental shortening increased significantly in the post-ischemic zone in response to systemic injection of norepinephrine. However, the post-ischemic response to stimulation of the cardiac nerves was impaired, and the authors concluded that nerve conduction had been disrupted during the period of ischemia (13). One problem with Ciuffo's study was that the placement of the crystals in the midwall would not allow separation of function of the potentially ischemic endocardium from the epicardium. Another problem is that the LAD region does not respond as markedly to stimulation of the left cardiac nerves as does the circumflex region. Finally, regional blood flow assessed by microspheres did not show any difference in the response to nerve stimulation or systemic norepinephrine. A study by Barber et al. provided evidence for loss of catecholamines and nerve activity following the injection of latex into a branch of the LAD (3). In the study by Ciuffo et al., the period of occlusion was 25 minutes, and the study by Barber et al., the infarction was

transmural. Neither of these conditions is applicable for the present study since the period of occlusion was not long enough to cause transmural infarction, and in any case sympathetic fibers are thought to travel in the epicardium (18), which did not become severely ischemic in this study.

The endo/epi ratios did not show any significant changes except in Group 1. During combined LSS and CAO, the endo/epi ratio dropped significantly from control in the posterior wall, due probably to a small amount of vasoconstriction in the endocardium superimposed on the existing level of decreased flow. What is interesting is that prazosin did not alter the endo/epi ratio in favor of increased flow to the epicardium. As Schaper remarked, increasing collateral flow to an ischemic region will result in decreased endo/epi ratios, with more flow going to the epicardium (40). This allows salvage of the epicardium at the expense of the endocardium. Reduction of tissue damage depends on limiting the wavefront of necrosis (37), and salvaging the epicardium at the expense of leaving a core of endocardium damaged.

GROUP 2:

In Group 2 (Praz:pre-CAO), prazosin did not increase blood flow to the occluded region, even when given before coronary artery occlusion. However, prazosin caused an increase in flow and a decrease in resistance to the anterior wall compared to control. If prazosin had acted merely by removing vasoconstriction imposed by LSS and beta-blockade, then it is interesting that it actually increased blood flow significantly from control levels. It is conceivable that the increase in flow mediated by

prazosin was not via its adrenergic blocking actions, but via a non-specific effect on the coronary vascular smooth muscle. Although an initial report by Constantine supported the idea that prazosin acted directly on smooth muscle to cause vasodilation (14), subsequent studies have shown that prazosin's actions are alpha-receptor mediated (9,20,43). The most probable explanation is that prazosin blocked coronary vascular alpha-1 receptors and that the flow increase was due to removal of alpha-vasoconstriction.

It is also possible in Group 2 (Praz:Pre-CAO) that prazosin's actions were due primarily to a release of vasoconstrictor tone imposed on anterior wall blood vessels by circulating catecholamines. The contribution of circulating catecholamines to increases in blood flow during mild exercise were examined in a recent investigation by Chilian et al., (10). Using conscious dogs, with regional denervation of the posterior wall, they found that alpha receptors limit augmentation of blood flow during exercise in the dog. This vasoconstriction appeared to be mediated primarily by circulating catecholamines, not by neurally released catecholamines. Their findings may explain why, contrary to our expectations, stimulation of the left stellate did not decrease flow significantly from control in either Group 1 or Group 2. Moreover, the left-side cardiac nerves do not innervate the anterior wall as uniformly as the posterior wall (36). Rinkema et al. demonstrated that in the presence of beta-blockade with propranolol, left stellate stimulation produced a 28% reduction in regional myocardial blood flow to the posterior LV, and only a 16% reduction in flow to the anterior wall (38). These results agree with an earlier study by Ross and Mulder. During LSS

in the study by Ross and Mulder, the resistance in the circumflex artery during beta blockade increased by 75% compared to a 19% increase in the LAD artery (39).

Significant differences in resistance between Control, LSS, Prazosin and CAO-LSS, in the anterior wall were evident in Group 2 (Praz;Pre-CAO) although flow had not changed significantly, due to a significant drop in blood pressure following the administration of prazosin. This result concurs with findings by Macho and Vatner in which prazosin did not increase flow significantly but may have evinced more of an effect hemodynamically (30).

The resting level of resistance in the heart may account for differences in the endocardial and epicardial response to prazosin. Although the resistance was decreased in comparison to control and LSS, the endocardial resistance was not significantly lower than that during coronary artery occlusion. The obvious explanation is that the endocardium was slightly more dilated than the epicardium, hence the response to additional vasodilation with prazosin was not as great. In the posterior wall, there were no differences between interventions. This may be due to the fact that blood flows were reduced so much following CAO, that slight differences were not demonstrable between the subsequent interventions.

SUMMARY OF GROUPS 1 AND 2

The differences in results between Group 1 (Praz;Post-CAO) and Group 2 (Praz;Pre-CAO) regarding the anterior wall can be ascribed to differences in the experimental preparations. In Group 1, the period of

occlusion lasted for up to two hours, with no reperfusion. In addition, cardiac nerve stimulation was maintained for approximately 15 minute intervals, as opposed to five minutes in Group 2. The role of cardio-cardiac reflexes in the response of the anterior wall to posterior wall occlusion should not be ignored, as reflex arcs through the middle cervical ganglion have been demonstrated in decentralized preparations (1).

GROUP 3

In the third group of dogs, alpha-1 blockade did not exhibit a noticeable effect on blood flow. Without prior beta blockade, alpha receptors were not unmasked and hence the effects of beta receptors were predominant (5). It was also shown that elevated levels of sympathetics were needed to stimulate the alpha receptors. It is possible that the existing levels of circulating catecholamines were not adequate to result in alpha-mediated vasoconstriction without prior beta blockade.

In conclusion, contrary to what has been reported in the literature, we did not see an increase in blood flow to the ischemic region following alpha blockade with prazosin regardless of whether it was given before of after coronary occlusion. However, one disadvantage of using microspheres is that when blood flow is low, the resolution of the technique is limited. Flows of at least 20 ml/min/100 gm are necessary to deposit the required number of microspheres for reliable, reproduceable flow measurements. Thus, small changes in flow might have gone undetected because of the greater degree of variability and error in

measuring flow at such low levels. In addition, in comparison to control values of blood flow, the low flows would very large variations to have differences detected by analysis of variance and Tukey test statistical analysis. It is probable that alpha receptor blockade did not increase infarct size in the previous study by removing alpha mediated vasoconstriction in the ischemic zone. Although blockade of alpha receptors may alter the course of myocardial infarction, it is probably due to a mechanism that is not related to changes in myocardial blood flow. The possible involvement of alpha receptors in cardiac metabolism and function should be clarified in order to understand more clearly their involvement in the process of infarction.

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CHAPTER VI

EFFECTS OF REGIONAL DENERVATION ON MYOCARDIAL BLOOD FLOW DURING CORONARY ARTERY OCCLUSION

A. INTRODUCTION

Although cardiac denervation had been utilized as an experimental tool to investigate the innervation of the heart (30), the suggestion that cardiac denervation could be used clinically to alleviate angina was initially suggested by Leriche (19). Subsequent investigations extended the number of clinical settings in which denervation was proposed to be beneficial to include protection against lethal arrhythmias (8,25) and myocardial infarction (17). The mechanism of these protective effects is not well-established. It was thought by some investigators that the protection may be due to alteration of regional myocardial blood flow during ischemia. DuPont et al. showed better perfusion of the border zones during coronary artery occlusion in animals with sympathectomized hearts compared to non-cardiac sympathectomized animals (7). However, Barber et al. found that blood flow to the ischemic zone did not change significantly following cardiac denervation (1). Both Barber et al., and DuPont et al. suggested that the beneficial effects may be due to a lowering of oxygen consumption (1,7).

Previous investigations have demonstrated a role for alpha receptors in the modulation of coronary blood flow under resting conditions and during exercise. Alpha receptors have also been implicated in the

control of blood flow under conditions of ischemia severe enough to cause net lactate production (3). Holtz et al. demonstrated that the tonic level of sympathetic vasoconstriction mediated by alpha receptors was removed by regional denervation with 6-hydroxy dopamine (16).

Our hypothesis was that removal of alpha receptor mediated vasoconstriction in the ischemic zone, would enhance delivery of blood flow from the normal zone, via a 'reverse coronary steal'. Different techniques for denervation have been used, ganglionectomy (5), autotransplantation (29), chemical sympathectomy with 6-hydroxy dopamine (16), and intrapericardial denervation (23). Most of these studies utilized total cardiac denervation. The intrapericardial denervation technique, autotransplantation and mediastinal neural ablation interrupt parasympathetic as well as sympathetic input. The disadvantages of these techniques are the inability to localize denervation and use the normally innervated region as control. Recently the use of topical phenol has been used to facilitate the selective sympathetic denervation of the heart (2). This technique was originally utilized for mapping of sympathetic distribution of the left ventricle (12,22). We combined the technique of isolation and severing of the ventrolateral cardiac nerve, which innervates the posterior left ventricle with application of topical phenol to denervate the posterior wall.

The second aim of this study was to determine whether sectioning of the VLCN as well as application of phenol in a ring circumscribing the posterior wall would eliminate sympathetic fibers which exert vasomotor control.

B. METHODS

1. General Preparation

Mongrel dogs of either sex were premedicated with morphine sulfate (2.5 mg/kg, s.c.) or xylazine (2 mg/kg, s.c.) approximately 15-20 minutes before being given alpha-chloralose (50-100 mg/kg, i.v.). A polyethylene catheter (PE-260 tubing) was placed in the right femoral artery and connected to a Statham P23dB pressure transducer for continuous monitoring of arterial blood pressure. The right femoral vein was catheterized with PE-260 tubing for fluid and drug administration. The trachea was cannulated with a glass Y-tube which was connected to a Harvard respirator. Each dog was ventilated with room air at a positive end-expiratory pressure of 5 cm of water. Both cervical vagi were isolated and severed. Succinylcholine (20 mg, i.v.) was administered as a muscle relaxant. The chest was opened in the 4th left intercostal space. Both the right and left ansae subclavia of the stellate ganglia were isolated and stimulated (7-10 Hz, 5 msec, 5-7 V) using bipolar stainless steel electrodes. Square wave pulses were delivered by a Frederick Haer stimulator and monitored on a calibrated oscilloscope. During stimulation, changes in the heart rate, and Lead II electrocardiogram, specifically the P-R interval and T-wave configuration confirmed that both the anterior and posterior limbs of the right and left ansae subclavia had been dissected out (11,31). Cutting of the right and left ansae subclavia, along with section of the cervical vagus, completed neural decentralization of the heart to prevent any reflexes from arising following coronary artery occlusion, and to insure against afferent stimulation of the cardiac nerves. The pericardium was incised and sutured to the walls

of the chest to form a pericardial cradle. No more than .5 cm of the circumflex artery (CX) was isolated distal to the first marginal branch. Blood gases and pH were monitored throughout the experiment and maintained within normal limits (pH 7.38-7.45, pO2 80-120 mm Hg, pCO2 30-40 mm Hg, HCO3 23-28 mm Hg) by adjusting the rate and/or volume of the Harvard respirator, with infusions of sodium bicarbonate (20 mEq/ml), when necessary, to maintain pH normal. Core temperature, measured in the thoracic cavity by a YSI probe, was kept between 37-39° C by means of a heating pad beneath the dog, and an infrared heating lamp above the dog when necessary. Succinylcholine (20 mg, i.v.) and additional anesthetic were administered as needed. The heart was kept moist with sponge soaked in saline, and plastic wrap was kept over the open chest to retain moisture and heat.

Blockade by timolol (0.2 mg/kg, i.v.) was considered to be adequate when stimulation of the left ansae subclavia produced no changes in heart rate or the Lead II electrocardiogram. Maintenance doses of timolol (0.08 mg/kg/hr, i.v.) were given to insure blockade of beta-receptors throughout the experiment. Lidocaine was given if there was an increased incidence of arrhythmias.

2. Regional Blood Flow Determination

Four of the following species of radioactive labeled microspheres (15 m) were used in random order: Ce-141, Cr-51, Sr-85 or Ru-103, Sc-46, Nb-95. The spheres were suspended in a 5% dextran solution with 0.05% Tween 80. The vials containing the spheres were sonicated for at least 20 minutes prior to microsphere injection, and agitated in a vortex mixer

immediately prior to use. For 30 seconds before microsphere injection, a reference blood sample was withdrawn from the femoral artery at a constant rate, and reference blood withdrawal continued for at least 90 seconds following microsphere injection. The withdrawal rate of the pump was calibrated at the end of the experiment to determine the exact rate of withdrawal. Microspheres were injected into the left atrium via a catheter over a period of 30-40 seconds and were flushed with 10 ml of warm saline. At the end of the experiment, the hearts were excised and the circumflex artery injected distal to the site of occlusion with Microfil to delineate the perfusion area of the circumflex artery. The hearts were fixed in formalin for 24-48 hours, after which samples of the myocardium were cut from both the stained and unstained regions, weighed, and placed in labeled plastic tubes. The reference blood withdrawal samples were also put into labeled plastic tubes, the syringes containing the blood samples were rinsed with saline, and the rinse put into the respective labeled counting tubes. Heart and reference organ samples were counted by a TM Analytic gamma counter (model 1185) and data were processed by an APPLE computer. Flows were calculated using standard equations with corrections for interference coefficients and background counts. The equation used to calculate blood flows was: MBF= (Ct/TW) x (RBW/Cb) x 100, where MBF = myocardial blood flow in ml/min per 100 gm, Ct = tissue radioactivity in counts/min, TW = tissue sample weight in grams, RBW = reference blood withdrawal rate in ml/min, and Cb = total radioactivity in reference blood sample.

3. Regional Denervation

Denervation of the posterior wall was accomplished by cutting the ventrolateral cardiac nerve where it courses over the pulmonary veins, and by stripping the adventitia of the pulmonary veins, cutting any small nerves located in the vicinity of the ventrolateral cardiac nerve, using a technique developed in this laboratory. Regional denervation was further insured by painting a thin ring of phenol around the perimeter of the posterior wall distal to the site of circumflex isolation and occlusion. Use of the blunt end of a cotton swab ensured that phenol did not spread into the anterior region. Phenol was also applied to the interventricular sulcus, along the fat of the atrioventricular groove, and in the region of the original dissection of the ventrolateral cardiac nerve. Phenol has been shown to penetrate approximately .25 mm into the epicardium thus destroying sympathetic fibers which travel superficially before penetrating the myocardium to innervate deeper layers (2,22).

4. Experimental Protocol

a. Group 1 (Ctrl-Lss-PhnLss-CaoPhnLss):

The purpose of this group was to demonstrate that alpha-vasoconstriction in the posterior wall following beta blockade would be eliminated after regional denervation. Figure 6-1 illustrates the protocols used in this study. In Group 1 (n=7), the first flow measurement was made following beta-blockade with timolol, and served as control. Stimulation of the left ansae subclavia was begun (LSS:8 Hz, 5 msec, 5-7 V), and the second injection of microspheres was made during the period of stimulation. Following completion of the microsphere injection,

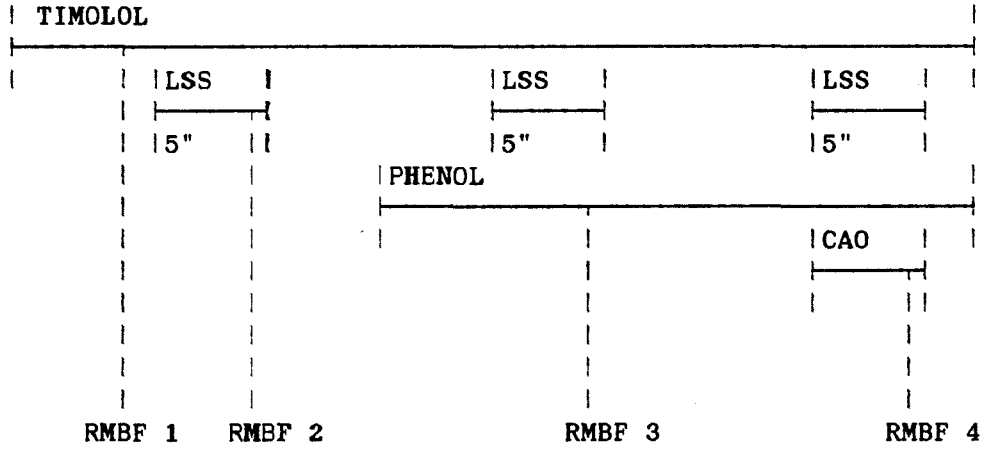
FIGURE 6-1

PROTOCOLS USED IN GROUPS 1 AND 2

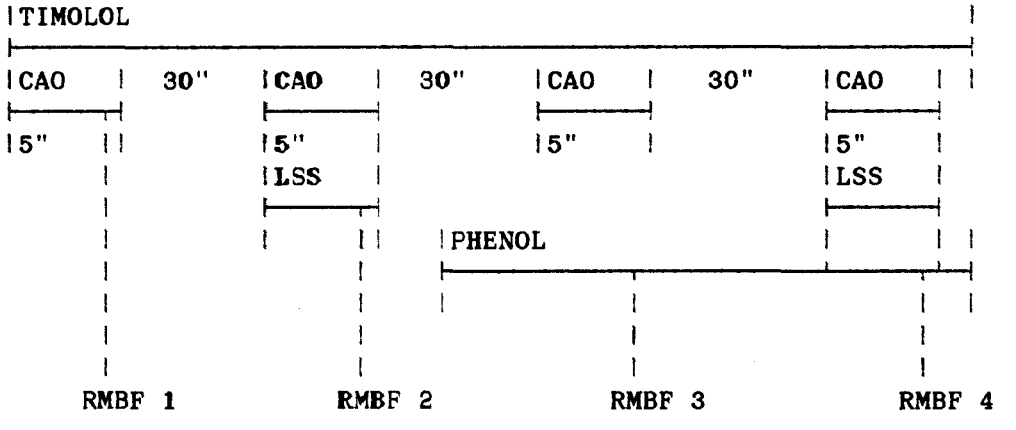
The abbreviations denote the following: Timolol: timolol was administered before beginning the sequence of microsphere injections; RMBF: regional myocardial blood flow determination; LSS: left ansae subclavia stimulation. Phenol: posterior wall denervation with topical phenol; CAO: circumflex artery occlusion.

FIGURE 6-1

GROUP 1 (N=7)



GROUP 2 (N=7)



stimulation was terminated, and the posterior wall was denervated. The third flow measurement was then made during a second period of left ansae subclavia stimulation (LSS-Phen). After the third injection period was completed, the stimulation was stopped, and the circumflex artery was ligated at the point of isolation, distal to the first marginal branch. After five minutes of coronary artery occlusion left stellate stimulation was again initiated, and the fourth flow determination was made (CAO-LSS-Phen).

b. Group 2 (CAO/CAO-LSS/CAO-Phn/CAO-LSS-Phn)

In Group 2 (n=8), it was our aim to demonstrate that alpha-mediated vasoconstriction in the ischemic posterior wall would be eliminated following application of phenol. Four blood flow determinations were made in each dog. All animals received timolol (0.2 mg/kg) prior to starting the regional blood flow determinations. Regional denervation was done via the methods described above. The model for coronary artery occlusion was a 7-8 minute occlusion with injection of the microspheres after five minutes of CAO, followed by release of the occlusion and reperfusion for thirty minutes. The circumflex artery was occluded distal to the first marginal branch using an atraumatic clamp. Microspheres were injected after five minutes of occlusion, and following completion of the injection, the occlusion was released, so that total occlusion time was between seven and eight minutes.

The four experimental conditions were: 1) a control blood flow determination in which the circumflex artery was occluded for five minutes (CAO), 2) during coronary artery occlusion and simultaneous

stimulation of the left ansae subclavia (CAO-LSS) (LSS was begun immediately after the clamp was placed on the circumflex, and both the occlusion and the stimulation were stopped at the end of the injection period), 3) During coronary artery occlusion following regional denervation with topical application of phenol (CAO-Phen), and 4) During coronary artery occlusion with stimulation of the left ansae subclavia following the topical application of phenol (CAO-LSS-Phen).

5. Data Analysis

Flows were expressed in ml/min/100 gm. Resistance was calculated as the quotient of mean arterial pressure and regional blood flow (MAP/MBF). In Group 1 the percent change in flow from control was calculated for each of the experimental protocols for both the endocardium and the epicardium. In Group 2, blood flow during the first coronary artery occlusion served as the control blood flow determination. Percent changes in flow for the subsequent blood flow measurements in Group 2 were expressed as change from the first flow determination. Blood flows, resistance, percent change in blood flow, endo/epi ratios and blood pressures during each of the experimental conditions were analyzed separately. Analysis of variance for repeated measures was used to test for differences between interventions, and Tukey's test was employed to isolate the differences within groups. Statistical significance was reached at a p value of <0.05.

C. RESULTS

1. Group 1

a. Hemodynamic Data:

In the presence of beta blockade, blood pressure in Group 1 was significantly elevated during LSS (160.3 ± 14.4) compared to control (132.6 ± 9.06), LSS following phenol (LSS-Phen: 123.3 ± 10.2) and CAO-LSS following phenol (CAO-LSS-Phen: 115.4 ± 8.73) ($p<.001$). Heart rates during each of the blood flow measurements were not significantly different.

b. Blood Flow Data:

Table 6-1 lists the blood flow values for Group 1 (Ctrl/LSS/Phn-LSS/CAO-Phn-LSS). Blood flow during LSS was significantly elevated from the other treatment groups in the anterior endocardium ($p<.0001$) and anterior epicardium ($p<.001$). In the posterior endocardium, blood flow during LSS was significantly higher than Control, LSS-Phen, and CAO-LSS-Phen ($p<.0001$). Blood flow during LSS was significantly different from control and CAO-LSS in the posterior epicardium ($p<.0001$). Flow was not significantly different during LSS and during LSS following phenol application in the posterior epicardium. Blood flow fell significantly from Control and LSS following CAO in the posterior endocardium and epicardium ($p<.0001$).

Percent changes in blood flow in Group 1(Ctrl/LSS/Phn-LSS/CAO-Phn-LSS) are shown in Figure 6-2. In the anterior wall, the changes in flow during LSS-Phen and CAO-LSS-Phen were significantly different from the change in flow during LSS in the endocardium (top left panel $p<.0001$), and in the epicardium (bottom left panel $p<.002$). In the posterior

TABLE 6-1

Regional blood flow values for Group 1 (n=7) in (ml/min/100 gm). Data are listed as mean flow ± SEM. Flows are divided into anterior and posterior wall blood flows, and for each region endocardial and epicardial blood flows are listed. Blood flow values for each of the interventions are listed. Control: blood flow during control conditions, with beta blockade; LSS: flow during left ansae subclavia stimulation; Phenol-LSS: flow during LSS following posterior wall denervation with topical phenol; CAO-Phenol-LSS: flow during LSS and circumflex artery occlusion, following posterior wall denervation. * indicates a significant difference from control, ☆indicates significant difference from LSS, *indicates significant difference from LSS-Phen and †indicates significant difference from CAO-LSS-Phen ($p<.0001$).

TABLE 6-1

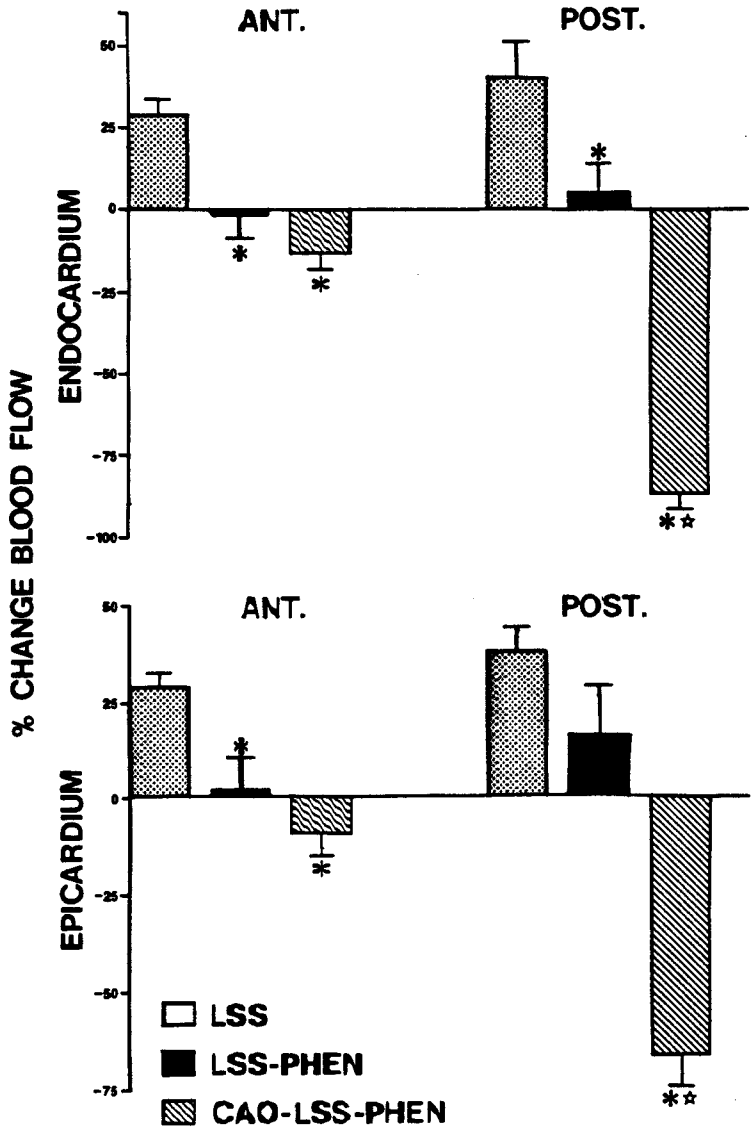
REGIONAL MYOCARDIAL BLOOD FLOWS DURING PERMANENT CORONARY OCCLUSION BEFORE AND AFTER REGIONAL DENERVATION (GROUP 1) (ML/MIN 100 GM)

ANTERIOR WALL			
ENDOCARDIUM			
CONTROL	LSS	PHENOL-LSS	CAO-PHENOL-LSS
92.41 ± 7.33	118.18 ± 9.87**†	89.96 ± 8.81	79.43 ± 8.47
EPICARDIUM			
71.25 ± 4.88	92.09 ± 8.78**†	71.33 ± 6.37	63.58 ± 5.31
POSTERIOR WALL			
ENDOCARDIUM			
CONTROL	LSS	PHENOL-LSS	CAO-PHENOL-LSS
84.84 ± 6.38	118.35 ± 12.80**†	88.29 ± 8.70	14.88 ± 6.78☆
EPICARDIUM			
62.75 ± 4.49	86.01 ± 7.40*†	69.57 ± 5.43	20.45 ± 6.34☆

(sig. diff. from control, sig.diff. from LSS, sig.diff. from phenol-LSS, $p<0.0001$)

FIGURE 6-2
PERCENT CHANGE IN BLOOD FLOW FROM CONTROL DURING
PERMANENT OCCLUSION BEFORE AND AFTER REGIONAL DENERVATION

Percent change in blood flow from control for group 1 (n=7). Data are shown as mean % ± SEM. The top panel illustrates endocardial flows for the anterior and posterior LV. The bottom of the graph are the epicardial flows for the anterior and posterior LV. *indicates significant difference from LSS ($p<.0001$), ☆indicates significant difference from LSS-Phen ($p<.0001$). See Table 6-1 for a description of the group labels.


ANT.
POST.
ENDOCARDIUM
EPICARDIUM
% CHANGE BLOOD FLOW
LSS
LSS-PHEN
CAO-LSS-PHEN

endocardium (top right panel), the percent change in flow during LSS-Phen was significantly different from LSS ($p<.0001$). In addition, the percent change in flow during CAO-LSS-Phen was significantly different from LSS and LSS-Phen ($p<.0001$). In the posterior epicardium, the percent change in flow during CAO-LSS-Phen was significantly lower than LSS and LSS-Phen ($p<.0001$). There were no differences between LSS, and LSS-Phen in the posterior epicardium.

There were no significant differences in the endo/epi ratios of the anterior wall in Group 1 (Ctrl/LSS/Phn-LSS/CAO-Phn-LSS). The endo/epi ratio dropped significantly to $0.64 \pm .10$ in the posterior wall during combined CAO-LSS following phenol application compared to values of $1.39 \pm .06$, $1.42 \pm .14$ and $1.30 \pm .08$ during control, LSS and LSS following phenol, respectively ($p<.001$). There were no other differences between groups. There were no significant differences between groups in either the anterior or posterior wall resistances.

In summary, in Group 1 (Ctrl/LSS/Phn-LSS/CAO-Phn-LSS), LSS in the presence of timolol increased blood pressure and blood flow to both the anterior and posterior wall even in the presence of beta blockade. These increases in blood pressure and blood flow to both the anterior and posterior left ventricle were eliminated by the regional denervation of the posterior wall.

2. Group 2

a. Hemodynamic Data:

Blood pressure during CAO following phenol application (112.50 ± 6.27) and during combined CAO-LSS following phenol application ($112.76 \pm$

6.82) decreased significantly from CAO (132.88 ± 5.36) and CAO-LSS (129.00 ± 6.83) ($p<.0002$). There were no statistical differences between blood pressure during CAO and combined CAO-LSS or between blood pressure during either intervention following phenol application.

b. Blood Flow Data:

Blood flow values for Group 2 (CAO/CAO-LSS/CAO-Phn/CAO-LSS-Phn) are listed in Table 6-2. Blood flow during CAO-Phen was significantly decreased from CAO and CAO-LSS ($p<.02$) in the anterior endocardium. There were no other significant differences between groups in the anterior epicardium or in the posterior endocardium and epicardium.

Figure 6-3 depicts the data for Group 2 (CAO/CAO-LSS/CAO-Phn/CAO-LSS-Phn). The data are represented as percent change from initial blood flow values during circumflex artery occlusion. In the anterior endocardium (top left panel), the change in blood flow during CAO-Phen was significantly different from the change in blood flow during CAO-LSS and CAO-LSS-Phen ($p<.002$). CAO-LSS was also statistically different from the change in flow during combined CAO-LSS-Phen ($p<.002$). There were no significant differences between groups in the anterior epicardium (bottom left panel) or posterior endocardium (top right panel). In the posterior epicardium (lower right panel), the percent change in blood flow during CAO-LSS-Phen was significantly different from the percent change in blood flow during CAO-Phen ($p<.002$).

There were no significant differences in the endo/epi ratios for either the anterior or the posterior wall. There were no significant differences in calculated resistance for either the anterior or posterior

TABLE 6-2

Regional blood flow values (in ml/min/100 gm left ventricle) for group 2 (n=7). Data are represented as mean ± SEM. CAO: blood flow during circumflex artery occlusion; CAO-LSS: flow during CAO and left ansae subclavia stimulation; CAO-Phenol: flow during CAO following posterior wall denervation with topical phenol; CAO-LSS-Phenol: flow during CAO and LSS following regional denervation with phenol. * indicates significant difference from blood flow during coronary artery occlusion (CAO), † indicates significant difference from blood flow during CAO-LSS ($p<.0.02$).

TABLE 6-2

REGIONAL MYOCARDIAL BLOOD FLOWS DURING REPETITIVE CORONARY ARTERY OCCLUSION BEFORE AND AFTER REGIONAL DENERVATION (GROUP 2)

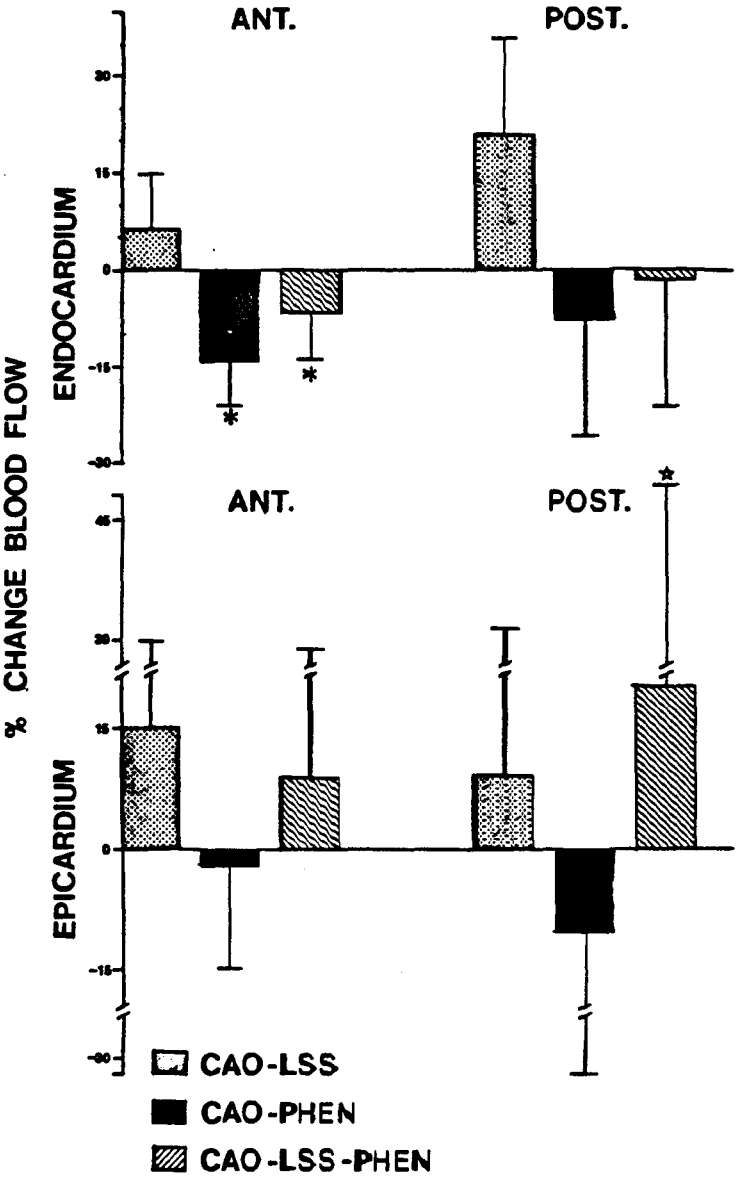
ANTERIOR WALL			
ENDOCARDIUM			
CAO	CAO-LSS	CAO-PHENOL	CAO-LSS-PHENOL
97.84 ± 10.48	100.38 ± 9.81	81.29 ± 7.30*†	91.72 ± 8.87
EPICARDIUM			
85.92 ± 10.14	91.48 ± 8.69	77.38 ± 6.54	87.17 ± 9.11
POSTERIOR WALL			
ENDOCARDIUM			
CAO	CAO-LSS	CAO-PHENOL	CAO-LSS-PHENOL
8.97 ± 2.87	9.28 ± 1.86	6.89 ± 1.55	7.63 ± 1.82
EPICARDIUM			
17.55 ± 4.54	17.45 ± 4.67	13.46 ± 2.68	16.20 ± 3.41

(*sig. diff. from control, †sig. diff. from CAO-LSS, $p<0.02$)

FIGURE 6-3

PERCENT CHANGE IN BLOOD FLOW FROM CONTROL CAO DURING REPETITIVE CORONARY OCCLUSION BEFORE AND AFTER DENERVATION
GROUP 2 (CAO/CAO-LSS/CAO-PHEN/CAO-LSS-PHEN)

Percent change in blood flow from flow measured during coronary artery occlusion alone. Data represent mean ± SEM. (See Table 6-2 for explanation of group names). *indicates significant difference from LSS, ☆indicates significant difference from CAO-Phen ($p<.002$).


ANT.
POST.
ENDOCARDIUM
ANT.
POST.
EPICARDIUM
% CHANGE BLOOD FLOW
CAO-LSS
CAO-PHEN
CAO-LSS-PHEN

endocardium or epicardium. The results from this second group indicate that regional denervation of the posterior wall does not increase blood flow to the ischemic area.

D. DISCUSSION

GROUP 1 (Ctrl/LSS/Phn-LSS/CAO-Phn-LSS)

One of the aims of this study was to determine whether denervation of the posterior left ventricle would increase blood flow to that region by removing alpha vasoconstriction. An interesting aspect of this study was that blood flow to the anterior and posterior wall and systemic blood pressure in Group 1 were significantly elevated from control during LSS even after beta-adrenergic blockade with timolol. This contradicts the results of previous investigations in which sympathetic input during beta blockade resulted in a decrease in blood flow (24). Feigl observed that during LSS following beta-blockade with propranolol, blood flow decreased by 18% from control. This decrease in blood flow was blocked by administration of phenoxybenzamine (9). In a later study, Feigl demonstrated that during a 90 second stimulation of the left stellate ganglion, blood pressure and blood flow increased but coronary sinus oxygen tension decreased from 19 to 15 mm Hg. Following beta-receptor blockade with propranolol, these changes were blunted and coronary sinus oxygen tension fell from 17 to 11 mm Hg. The administration of dibozane blocked the sympathetically mediated increase in coronary vascular resistance and decrease in coronary sinus oxygen tension (10). Other studies have demonstrated a similar response of coronary vascular resistance during nervous stimulation following beta blockade (13). In addition, coronary artery vasoconstriction has been demonstrated during stimulation of alpha receptors with methoxamine (27).

It should be emphasized that the duration of stimulation and the time course of blood flow measurements in the present study differs from

the studies cited above. In Feigl's studies, stimulation of the left stellate ganglion was of brief duration (90 seconds) (10), while in the present study the left ansae subclavia were stimulated for five minutes. Rinkema et al. injected microspheres immediately after beginning stellate stimulation (24). In addition, the stimulation of cardiac nerves is different from the systemic injection of exogenous adrenergic agonists. An investigation by Williams and Most reported that during brief stimulation of alpha receptors via a bolus dose of phenylephrine, vasoconstriction was elicited. However, with prolonged stimulation of alpha receptors, the constriction gradually disappeared, and flow returned to baseline levels (28). It should be noted that the reduction in vessel diameter reported by Vatner was maintained over a sustained injection of methoxamine. In that preparation, the heart was not decentralized, nor had ganglionic blockade been administered (27). Hence the possibility exists that the vasoconstriction in Vatner's study was in part the result of withdrawal of sympathetic tone in the face of sustained elevated peripheral pressure.

Thus, alpha-mediated vasoconstriction may be of a transient nature, whereas with longer periods of sympathetic stimulation, stimulation of alpha receptors no longer evokes vasoconstriction. There is evidence to suggest that alpha receptors on the myocardium can contribute to metabolism (21). Therefore the role of alpha-receptors on myocardial metabolism and the indirect effects on blood flow need to be established. The mechanism whereby blood pressure increased in Group 1 (Ctrl/LSS/Phn-LSS/CAO-Phn-LSS) even though myocardial beta-receptors were blocked in unclear. It is possible in the present study that beta-blockade was

incomplete. However, changes in HR and the Lead II EKG were absent during supramaximal left stellate ansae subclavia stimulation. In addition, we found that increasing the dose of timolol did not eliminate the rise in arterial pressure during stimulation. Thus, the increase ion blood pressure was not mediated by beta receptors. Alpha-1 receptors have been localized to the myocardium (6,14), and Starke demonstrated that phenylephrine which is fairly selective for alpha-1 receptors, caused an increase in blood pressure which was not due to increased norepinephrine release (26). It is therefore possible that stimulation of alpha receptors led to the increase in blood pressure.

We found in several animals, that the rise in arterial pressure was eliminated only when the application of phenol was extended from the point of the coronary sinus down the interventricular groove. Studies by Randall et al. which utilized the phenol denervation technique noted that nearly all of the inotropic responses of the anterior wall were eliminated when a line of phenol was extended along the A-V groove to the coronary sinus. However, they found that the rise in arterial pressure elicited by LSS remained present even when phenol had been extended along the base of the heart (22). In the present study, it seems as though a few nerve fibers reach the anterior left ventricle via the interventricular sulcus. The fact that flow was increased significantly in the anterior wall during LSS and dropped following application of phenol to the posterior wall suggests several possibilities. First, the presence of significant innervation of the anterior wall by the ventrolateral cardiac nerve or other nerves. Geis and Kaye found that fibers from the left stellate ganglion reach the anterior left ventricle after entering

the epicardial plexus along the A-V groove near the marginal artery. The origin of these fibers was believed to be in the ventrolateral cardiac nerve (12). Application of phenol decreased flow in Group 1 and dropped the flow in Group 2 from control and LSS. Thus phenol either interrupted the fibers to the anterior and posterior wall, or it dropped function in the posterior wall enough to affect the overall function of the heart and drop blood pressure. The second, more likely possibility is that since resistance did not change significantly it appears that the changes in flow were passive, and due merely to changes in blood pressure. Finally, it is also possible that LSS in some way can affect the capacitance of the proximal aorta and alter the afterload of the heart via non beta-adrenergic mechanisms or by alpha mediated increases in myocardial contractility.

In Group 1 (Ctrl/LSS/Phn-LSS/CAO-Phn-LSS) regional denervation dropped blood flow to both the endocardium and epicardium of the anterior and posterior wall. Thus while regional denervation has been reported to be beneficial to the heart during ischemia, it may not be due to release of sympathetic tone as Holtz has suggested (16). Rather, it is more likely to be due to a drop in overall myocardial metabolism as was originally suggested by Gregg et al.(15). The results of this study confirm previous findings of Chilian et al. (4) in which phenol did not increase blood flow to the denervated region with intact sympathetics. The discrepancy between the studies by Holtz et al. and Chilian et al. is not easily reconciled, since the differences in results may be due entirely to the mode of regional denervation employed in each study. It is reasonable to postulate that the changes in flow in the present study

may have been due to the changes in pressure since an autoregulatory response might not have been expected to occur within the time frame of the blood flow measurement.

GROUP 2 (CAO/CAO-LSS/CAO-Phn/CAO-LSS-Phn)

In Group 2, LSS did not increase flow to the anterior wall compared to control. However, in that group of animals, occlusion of the blood supply to the posterior wall was a confounding factor in interpreting alterations in blood flow to the anterior wall. Indeed, in another study from this laboratory (Chapter V), LSS did not change flow significantly from control although administration of prazosin did increase flow significantly from control but not from flow during left stellate stimulation. It is possible that in the present study following occlusion in the posterior wall, function was impaired enough to limit the increase in blood pressure during LSS. It is unlikely that the nerves to the posterior wall were in some way impaired by the period of ischemia. Phenol application to the posterior wall dropped blood pressure even during stellate stimulation so that the nerves were in all probability functioning during the previous bouts of ischemia, particularly the nerves to the anterior wall which may have passed through the posterior wall.

SUMMARY OF GROUPS 1 AND 2

In conclusion, this study did not find evidence in support of sympathetically mediated vasoconstriction in an ischemic region which could be eliminated by regional denervation. We found no evidence to

suggest that denervation of the ischemic zone would enhance blood flow to that region by a 'reverse coronary steal' mechanism. However, this study did find that posterior left ventricular regional denervation did produce a drop in blood pressure in Group 2 (CAO/CAO-LSS/CAO-Phn/CAO-LSS-Phn), and a drop in blood flow to the anterior wall in Group 2 (CAO/CAO-LSS/CAO-Phn/CAO-LSS-Phn). In Group 1 (Ctrl/LSS/Phn-LSS/CAO-Phn-LSS), the blood pressure and blood flow during left ansae subclavia stimulation in the presence of beta blockade increased. In this group, posterior wall denervation eliminated the response to sympathetic stimulation. However, since there was no change in resistance, the increase in blood flow was probably of a passive nature and reflected the rise in arterial pressure during LSS. The increase in blood pressure during LSS appears to non-beta in origin, and might possibly be due to stimulation of myocardial alpha receptors. Furthermore, the drop in blood pressure following application of phenol indicates several possibilities. Either phenol interrupted nervous transmission to the entire left ventricle by denervation of the posterior wall, or posterior wall application of phenol caused an overall decrease in cardiac function and metabolism, thus dropping blood pressure and blood flow.

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CHAPTER VII

INFLUENCE OF THE CARDIAC NERVES ON BLOOD FLOW TO THE CIRCUMFLEX BED

A. INTRODUCTION

Since the early part of this century it has been well-documented that coronary arteries can constrict in response to adrenergic agents (31). Brodie and Cullis postulated the existence of both vasoconstrictor and vasodilator fibers to the coronary arteries (1). Studies undertaken by Randall et al. investigated the cardiac nerve distribution to the heart and its functional importance (22), thus extending earlier anatomic studies on the pathways of autonomic nerves to the heart (18,19). The studies by Randall et al. involved mapping the distribution of nerves to the left and right ventricles and atria. However, in those studies, and others from the same laboratory, the investigations concentrated primarily on the chronotropic and inotropic effects of sympathetic stimulation, and did not address the specific innervation of coronary arteries (7). In addition, due to technical limitations, innervation of the posterior wall was not as well defined as the anterior wall. Thus, it was not known whether each of the major cardiac sympathetic nerves exerted discrete control over specific coronary arteries as well as having localized influence on ventricular function. Other studies which have investigated cardiac sympathetic effects on blood flow have stimulated either the stellate ganglia (29) or thoracic roots (8) and not specific cardiac nerves, to investigate the regional distribution of blood flow in

response to nerve stimulation.

Although under normal conditions, local control of blood flow predominates, it is also of interest to determine whether the cardiac accelerator nerves supplying local left ventricular regions also carry fibers to the coronary arteries, and which type of nerve fiber has greater influence on coronary blood flow during myocardial ischemia. By blocking beta receptors, it is possible to determine direct innervation of the circumflex artery due to vasoconstrictor effects. Thus one aim of this study was to determine the specific neural control of blood flow through the circumflex artery.

The vasoconstrictor actions of nerve stimulation on coronary blood flow are well established in both experimental (5) and clinical situations (13). It is believed that the sympathetic nervous system exerts a tonic level of vasoconstriction (11,27) and that even under conditions of increased metabolic activity (19,20), alpha-receptors can limit increases in blood flow. Most previous studies did not differentiate between alpha-1 and alpha-2 receptors (5,30). However, results from some recent studies suggests that not only do pre-junctional alpha receptors modulate coronary vasoconstriction by exerting influences on norepinephrine release (10), but that post-junctional alpha-2 receptors also modulate coronary blood flow (4,12,26). The second goal of this investigation was to determine whether alpha vasoconstriction reported in previous studies is mediated primarily by alpha-1 or alpha-2 receptors or whether both receptor subtypes exert equivalent influences on restricting coronary flow.

B. METHODS

1. General Preparation

Mongrel dogs of either sex were premedicated with morphine sulfate (2.5 mg/kg, s.c.) or xylazine (2 mg/kg, s.c.) approximately 15-20 minutes before being given alpha-chloralose (100 mg/kg, IV). A polyethylene catheter (PE-260 tubing) was placed in the right femoral artery and connected to a Statham P23dB pressure transducer for continuous monitoring of arterial blood pressure. The right femoral vein was catheterized with PE-260 tubing for fluid and drug administration. The trachea was cannulated with a glass Y-tube which was connected to a Harvard respirator. Each dog was ventilated with room air at a positive end-expiratory pressure of 5 cm of water. Both cervical vagi were isolated and severed. The left carotid artery was cannulated with surgical grade Tygon tubing. This served as one limb of the extracorporeal shunt delivering blood to the circumflex artery, distal to the site of occlusion. Succinylcholine (20 mg, IV) was administered as a muscle relaxant. The chest was opened in the fourth left intercostal space. Both the right and left ansae subclavia of the stellate ganglia were isolated and stimulated (7-10 Hz, 5 msec, 5-7 V) using bipolar stainless steel electrodes. The three major left side cardiac sympathetic nerves were isolated, (Ventrolateral, ventromedial, innominate) in addition to the left anterior (AAS) and posterior ansa subclavia (PAS). Square wave pulses (8 Hz, 5 msec, 5-7 V) were delivered by a Frederick Haer Pulsar 4i constant voltage stimulator, and voltage and frequency of the delivered pulse were monitored throughout the experiment on a Tektronix T922R calibrated oscilloscope. Changes in the heart rate, and

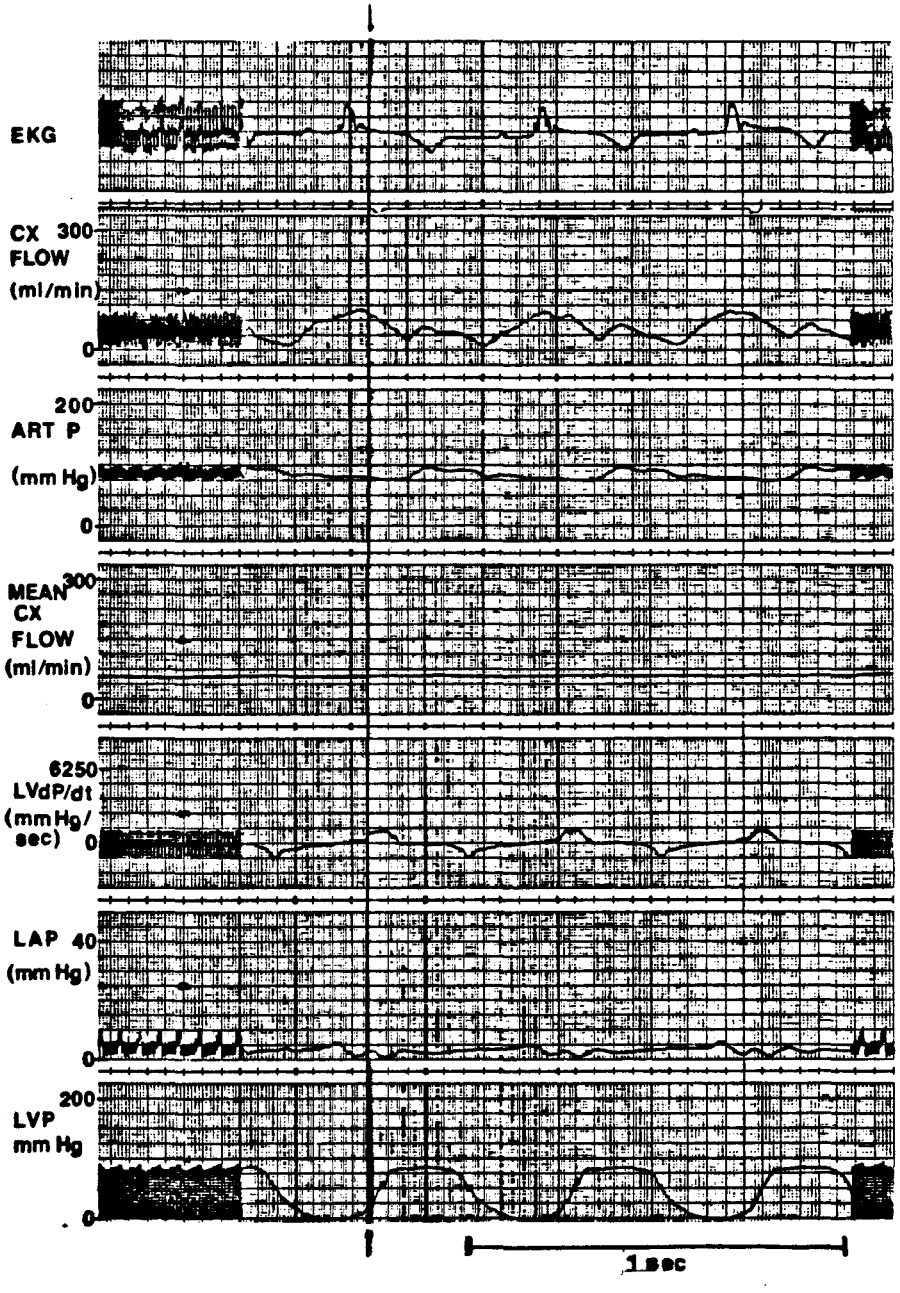
Lead II electrocardiogram, specifically the P-R interval and T-wave configuration during stimulation of the left and right stellate ganglia confirmed that both the anterior and posterior limbs of the right and left ansae subclavia had been dissected out (6,33). The right and left ansae subclavia were cut distal to the stellate ganglia, and both cervical vagi were cut, thus completing neural decentralization of the heart and insuring against afferent stimulation of the cardiac nerves. The pericardium was incised and sutured to the walls of the chest to form a pericardial cradle.

A catheter was placed in the left atrium for measurement of left atrial pressure. A Konigsberg high fidelity pressure transducer (P-22) was inserted into the left ventricular chamber via a stab wound in the apex, and tied securely in place with purse string sutures. The Konigsberg transducer was cross-calibrated with the systolic pressure of the thoracic aorta, and the diastolic pressure recorded from the left atrium. The left ventricular pressure signal was differentiated and recorded throughout the experiment. End diastole was determined as the start of the positive slope of the LV dP/dt signal (See Figure 7-1). All measurements in this series of experiments were recorded on an 8-channel Gould recorder with a frequency response of 125 Hz $\pm$ 1 div; 10 div @ 10 mm. Calculation of resistance was determined as the quotient of the pressure gradient between the aorta and the left ventricle at end-diastole, and coronary blood flow at end diastole.

The circumflex artery was isolated close to its origin, ligated with a piece of 2-0 silk, and a piece of adult feeding tubing (8-F) was inserted into the artery, and tied securely in place. The circumflex

FIGURE 7-1
REPRESENTATIVE TRACING OF MEASURED VARIABLES
DURING CONTROL CONDITIONS PRIOR TO CARDIAC
SYMPATHETIC NERVE STIMULATION

A representative tracing of hemodynamic recordings during a control run. The heavy black line at the arrows, shows where the actual measurements were taken for calculation of blood flow and resistance. EKG: Lead II electrocardiogram; CX FLOW: circumflex blood flow; ART P: arterial pressure; MEAN CX FLOW: mean circumflex blood flow; LV dP/dt: first derivative of left ventricular pressure; LAP: left atrial pressure, LVP: left ventricular pressure.


EKG
CX FLOW (ml/min)
300
0
ART P (mm Hg)
200
0
MEAN CX FLOW (ml/min)
300
0
LVdP/dt (mm Hg/ sec)
6250
0
LAP (mm Hg)
40
0
LVP mm Hg
200
0
1 sec

artery was perfused with blood from the carotid artery which was cannulated with surgical grade Tygon tubing. The blood from the carotid artery flowed through an In Vivo extracorporeal flow probe and was measured on a Carolina square wave electromagnetic analog flowmeter. A T-tube was connected to the extra-corporeal shunt and the side arm was used to facilitate flushing of the shunt system with heparinized saline, which was necessitated at frequent intervals to prevent the formation of clots. Prior to insertion of the shunt, the dog was heparinized with 200 units of heparin and the Tygon tubing was soaked in heparinized saline. The flowmeter was periodically calibrated by running saline through the flow probe at known rates via a Harvard perfusion pump. The probe factor was adjusted accordingly. Flow was zeroed electrically and mechanically at the beginning of each set of nerve stimulations by clamping the tubing distal to extracorporeal flow probe.

Blood gases and pH were monitored throughout the experiment and maintained within normal limits (pH 7.38-7.45, pO2 80-120 mm Hg, pCO2 30-40 mm Hg, HCO3 23-28 mm Hg) by adjusting the rate and/or volume of the Harvard respirator, with infusions of sodium bicarbonate (20 meq/ml), when necessary, to maintain pH normal. Core temperature, measured in the thoracic cavity by a YSI probe, was kept between 37-39 C° by means of a heating pad beneath the dog, and an infrared heating lamp above the dog when necessary. Succinylcholine (20 mg, IV) and additional anesthetic were administered as needed. The heart was kept moist with sponge soaked in saline, and plastic wrap was kept over the open chest to retain moisture and heat. Lidocaine was administered intravenously if the heart became arrhythmic, particularly during clamping of the circumflex artery

and insertion of the shunt.

2. Adrenergic Receptor Blockers

The adrenergic blockers used in this study are listed in Table 7-1. Blockade by timolol (0.2 mg/kg, IV) was considered to be adequate when stimulation of the left ansae subclavia produced no changes in the Lead II electrocardiogram. Maintenance doses of timolol (0.08 mg/kg/hr, IV) were given to insure blockade of ß-receptors throughout the experiment. Alpha-1 blockade was achieved with prazosin hydrochloride (0.5 mg/kg, IV), a dose which was sufficient to attenuate the rise in arterial pressure elicited by a 5 g/kg dose of phenylephrine (17). Phenylephrine was chosen to test the efficacy of alpha-1 blockade since it was considered to exert its agonist effects preferentially on post-junctional or alpha-1 receptors. Rauwolscine (0.3 mg/kg, IV) was considered to be a reasonably pure alpha-2 blocking agent (14). Blockade by rauwolscine was challenged by 5-10 g/kg dose of BHT-920. The adequacy of blockade was challenged in each dog. Atropine was administered prior to recordings of nerve stimulation, to prevent the stimulation of muscarinic receptors during stimulation of the cardiac sympathetic nerves.

3. Experimental Protocol

Figure 7-2 shows in diagram form the protocols used in this study. This study was comprised of four different protocols for investigation of the role of alpha receptors during cardiac sympathetic nerve stimulation. Stimulation of the cardiac nerves was carried out under four separate experimental conditions, all of which were done in the presence of

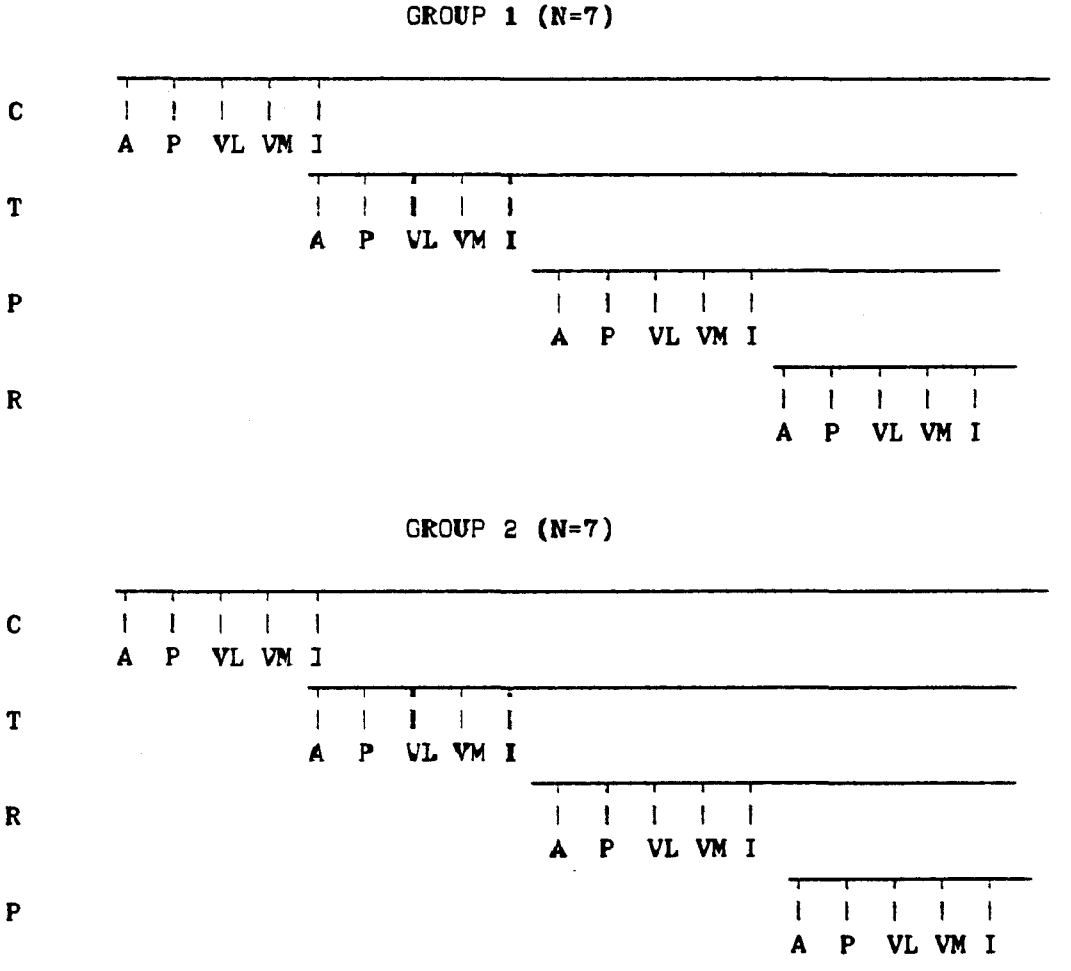
TABLE 7-1

ADRENERGIC AND CHOLINERGIC AGONISTS AND ANTAGONISTS USED DURING CARDIAC SYMPATHETIC NERVE STIMULATION

SYMPATHETIC AGENT	SITE OF ACTION	DOSE USED
Timolol	ß-1,ß-2 Blockade	0.2 mg/kg,LD, 0.8mg/kg/h,MD
Prazosin	alpha-1 blockade	0.5 mg/kg
Rauwolscine	alpha-2 blockade	0.3 mg/kg
Phenylephrine	alpha-1 stimulant	5 g/kg
BHT-920	alpha-2 stimulant	5-10 g/kg
PARASYMPATHETIC AGENT		
Atropine	muscarinic blockade	0.2 mg/kg

FIGURE 7-2

PROTOCOLS USED FOR GROUPS 1 AND 2 DURING CARDIAC NERVE STIMULATION



Protocols for Groups 1 and 2. C: Control conditions of no adrenergic blockade; T: Stimulations done during ß-blockade with timolol; P: stimulations done during alpha-1 blockade with prazosin; R: stimulations done during alpha-2 blockade with rauwolscine. A: Anterior ansa subclavia stimulation; P: posterior ansa subclavia stimulation; VL: ventrolateral cardiac nerve stimulation; VM: ventromedial cardiac nerve stimulation; I: innominate cardiac nerve stimulation.

atropine (0.3 mg/kg, I.V.) with hemodynamic responses recorded before, during and after each nerve stimulation, at a paper speed of 100 mm/sec. 1) nerve stimulations were carried out under control conditions in which there was no adrenergic blockade, 2) during ß-blockade with timolol, 3) during alpha-1 blockade with prazosin, and 4) during alpha-2 blockade with rauwolscine. The sequence of adrenergic blockade was randomized. The adrenergic blocking agents used were not administered independently of one another but were added in combination to the previous drug.

In Group 1 (n=7) the order of adrenergic blockade was 1) no blockade (Control); 2) Timolol, 3) Prazosin, 4) Rauwolscine. In Group 2 (n=7) the order of the blockade was 1) no blockade, 2) Timolol, 3) Rauwolscine, 4) Prazosin. The purpose of the first two groups was to determine which of the five isolated cardiac nerves caused significant vasoconstriction in the presence of beta blockade, and to determine whether alpha-1 blockade or alpha-2 blockade would reverse the vasoconstriction thus elicited.

In Group 3 (n=5) the order of blockade was 1) Control, 2) Prazosin, 3) Rauwolscine and 4) Timolol. In Group 4 (n=5) the order of the alpha blocking agents was reversed: 1) Control, 2) Rauwolscine 3) Prazosin and 4) Timolol. The reason for these last two protocols was to determine whether the order of alpha blockade resulted in any significant differences in blood flow and resistance, and whether either alpha-1 or alpha-2 receptor blockade caused a larger restricting influence on ß-receptor metabolically mediated increases in blood flow.

4. Data Analysis

All measurements were read from a fast tracing (100 mm/sec). Flow was measured at end diastole which was taken at the point of the beginning of the positive slope of the dP/dt signal (Figure 7-1). Resistance was calculated as the difference between end diastolic arterial pressure and end diastolic left ventricular pressure, divided by the end diastolic circumflex flow. The difference between prestimulation baseline flow values and the flow response to nerve stimulation in each dog was used in the analysis of data (delta). The percent change in flow during nerve stimulation for each dog was also used in the analysis of data, and was also based on prestimulation flow and flow during nerve stimulation. The delta and percent change for each nerve (AAS, PAS, VLCN, VMCN, ICN) were analyzed independently of one another. Analysis of variance was used to determine whether there were significant differences between any of the treatment groups (Control, Timolol, Prazosin, Rauwolscine), and Tukey's test was used to isolate the differences at a p value of 0.05. This procedure was followed for Groups 1 through 4.

C. RESULTS

1. Group One

In each group of dogs, all five of the major left side cardiac nerves were stimulated and the responses to stimulation recorded and subjected to statistical analysis. The data for VMCN and ICN are not included, as these nerves had no major vasoconstrictor action following adminstration of timolol.

The blood flow values during control and during stimulation of the AAS, PAS and VLCN are shown in Table 7-2. The changes from resting levels are listed during each of the drug administrations. Stimulation of the AAS following timolol and prazosin resulted in a significantly smaller change in blood flow compared to control stimulation of the AAS ($p<0.025$). Stimulation of the PAS following timolol, prazosin and rauwolscine all resulted in significantly smaller changes in blood flow compared to control ($p<0.01$). There were no significant differences between different groups during stimulation of the VLCN in Group 1.

Figure 7-3 illustrates the percent change in blood flow during stimulation of the AAS, PAS, and VLCN in each of the control conditions and following the adminstration of the adrenergic blocking agents. In Group 1 during stimulation of the AAS (top left panel), the change in flow following timolol was significantly lower than control ($p<0.02$). The change in flow following prazosin remained significantly lower than control ($p<0.02$). Following administration of rauwolscine, the percent change in flow was no longer significantly different from control. During stimulation of the PAS (middle left panel), the change in flow following timolol and subsequent alpha blockade were significantly lower

TABLE 7-2

Blood flow data in ml/min for Group 1 (n=7). Data are listed as mean ± SEM. Shown are the data for prestimulation baseline flow value, response to stimulation of each of the three major nerves studied, and the change in blood flow from prestimulation blood flow (delta). Results are shown for nerve stimulation during each of the stimulation protocols, 1) Control: during control (conditions of no adrenergic blockade), 2) Timolol: following beta-blockade, 3) Prazosin: following prazosin (alpha-1 blockade) and 4) Rauwolscine: following rauwolscine (alpha-2 blockade). All significant changes shown are differences from control response (no adrenergic blockade) to nerve stimulation. * indicates $p<0.02$, ☆indicates $p<0.01$.

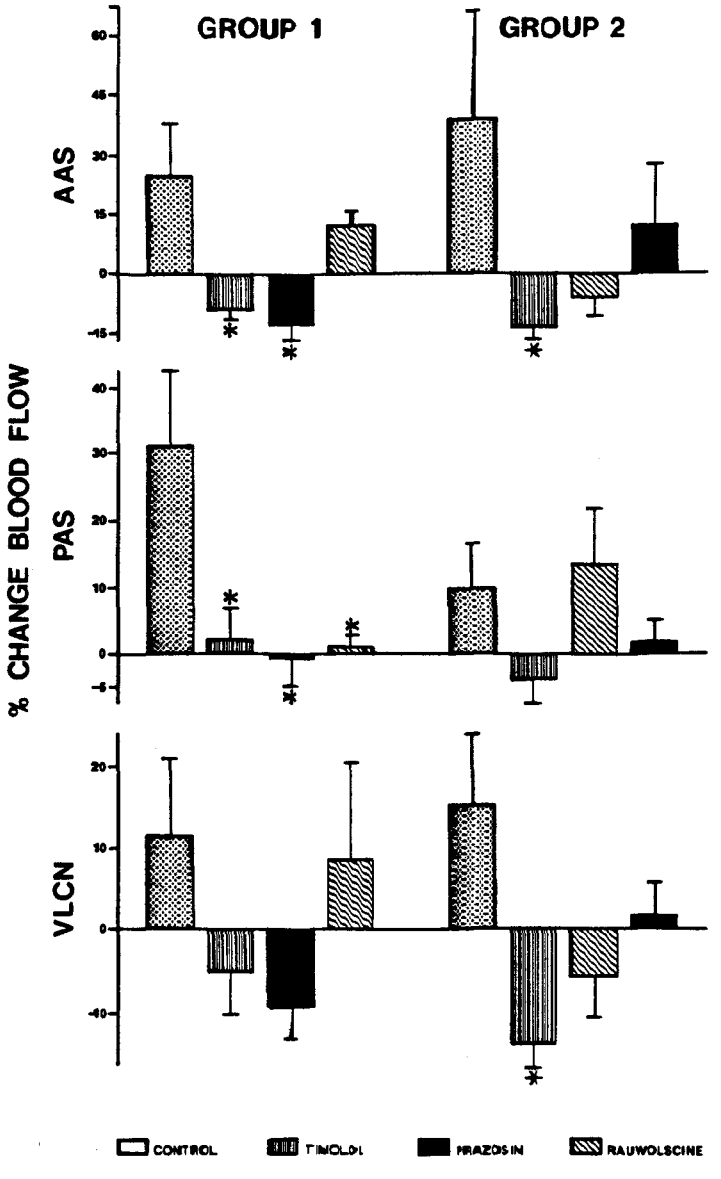
TABLE 7-2

CIRCUMFLEX ARTERY BLOOD FLOW DURING STIMULATION OF THE CARDIAC SYMPATHETIC NERVES IN GROUP 1

GROUP 1	BASELINE	AAS	DELTA
CONTROL	46.66 ± 2.75	57.04 ± 5.05	10.37 ± 5.89
TIMOLOL	42.69 ± 3.47	39.06 ± 3.94	-3.63 ± 0.61*
PRAZOSIN	34.40 ± 5.88	30.63 ± 5.87	-3.78 ± 1.23*
RAUWOLSCINE	37.86 ± 6.09	39.52 ± 5.66	1.67 ± 1.51
	BASELINE	PAS	DELTA
CONTROL	45.37 ± 3.28	53.95 ± 4.9	12.87 ± 4.72
TIMOLOL	41.91 ± 3.39	42.90 ± 4.13	1.00 ± 1.84☆
PRAZOSIN	33.01 ± 5.41	31.65 ± 5.49	0.07 ± 1.31☆
RAUWOLSCINE	36.47 ± 5.62	36.36 ± 5.56	-0.11 ± 0.58☆
	BASELINE	VLCN	DELTA
CONTROL	49.91 ± 3.62	54.87 ± 5.15	4.96 ± 4.82
TIMOLOL	40.76 ± 1.69	38.62 ± 2.67	-2.14 ± 2.03
PRAZOSIN	33.81 ± 5.45	29.81 ± 5.55	-2.58 ± 1.19
RAUWOLSCINE	35.49 ± 5.80	35.53 ± 5.40	0.04 ± 1.15

FIGURE 7-3
PERCENT CHANGE IN BLOOD FLOW DURING CARDIAC NERVE STIMULATION FOR GROUPS 1 AND 2

Percent change in blood flow (mean ± SEM) from prestimulation baseline flow for Groups 1 and 2 during stimulation of the AAS, PAS, and VLCN. All significant changes shown are significant differences from the percent change during control conditions of no adrenergic blockade. Group 1 (n=7) (Blocking order: Control, timolol, prazosin, rauwolscine) is depicted on the left. Group 2 (n=7) (Blocking order: Control, timolol, rauwolscine, prazosin) is depicted on the right. * indicates $p<0.05$, *indicates $p<0.02$.


GROUP 1
GROUP 2
AAS
PAS
VLCN
% CHANGE BLOOD FLOW
CONTROL
TIMOLOL
PRAZOSIN
RAUWOLSCINE

than control ($p<0.02$). No significant changes were evident during VLCN stimulation in Group 1 (bottom left panel).

Table 7-3 lists the values of resistance before and during stimulation of the AAS, PAS, and VLCN and the change in resistance for Group 1. During stimulation of the AAS resistance did not change significantly following either ß- or alpha blockade. During stimulation of the PAS resistance was significantly lower following timolol and combined timolol and prazosin compared to control ($p<0.005$). There were no significant differences between treatments during VLCN stimulation in Group 1.

Figure 7-4 illustrates the % change in resistance from resting levels. During AAS (top left panel), the change in resistance was significantly higher during timolol and timolol and prazosin ($p<0.01$). Following alpha-2 blockade with rauwolscine, the change in resistance was no longer statistically different from control.

During stimulation of the PAS (middle left panel), the change in resistance was significantly higher during beta blockade and combined alpha-1 and beta blockade, from control ($p<0.002$). Administration of rauwolscine resulted in a change in resistance which was no longer significantly different from control.

During VLCN stimulation (bottom left panel) with combined alpha and beta blockade, the resistance was significantly higher than control ($p<0.05$). Note that timolol administration did result in significant increases in resistance during VLCN stimulation. Rauwolscine returned the response to VLCN toward control levels.

TABLE 7-3

Calculated resistances (in mm Hg/ml/min) for Group 1 (n=7). Shown are the data (mean ± SEM) for the prestimulation baseline resistances, response to stimulation of each of the three major nerves, and the change in resistance from prestimulation values (delta). Results are shown for response to nerve stimulation during each of the stimulation protocols used. All significant changes shown are differences from control response (no adrenergic blockade) to nerve stimulation. *indicates $p<0.005$.

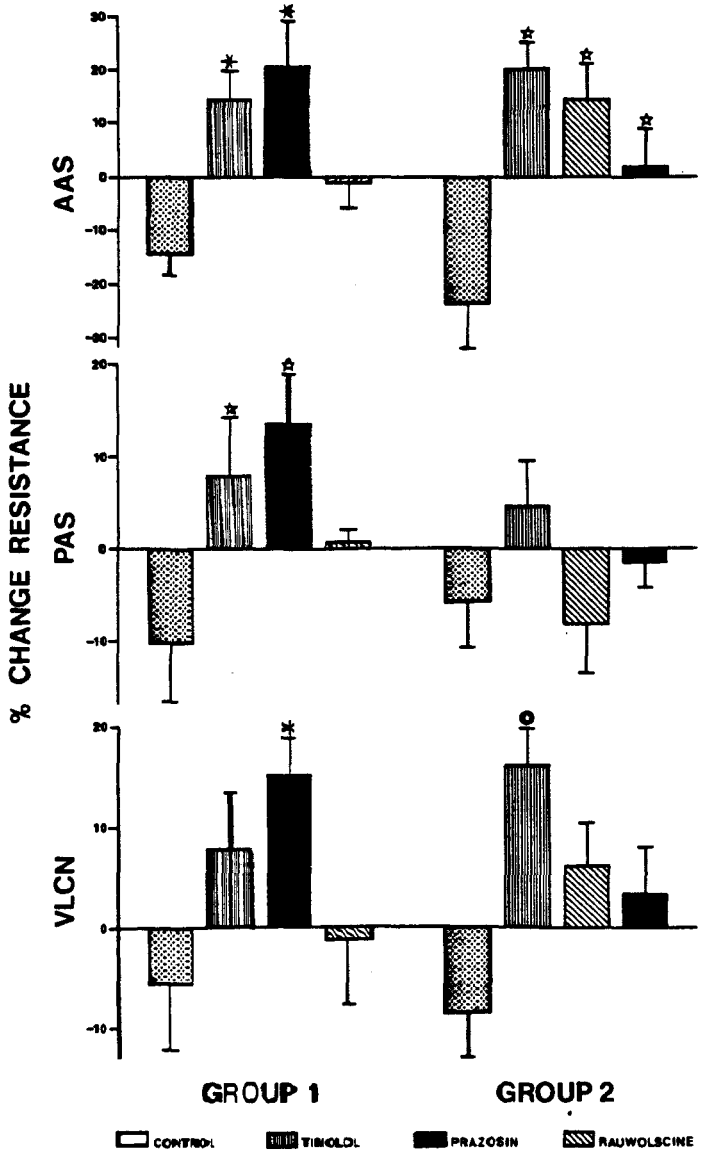
TABLE 7-3

CALCULATED RESISTANCE OF THE CIRCUMFLEX ARTERY DURING CARDIAC NERVE STIMULATION FOR GROUP 1

GROUP 1	BASELINE	AAS	DELTA
CONTROL	2.57 ± 0.21	2.20 ± 0.24	-0.37 ± 0.16
TIMOLOL	2.78 ± 0.28	3.23 ± 0.42	0.44 ± 0.18
PRAZOSIN	3.05 ± 0.84	3.92 ± 1.35	0.87 ± 0.53
RAUWOLSCINE	2.06 ± 0.42	1.95 ± 0.27	-0.12 ± 0.17
	BASELINE	PAS	DELTA
CONTROL	2.55 ± 0.23	2.25 ± 0.18	-0.31 ± 0.17
TIMOLOL	2.86 ± 0.29	3.12 ± 0.32	0.26 ± 0.16*
PRAZOSIN	2.95 ± 0.74	3.36 ± 0.83	0.40 ± 0.15*
RAUWOLSCINE	2.27 ± 0.44	2.27 ± 0.42	0.00 ± 0.03
	BASELINE	VLCN	DELTA
CONTROL	2.39 ± 0.21	2.20 ± 0.20	-0.18 ± 0.12
TIMOLOL	2.96 ± 0.22	3.19 ± 0.30	0.24 ± 0.18
PRAZOSIN	2.98 ± 0.63	3.48 ± 0.80	0.50 ± 0.18
RAUWOLSCINE	2.65 ± 0.82	2.33 ± 0.43	-0.32 ± 0.42

FIGURE 7-4
PERCENT CHANGE IN CALCULATED RESISTANCE DURING CARDIAC NERVE STIMULATION FOR GROUPS 1 AND 2

Depicted are the percent changes in resistance from prestimulation baseline resistance (mean ± SEM) during stimulation of the AAS (top panel), PAS (Middle panel) and VLCN (bottom panel) for Group 1 (n=7) (left side of graph) and Group 2 (n=7) (right side of graph). *p less than .05, *p less than .01, ☆p less than .002 and ✪p less than .005. All changes shown are in comparison to percent change during control conditions of no adrenergic blockade.


% CHANGE RESISTANCE
AAS
PAS
VLCN
GROUP 1
GROUP 2
CONTROL
TIMOLOL
PRAZOSIN
RAUWOLSCINE

2. Group Two

The blood flow values for Group 2 during control and during stimulation of the AAS, PAS and VLCN are shown in Table 7-4. The changes from baseline levels are listed during each of the drug administrations. Stimulation of the AAS following timolol and combined timolol and rauwolscine resulted in a significantly smaller change in blood flow compared to control stimulation of the AAS ($p<0.01$). There were no changes between groups during stimulation of the PAS. During stimulation of the VLCN, flow changes were significantly reduced ($p<0.005$) following timolol and after rauwolscine. After prazosin, the changes during nerve stimulation were not significantly different from control. Figure 7-3 illustrates the percent change in blood flow during stimulation of the AAS, PAS, and VLCN in each of the control conditions and following the adminstration of the adrenergic blocking agents. In Group 2 during stimulation of the AAS (top right panel, Fig. 7-3), the change in flow following timolol was significantly lower than control ($p<0.05$). The change in flow following rauwolscine was not significantly different from control. During PAS (middle right panel of Fig. 7-3), there were no changes in flow following timolol and subsequent alpha blockade. During stimulation of the VLCN (bottom right panel, Fig 7-3), after timolol the percent change in flow was significantly different from control ($p<0.02$). After rauwolscine the percent change was no longer different from control. Thus, Figure 7-3 illustrates that stimulation of each of the major cardiac nerves in the presence of ß-blockade causes a significant vasoconstriction compared to the change in flow when no ß-blocking agent was given. Administration of alpha-1 blockade in addition to ß-blockade

TABLE 7-4

Blood flows (m/min) for Group 2 (n=7) (Blocking order: control, timolol, rauwolscine, prazosin). Shown are the values of blood flow before and during stimulation of the AAS, PAS, and VLCN. Delta is the change from prestimulation flow during each of the interventions. Statistical comparisons were made between interventions. Data are shown as mean ± SEM. *p less than .01, compared to control response.

TABLE 7-4

CIRCUMFLEX ARTERY BLOOD FLOW DURING STIMULATION OF THE CARDIAC SYMPATHETIC NERVES IN GROUP 2

GROUP 2	BASELINE	AAS	DELTA
CONTROL	36.16 ± 5.14	47.28 ± 5.65	11.11 ± 5.57
TIMOLOL	34.94 ± 4.01	29.85 ± 3.42	-5.20 ± 1.29*
RAUWOLSCINE	39.57 ± 3.25	37.92 ± 2.61	-1.66 ± 1.29*
PRAZOSIN	24.29 ± 2.90	25.89 ± 2.77	1.61 ± 2.33
	BASELINE	PAS	DELTA
CONTROL	36.04 ± 6.23	38.82 ± 6.18	2.78 ± 1.66
TIMOLOL	33.54 ± 3.48	32.05 ± 3.18	-1.49 ± 1.21
RAUWOLSCINE	33.75 ± 2.51	38.24 ± 4.26	4.49 ± 3.09
PRAZOSIN	25.21 ± 4.53	25.18 ± 3.88	-0.03 ± 0.94
	BASELINE	VLCN	DELTA
CONTROL	36.58 ± 4.77	40.83 ± 4.25	4.25 ± 2.12
TIMOLOL	34.04 ± 4.39	29.12 ± 3.93	-4.92 ± 1.13*
RAUWOLSCINE	34.40 ± 2.29	32.42 ± 2.74	-1.97 ± 1.76*
PRAZOSIN	22.05 ± 3.00	22.32 ± 2.86	0.27 ± 0.82

did not alter the vasoconstriction. However, alpha-2 blockade in addition to alpha-1 blockade or even prior to administration of alpha-1 blockade returned blood flow values toward control.

Table 7-5 lists the values of resistance before and during stimulation of the AAS, PAS, and VLCN and the change in resistance (delta). During stimulation of the AAS resistance changed significantly following timolol and remained lower even after both alpha-1 and alpha-2 blockade ($p<0.002$). During stimulation of the PAS resistance did not change significantly. During VLCN stimulation, the change in resistance was significantly increased during timolol ($p<0.01$) but decreased after alpha-2 blockade so that resistance was no longer significantly different from control.

The right hand side of Figure 7-4 illustrates the percent change in resistance for Group 2. During AAS stimulation (Top right panel), the change in resistance was significantly higher during timolol, rauwolscine and prazosin ($p<0.002$). There were no changes in resistance during stimulation of the PAS (middle right panel). Timolol produced increased resistance from control during stimulation of the VLCN, $p<.005$, (bottom right panel), but following rauwolscine, the resistance decreased back toward control.

In summary, ß-blockade during cardiac sympathetic stimulation resulted in increases in coronary resistance which were not affected by the addition of alpha-1 blockade. Alpha-2 blockade resulted in reductions in resistance to levels comparable to control conditions of no adrenergic blockade. Resistance did not return to control levels following alpha-1 blockade in combination with ß-blockade.

TABLE 7-5

Resistances (mm Hg/ml/min) for Group 2 (n=7) during stimulation of the AAS, PAS, and VLCN. Delta is the change from baseline resistance during nerve stimulation. All statistical comparisons are based on the control response to nerve stimulation. Data are listed as mean ± SEM. *indicates $p<.002$, †indicates $p<.01$.

TABLE 7-5

CALCULATED RESISTANCE OF THE CIRCUMFLEX ARTERY DURING CARDIAC NERVE STIMULATION FOR GROUP 2

GROUP 2	BASELINE	AAS	DELTA
CONTROL	3.21 ± 0.31	2.35 ± 0.22	-0.87 ± 0.35
TIMOLOL	3.30 ± 0.33	3.93 ± 0.34	0.63 ± 0.15*
RAUWOLSCINE	3.14 ± 0.27	3.42 ± 0.19	0.27 ± 0.16*
PRAZOSIN	2.85 ± 0.35	2.97 ± 0.53	0.12 ± 0.24*
	BASELINE	PAS	DELTA
CONTROL	3.19 ± 0.33	2.97 ± 0.27	-0.22 ± 0.17
TIMOLOL	3.48 ± 0.29	3.58 ± 0.21	0.10 ± 0.20
RAUWOLSCINE	3.78 ± 0.26	3.37 ± 0.19	-0.33 ± 0.20
PRAZOSIN	2.96 ± 0.58	2.86 ± 0.49	-0.10 ± 0.13
	BASELINE	VLCN	DELTA
CONTROL	3.09 ± 0.28	2.82 ± 0.31	-0.27 ± 0.14
TIMOLOL	3.43 ± 0.33	3.97 ± 0.38	0.54 ± 0.11†
RAUWOLSCINE	3.56 ± 0.17	3.74 ± 0.12	0.18 ± 0.15
PRAZOSIN	3.12 ± 0.56	3.14 ± 0.47	0.02 ± 0.16

In Groups 3 and 4, the sequence of adminstration of prazosin and rauwolscine did not result in differences in the change in flow with and of the major nerves. Combined alpha-1 and alpha-2 blockade did not alter the response to nerve stimulation significantly from the control response, regardless of which agent was administered first.

Comparison of the prestimulation flow values for Groups 1 through 4 with analysis of variance, determined that there were no significant differences for prestimulation flow values between groups.

In summary in Groups 1 and 2, the nerves which have a significant influence on circumflex blood flow are the AAS, PAS, and the VLCN. These nerves had not previously been shown to specifically cause vasoconstriction of the circumflex artery. These nerves can cause significant reductions in blood flow and increases in resistance during beta blockade. The mechanism of this vasoconstrictor action appears to be alpha mediated.

D. DISCUSSION

The aims in this study were to determine which of the major left side cardiac nerves influence circumflex blood flow, whether they can elicit significant vasoconstriction when stimulated separately in the presence of beta blockade, and whether the vasoconstriction is alpha-1 or alpha-2 mediated.

Studies by Randall et al. addressed the question of specific neural innervation of the heart (22). They stimulated cardiac nerves and recorded the changes in contractile force measured by Walton-Brodie strain gauge arches. In this way the patterns of sympathetic distribution to the heart were determined. The ICN increased the contractile force of the right ventricle and left ventricular apex. The VMCN increased the contractility of the right ventricular conus. The VLCN increased contractility in the dorsal lateral aspect of the left ventricle and slightly in the anterior wall. Geis and Kaye extended these findings by measuring the contractile changes of the posterior left ventricle (7). Both of these studies concurred that stimulation of the left stellate ganglion produced profound increases in the contractile function of all segments of the left ventricle. Randall and Armour also noted that stimulation of more distal branches caused more discrete areas to be affected by the nerves (23).

Later studies investigated the influence of the sympathetics on myocardial blood flow. Rinkema et al. stimulated the left and right stellate ganglia and injected microspheres to determine the changes in regional blood flow. Stimulation of the left stellate ganglia produced pronounced changes in posterior wall blood flow as well as anterior wall

blood flow (24). Ross and Mulder placed electromagnetic flow probes on the LAD, right coronary artery and left coronary artery, and stimulated the ansae subclavia of the left stellate ganglion. Their results were similar to those of Rinkema et al., in that large increases in blood flow were produced in both areas, with the circumflex artery responding to stimulation of the left stellate with greater increases in flow than the left anterior descending artery (25).

In the present study, there was an increase in blood flow during stimulation of the AAS, PAS, and VLCN. The response to stimulation of these three nerves during timolol was significantly different from the response to nerve stimulation under control conditions. Only these three major nerves actually had this effect. The VMCN and ICN did not actually increase flow during stimulation under control conditions. There are two plausible explanations for this. First the distribution of these nerves is not such as would cause major increases in left ventricular metabolism. Second, both of these nerves contain proportionately more vagal fibers than the other three cardiac nerves. Hence it is not surprising that following ß-blockade neither nerve would effect significant vasoconstriction of the circumflex bed, since they usually have large parasympathetic influences, which were not elicited in this study due to prior muscarinic blockade with atropine.

A problem with this method of analysis is that nerves which do not innervate a significant portion of the left ventricle and hence do not cause significant metabolic vasodilation are excluded from the study. It is possible that such nerves may in fact contribute to reduction in blood flow when stimulated, but the response would be significant only when

compared to resting blood flow during beta blockade. One possibility for assessing the influence of all of the cardiac nerves on blood flow would be to stimulate the nerves in an arrested or fibrillating heart preparation to avoid the confounding influence of neurally mediated metabolic changes. An additional disadvantage of this method is that it would appear that the nerves supplying the myocardium also supply the arteries in the vicinity. While it is possible that a large nerve such as the VLCN has separate vasomotor and accelerator fibers, the separation of vasomotor and inotropic effects during stimulation of this nerve would be difficult. Without a separation of myocardial influences from purely vasomotor effects, the determination of a regional distribution and pattern of innervation on coronary blood flow is hindered.

Only the AAS showed consistent vasoconstriction in Group 1 and Group 2. The reason for the non-uniformity of results between nerves is most likely due to the amount of myocardium innervated. Stimulation of the left stellate ganglion causes large increases in contractile force (7,22), and blood flow throughout the entire left ventricle (24). Stimulation of the cardiac nerves more distally produces more discrete changes in contraction and presumably blood flow. If a large amount of myocardium is innervated by one nerve, the response to nerve stimulation will be more consistent between animals. Smaller nerves which innervate less myocardium will probably result in more variability of response between animals, particularly if the site of stimulation is varied distal to the stellate ganglion.

Following beta blockade with timolol the stimulation induced change in flow decreased compared to control. This indicates a vasoconstriction

which is most likely due to the unmasking of alpha receptors, and is in accord with previous studies which demonstrated a vasoconstriction following beta blockade (5). In a non-beating preparation, Gerova et al. demonstrated coronary vasoconstriction of the LAD during stimulation of the left stellate ganglia which was eliminated by adminstration of phentolamine (8). Many previous investigations have demonstrated vasoconstriction which limits metabolic vasodilation (9), or is unmasked by beta blockade (2), but most of those studies utilized phenoxybenzamine or phentolamine which block both alpha-1 and alpha-2 receptors. The results from such studies would not clarify the separate influences of both receptor subtypes.

Although alpha-2 receptors have been demonstrated to modulate norepinephrine release (15,16,28), only recently have studies been done to determine whether alpha-2 receptors also control coronary blood flow. Investigations in recent years have proposed that it is actually alpha-2 receptors which mediate coronary vasoconstriction (10,12).

It is difficult to determine from the present studies whether alpha-1 or alpha-2 receptors predominate in mediating coronary vasoconstriction. Although timolol in Group 1 reduced flow and increased resistance probably by unmasking alpha receptors, this reduction in flow was not blocked by prazosin. Subsequent adminstration of rauwolscine eliminated the vasoconstriction.

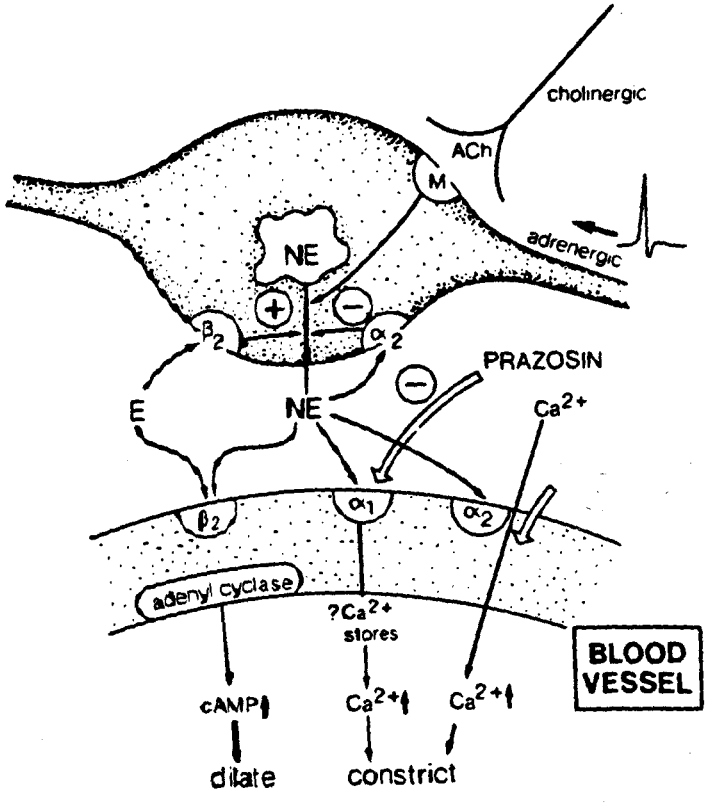
In part, these effects of rauwolscine in Group 2 may be ascribed to its presynaptic action. By removing the negative feedback inhibition of norepinephrine (NE), more NE might be expected to have stimulated the unblocked alpha-1 receptors causing vasoconstriction following admini-

stration of rauwolscine (See Figure 7-5). In addition the peripheral adminstration of rauwolscine may have resulted in a larger release of the NE due to the open chest anesthetized preparation. This higher background level of NE plus background levels of epinephrine which is taken up by nerve terminals and released, may have caused a higher preexisting level of vasoconstriction which would appear to cause of blunting of the effect of NE during stimulation. Thus changes in blood flow during stimulation would not be as apparent due to a high existing level of circulating catecholamines. Separation of the pre and post synaptic actions of alpha-2 receptors would clarify the results of this study and also elucidate the respective roles of alpha-1 and alpha-2 receptors.

An unexpected result of this study was that blockade of alpha-1 receptors in Group 1 resulted in significantly higher resistance and lower flows compared to control. There are several possibilities for this. First, it may be that alpha-2 receptors are responsible for the vasoconstriction. This conclusion would be supported by the fact that subsequent alpha-2 blockade brought the change in resistance back toward control. Another possibility for this action of prazosin may be that alpha-1 receptors on the myocardium mediate some form of metabolic vasodilation and that blockade of these receptors unmasks alpha- 2 receptors located on the vasculature. This conjecture has some basis in recent studies on the importance of myocardial alpha-1 receptors by Corr (3). Corr has proposed that alpha-1 receptors can influence electrical activity in the myocardium, and can also mediate the rise in blood pressure elicited with stellate stimulation in the presence of beta blockade. Corr has also shown that myocardial alpha-1 receptors increase

FIGURE 7-5
SCHEMATIC OF PRESYNAPTIC ADRENERGIC RECEPTOR MODULATION
OF NEURONALLY RELEASED NOREPINEPHRINE

Schematic of the modulation of norepinephrine release from a sympathetic nerve terminal by adrenergic receptors and muscarinic receptors and the effects of adrenergic nerve stimulation on a blood vessel. (-) indicates a negative feedback effect on norepinephrine release and (+) indicates positive feedback on release of norepinephrine. (Modified from: Opie,L. The Heart. Ed. by L. Opie. New York: Grune and Stratton, 1982, pp.234.)


cholinergic
ACh
M
NE
adrenergic
β2
α2
PRAZOSIN
E
NE
Ca2+
β2
α1
α2
adenyl cyclase
?Ca2+
stores
BLOOD
VESSEL
cAMP
Ca2+
Ca2+
dilate
constrict

significantly in number during myocardial ischemia.

The time course of stimulation of the nerves might be important in interpreting the results from this and previous studies. In our study the measurements were taken during a maximal response. Other studies which have demonstrated alpha-vasoconstriction have also measured either maximal response, or stimulated for less than 2 minutes. However, in the study by Ross and Mulder (25), a maximum response to stimulation following beta blockade was reached at 10-15 seconds and slowly subsided after 1-2 minutes of duration. Williams and Most demonstrated that bolus injections of phenylephrine caused vasoconstriction, but that with prolonged administration of the alpha-1 agonist, the vasoconstriction subsided (32). The possibility of an overriding metabolic influence on vasoconstriction during prolonged sympathetic stimulation cannot be ruled out.

In conclusion, the major nerves which innervate the posterior wall also influence coronary blood flow. Furthermore, the traditional concept of alpha-1 receptors innervating the vasculature needs to be reexamined. It appears from the results of this study that alpha-2 receptors play as important, it not more so, a role in constricting the coronary vasculature.

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CHAPTER VIII

SUMMARY

1. Stimulation of cardiac sympathetic nerves during beta blockade increased infarct size following coronary artery occlusion, compared to when the nerves were not stimulated, and compared to when the sympathetic nerves were stimulated without prior beta blockade.
2. Blockade of alpha receptors in addition to beta receptor blockade with sympathetic stimulation resulted in a smaller infarct size compared to infarct size in the presence of sympathetic stimulation and beta blockade alone.
3. Blockade of alpha receptors did not increase blood flow to the posterior wall following circumflex artery occlusion.
4. Regional denervation of the posterior wall did not increase blood flow to the occluded region.
5. The left anterior ansa subclavia, left posterior ansa subclavia, and ventrolateral cardiac nerve induce significant vasoconstriction of the circumflex artery.
6. Alpha-2 adrenergic receptor blockade effectively blocked the vasoconstriction produced during stimulation of the left side cardiac nerves, in the presence of beta-blockade. Alpha-1 receptor blockade did not eliminate the vasoconstriction induced under similar conditions.

CHAPTER IX

CONCLUSIONS

This dissertation began as an attempt to define the role of alpha receptors in the evolution of a myocardial infarction and then broadened to encompass an investigation into how alpha receptors altered blood flow during coronary artery occlusion, how removal of the sympathetics affected blood flow during coronary artery occlusion, and how specific cardiac sympathetic nerves modulated blood flow to the posterior wall.

It was initially hypothesized that stimulation of the left stellate ganglion in the presence of ß-blockade would cause an increase in infarct size due to alpha receptor stimulation (Chapter IV). That study showed that administration of timolol prior to a coronary artery occlusion did not result in a smaller infarct size when compared to control. The fact that there was no difference in the mean size of the infarctions between the control dogs, and dogs given timolol, suggested that circulating catecholamines were not as important as neurally released catecholamines. However, when the left stellate ganglion was stimulated in the presence of beta blockade, the size of the infarcts were significantly larger than control. When alpha-blockade was given in addition to ß-blockade prior to coronary artery occlusion, left stellate stimulation did not increase the size of the infarcts compared to control. Moreover, the size of the infarcts were significantly smaller than when the left stellate was stimulated in the presence of ß-blockade alone.

Thus, it was postulated that the deleterious effect on infarct size of stellate stimulation in the presence of ß-blockade, was due to a reduction in blood flow to the ischemic zone via unopposed alpha receptor stimulation.

In the next study (Chapter V), the possibility that alpha receptors reduced blood flow to the ischemic zone during stellate stimulation was tested as an explanation for the findings from the first study. In the second study, the administration of prazosin either before or after coronary occlusion did not result in increased blood flow to the ischemic region (Chapter V). However, prazosin did increase blood flow to the normal region when given prior to coronary artery occlusion. Furthermore, not only did prazosin cause a vasodilation in the normal zone, it actually increased blood flow above control levels, which was somewhat surprising since blockade of alpha receptors would have been expected to have merely removed the vasoconstrictor effects of left ansae subclavia stimulation in the presence of ß-blockade. A possible explanation for the large vasodilation elicited following prazosin, is that removal of the influence of circulating catecholamines might account for the large increase in blood flow following alpha blockade (See Chapter V). In the study in which infarct sizes were measured (Chapter IV) circulating catecholamines may not have contributed as much to infarct size, due to the limitation in blood flow to the posterior wall. However, circulating catecholamines were not measured in either study.

Even more surprising in the prazosin study than the increase in flow to the normal zone during alpha-1 blockade, was the fact that left ansae subclavia stimulation in the presence of ß-blockade did not

decrease blood flow from control. Other investigators had reported a potent vasoconstriction via alpha receptors when unopposed by ß-blockade. The posterior coronary artery occlusion in the prazosin study in all probability confounded the results of anterior wall blood flow measurements.

Whereas blockade of alpha receptors was involved in the evolution of a myocardial infarction in the infarct study, that effect did not appear to be mediated by a reduction in blood flow to the ischemic zone based on the results from the second study (Chapter V). In light of the fact that prazosin increased blood flow to the normal zone, the possibility exists that prazosin also increased border zone flow, and thus retarded the extension of the infarction. Alpha-blockade might have accomplished this by blockade of peripheral alpha receptors resulting in a reduction of total peripheral resistance, with a consequent drop in afterload. With a drop in afterload, the reduction in myocardial oxygen consumption would have allowed salvage of some marginally ischemic zones, by returning the balance between oxygen supply and demand toward normal levels.

The possible contribution of myocardial alpha receptors to the development of ischemia should not be overlooked. It is conceivable that myocardial alpha-1 receptors alter cardiac metabolism and thus oxygen consumption. Thus, in the infarct study, blockade of myocardial alpha-receptors might have removed the detrimental influence of increased myocardial metabolism. During a coronary artery occlusion, the oxygen supply/demand balance is upset. Under such conditions alpha receptors may not be expected to cause noticeable changes in metabolism, due to the

overwhelming influence of ß-receptors. However, if ß-receptors are blocked, and the amount of coronary blood flow is decreased, a small increment in oxygen consumption (via alpha-1 receptor stimulation) may tip the balance on the side of increasing oxygen debt. A vicious cycle could result, with the increased demand outstripping the restricted blood supply. Blockade of alpha-1 receptors could then return the balance toward normal. It is likely that such a small change in blood flow could not be detected in these studies in which microspheres were utilized. Blood flows in the posterior wall following coronary occlusion dropped down to the limit of the blood flow necessary to deposit the requisite number of microspheres per tissue sample for accurate blood flow calculation. Thus, the limited resolution of the microsphere technique at low blood flows probably contributed to the lack of positive findings during the treatment with prazosin.

Several possibilities exist for prazosin's beneficial effects on the extent of myocardial infarction. Prazosin may favorably alter hemodynamics by reducing afterload via peripheral alpha blockade and it may lower oxygen consumption by blocking myocardial alpha-1 receptors. Furthermore, as was suggested in the study investigating the effects of prazosin on blood flow, while it does not alter flow to the ischemic zone, it may increase border zone flow and salvage tissue in that manner.

The results from the second study in which blood flow to the myocardium was assessed following prazosin (Chapter V) indicated that alpha receptors did not influence blood flow to the ischemic zone. The third study (Chapter VI) was included to determine whether regional denervation of the posterior wall might influence blood flow to the

ischemic zone. It was originally hypothesized that removal of the sympathetic nerves to that region would entail removal of the 'tonic' level of vasoconstriction previously reported, and thus increase blood flow to the ischemic zone. This study demonstrated that posterior wall denervation did not result in increased blood flow to the ischemic zone by removing sympathetic vasoconstriction. However, posterior left ventricular denervation did drop blood flow to both the anterior and posterior wall and resulted in a reduction in mean arterial pressure.

During LSS, in the presence of ß-blockade, blood pressure and blood flow increased. The fact that resistance did not change during LSS suggests that the change in blood flow was not vasomotor in origin, but was passive and reflected the rise in arterial pressure. It was found that increasing the dose of timolol did not eliminate the rise in arterial pressure during stellate stimulation. These effects indicate the involvement of non-beta mediated effects, possibly via alpha-1 receptors on the myocardium. However, alpha blockade was not administered in this study, to determine whether alpha-1 receptors would indeed have blocked the increase in blood pressure elicited by stellate stimulation.

One of the intriguing findings in the phenol study, was that regional denervation did not increase blood flow via removal of 'tonic' vasoconstriction. In fact, posterior wall denervation actually decreased blood flow to both the posterior and anterior left ventricle. In addition, regional denervation eliminated the response to left stellate stimulation in the innervated region. Prior to application of phenol to the posterior wall, LSS had increased blood pressure and blood flow even

in the presence of beta blockade. Following the regional denervation, both responses to LSS were eliminated. By removing the sympathetic influences to both the anterior and posterior wall, cardiac metabolism probably decreased as did cardiac function, with a consequent drop in blood pressure and a passive drop in blood flow. In addition, the possibility exists that denervation of the posterior wall interrupted the innervation of the anterior wall. Previously it had been thought that cardiac nerves travel in a base to apex fashion and follow the coronary arteries to innervate the adjacent myocardium. Finally, it cannot be ruled out that acute application of phenol caused a stunning of the myocardium, and decreased function possibly by a cardio-cardiac depressor effect due to stimulation of posterior wall receptors.

In conclusion, the phenol study suggests that the previously reported tonic level of alpha-vasoconstriction does not appear to be of as much importance during coronary artery occlusion, as it might be during normal physiologic conditions. The results from the phenol study (Chapter VI) indicate that an important pathway for nervous innervation to the anterior wall is via fibers originating in the posterior region which cross over the posterior epicardium to innervate the anterior wall. Finally, there is possibility not tested in this study, that alpha receptors have inotropic influences which can be eliminated by posterior wall denervation.

The actual cardiac nerves mediating coronary vasoconstriction have not been determined. It was found in the last study, that the VLCN, AAS, and PAS, all cause significant vasoconstriction of the circumflex artery but only with prior ß-blockade. Many investigations have reported that

alpha receptors mediate potent coronary vasoconstriction, but these studies did not differentiate between alpha-1 and alpha-2 receptors. Only recently have alpha-2 receptors been found to mediate vasoconstriction. The vasoconstriction elicited by stimulation of the AAS, PAS, and VLCN was eliminated more effectively by blockade of alpha-2 receptors with rauwolscine, than by alpha-1 blockade with prazosin. Thus, alpha-2 receptors appeared to play a slightly greater role in vasoconstriction of the circumflex artery than did alpha-1 receptors. However, as was remarked upon, the pre- and post-junctional actions of alpha-2 receptors could not be separated in this study (See Discussion, Chapter VI).

It would appear that alpha-mediated vasoconstriction does not play a major role in altering blood flow to ischemic regions. Alpha vasoconstriction can be elicited by sympathetic stimulation, but only when the overriding influence of ß-mediated vasodilation is eliminated. The time course of stimulation used may have led to some conflicting results when compared to earlier studies. In Chapter VII, vasoconstriction in the presence of ß-blockade was readily elicited, but stimulations did not last for more than 90 seconds. This period of stimulation was comparable to that used in other studies demonstrating alpha-mediated vasoconstriction. However, in the first three studies presented, stellate stimulation was continued for much longer durations, 3-5 minutes before beginning microsphere injections. It is possible that alpha-vasoconstriction is transient and of insignificant importance during longer periods of sympathetic input. If alpha receptors do mediate metabolic increases in flow, however slight, then these effects may not be of apparent immediately. This would be of particular importance during the course of

ischemia, when regardless of whether alpha receptors mediate noticeable vasomotor effects, the possibility of alpha-1 mediated increases in metabolism may be enough to initiate significant myocardial damage.

It should not be disregarded that the open chest anesthetized preparation utilized in this dissertation does not facilitate the responses of adrenergic receptors to stimulation. Therefore the activity of alpha receptors may be of more importance in studies done in conscious animals.

In conclusion, I would like to put into writing a few closing comments on this area of research based on what I have found in my studies. I started this project with the concept that neural stimulation of alpha receptors would cause vasoconstriction, which would worsen the course of myocardial infarction. However, this rather simple assumption that alpha receptors vasoconstrict arteries during normal and abnormal conditions has evolved into an appreciation of the complex interactions between alpha-1 and alpha-2 receptors on blood flow and cardiac function.

I assumed that alpha receptor stimulation would decrease blood flow to an ischemic bed and that this could be eliminated by alpha-1 blockade or regional denervation. Until recently alpha-1 receptors were thought to be the primary mediator of vasoconstriction. This was not seen in our study, contrary to other studies, in part because the duration of stimulation was longer than those used by other investigators. Thus it would appear that if alpha receptors induce coronary vasoconstriction it is probably transient in nature.

However, if alpha receptors exert only transient influences on blood flow, this does not mean that they are a receptor of only inconsequential importance or interest. Our studies point toward the possible

role of alpha receptors in other aspects of neural regulation of the heart. It is possible that alpha receptors have influences on function and metabolism, but these effects have yet to be elucidated. Thus the concept of neural stimulation of alpha receptors causing coronary vasoconstriction does not appear to be as important as has been written in the literature. Instead, I have come around full circle, to stress the predominance of cardiac metabolism on blood flow. In this picture of blood flow regulation, the sympathetic nerves are even more important because of their complex influence on myocardial and vascular beta and alpha receptors.

APPENDIX A

RETROSPECTIVE ANALYSIS OF BLOOD FLOW

In order to check the accuracy of flows in the ischemic region, endocardial and epicardial samples from the same slice were combined to estimate flow retrospectively thus achieving sample weights of close to 1 gram.

Initial blood flow analysis:

	Counts/min				Weight	FLows (ml/min/gm)			
Sample#	CTRL	CAO	CAO-LSS	CAO-LSS-PRAZ	(gm)	CTRL	CAO	CAO LSS	CAO LSS-PRAZ
Endo									
5	2685	1746	710	451	.495	.549	.636	.507	.483
7	4374	969	500	726	.540	.819	.324	.328	.712
9	3981	1328	568	764	.587	.686	.408	.342	.690
Blood Flow.....						0.685	0.456	0.392	0.628
Epi									
6	5920	1818	779	846	.689	.869	.476	.400	.651
8	3545	2075	1009	577	.555	.646	.674	.643	.551
10	4621	2154	918	743	.720	.649	.539	.451	.547
Blood Flow.....						0.721	0.546	0.498	0.583

* Blood flows were initially calculated per sample as shown above. The flows were separated into endocardial and epicardial flows, for each of the four experimental conditions. The final value of blood flow used in the analysis of data represents the average of all the samples in the left ventricular ischemic zone.

Flows estimated retrospectively:

	Counts/min				Weight	Blood Flow (ml/min/gm)			
Sample#	CTRL	CAO	CAO-LSS	CAO-LSS-PRAZ	(gm)	CTRL	CAO	CAO-LSS	CAO-LSSPRAZ
endo									
5	2685	1746	710	451	0.50				
7	4374	969	500	726	0.54				
9	3981	1328	568	764	0.59				
Totals	11040	4043	1778	1941		1.62	.732	.478	.412 .674
epi									
6	5920	1818	779	846	0.69				
8	3545	2075	1009	577	0.56				
10	4621	2154	918	743	0.72				
Totals	14086	6047	2706	2166		1.96	.771	.590	.518 .621

**The samples of tissue in each slice of the left ventricular ischemic zone were first separated into endocardial and epicardial samples. The counts/min and weight of each sample were summed. Blood flow was calculated using the summed counts/min and weights for each slice. The final blood flow value for the left ventricular ischemic zone represented the average of the blood flow per slice of LV, not per sample of LV as described in the first section of the table.

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The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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