THE EFFECTS OF CORONARY α_1-ADRENERGIC STIMULATION ON CORONARY BLOOD FLOW AND LEFT VENTRICULAR FUNCTION

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Previous studies indicate that an α-adrenergic constrictor tone limits myocardial flow in exercising dogs and, as a result, imposes a limitation on myocardial contractile function. Study 1 examined whether an α-adrenergic constrictor tone varies with intensity of exercise. In 6 dogs, the specific α_1-adrenergic receptor blocker prazosin (or its vehicle) was infused into the circumflex artery during a submaximal exercise test. Removing the α_1-constrictor tone resulted in a greater increase in coronary flow at the 3 highest exercise workloads, which was associated with an increase in regional and global left ventricular contractile function. These studies indicated that a coronary vascular α_1-constrictor tone increases in magnitude with increased exercise intensity, and imposes a significant limitation on myocardial function.

In Study 2, the effects of coronary α_1-adrenergic blockade on left ventricular contractile function and regional myocardial perfusion were examined during

stimulation of the left stellate ganglion in anesthetized, open-chest dogs. Selective α_1-adrenergic receptor blockade using intracoronary prazosin during maximal stellate stimulation caused significant increases in global and subendocardial contractile function. No changes were observed in subepicardial function. Regional myocardial perfusion was measured using tracer microspheres. Prazosin increased both subepicardial and subendocardial perfusion equally. Thus, during sympathetic stimulation, a coronary α_1-constriction limits flow uniformly across the left ventricular wall. However, blockade of this constriction increases contractile function in the deeper muscle layers only.

Study 3 compared the effects of increasing coronary blood flow by removing α_1-constrictor tone using prazosin with the effects of increasing coronary flow by direct coronary vasodilation using intracoronary adenosine in exercising dogs. Similar increases in coronary flow were obtained with both agents. These increases in coronary flow were followed by significant increases in region and global left ventricular contractile function. These results suggest that myocardial contractile function may be flow-limited under conditions of submaximal exercise.

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CHAPTER I

INTRODUCTION

Regulation of coronary blood flow is tied to the nutrient need of the myocardium and is primarily due to local metabolic mechanisms. Additionally, many external factors are superimposed on the local regulatory mechanisms and can substantially modify coronary flow. These external influences include driving forces, resistive forces, and vasoactive forces.

Autonomic nerves, especially sympathetic nerves, are closely associated with the larger coronary vessels. The coronary vascular wall contains adrenergic receptors which are responsive to adrenergic agonists. It appears that the primary response of coronary vessels to adrenergic stimulation is vasoconstriction. It has been demonstrated that an adrenergic coronary vasoconstriction can compete with, and limit, local metabolic vasodilation (Mohrman and Feigl, 1978). The precise nature of the physiological function served by sympathetic modulation of myocardial perfusion is controversial. However, current literature

suggests that sympathetic vasoconstriction of the coronary vessels may, under certain conditions, impose an impediment to adequate supply of nutrition to the myocardium (Gwirtz et al, 1986; Strader et al, 1988).

Evidence indicates that, in a conscious resting subject, sympathetic stimulation of the heart and coronary vasculature is minimal or nonexistent (Chilian et al, 1981; Gwirtz et al, 1986). However, many conditions which cause an increased sympathetic stimulation of the heart are associated with coronary vasoconstriction. Thus, an α-adrenergic receptor mediated vasoconstriction imposing limitation to metabolic dilation has been demonstrated during conditions of hemorrhagic hypotension (Jones et al, 1983), partial coronary stenosis (Heusch and Deussen, 1983, Jones et al, 1986), stellate ganglion stimulation (Heusch and Deussen, 1983; Giudicelli et al, 1980), and exercise (Bache et al, 1987; Gwirtz and Stone, 1981; Gwirtz et al, 1986; Heyndrickx et al, 1982; Heyndrickx et al, 1984; Huang and Feigl, 1988, Murray and Vatner, 1979, Strader et al, 1988; Dai et al, 1989). These studies indicate that many perturbations which result in generalized sympathetic adjustments of the cardiovascular system also result in sympathetic stimulation of the coronary vasculature, resulting in vasoconstriction. Because of the potential importance of this adrenergic effect, further evaluation of

this coronary α-adrenergic constrictor tone and its influence upon the heart is warranted. Studies were designed to examine the following hypotheses:

1) An adrenergic coronary constriction mediated by α_1-adrenergic receptors persists in the presence of physiologic quantities of metabolic dilators.

2) A specific α_1-adrenergic receptor mediated coronary constriction varies with intensity of exercise. During low levels of physical stress, this α_1-adrenergic tone acts to shunt blood to the subendocardium, where energy demands are greatest. By shunting blood toward the subendocardium, the α_1-adrenergic tone improves subendocardial function without compromising subepicardial function. As a result, global contractile function is improved by α_1-adrenergic coronary tone during low levels of physical stress. At higher levels of physical stress, this α_1-adrenergic tone restricts total transmural flow to the point that the transmural energy supply:demand ratio is deleteriously effected. Under these conditions, blockade of the α_1-adrenergic coronary tone increases flow and improves performance of all layers of the ventricle.

3) The changes in contractile function which result from α_1-adrenergic blockade are an effect of the blood flow alterations, and not a manifestation of an intrinsic effect of the vasodilator used.

Three studies were performed to test these three hypotheses.

STUDY 1: An α_1-Adrenergic Coronary Constrictor Tone Varies With Exercise Intensity.

The degree of coronary vasodilation during any condition is determined by the balance between vasodilatory and vasoconstrictor influences. An α-adrenergic vasoconstrictor tone is a major factor opposing metabolic vasodilation. Previous studies have only examined the influence of an α-adrenergic constrictor tone on coronary flow and ventricular performance at strenuous levels of exercise. At these levels, the restriction of coronary vasodilation is associated with a limitation of myocardial contractile function in the left ventricular subendocardium (Gwirtz et al, 1986; Strader et al, 1988). The aims of this study were to examine the hypothesis that a specific α_1-adrenergic receptor mediated coronary constrictor tone varies with intensity of exercise. This study determined the presence of an α_1-adrenergic coronary constrictor tone at various intensities of submaximal exercise. The expression of this constrictor tone was evaluated by its effect both on

coronary flow and on left ventricular contractile performance. If a constrictor tone was present, we distinguished whether its influence on contractile function was unidirectional (i.e. consistently improves or attenuates contractile function at all levels of physical stress), or whether its effect on function was bidirectional (i.e., improved contractile function at low levels of stress and was associated with attenuated contractile function only at high levels of stress).

STUDY 2: Effects of a Coronary α_1-Constrictor Tone on Transmural Left Ventricular Flow and Contractile Function During Left Stellate Ganglion Stimulation.

Previous studies have suggested that an α_1-adrenergic constrictor tone during exercise limits myocardial perfusion and oxygen delivery, and therefore may also limit myocardial contractile function (Gwirtz et al, 1986; Strader et al, 1988). In these studies, only regional left ventricular subendocardial contractile function was measured. No analysis of regional contractility in the more superficial layers was made. Thus, earlier studies were unable to evaluate the possibility of a differential effect of the α-adrenergic constrictor tone on contractility throughout the various layers of the heart. In addition, no evaluation regarding the transmural nature of the α-adrenergic

constrictor tone was made. Studies in the literature conflict with regard to the transmural distribution of a coronary α-adrenergic tone (Johannsen et al, 1982; Buffington and Feigl, 1983; Giudicelli et al,1980). The aims of this study were two-fold. First, to examine the possibility of a differential effect of an α-adrenergic constrictor tone on left ventricular subendocardial vs subepicardial contractile function during cardiac sympathetic stimulation. To accomplish this, contractile function of the left ventricular subendocardial and subepicardial layers were each evaluated independently using a pair of 5 MHz piezoelectric crystals placed in each layer. Measurements were taken at rest and during stimulation of the left stellate ganglion. Second, we determined if any differential effects of α_1-vasoconstriction on contractile function in subendocardial and subepicardial layers are associated with a nonuniform influence of this vasoconstriction on perfusion across the ventricular wall. For this purpose, transmural coronary blood flow was measured using tracer microspheres at rest and during left stellate ganglion stimulation. The effects of left stellate ganglion stimulation on the left ventricular wall contractile function gradient and flow gradient were then correlated to determine if any changes in the transmural

flow gradient were associated with parallel changes in the transmural myocardial contractile function gradient.

STUDY 3: Augmentation of Coronary Blood Flow Improves Myocardial Contractile Function During Exercise.

Previous studies suggest that removal of a coronary α-adrenergic constrictor tone during exercise results in a substantial increase in both coronary blood flow and myocardial contractile function (Gwirtz et al, 1986; Strader et al, 1988; Dai et al, 1989). These data suggest that an α-adrenergic constrictor tone limits myocardial perfusion and oxygen delivery in the exercising dog and, as a result, is associated with a limitation to myocardial contractile function. Study 3 examined whether the increase in contractile function following α_1-adrenergic blockade is due to an increase in myocardial perfusion or to some direct myocardial action of the α_1-adrenergic antagonist. For this purpose, the effects of α-adrenergic blockade on coronary flow and myocardial function during exercise was compared to those observed during direct coronary vasodilation with adenosine.

CHAPTER II

REVIEW OF RELATED LITERATURE

A. Local Control of Coronary Blood Flow - Metabolic Factors

Coronary blood flow is dependent on driving force, resisting forces, and vasoactive forces. Driving force equals aortic root pressure unless a stenotic lesion imposes a resistance after entrance into the left or right coronary artery. Resisting forces are intramyocardial stresses compressing the vasculature and increasing resistance. Vasoactive forces are metabolic, myogenic, and neural influences which manipulate the degree of coronary vascular dilation.

Investigation of a metabolite-dependent alteration of coronary vascular tone results from the finding of a high degree of covariance between coronary flow and myocardial oxygen utilization (Khouri et al, 1965). These findings suggest that, as a result of an oxygen supply inadequate to accommodate the needs of either the working myocardium or of the vasculature supplying it, a change occurs in the local milieu of the involved vasculature. This change in local milieu, then, results in a relaxation of the affected vasculature, an increase in blood flow to the working myocardium, and an alleviation of the discrepancy between

myocardial oxygen demands and oxygen supply. The greater the initial discrepancy between oxygen supply and oxygen demand, the greater the change in local milieu, and the greater the influence tending toward a relaxation of the coronary vasculature.

The local milieu of the myocardium or its vasculature can change by the addition and/or removal of some factor. The factor whose local concentration changes in this situation is often referred to as the metabolic dilator. Berne (1980) proposed the following criteria for establishing a compound to be a metabolic dilator: (a) the substance must have intrinsic activity as a dilator on resistance vessels; (b) the substance must be produced locally in a quantity sufficient to have a vasodilatory effect; (c) the substance must be available to the resistance vessels after its production; (d) the effect of the substance must be reproducible with an exogenous analogue; and (e) the effect of the substance must be reproducible when normal breakdown of the substance is interrupted. Using these criteria, many substances have been proposed as possible metabolic dilators. These include decreased oxygen tension, carbon dioxide, acetate, potassium, adenosine, prostaglandins, and fluctuations in osmolality. Though some are more attractive candidates than

others, none of these fully satisfies all of Berne's criteria.

In this regard, decreased oxygen tension has been hypothesized to be a metabolic dilator because of the inverse relationship between oxygen supply and degree of metabolic dilation (Khouri et al, 1965). It can be reasoned, though, that in order for low oxygen tension to have a direct vasodilatory effect capable of preventing hypoxic damage to the myocyte, the precapillary sphincter must be more sensitive than the myocyte to low oxygen tension (Olsson, 1981). This has not been proven or disproved. However, studies in large vessels show that the vasodilatory effect of hypoxia is probably not manifested until reaching oxygen tensions lower than those likely to be found in the functioning heart (Gellai et al, 1973). Additionally, the use of cyanide to block cytochrome α_3-receptor, (which is most likely the receptor stimulated by oxygen when present) does not result in vasodilation to the degree expected under conditions of a functional anoxia (Coburn, 1977).

Similarly, increased local concentrations of carbon dioxide have been proposed to act as metabolic dilators. Carbon dioxide, a metabolic byproduct of the tricarboxylic acid (TCA) cycle and aerobic metabolism, is a highly diffusible compound whose presence has been shown to be related to increased blood flow (Feigl, 1983). However, no

studies exist relating endogenous carbon dioxide to blood flow. Acetate, an intermediate in the glycolytic pathway, has been shown to have vasodilator effects (Liang and Lowenstein, 1978; Molnar et al, 1962). Nevertheless, the mechanism mediating this vasodilation is unclear, and it is questionable whether local acetate concentrations ever rise to the millimolar levels required to produce vasodilation as shown in previous studies (Olsson and Bugni, 1986). Potassium is another compound found to increase flow when infused intracoronarily (Bugner et al, 1976; Scott et al, 1961). It leaves the myocyte during contraction, and its extracellular concentration is therefore directly related to heart rate. In this regard, its extracellular concentration would increase during tachycardia, when oxygen requirements are greater. However, there is question as to the ability of high concentrations of extracellular potassium to maintain coronary vasodilation for other than very short durations (Murray et al, 1979). Those investigators supporting prostaglandins as having a role in coronary vasodilation cite powerful coronary vasorelaxant effects in vitro (Needleman et al, 1975). Still, no studies can show any relationship between tissue prostaglandin levels and coronary resistance. Likewise, coronary vasomotion is not affected by prostaglandin synthesis inhibitors (Hintze and Kaley, 1977).

Another possible metabolic vasodilator is adenosine. This highly diffusible compound is a breakdown product of adenosine monophosphate (AMP), and there is evidence that the 5'-nucleotidase required to catalyze this reaction exists in the mitochondria (Bukoski et al, 1984), where 90% of cardiac AMP is found (Bunger et al, 1983; Sobol and Bunger, 1981). The vasorelaxing properties of adenosine have long been recognized (Drury and Szent-Gyorgyi, 1929). It is now apparent that this vasoactive effect is not endothelium dependent (Furchgott, 1984), but rather mediated through specific adenosine receptors (Olsson et al, 1976; Schrader et al, 1977). Still, undeniable demonstration that adenosine is a physiologically significant metabolic dilator suffers from the limitations in studies directly measuring interstitial concentrations of adenosine and demonstrating that these concentrations change directly with degree of muscle use. (Discussion of adenosine receptors in presented in Appendix C).

B. Local Control of Coronary Blood Flow - Myogenic Factors

Myogenic tone refers to the intrinsic ability of all smooth muscle to contract in response to stretch. The stimulus for this tone appears to be the transmural pressure gradient developed across the vascular smooth muscle (Olsson and Bugni, 1986), and the strength of the myogenic tone is inversely related to the amount of extracellular calcium

available for entrance into the smooth muscle cell (Harder, 1984). Additionally, this myogenic tone on coronary vessels can be minimized by a combination of high dose adenosine and the non-selective α-adrenergic blocker phentolamine (Vlahakes et al, 1982).

Autoregulation, or the ability of a vascular bed to adjust resistance in order to maintain a constant blood flow in the face of changing perfusion pressure, is a manifestation of vascular myogenic tone. In their review, Olsson and Bugni (1986) refer to the capacity of the coronary circulation exhibit autoregulation between perfusion pressures of 60 mmHg and 140 mmHg . Thus, these authors feel that myogenic tone is a major contributor to coronary tone in the resting state (Olsson and Bugni, 1986).

C. Neural Factors Modulating Coronary Blood Flow

Another modulatory influence on coronary blood flow is the autonomic nervous system. Autonomic nerves especially sympathetic nerves, are closely associated with the large conduit vessels as well as with the smaller coronary resistance vessels (Dolezel et al, 1978). The coronary vascular smooth muscle contains adrenergic receptors that are responsive to adrenergic agents. The primary response of the coronary vasculature to adrenergic stimulation is one of vasoconstriction. It has been clearly demonstrated by Mohrman and Feigl (1978) that an adrenergic coronary

constriction may compete with, and limit, local metabolic vasodilation.

Although the existence of a sympathetic coronary vasoconstrictor tone has long been recognized, the quantitative influence of this constrictor tone relative to vasodilatory forces has remained unclear. In this regard, Brachfeld et al (1960) demonstrated that left pericoronary neurectomy increased left coronary blood flow by 13% and decreased left ventricular oxygen extraction by 21% in open chest, pentobarbital-anesthetized dogs. Coronary sympathectomy achieved by intracoronary injection of 6-hydroxydopamine has been shown to increase left ventricular myocardial flow by as much as 64% in resting dogs (Holtz et al, 1977). Schwartz and Stone (1977) showed that left stellate ganglionectomy increases the reactive hyperemic response to a 10-second occlusion of the left circumflex artery in conscious dogs at rest. They also showed that an increase in reactive hyperemia similar to that produced by left stellate ganglionectomy could be achieved in stellate-intact dogs by administration of phentolamine. Furthermore, phentolamine administration had no effect upon reactive hyperemia in resting dogs following removal of the left stellate ganglion. Finally, Drake et al (1978) reported that cardiac denervation by extrinsic regional neural ablation increased both oxygen extraction and lactate utilization in

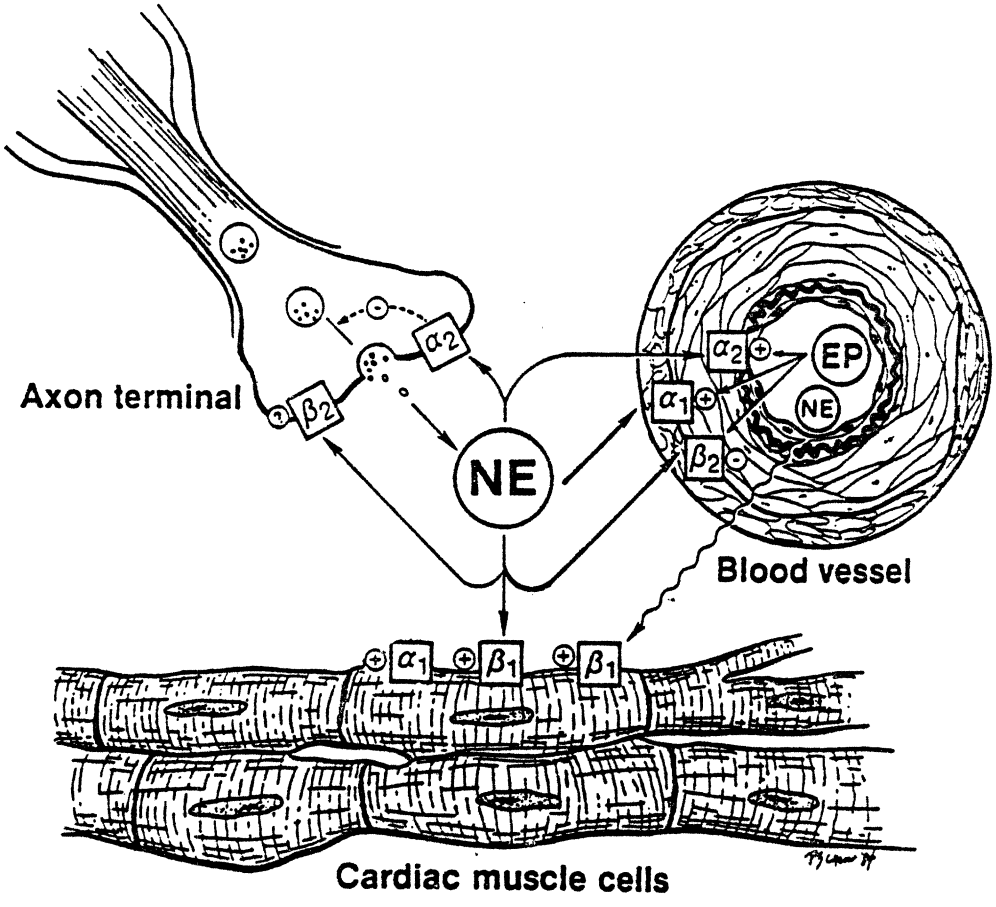
anesthetized dogs. This indicated that sympathetic tone may actually impede the ability of the myocardium to produce mechanical energy.

D. Vascular α-Adrenergic Receptors

The pre- and postsynaptic vascular α-adrenergic receptors consist of two major subtypes, the α_1- and the α_2-receptor. This classification has been based on the vascular response to α_1-receptor activation or inhibition by specific pharmacological agents. The location of these receptor subtypes in the neuroeffector junction are schematically depicted in Figure 1. Thus, α-adrenergic receptors are found postsynaptically, in the membranes of cardiac muscle cells and coronary blood vessels (Langer et al, 1985). However, the α_2-adrenergic receptors are found postsynaptically in the membrane of the coronary smooth muscle cells but not in the membrane of the cardiac muscle cell (Langer et al, 1985). In addition, the α_2-adrenergic receptors are found presynaptically in the membrane of the axon nerve terminal (Langer et al, 1985). The α-adrenergic receptor subtypes are integral membrane proteins and activation of either subtype on the coronary vessel results in an increase vascular smooth muscle constriction. (Langer et al, 1985). Activation of α_1-adrenergic receptors on the cardiac muscle cell results in a positive inotropic effect, and activation of presynaptic α_2-adrenergic receptors on the axon nerve

Figure 1 - Neuroeffector Junction

Figure 1 depicts the location of the α_1- and α_2-adrenergic receptors at the neuroeffector junction. The α_1-adrenergic receptors are found postsynaptically at the adventitia-media border of the coronary vessel and on the cardiac muscle cell. The α_2-adrenergic receptors are found presynaptically on the axon terminal and postsynaptically on the media-intima border of the coronary vessel.


Axon terminal
α2
β2
NE
α2
EP
α1
NE
β2
Blood vessel
α1
β1
β1
Cardiac muscle cells

terminal inhibits further release of norepinephrine from that nerve terminal (Langer et al, 1985).

The signal transduction cascade activated by the stimulation of these two receptor types have also been found to be different. Activation of α_1-adrenergic receptors initiates degradation of membrane phospholipids resulting in: a) the activation by diacylglycerol of a protein kinase, PKC, which both mediates an influx of extracellular calcium through cellular membrane channels and phsoporylates myosin light chains to directly produce contraction; b) an increase in intracellular myoinositol-1,4,5-triphosphate which mediates the release of calcium from an intracellular store; and c) activation by high intracellular calcium concentration of myosin light chain kinase, with resultant phosphorylation of intracellular myosin and the promotion of muscle contraction (Graham and Lanier, 1986). In contrast, activation of α_2-adrenergic receptors results in: a) a decrease in the activation of adenyl cyclase by a G-protein mediated process with resultant decreased hydrolysis of intracellular ATP to cAMP; b) increased permeability of membrane calcium channels probably via a G-protein mediated process involving activation of sodium ion/hydrogen ion exchange (leading to intracellular alkalinization) (Insel, 1989).

Many investigators have recently devoted much effort to characterizing the nature of an α-adrenergic coronary constrictor tone in the left ventricle. Initially, controversy existed over which α-adrenergic subtype is responsible for the coronary vasoconstriction during adrenergic stimulation. Some researchers using exogenous agonists have provided evidence for the existence of both α-receptor subtypes in large coronary arteries (Young et al, 1988) and small resistance vessels (Woodman and Vatner, 1987). Others have reported that only the α_2-receptor subtype mediated a coronary constriction during cardiac nerve stimulation (Heusch and Deussen, 1983). Still others have reported that the α_1-receptor subtype predominated in the large coronary arteries and that the α_2-subtype predominated in the smaller resistance vessels (Heusch et al, 1984). Finally, more recent research has indicated that during exercise or myocardial ischemia, only the α_1-subtype is responsible for the observed coronary adrenergic constriction (Liang and Jones, 1985; Gwirtz et al, 1986; Strader et al, 1988; Laxson et al, 1989; Dai et al, 1989). The distinction between the roles of α_1- and α_2-receptor subtypes in mediating adrenergic coronary constriction is of obvious pharmacological and physiological importance. However, the results of a multitude of animal studies remain dilemmatic.

Some clarification of the role of each α-receptor subtype in mediating coronary constriction may be found in the recent studies showing that one α-receptor subtype (α_1) is found on one surface of the vessel, and that the other α-receptor subtype (α_2) is found on the luminal surface of the vessel. The α-receptors near the media-adventitia border are primarily sensitive to specific α_1-adrenergic agonists (Langer et al, 1985). As a result, activation of these α_1-adrenergic receptors results in the release of intracellular stores of calcium, and their function is only partially dependent upon extracellular calcium (Langer and Shepperson, 1982). In addition, the α-adrenergic receptors at the media-adventitia border are anatomically more proximal to catecholamines released from sympathetic nerves terminating on the adventitial surface of vessels than are α-adrenergic receptors located at the media-intima border of the coronary vessels. In contrast, the α-adrenergic receptors near the media-intima border are primarily activated by α_2-adrenergic agents (Langer et al, 1985). Activation of these α_2-adrenergic receptors does not result in the release of intracellular calcium stores, and the response to activation of these receptors is totally dependent upon the availability of calcium from the extracellular medium (Langer and Shepperson, 1982). The α_2-adrenergic receptors located at the media-intima border are primarily responsive

to circulating catecholamines (Langer and Shepperson, 1982), and pharmacologic methods of adrenergic receptor activation may better represent the effects of hormonal receptor stimulation. In contrast, physiologic stress activates both hormonal and neural receptors. Thus, compared to the postsynaptic α-adrenergic receptors on the media-adventitial border of coronary vascular smooth muscle, the postsynaptic adrenergic receptors on the smooth muscle along the media-intima border may be stimulated by different mechanisms and by different pharmacological agonists, function by a different receptor-coupling system, and subserve different functions (Langer and Shepperson, 1982; Langer et al, 1985).

It is also known that α_2-receptors are located presynaptically in sympathetic nerve terminals. These receptors may be involved in a negative-feedback loop. It appears that their activation by neurally released norepinephrine attenuates further release of the sympathetic neurotransmitter (Shepherd and Vanhoutte, 1985). This negative feedback mechanism for modulation of norepinephrine release by sympathetic nerve terminals may be of importance in many physiological and pathological conditions, but it is also a complicating factor in studying the vascular roles of α-adrenergic receptors. In this regard, it is recognized that the use of α-adrenergic receptor antagonists, especially α_2-adrenergic receptor antagonists, in the intact

experimental animal may elicit coronary vasodilation by a direct mechanism as well as by an indirect mechanism. The direct mechanism involves inhibition of the vasoconstrictor action of the postsynaptic receptor. The indirect mechanism involves inhibition of the presynaptic receptor. This results in an increase in the neural release of norepinephrine. This increased norepinephrine may then cause an increase in the activation of myocardial β-adrenergic receptors with a resulting increase in cardiac activity and oxygen demand. In turn, the increases in cardiac activity and oxygen demand will elicit coronary vasodilation by local metabolic mechanisms.

E. α-Adrenergic Coronary Vasoconstrictor Tone at Rest

Evidence indicates that in the conscious, quiescent subject, sympathetic stimulation of the heart and coronary vasculature is low or nonexistent. Schwartz and Stone (1977) reported that nonspecific α-adrenergic blockade with phentolamine did not change mean coronary blood flow in seven conscious dogs. Likewise, in a sophisticated set of experiments, Chilian et al (1981) used topically applied phenol to chemically sympathectomize a portion of the left ventricle, while innervation to the remainder of the ventricle remained intact. Tracer microspheres were used to measure regional left ventricular blood flow while the dogs were in the conscious resting state. No differences in

perfusion of the sympathectomized and innervated regions were observed, and the subendocardial-to-subepicardial blood flow ratio was not different between the two regions. Similarly, Gwirtz et al (1986) observed that in conscious instrumented dogs trained to lie quietly, intracoronary administration of the specific α_1-adrenergic antagonist prazosin, at a dose which abolished the constrictor response to phenylephrine, cause no observable changes in coronary inflow.

In contrast, Murray and Vatner (1979) noted that nonspecific α-adrenergic blockade with phentolamine resulted in an increase of approximately 33% in mean left circumflex blood flow in dogs resting prior to exercising. These contradictory results may be explained by a certain degree of anxiety in the dogs anticipating their imminent run. In this regard, psychological stress has been associated with sympathetic stimulation of the heart and coronary circulation (Billman and Randall, 1981). Specifically, evidence exists that dogs anticipating exercise can demonstrate splenic contracture during the rest period prior to a run (Ordway et al, 1984).

F. α-Adrenergic Coronary Vasoconstrictor Tone During Pathological Cardiac Stress

Although there may be little sympathetic constriction of the coronary circulation at rest, a multitude of factors

which increase sympathetic stimulation of the heart are associated with increased adrenergic coronary constriction. For example, Mohrman and Feigl (1978) found that cardiac sympathetic activation by intracoronary norepinephrine infusion led to an increase in oxygen delivery resulting from the combined effects of three factors: a) a minimal increase in coronary blood flow; b) an increase in myocardial oxygen extraction; and c) a decrease in coronary venous oxygen content. When preceded by either systemic α-adrenergic receptor blockade using dibozane or intracoronary α-receptor blockade using phenoxybenzamine, the same degree of cardiac sympathetic activation increased oxygen delivery by an additional 30%. In this model, the improved oxygenation resulted primarily from a greater coronary dilation with minimal alteration in myocardial oxygen extraction or coronary venous oxygen content. These studies were performed in anesthetized dogs under constant perfusion pressure of the left coronary artery.

An adrenergic coronary constriction exists in the anesthetized, open-chest preparation as well. Thus, Brachfeld et al (1960) demonstrated that left pericoronary neurectomy increased left coronary blood flow by 13% in pentobarbital-anesthetized dogs. The elevated heart rate of these dogs suggests that the conditions of this experiment did not accurately depict the basal state. Similarly, Jones

et al (1987) observed that intracoronary administration of prazosin led to a significant increase in coronary inflow when the coronary perfusion pressure was 100 mmHg. A combination of morphine sulfate and α-chloralose was used as anesthesia.

A variety of specific cardiovascular reflexes also have a significant effect on the coronary vasculature. For example, the arterial baroreflex, which is initiated by a decrease in arterial blood pressure and which is associated with vasoconstriction in many vascular beds, elicits an adrenergic coronary constriction (Feigl, 1968). When the sympathetic nervous system was activated by carotid sinus stimulation (carotid artery occlusion), α-receptor blockade was found to increase oxygen delivery by causing coronary vasodilation with minimal alteration in myocardial oxygen extraction or coronary venous oxygen content (Mohrman and Feigl, 1978). These studies were performed in anesthetized dogs under constant pressure perfusion of the left coronary artery. Other investigators also found that carotid sinus stimulation will increase coronary vascular resistance by 21% in the unblocked circulation, but by only 5% after α-adrenergic receptor blockade using intracoronary dibozane (Powell and Feigl, 1979). These findings and those of others (Ely et al, 1981) suggest a role of α-adrenergic receptor activation in limiting the coronary vasodilation associated

with reflex activation of the sympathetic nervous system by carotid sinus stimulation. An α-adrenergic constriction has also been observed to compete with vasodilatory influences when sympathetic stimulation is caused by hemorrhagic hypotension (Carlson et al, 1976; Birinyi et al, 1977; Jones et al, 1983), partial coronary stenosis (Buffington and Feigl, 1981; Heusch and Deussen, 1983), and coronary hypotension (Liang and Jones, 1985; Jones et al, 1987).

The chemoreflex may also have coronary effects. Researchers have noted that, during arterial hypoxemia, an adrenergic constriction exists much like that seen with the baroreflex. In a complex experimental protocol, Williams et al (1988) perfused the coronary circulation of dogs with normoxic blood while producing systemic hypoxemia by respiring the animals with low oxygen gas mixtures. Following β-adrenergic blockade to prevent a reflex increase in cardiac activity and oxygen demand, peripheral hypoxemia produced a significant reduction in coronary inflow of 20%. This reflex reduction in coronary inflow was totally abolished by intracoronary administration of prazosin, indicating that it was mediated by α_1-adrenergic receptors.

Kelley and Feigl (1978) examined the effects of α-adrenergic receptor activation on the coronary circulation caused by left stellate ganglion stimulation in dogs while minimizing changes in other factors known to control

coronary flow. Thus, during conditions of β-adrenergic receptor and parasympathetic blockade (to minimize changes in contractility, heart rate, myocardial tension development), electrical stimulation of the left stellate ganglion decreased coronary flow and increased coronary vascular resistance in anesthetized, open-chest dogs. Similarly, Giudicelli et al (1980) found that left stellate ganglion stimulation increased coronary flow in open chest dogs with an intact adrenergic nervous system, decreases coronary flow in dogs after β-adrenergic blockade, and has no effect on coronary flow in dogs after combined α- and β-adrenergic receptor blockade. These studies suggested that sympathetic stimulation activated α-adrenergic receptors which mediate a direct coronary vasoconstriction and restricted metabolic vasodilation. When carotid chemoreceptor stimulation by intra-carotid nicotine administration was used to activate cardiac sympathetic nerves, a bidirectional coronary response was seen whereby an initial vasodilation (mediated by cholinergic activation) was followed by a phase of reduced coronary blood flow, increased coronary vascular resistance, and reduced coronary sinus oxygen content (Vatner, 1983). This late phase coronary vasoconstriction was attenuated in dogs with controlled respirations by either total cardiac denervation or by adrenalectomy (Murray et al, 1984). It was also

abolished by intravenous administration of phentolamine (Murray et al, 1984). Similarly, Williams et al (1988) demonstrated that intracoronary prazosin will prevent the coronary vasoconstriction which follows carotid chemoreceptor stimulation from systemic hypoxia. In this study, the coronary vasculature was perfused with normoxic blood, left ventricular afterload was held constant, propranolol and atropine were used to block vasodilatory and chronotropic changes of β-adrenergic receptor and parasympathetic etiology, respectively.

G. a-Adrenergic Coronary Vasoconstrictor Tone During Physiologic Cardiac Stress: Exercise

The studies described above suggested the existence of a sympathetic-mediated coronary constrictor tone mediated by vascular α-adrenergic receptors. Other studies have been directed at examining the influence of this constrictor tone during the physiologic stress of exercise. During exercise, the work output of the normal heart may increase several-fold. Intimately involved in this increased pumping capacity of the heart is a sympathetic stimulation which increases both heart rate and the inotropic state of the myocardium. Consequent to the increased work output of the heart, myocardial oxygen and blood flow demands increase. Because of local metabolic influences on the coronary circulation, coronary resistance vessels undergo a substantial dilation,

and coronary blood flow may increase by as much as five-fold or more during maximal exercise. Due to the substantial increase in coronary blood flow observed during exercise, and because coronary flow is closely correlated with myocardial oxygen consumption over a wide range of oxygen consumptions, it has long been felt that adrenergic constrictor influences on the coronary circulation during exercise are relatively unimportant and may actually be overridden by the more powerful local metabolic mechanisms. However, adrenergic constrictor influences may increase concomitantly with the local metabolic dilatory influences during exercise. With this in mind, it becomes easier to appreciate that an adrenergic coronary constrictor tone may exist which limits the increase in coronary blood flow during exercise.

In this regard, Murray and Vatner (1979) found a decrease in diastolic coronary resistance after systemic administration of phentolamine to running dogs. This effect persisted after systemic β-adrenergic blockade (propranolol), suggesting little likelihood that the reduction in coronary resistance resulted from increased myocardial metabolic demand (associated with a raised level of circulating catecholamines after blockade of autocrine presynaptic α_2-adrenergic receptors). Furthermore, others have demonstrated that systemic phentolamine administration

can: 1) increase coronary flow at any given myocardial oxygen consumption ($M\dot{V}O_2$) (Gwirtz and Stone, 1980); 2) increase coronary sinus O_2 at any given $M\dot{V}O_2$ (Bache et al, 1987); and 3) increase coronary blood flow without lowering myocardial oxygen extraction during exercise (Heyndrickx et al, 1982; Gwirtz et al, 1986). Altogether, these studies demonstrated that a coronary vascular sympathetic constrictor tone may actually limit oxygen delivery to the heart during exercise in the normal dog.

H. Transmural Nature of the Coronary a-Adrenergic Tone

While the above studies strongly suggest the existence of an α-adrenergic receptor mediated vasoconstriction which modulates flow during conditions of sympathetic activation, its effect upon contractile function is unclear. Teleologically, the existence of a vasoconstrictor tone during sympathetic stimulation could be justified if it were to more efficiently distribute the limited coronary flow to regions of the heart most prone to ischemia. In this regard, a sympathetic vasoconstriction may act to shunt blood to the subendocardial layer of the heart under conditions when blood flow and energy supply are theoretically in greatest demand. The subendocardial myocytes contract more completely and from a shorter initial starting length than do the subepicardial myocytes (Stroebec et al, 1986). This limitation to the Starling forces in the subendocardium may

result in a greater energy demand per unit force development by these cells when compared to the subepicardial myocytes. In addition, an increasing systolic pressure gradient from subepicardial to subendocardial layers restricts subendocardial perfusion to the diastolic phase of each cardiac cycle (Stein et al, 1980). This is not the case in the subepicardial layers, where blood flow perfusion can continue during systole (Olsson and Bugni, 1986). This implies that as heart rate increases and time spent in diastole decreases, subendocardial perfusion time may become compromised. In spite of the relatively limited perfusion time of the subendocardium, the combination of a reversal of this transmural pressure gradient during diastole (Stein, 1980) along with a preferential dilation of the subendocardial vessels during diastole, results in an subendocardial-to-subepicardial flow ratio of approximately 1.25:1 averaged throughout the entire cardiac cycle in the resting normal heart (Braunwald and Sobel, 1988). However, the vasodilatory reserve of the subendocardium is less than that of the subepicardium (Braunwald and Sobel, 1988). This limited vasodilatory reserve of the subendocardium compared to the subepicardium, in combination with a decreased time for subendocardial perfusion as duration of diastole is shortened by tachycardia, may cause the preferential subendocardial flow seen at normal heart rates to be altered

as heart rate increases (Braunwald and Sobel, 1988). Thus, a constrictor tone could more efficiently distribute blood flow across the ventricular wall during conditions of sympathetic stimulation by shunting blood toward the subendocardium (where energy demands are greater and perfusion times are limited). A vital role for this vasoconstrictor tone during conditions of tachycardia or sympathetic stress would be obvious if it improved subendocardial function with minimal or no compromise to subepicardial performance.

Thus, the possibility that coronary α-adrenergic receptors are more densely concentrated in the subepicardial layers was demonstrated by Nathan and Feigl (1986). In open-chest, anesthetized dogs, two regions of the left ventricle were perfused separately. In one region, α-receptors were blocked with the nonspecific antagonist phenoxybenzamine. Norepinephrine was infused into both regions of the ventricle while coronary inflow to both regions was reduced progressively from 100% to 50% or normal. Regional flows in each region were measured using tracer microspheres. It was observed that at low coronary flow levels, subendocardial perfusion was greater in the ventricular region in which α-receptors were intact. These results are consistent with those of Giudicelli et al (1980) who reported a greater α-adrenergic vasoconstriction in the subepicardium during

sympathetic nerve stimulation of β-adrenergic blocked, nonischemic hearts. In addition, Johannsen et al (1982) reported that during maximal adenosine-induced coronary dilation following β-adrenergic blockade in dogs, a condition which may simulate the high metabolic vasodilatory signal thought to exist in myocardial ischemia, sympathetic nerve stimulation caused vasoconstriction in the subepicardium only.

Few studies have examined the transmural distribution of the coronary adrenergic constrictor tone during exercise. Huang and Feigl (1988) proposed that the adrenergic coronary constriction is not uniform across the ventricular wall but may be more intense in the subepicardial muscle layers and, as a result, divert blood flow to the more vulnerable deeper layers during exercise. To test this hypothesis in dogs, these investigators selectively blocked α-receptors in one myocardial region using the nonspecific antagonist phenoxybenzamine, and measured regional myocardial perfusion using microspheres during graded treadmill exercise. They observed that average transmural flow was less in the unblocked region than in the blocked region. This would be expected from the results of other investigators. However, they also observed that the ratio of subendocardial-to-subepicardial perfusion was better maintained in the region in which α-receptors were intact. Although the mechanism for

the apparent preservation of uniform transmural flow by α-adrenergic coronary constriction is uncertain, it may provide a physiological basis for this constriction. It should be noted, however, that no evidence exists for a nonuniform distribution of α_1-adrenergic receptors in the left ventricular coronary vessels. In this regard, Saffitz (1989) using receptor binding techniques found no difference in the density of α_1-receptors between subepicardial and subendocardial coronary arterioles. Recognizing the absence of data indicating a nonuniform distribution of α-receptors across the ventricular wall, Huang and Feigl (1988) suggested the possibility that the endogenous vasodilator adenosine is released from myocardial myocytes preferentially in the deeper layers, where contractile function may be more flow-limited. Since adenosine is known to attenuate release of norepinephrine from sympathetic nerve terminals, it is then feasible that neuronal release of norepinephrine during exercise is less in subendocardial layers than in subepicardial layers. Such a mechanism would explain a greater adrenergic vasoconstriction in subepicardial layers. A similar nonuniform adrenergic constriction across the ventricular wall has also been reported by Chilian and Ackell (1986) during exercise in dogs with a coronary stenosis.

Experiments by others suggested a more uniform transmural distribution of the α-adrenergic constrictor effect in the coronary vasculature. The idea that there was a uniform distribution of coronary α-adrenergic receptors was introduced when Holtz et al. (1977) reported no change in transmural distribution following chemical sympathectomy with 6-hydroxydompamine. Johannsen et al (1982) measured coronary flow with labeled microspheres and observed that intracoronary phenylephrine did not alter the diminished subendocardial-to-subepicardial flow ratio resulting from intravenous adenosine infusion. Furthermore, Rinkema et al (1982) saw no change in transmural distribution of flow during stellate ganglion stimulation. The potential for a uniform transmural distribution of receptors was also demonstrated in studies by Buffington and Feigl (1983). Using 9 μm microspheres to measure regional coronary blood flow in an open chest preparation at a perfusion pressure of 100 mmHg and during conditions of intracoronary norepinephrine infusion, they found that intracoronary phenoxybenzamine improved coronary flow equally in all layers of the left ventricle. Similarly, Chen et al (1988) noted no transmural redistribution of myocardial perfusion after left circumflex artery perfusion with either the α_1-agonist phenylephrine or the α_2-agonist BHT-933. Likewise, Williams et al (1988) showed that an α_1-adrenergic receptor

mediated coronary vasoconstrictor reflex caused by acute systemic hypoxemia was uniform across the left ventricular free wall. As mentioned above, Saffitz (1989) used light microscopic autoradiography of radioligand binding sites ([H^3]-prazosin) and found no difference in the density of α_1-receptors between coronary arterioles of the subepicardium and those of the subendocardium in the cat ventricle. However, no studies were performed of the functional significance or of the potential for these α_1-receptors to initiate Ca^{2+} flux and inositol phospholipid turnover in this experiment.

I. Rationale for Specific Studies

Few studies have examined the question of whether ventricular function is affected by the coronary vascular constrictor response to sympathetic neural activation. It has been suggested by Gwirtz et al (1986) and Strader et al (1988) that a coronary α_1-adrenergic constriction not only limits myocardial perfusion and oxygen delivery during exercise, but that these limitations in perfusion and energy supply impose a restriction on myocardial contractile function. Gwirtz et al (1988) conducted experiments in which both the nonspecific α-adrenergic receptor antagonist phentolamine and the specific α_1-adrenergic receptor antagonist prazosin were intracoronarily administered during exercise in dogs. The injection of prazosin directly into

the left circumflex artery during exercise resulted in a 21% increase in circumflex inflow, and this increase in flow was followed by a 37% increase in the maximal rate of segmental shortening in the circumflex perfusion territory as well as a 21% increase in dP/dt_{max} of the left ventricle. In the study by Gwirtz et al (1986), it was proposed that an adrenergic vasoconstriction was mediated primarily by the α_1-receptor subtype since effects of phentolamine were not significantly different from those observed with prazosin. Additional studies were performed to more systematically examine this proposal (Strader et al, 1988). At the peak level of exercise, the effect of the α_1-adrenergic receptor antagonist prazosin injected intracoronarily was compared to that of the specific α_2-adrenergic antagonist yohimbine. Prazosin caused a significant increase in coronary flow of 28%, while yohimbine caused no change in coronary inflow. Results similar to these have recently been reported by Dai et al (1989). They noted that following α_1-adrenergic blockade with prazosin, coronary blood flow during exercise was significantly greater compared to control. α_2-adrenergic blockade with idazoxan did not alter the coronary response to exercise. They also noted that combined α_1- and α_2-adrenergic blockade was not more effective in increasing blood flow during exercise than was α_1-adrenergic blockade alone. Laxson et al (1989) noted that the α_1-receptor

subtype is primarily responsible for adrenergic coronary constriction during exercise and ischemia. These researchers occluded the circumflex artery in dogs until distal pressure fell to 40 mmHg. During exercise, intracoronary prazosin increased subendocardial flow by 87% and mean transmural flow by 49%. A significant increase in segmental shortening in subendocardium also occurred. Specific α_2-adrenergic blockade with intracoronary idazoxan was without effects.

The results of these previous studies, then, appear to suggest a paradox between a vasoconstrictor tone whose existence can teleologically be understood only if it improves myocardial function, but whose potential for a deleterious effect upon ventricular performance can be demonstrated because it imposes a limitation or restriction on the increase in contractile function at this level of stress. Because of this paradox, it is important to keep in mind that the studies regarding the effect of α_1-adrenergic constriction on ventricular function examined the effects of α-adrenergic constrictor tone at only one level of strenuous exercise (Gwirtz et al, 1986, Strader et al, 1988). One must therefore consider the possibilities of a functional effect of α_1-adrenergic vasoconstriction which is bidirectional in nature. In other words, the α-adrenergic constrictor tone may augment the increase in contractile function at low levels of exercise, but may impose a limitation on the

increase in contractile function at higher degrees of physical stress). Thus, at lower levels of stress, an α-adrenergic constrictor tone may cause a redistribution in coronary transmural flow and, as a result, prevent a decrease in subendocardial performance without precipitating a decline in subepicardial contractile function. However, as the level of sympathetic stimulation increases α-adrenergic receptor activation with increasing exercise workload, a coronary vasoconstriction may produce a significant flow limitation and a decrease in the energy supply:demand ratio. In turn, this restriction on coronary flow may impose a limitation on myocardial contractile function.

Such a bidirectional effect of vascular α_1-adrenergic receptor activation on myocardial contractile function can go undetected unless ventricular performance is evaluated throughout a continuum of increasing levels of adrenergic stimulation. Thus, one must compare the exercise load or sympathetic stress level at which α_1-adrenergic receptor stimulation first begins to affect coronary flow with the level at which it first begins to affect contractile function. If these are two different levels of physical stress, or if the affect upon contractile function varies with increasing levels of exercise stress, then the possibility of a bidirectional effect of α_1-adrenergic receptor mediated vasoconstriction upon myocardial

contractile function must be considered. In this regard, a recent study by Dai et al (1989) reports that intracoronary administration of prazosin improves coronary blood flow at all measured levels of exercise but improves global ventricular function only at 4 mph, 15% incline. This work load corresponds to 70% $M\dot{V}O_{2max}$ in the dog, which is considered a strenuous level of exercise (Musch et al, 1986). In this study, prazosin was administered as a continuous intracoronary infusion as dogs underwent an exercise protocol in which treadmill speed was increased in stages from rest (0 mph, 0% incline) to 4 mph, 20% incline. At all treadmill inclines below and above 15%, global function during prazosin administration was unchanged in spite of increased coronary flow. No measure of oxygen extraction or of regional contractile function are provided. It is interesting that the level at which these investigators found an effect of coronary α_1-adrenergic receptor blockade on global contractile function is similar to the exercise workload used in the studies by Gwirtz and coworkers (Gwirtz et al, 1986; Strader et al, 1988).

To resolve the question of a possible intensity-dependent effect of α_1-adrenergic coronary tone upon left ventricular contractile function, Study 1 evaluated global and regional ventricular function as well as $M\dot{V}O_2$ during increasing intensities of dynamic exercise. Contractile

function, coronary flow, and $\dot{M}VO_2$ were evaluated under conditions of α_1-adrenergic receptor blockade using prazosin and during administration of the appropriate vehicle for this blocking agent. Contractile function, coronary flow, and $\dot{M}VO_2$ under these two conditions were compared at each level of dynamic exercise to determine whether blockade of the α_1-adrenergic vascular tone results in increased coronary flow, increased MVO_2, and improved ventricular function. Since the α_1-adrenergic receptor blocking agent was administered regionally through an indwelling coronary catheter, both regional and global contractile function was monitored. In this model, evaluation of regional contractile function was vital because improvements in regional performance can occur unaccompanied by changes in global performance (Kedem et al, 1989; Gwirtz et al, 1986). Thus, the beneficial effect upon contractile function resultant from regional coronary α_1-adrenergic receptor blockade could go unnoticed if global contractile function is monitored without evaluating regional contractile function.

As described above, studies have indicated that an α_1-adrenergic constriction limits the increase in coronary blood flow during severe exercise, and as a result, imposes a restriction on the increase in global and regional myocardial contractile function (Gwirtz et al, 1986; Strader and Gwirtz, 1988). Though intriguing, these studies leave

many questions unanswered. Regional contractile function was evaluated using piezoelectric crystals implanted only in the subendocardium. No analyses of regional contractile function in the more superficial subepicardial layers were made. Thus, the possibility of a differential effect of α_1-receptor antagonism upon contractility throughout the various layers of the heart was not evaluated. Similarly, flow was measured using a chronically implanted Doppler flow probe. Therefore, no information was gathered regarding the transmural nature of the α_1-adrenergic tone. Such analyses may be enlightening with regard to the existence of a transmural gradient of an α_1-adrenergic vasoconstrictor tone on the coronary vasculature, and to the degree with which this constrictor tone limits ventricular function in the sympathetically stimulated heart. Thus, Study 2 evaluated the effects of coronary α_1-adrenergic stimulation on the transmural gradient of coronary flow and of left ventricular contractile function. To focus upon neural effects and to minimize influence from circulating catecholamines released from the adrenal medulla, sympathetic activation was accomplished by electrical stimulation of the left stellate ganglion.

The precise basis for the increased contractile performance associated with the prazosin-induced increase in coronary flow is not certain. This effect cannot be

attributed to increased adrenergic stimulation of myocardial β-receptor since prazosin, in the doses employed, does not cause increased norepinephrine release from sympathetic nerve terminals (Heyndricks et al, 1984). Likewise, experiments with phentolamine showed that the increased flow and performance were not altered by β-adrenergic blockade with atenolol. Furthermore, the increased performance cannot be attributed to inhibition of postsynaptic myocardial α-adrenergic receptors, since if these receptors are of functional importance, their inhibition would cause a negative inotropic response (Rabinowitz et al, 1975). Also, the increased myocardial performance following prazosin was not due to a direct inotropic effect of the agent, but was dependent on a high sympathetic drive to the heart since prazosin did not elicit an increase in contractile performance in the resting dog (Schwartz and Stone, 1977; Chilian et al, 1981).

Stimulation of α_1-adrenergic receptors receptors by an agonist results in a triphasic effect upon inotropy (Osnes et al, 1978). This triphasic response consists of: 1) an initial transient positive inotropy, possibly produced by mobilization of intracellular calcium through the cleavage of phosphatidylinositol-4,5-biphosphate (PIP_2) to myoinositol-1,4,5-triphosphate (IP_3); 2) a transient negative inotropy; 3) followed by a more sustained positive

inotropy possibly resultant from the potentiation of slow calcium channels through activation of protein kinase C (Otari et al, 1988). In this regard, α_1-adrenergic receptors have been shown to mediate a positive inotropic response in the cat (Wagner and Brodde, 1978), the rat (Wenzel and Su, 1966; Nakashima et al 1971), the guinea pig (Govier, 1968), and the rabbit (Schumann et al, 1974). Additionally, Flavahan and McGrath (1981) provide evidence that α_1-adrenergic receptor activation can mediate a positive chronotropic effect in the rat. Prazosin administration has been shown to inhibit the positive inotropic effects associated with phenylephrine perfusion of isolated cardiomyocytes (Otani et al, 1988). Thus, if prazosin has any direct effect on cardiomyocytes, it would be a negative inotropic effect. However, it has also been shown that α_1-adrenergic receptors to not exist on the canine ventricular myocyte (Endoh et al, 1978), and that intravenous injection of α_1-adrenergic receptor agonists may decrease heart rate in the rat indirectly through parasympathetic activation (Flavahan and McGrath, 1982).

The findings reviewed above indicate that there is no direct effect of α_1-adrenergic receptor blockade upon contractile function of the canine ventricle. It is attractive to propose that the prazosin-induced increase in contractile performance of the myocardium was a direct

result of the increase in coronary blood flow. To explore this possibility that the changes in ventricular contractile function associated with intracoronary administration of prazosin are due to an increase in coronary blood flow rather than to some direct action of the α_1-adrenergic receptor blocker prazosin, Study 3 compared the effects of intracoronary prazosin on myocardial flow and function during exercise to those observed after direct coronary vasodilation with adenosine during exercise. If the increase in contractile function following prazosin were secondary to an increase in flow, then similar results should have been obtained when the same degree of flow increase was provided by intracoronary infusion of adenosine.

CHAPTER III

PROCEDURES AND METHODS

STUDY 1: a1-Adrenergic Coronary Constrictor Tone at Various Intensities of Submaximal Exercise. This study examined the presence of an α_1-adrenergic coronary constrictor tone at various intensities of submaximal exercise. The expression of this constrictor tone was evaluated by its effect both on coronary flow and on ventricular performance. When a significant coronary constrictor tone could be shown to affect left ventricular contractile function, we distinguished whether the effect on function was unidirectional (i.e. consistently limiting the increase in ventricular contractile function at all levels of physical stress), or whether the effect was bidirectional (i.e., augmenting the increase in ventricular contractile function only at low levels of stress and attenuating ventricular performance only when exercise stress was increased).

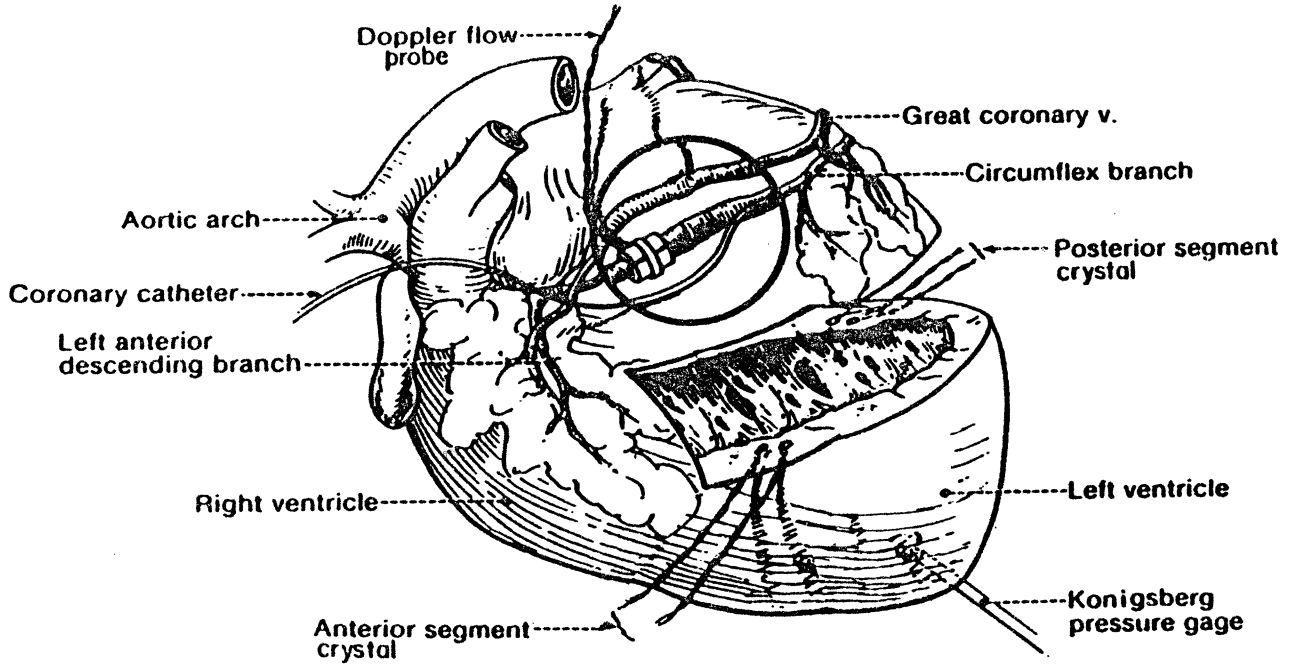
SURGICAL PREPARATION: Six healthy, untrained mongrel dogs of either sex and weighing between 25-35 kg were anesthetized using acepromazine (5-10 mg, s.c.) and sodium pentobarbital (30 mg/kg iv). The trachea was intubated, and each dog was ventilated using a Harvard Model 614

Respirator. Using sterile technique, a thoracotomy was performed through the left 5th intercostal space and the dog was instrumented as shown in Figure 2. To monitor arterial pressure (AP) and obtain arterial blood samples, the aorta was exposed and cannulated with a Silastic catheter (0.94 mm O.D.) distal to the aortic arch using the Herd-Barger technique (Herd and Barger, 1964). The heart was then exposed and suspended in a pericardial cradle. To measure left ventricular pressure (LVP), a solid-state micromanometer (Konigsberg P-6.5) and a fluid-filled Tygon catheter (1.27 mm OD) was inserted into the left ventricle through a stab wound in the apex. At the beginning of each experiment, the tygon catheter was connected to an Isotec pressure transducer and the Konigsberg micromanometer was calibrated against the catheter pressure. The Isotec pressure transducer was calibrated using a mercury manometer. The circumflex artery was dissected free of the surrounding tissue for a distance of approximately 3 cm beginning at the origin of the vessel. For measurement of left circumflex flow velocity (CFV), a 4 mm ID 10 MHz Doppler flow probe was placed around the circumflex artery. To check the zero flow reference, a pneumatic occluder was placed around the circumflex artery immediately distal to the Doppler flow transducer such that there was no vessel branch between the two. For injection of the solutions into

Figure 2 - Experimental Instrumentation of the Heart

Figure 2 illustrates the experimental instrumentation used. To monitor left ventricular pressure, a Konigsberg micromanometer pressure gauge and a Tygon catheter (not shown) were placed in the left ventricle. To measure regional shortening, one pair of 5 MHz piezoelectric crystals was placed in both the anterior (control territory perfused by the left anterior descending artery) and the posterior (α_1-adrenergic receptor blocked territory perfused by the left circumflex artery) region of the left ventricle. To measure left circumflex flow velocity, a 10 MHz Doppler flow probe was placed around the left circumflex artery, with a pneumatic occluder cuff placed distal to the flow probe to check the zero flow reference. For the injection of vehicle or prazosin, a catheter was inserted into the left circumflex artery distal to the occluder cuff. To obtain venous blood samples, a catheter was placed in the coronary sinus.

Instrumentation



the circumflex artery, a heparin-filled Silastic catheter (0.12 mm ID and 0.6 mm OD) was inserted into the circumflex artery distal to the occluder (Gwirtz, 1986). This catheter has been shown not to interfere either with flow in the circumflex artery or with its distribution during conditions of rest or exercise (Gwirtz, 1986). To permit coronary venous blood sampling, a Silastic catheter (0.94 mm O.D..) was placed in the coronary sinus (Young and Stone, 1974). For measurement of regional myocardial contractile function, 2 pairs of opposing 5 MHz ultrasonic crystals were placed in the circumflex (posterior) perfusion territory, and one pair was placed in the left anterior descending (LAD, anterior) perfusion territory. Each pair of crystals was implanted 0.5-1.0 cm apart and 0.5-0.7 cm below the subepicardial surface to monitor contractile function by measuring changes in segment length (SL) in the subendocardial layer (Theroux et al, 1974).

After instrumentation was completed, a chest tube was placed in the thoracic cavity to evacuate the pneumothorax and any post-surgical intrathoracic transudate accumulation. The chest tube was exited through the left 6th intercostal space and was removed on the first post-surgical day. All wires and catheter tubing from the instrumentation were passed subcutaneously to the back of the dog and externalized between the scapulae. The indwelling catheters

were maintained patent by daily flushings with heparinized saline. The surgical wounds were cleaned daily. Post-operative analgesics (Nubain), antibiotics (penicillin, streptomycin, or chloramphenicol), and antipyretics (aspirin) were given as needed. No experiments were run within 72 hrs of the most recent administration of narcotics.

At the conclusion of all experimentation, the dogs were sacrificed using an overdose of sodium pentobarbital and potassium chloride (KCl). Injection of Evans blue dye into the circumflex catheter while the occluder was inflated delineated the posterior perfusion territory in order to verify that the ultrasonic crystals were indeed placed in the representative anterior and posterior perfusion regions. Postmortem examination showed no evidence of thrombus formation, local inflammation, or scarring from implanted crystals. Postmortem determinations of the mass of the posterior perfusion territory and the cross-sectional area of the circumflex artery allowed conversion of Doppler flow velocity data to a volume-per-unit mass value.

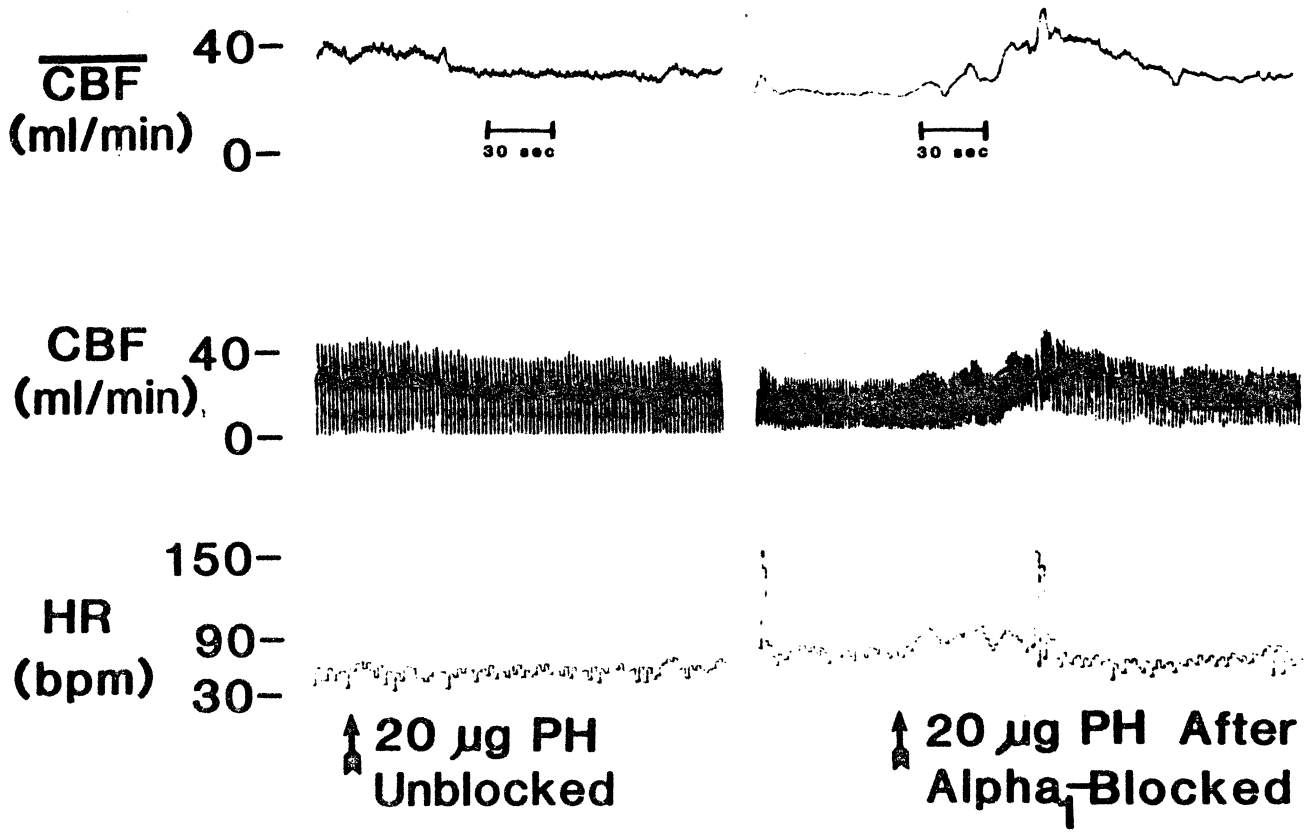
EXPERIMENTAL PROTOCOL: Experimentation began 10-14 days following surgery. Coronary blood flow and global and regional myocardial function were evaluated while the dog was subjected to a standardized submaximal exercise regimen (Tipton et al, 1974). This regimen consists of six 3-min

intervals of increasing workload. The dog first ran a 3-min warm-up at 4.8 kilometers per hour (kph), 0% grade. The treadmill speed and grade were progressively increased to encompass: Stage 2) 6.4 kph, 0% grade; Stage 3) 6.4 kph, 4% grade; Stage 4) 6.4 kph, 8% grade; Stage 5) 6.4 kph, 12% grade; and Stage 6) 6.4 kph, 16% grade. Each dog performed this regimen twice. During one exercise test, the α_1-antagonist prazosin was infused into the left circumflex artery catheter. During the other exercise test, the vehicle (sterile water) was infused. Prior to α_1-adrenergic blockade, 20 μg-40 μg phenylephrine was administered into the circumflex artery using the indwelling catheter in order to elicit an α_1-adrenergic receptor mediated coronary constrictor response. A 20 μg dose of phenylephrine administered intracoronarily is known to normally elicit a substantial circumflex constriction (Gwirtz and Stone, 1982). α_1-Adrenergic receptor blockade of the circumflex perfusion territory was then accomplished with an initial prazosin dose of 0.5 mg infused directly into the circumflex artery over 3.5 minutes until the α_1-adrenergic receptors were blocked. This dose of prazosin has previously been shown to abolish, for at least 8 minutes, the vasoconstrictor response to intracoronary injection of 20 μg phenylephrine without indication of peripheral circulatory spillover effects (Gwirtz et al, 1986). In the present

experiments, the effectiveness of α_1-adrenergic blockade was verified in each dog by loss of constrictor response to intracoronary administration of the 20-40 μg phenylephrine dose which produced a constrictor response prior to α_1-adrenergic blockade. Representative tracings of CBF are shown in Figure 3. The left panel of Figure 3 demonstrates a decrease in mean CBF following administration of 20 μg phenylephrine. This is indicative of an α-adrenergic receptor mediated vasoconstriction of the left circumflex artery following intracoronary administration of 20 μg phenylephrine. In the right panel, intracoronary administration of the same dose of phenylephrine following blockade of α_1-adrenergic receptors by prazosin is not followed by a vasoconstrictor response. Blockade of α_1-adrenergic receptors was maintained during the exercise regimen by continuous infusion of prazosin at a rate which did not exceed 1 $\mu g\ kg^{-1}\ min^{-1}$. Infusion of this α_1-antagonist at a similar rate was shown by Dai et al (1989) to maintain effective blockade until completion of this exercise protocol. In the present experiments, the effectiveness of α_1-adrenergic blockade at the end of the exercise protocol was verified in each dog by loss of constrictor response to intracoronary administration of the

Figure 3 - Verification of Effective Blockade of Coronary α1-Adrenergic Receptors

Figure 3 illustrates verification of effective blockade of coronary α_1-adrenergic receptors, as demonstrated by the abolishment of vasoconstrictor response to phenylephrine. In the left panel intracoronary administration of a test dose of 20-40 μg phenylephrine is followed shortly by a decrease in mean coronary blood flow. In the right panel, intracoronary administration of the same dose of phenylephrine after effective blockade of α_1-adrenergic receptors is not followed by a vasoconstrictor response. Rather, an unexplained rise in coronary blood flow is seen with injection.


CBF
(ml/min)
40–
0–
30 sec
30 sec
CBF
(ml/min)
40–
0–
150–
HR
(bpm)
90–
30–
20 µg PH
Unblocked
20 µg PH After
Alpha1-Blocked

20-40 μg phenylephrine dose which produced a constrictor response prior to α_1-adrenergic blockade. In the "vehicle" study, sterile water was infused at the same rate as described for prazosin above. In order to avoid volume effects on coronary blood flow, the infusion rate of prazosin or sterile water vehicle never exceeded 0.50 ml min-1, and was actually 0.25 ml min-1 during the exercise regimen.

In each study, control data consisting of LVP, systemic AP, pulsatile and mean CFV, and anterior and posterior SL were recorded with the animal standing at rest on a Quinton model 18-60 motor-driven treadmill prior to administration of prazosin or vehicle. Data were again recorded with the animal standing at rest on the treadmill 45-60 sec following initial prazosin or vehicle administration. Measurements were repeated during the final 30 seconds of any stage of the regimen. Also during the control period and during the final 30 seconds of each of the six exercise stages, coronary venous blood samples (coronary sinus) were obtained. Arterial blood samples (aortic artery) were obtained during the first and final stages of the exercise regimen. Because arterial blood does not desaturate in untrained, healthy individuals even at high levels of exercise (Rowell, 1986), acquisition of arterial samples at every level for analysis of oxygen saturation was not

mandatory. The order of the runs (with prazosin or with vehicle) was random.

DATA COLLECTION AND ANALYSIS: On line variables were recorded on a Coulbourn 8-channel chart recorder. These variables included LVP, SL in the posterior (posterior SL) and anterior (anterior SL) perfusion territories, AP, and CFV. Heart rate (HR) was derived from the LVP signal by use of a cardiotachometer. The LVP and SL signals were differentiated to obtain the rate of left ventricular pressure development (dP/dt) and rate of segment length change (dL/dt) in each region. This was accomplished using an active differentiating circuit which converts slope to vertical displacement from horizontal. All these data were recorded on an 8-channel Hewlett-Packard model 3968A tape recorder. Data from the magnetic tape was then analyzed using an Apple IIe computer and a software program previously developed in this laboratory (Mass et al, 1987). The program samples recorded data at 2 msec intervals over 10 consecutive beats. The program determines dP/dt and dL/dt, and then derives the maximal rate of pressure generation (dP/dt_{max}) and of systolic SL shortening (dL/dt_{max}). The end-diastolic length (EDL) and end-systolic length (ESL) were derived by referring to the dP/dt signal as described by Theroux (Theroux et al, 1974). Percent segment length shortening (%SL) was calculated as 100 x [EDL

- ESL/EDL]. The program also provides values for peak left ventricular systolic (LVSP) and end-diastolic pressure (LVEDP), HR, mean AP, and mean CFV over the 10 beat period.

CFV was measured using the Doppler technique (Franklin et al, 1961). From a piezoelectric crystal, the Doppler flow probe emits sound waves of known frequency. These waves are reflected off traveling red blood cells (RBC), and the frequency of the reflected waves is determined. In relation to the direction of RBC flow, the angle at which wound waves approach the RBC from the crystal is known. Mean flow velocity (v) can then be determined using the formula:

$$v = \frac{(\text{change in F})\ (C)}{(2)\,(F_e)\,(\cos a)}$$

Where v = mean flow velocity

(change in F) = absolute difference in frequency (KHz) between the emitted frequency (F_e) and the received frequency

C = velocity of sound in blood

$=(1.5)(10^5$ cm/sec)

F_e= emitted frequency = 10 MHz

cos a = cos (angle of crystal in probe)

$= \cos 45^{o} = 0.707.$

CFV measured using this technique has been shown to be linearly related to volume flow rate provided that the cross-sectional area of the blood vessel within the flow

probe remains constant (Vatner et al, 1970). The Doppler flow measurement has also been shown to be linearly correlated with volume flow rates measured directly (Gwirtz et al, 1986; Gwirtz, 1986). Mean CFV was converted to mean circumflex blood flow (CBF, volume flow rate) using the cross-sectional area of the vessel within the flow probe as determined by postmortem measurement. This equation is:

$$CBF = (\pi)(D^2)(v)(60 \text{ sec/min}) / 4$$

where CBF = coronary blood flow (ml/min)

D = diameter of vessel (cm)

v = mean flow velocity (cm/sec)

$(\pi)(D^2) / 4 = (\pi)(r^2)$

= cross sectional area of vessel

(Hartley et al, 1982).

Myocardial oxygen consumption ($M\dot{V}O_2$) was determined using the Fick principle:

$$M\dot{V}O_2 \text{ (ml } O_2\text{/ min g)} = CBF \times [(A\text{-}V)\ O_2] \times Hgb \times [O_2 \text{ carrying capacity of Hgb}]$$

where CBF = coronary blood flow (ml/min g)

AO_2 = oxygen saturation of arterial blood

VO_2 = oxygen saturation of venous blood

Hgb = concentration hemoglobin in blood (g/ml)

average O_2 carrying capacity of Hgb

= 1.34 g O_2/ g Hgb.

The arterial and coronary venous oxygen saturations, as well as Hgb content were determined from the respective blood samples using a Radiometer OSM 2 hemoximeter. It should be noted that fluids were administered regionally into the posterior perfusion territory and that only the coronary blood flow to the posterior region of the left ventricle was measured, while coronary sinus blood reflects a mixture of blood from both the anterior and posterior regions of the ventricle. Though this method may underestimate O_2 consumption by the posterior region of the myocardium, an increased oxygen extraction would be expected if the increase in contractile performance were the primary event and precipitated an increase in circumflex blood flow. Likewise, a decrease in oxygen extraction would be expected if the increase in circumflex blood flow were the primary event and the increase in contractile function were the secondary event.

To determine the effect of an α_1-adrenergic constrictor tone upon coronary flow and ventricular function, flow and function values obtained with and without prior intracoronary administration of prazosin were compared. Each dog served as its own control. Within each exercise test, the measured variables were normalized to the resting control values for that run by dividing the value of a variable at a given workload by the value of that variable

at rest and then multiplying the quotient by 100%. The resultant value of that variable at a given workload is then expressed as the percent change from its value before exercise. At any given exercise level, normalized values obtained from the prazosin run were compared to normalized values obtained from the vehicle run using a Paired t-test. Within each run, the normalized values obtained at any given exercise level were compared to the normalized values obtained during control using Analysis of Variance with repeated measures (ANOVA) and Duncan's post hoc test. All values are expressed as mean ± standard error, and statistical significance was accepted at $P<0.05$.

STUDY 2: Effects of a Coronary α-Adrenergic Constrictor Tone on Transmural Left Ventricular Flow and Contractile Function During Left Stellate Ganglion Stimulation. This study examined the influence of selective sympathetic adrenergic neural stimulation on transmural blood flow and on myocardial contractile performance in the left ventricle. To produce selective neurally-mediated adrenergic effects on the heart and to minimize contributions associated with concurrent alterations in the remainder of the sympathetic nervous system (i.e., adrenal medulla), adrenergic activation was accomplished by direct stellate ganglion stimulation.

SURGICAL PREPARATIONS: Two groups of dogs were used for this study. To measure contractile function, nine mongrel dogs (Group I) of either sex, weighing 25-30 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The trachea was intubated, and positive pressure ventilation was maintained with room air using a Harvard Model 614 respirator. The chest was opened through the left fifth intercostal space, the stellate ganglion was carefully isolated, and a ligature was placed around it. The pericardium was opened, and the heart was instrumented for measurement of global and regional left ventricular contractile function, CFV and left ventricular oxygen and lactate extractions. For measurement of LVP, a solid state micromanometer (Konigsberg, P6.5) and a fluid-filled Tygon catheter (1.27 mm O.D..) were placed in the ventricular chamber through a stab wound in the apex. The Konigsberg micromanometer was calibrated against the pressure measured in the Tygon catheter, which was connected to an Isotec pressure transducer which was calibrated using a mercury manometer. The left ventricular pressure signal was differentiated to obtain the rate of pressure change (dP/dt). HR was derived from the LVP signal by use of a cardiotachometer. Regional myocardial SL was measured using 3 pairs of opposing 5 MHz piezoelectric crystals. One pair each was positioned in the subendocardium of both the left

anterior descending (anterior) and left circumflex (posterior) perfusion territories as illustrated previously in Figure 2 and described by Theroux et al (1974). In the posterior region one pair of crystals was also placed in the subepicardium as described by Gallagher et al (Gallagher et al, 1980). The left circumflex coronary artery was dissected free of surrounding tissue, and a 4 mm ID 10 MHz Doppler ultrasonic flow probe was placed around the circumflex artery for measurement of changes in CFV. When using the Doppler flow probe for acute preparation, a fibrotic contact between the flow probe and the coronary artery cannot be obtained as in the chronically instrumented preparation. Therefore, electrode paste was placed within the flow probe before it was placed around the vessel, so that a continuous ultrasonic conduction medium between the probe and vessel could be established. Qualitatively, the signals obtained were indistinguishable from those observed in a chronic preparation (Gwirtz and Stone, 1981; Gwirtz, 1986), but the absolute values of CFV may not accurately reflect the true values. For this reason, in the present experiments, the flow velocity data are reported as percent changes in velocity during stellate stimulation and during administration of the α_1- adrenergic antagonist. A fluid-filled Silastic catheter (0.6 mm O.D.) (Gwirtz, 1986) was inserted into the circumflex artery distal to the Doppler

flow probe for injection of solutions and coronary arterial blood sampling. Another Silastic catheter (0.94 mm O.D.) was positioned in the coronary sinus to permit coronary venous blood sampling (Young and Stone, 1981). AP was measured with a catheter placed in a femoral artery which was connected to an Isotec transducer. Upon termination of the experiment, Evan's blue dye was injected into the circumflex catheter to demonstrate that the segment length crystals were indeed placed in the representative locations of the anterior and posterior regions.

To measure transmural blood flow distribution, a separate group of five mongrel dogs (Group 2) of either sex, weighing 25-30 kg, were anesthetized and instrumented. A Konigsberg 6.5 micromanometer, a femoral artery catheter, and a left circumflex artery catheter were placed to monitor LVP, dP/dt, HR, and mean AP and for injection of solutions into the circumflex artery as described above. In addition, a catheter was introduced into the left atrial appendage for injection of 15 um tracer microspheres. A second catheter was inserted into the femoral artery; the end of this catheter was placed 2-3 cm beyond the end of the arterial blood pressure catheter and was used for withdrawal of reference blood samples.

EXPERIMENTAL PROTOCOL: In the nine Group 1 dogs, transmural contractile function was monitored. After a

period of stabilization following completion of the instrumentation, control data were collected. The left stellate ganglion was then stimulated using a Narco SI-10 stimulator which delivered square-wave pulses of 8-10 volts, 10 pulses/s and 5 ms duration. After attaining a steady state for all variables during stellate ganglion stimulation, data were again collected. The α_1-adrenergic receptor antagonist prazosin (0.5 mg) was then injected into the circumflex artery while stellate stimulation was continued. Changes in LVP, dP/dt, HR, AP, CFV, and SL were recorded. Previous studies have demonstrated that this dose of intracoronary prazosin abolishes the left coronary response to the α_1-receptor agonist phenylephrine without peripheral circulatory effects (See Figure 3; Gwirtz and Stone, 1982; Liang and Jones, 1985; Liang et al, 1986).

Blood samples were withdrawn from the coronary artery and coronary sinus catheters for determination of blood oxygen (6 dogs) and lactate concentrations (4 dogs) before stellate stimulation, during stellate stimulation, and after intracoronary prazosin while the left stellate ganglion was being stimulated. Oxygen concentration was calculated from oxygen saturation and hemoglobin content, which were determined using a Radiometer OSM 2 hemoximeter. Left ventricular oxygen extraction ratio was obtained by dividing the arterial-venous oxygen concentration difference by the

arterial oxygen concentration. Lactate concentration was determined in arterial and sinus coronary blood samples by ultraviolet absorption spectrometry as described by Marbach and Weil (1967). Lactate extraction ratio was obtained by dividing the arterial-venous lactate concentration difference by the arterial lactate concentration. It is recognized that these two variables are only qualitative indicators of changes which may occur specifically within the circumflex perfusion territory, especially within the subendocardial region. However, in these studies, it was felt that these variables may provide an important insight into the effects of stellate stimulation and α_1-adrenergic blockade on left ventricular perfusion and oxygenation.

In Group 2, transmural flow distribution was determined using three different species of 15 μm tracer microspheres (^{57}Co, ^{46}Sc, ^{113}Sn) in 10% dextran with 0.01% Tween 80 (New England Nuclear, DuPont, Wilmington, Delaware). Each microsphere dose (1-2 million spheres) was diluted with 8-9 ml normal saline, sonicated for no less than 30 min. and vortexed prior to infusion. After heparinization of the dogs (500 U heparin/kg), control readings were recorded. The stellate ganglion was then stimulated at 6 volts, 10 pulses/s, and 10 msec duration. The change in stimulation parameters from Group I dogs was required to maintain maximal sympathetic response and constant hemodynamic

parameters throughout the 4-5 minute stellate stimulation period. After 2-2.5 minutes of stellate ganglion stimulation, a bolus injection of 0.5 mg prazosin, i.c. was administered. Different species of microspheres were injected (followed by a 10 ml. normal saline flush) in random order during the three experimental conditions: 1)control conditions; 2)left stellate ganglion stimulation; and 3)left stellate ganglion stimulation after i.c. administration of prazosin. Reference sampling from the femoral artery at a constant rate of 3.5 ml min-1 was begun 10 sec before injection of the microspheres and continued for 2-min following administration of the set of microspheres. The blood samples were collected in separate vials at 1-min intervals using an Isomatic 7624-02 micropump (Cole-Parmer, Chicago, Ill.).

At the conclusion of the experiment, the heart was excised and divided horizontally into four rings of approximately equal height, with the rings numbered consecutively 1-4, from base to apex. Each ring was then cut in half such that the circumflex perfusion territory was bisected. The resulting halves were then bisected, and the process repeated until each of the 4 rings was divided into eight pieces of approximately equal mass. Each of these eight pieces was then divided into two layers (subendocardium and subepicardium) of approximately equal

thickness. The region designated "posterior perfusion territory" was the 4 subendocardial and 4 subepicardial tissue segments from rings 2 and 3 which had directly abutted the circumflex artery. The region designated "anterior perfusion territory" was the 4 subendocardial and 4 subepicardial tissue segments from rings 2 and 3 located directly opposite the posterior perfusion territory.

DATA COLLECTION AND ANALYSIS: LVP, AP, pulsatile and mean CFV, and anterior and posterior SL were recorded on a Coulbourn 8-channel chart recorder and on magnetic tape using an 8-channel Hewlett-Packard Model 3968A tape recorder for subsequent computer analysis of data. HR was derived from the LVP signal using a cardiotachometer. The LVP and SL signals were differentiated to obtain dP/dt and dL/dt, respectively. From these variables, dP/dt_{max} and dL/dt_{max} were derived (Theroux et al, 1974). EDL and ESL were identified on the records by relating the segment length signal to the rate of change of left ventricular pressure signal as described by Theroux et al (1974). %SL was calculated as 100 X [(EDL - ESL)/EDL]. Contractile function data were analyzed from the magnetic tape using an Apple IIe computer and associated software as described for Study 1.

Radioactivity of myocardial tissue samples and blood reference samples were analyzed with a gamma spectrometer (Packard Instrument, Downers Grove, Illinois). The three

different nuclide labels were separated and blood flow computations were performed using an IBM microcomputer and the following blood flow equation:

$$MBF = (F_{ref})(R_{tis}/R_{ref})$$

where MBF = mean blood flow (ml/min)

F_{ref} = flow rate (ml/min) of the reference blood samples

R_{tis} = radioactivity (cpm) of the tissue sample

R_{ref} = radioactivity (cpm) of the reference blood samples

(Heymann et al, 1977).

To determine mean myocardial blood flow to each individual subendocardial and subepicardial layer within a region, the myocardial blood flow from all four samples of a given layer within a region were averaged. To determine the subendocardial to subepicardial flow ratio, the flow ratio for each of the four samples was calculated separately, and the values obtained in each of the four samples were averaged.

To quantitate the response to stellate stimulation, the values obtained during the control period were compared to those obtained during stellate stimulation. To quantitate the response to prazosin during stellate stimulation, data

for mean AP, LVP, dP/dt_{max} in posterior and anterior regions during the peak response to prazosin were compared to those values immediately before administration of prazosin using an ANOVA and Duncan's post hoc test. In addition, those responses obtained in the posterior subendocardial region were compared to those obtained in the posterior subepicardial region. All values are expressed as mean ± S.E., and statistical significance was accepted at $P<0.05$.

To quantitate the regional perfusion response to stellate stimulation in Group 2 dogs, the regional flow values obtained during the control period were compared to those obtained during stellate stimulation before prazosin. To quantitate the response to prazosin during stellate stimulation, regional flow values during stimulation before prazosin were compared to those values obtained after administration of prazosin using an ANOVA and Duncan's post hoc test. Data obtained from subendocardial regions were compared to those from subepicardial regions, and data obtained from the posterior left ventricle were compared to those obtained from the anterior region. Data are reported as mean ± SE Significance was accepted at $P<0.05$.

STUDY 3: Effect of Direct Coronary Vasodilation on Myocardial Contractile Function During Exercise. This study was undertaken to evaluate whether the change in contractile function following α_1-adrenergic blockade is due to an

increase in perfusion or to some other action of the α_1-adrenergic receptor antagonist. For this purpose, an endogenous vasodilator with a mechanism of action distinct from that of prazosin and with (if any) a myocyte depressant effect was used to increase flow to a comparable level as seen with prazosin (our α_1-antagonist). If changes in contractile function resulting from intracoronary prazosin were equivalent to the changes in contractile function during intracoronary infusion of adenosine at a rate producing the same flow increases resulting from prazosin administration, this would support a flow-related etiology for the contractile function changes.

SURGICAL PREPARATION: Six mongrel dogs of either sex and weighing 25-35 kg were anesthetized using sodium pentobarbital (30 mg/kg, i.v.). The trachea was intubated, and each dog was ventilated with room air using a Harvard respiration pump. With sterile techniques, the heart was exposed through the left fifth intercostal space was suspended in a pericardial cradle.

For measurement of LVP, a solid-state micromanometer (Konigsberg P-6.5) and a fluid filled Tygon catheter (1.27 mm OD) were placed in the left ventricular chamber through a stab wound in the apex. At the beginning of each experiment, the Tygon catheter was connected to an Isotec pressure transducer, and the Konigsberg micromanometer was calibrated

against the pressure measured in the catheter. For measurement of CFV in the circumflex artery, the artery was dissected free, and a 10-MHz Doppler ultrasonic flow transducer (4 mm ID) was positioned around the vessel. The Doppler system used has been shown to have a reliable zero reference (Franklin et al, 1966). The relationship between CFV measured with this Doppler system and volume flow rate has also been shown to be linear provided that the cross-sectional area of the blood vessel within the flow probe remains constant (Vatner et al, 1970). In this regard, the Doppler flow measurement has been shown to be linearly correlated with directly measured volume flow rate (Gwirtz, 1986; Gwirtz et al, 1986). In a manner similar to that described in Study 1, CFV was converted to CBF (volume flow rate) in Study 3 using the cross-sectional area of the vessel within the flow probe as determined postmortem at the termination of all experiments in an animal. To occlude the circumflex artery to check the zero flow reference, a hydraulic occluder was placed around the circumflex artery immediately distal to the Doppler flow transducer. For injection of solution into the circumflex artery, a heparin-filled Silastic catheter (0.12 mm ID and 0.6 mm OD) was inserted into the circumflex artery distal to the occluder cuff as described by Gwirtz (1986). For measurement of regional myocardial subendocardial SL in both the circumflex

perfusion territory (posterior region) and the perfusion territory of the LAD coronary artery (anterior region), two pairs of opposing 5-MHz ultrasonic crystals were implanted 0.5-1.0 cm apart and 0.5-0.7 cm below the surface of the subepicardium. A catheter was also positioned in the aorta through the internal thoracic artery for measurement of AP.

After instrumentation of the heart was complete, the pneumothorax was evacuated, and all wires and tubing were passed subcutaneously to the back of the dog and externalized between the scapulas. In all cases, indwelling catheters were maintained patent for the duration of all experiments by daily flushing with fresh heparin solution. The surgical wounds were cleaned daily. Post-operative analgesics (Nubain), antibiotics (penicillin, streptomycin, or chloramphenicol), and antipyretics (acetaminophen) were give as needed. No experiments were run within 72 hrs of the most recent administration of narcotics. Postmortem examination showed no evidence of thrombus formation, local inflammation, or scarring from implanted crystals. Injection of Evans blue dye into the circumflex catheter demonstrated that the ultrasonic crystals were indeed placed in the representative locations in the anterior and posterior regions.

EXPERIMENTAL PROTOCOL: Experimentation began 10-14 days following surgery. In each study, data were recorded with

the animal standing at rest on a Quinton model 18-60 motor-driven treadmill. Each dog was then subjected to the standardized submaximal exercise regimen described by Tipton et al. (1974, described in PROTOCOLS of STUDY 1). After running at 6.4 kph, 16% grade, one of three agents was administered through the circumflex catheter of the dog. The agents were: 1) 2 ml saline flush (vehicle control); 2) 0.5 mg prazosin bolus; or 3) constant infusion of adenosine (10-100 μg min-1) titrated to produce approximately the same increase in CBF as observed with prazosin. Only one agent was administered per day to the animal to avoid any overlapping residual effects. Peripheral AP and HR were monitored during agent administration into the circumflex artery for signs of significant spill-over into the peripheral circulation.

DATA COLLECTION AND ANALYSIS: The basic data collection procedures paralleled those performed in STUDY 1. Data were collected at rest, after 3 min at peak level of exercise, and again 3 min following administration of the agent. At the termination of all experiments, the dog was anesthetized. With the pneumatic circumflex occluder inflated to prevent retrograde flow, Evans blue dye was administered through the circumflex artery catheter to stain the posterior perfusion territory. The stained area was then

be cut out and weighed so that CBF could be normalized per 100 g perfused myocardium.

To quantitate the response to an agent, the values of all variables during peak response were compared with those values immediately before administration of the agent by one-way ANOVA followed by Duncan's post hoc test, and by Student's Paired t-test. Data are reported as mean $\pm$ SE Significance was accepted at $P<0.05$.

CHAPTER IV

RESULTS

STUDY 1: α_1-Adrenergic Coronary Constrictor Tone at Various Intensities of Submaximal Exercise. This study determined the presence of an α_1-adrenergic coronary constrictor tone at various intensities of submaximal exercise. The expression of this constrictor tone was evaluated by its effect on coronary flow and on ventricular performance. When a sympathetic coronary constrictor tone affected ventricular contractile function, we distinguished whether this effect on function was unidirectional (i.e. consistently limiting the increase in ventricular contractile function at all levels of physical stress), or whether the effect was bidirectional (i.e., augmenting the increase in ventricular contractile function only at low levels of stress and attenuating this increase in ventricular performance only when stress was increased).

Table 1 shows the effects of intracoronary prazosin on HR and mean AP at each level of exercise. Resting HR while standing on the treadmill was somewhat elevated in each group. This likely reflects an anticipatory response to exercise. During intracoronary vehicle infusion, exercise significantly increased HR above resting conditions.

Heart rate at the peak of exercise (6.4 kph/16% grade) was 235±7 bpm in this group, an increase of 87±13% over resting values. Similar increases in HR during submaximal exercise were observed in the prazosin treated dogs. Exercise was associated with an increase in mean AP at the three most strenuous levels during intracoronary vehicle infusion, but only at the most strenuous level during infusion of prazosin. There was no difference in HR or mean AP at any level of exercise during intracoronary prazosin infusion as compared to intracoronary infusion of the vehicle.

Table 2 shows the effects of intracoronary prazosin on mean CBF, left ventricular oxygen extraction, and MVO_2 during exercise in six dogs. During intracoronary administration of the vehicle, exercise was associated with an increase in oxygen extraction above resting values. During intracoronary prazosin infusion, similar increases in oxygen extraction were noted with exercise. Note that there was no difference in oxygen extraction during prazosin administration compared to vehicle administration at any level of exercise. Exercise was associated with an increase in mean CBF and $M\dot{V}O_2$ above resting in both groups at all levels of exercise. Compared to vehicle administration, intracoronary administration of prazosin resulted in a greater increase in CBF of 15±7%, 24±9%, and 35±10%, respectively, at the three most strenuous levels of

TABLE 1

EFFECT OF INTRACORONARY PRAZOSIN ON HEART RATE AND MEAN ARTERIAL PRESSURE DURING EXERCISE

Exercise Speed/Grade	HR (beats per min)		MAP (mmHg)	
(kph)/(%)	Vehicle	Praz	Vehicle	Praz
0/0	128 ± 10	133 ± 4	89 ± 2	88 ± 3
4.8/0	174 ± 6*	174 ± 4*	93 ± 2	89 ± 1
6.4/0	180 ± 10*	176 ± 3*	91 ± 2	92 ± 2
6.4/4	193 ± 9*	196 ± 6*	93 ± 1	89 ± 2
6.4/8	207 ± 5*	208 ± 7*	95 ± 2*	97 ± 5
6.4/12	224 ± 3*	220 ± 6*	99 ± 2*	103 ± 5
6.4/16	235 ± 2*	232 ± 13*	106 ± 4*	113 ± 6*

Abbreviations: HR, heart rate; MAP, mean arterial pressure; kph, kilometers per hour; Praz, Prazosin
Values are means ± SE for 6 dogs.
*P < 0.05 vs value at 0 kph/0% grade by Student Neuman Keuls post-hoc test.
†P < 0.05 vs vehicle at matched exercise level by Paired t-test.

TABLE 2

EFFECTS OF INTRACORONARY PRAZOSIN ON MEAN CORONARY BLOOD FLOW, LEFT VENTRICULAR OXYGEN EXTRACTION AND LEFT VENTRICULAR MVO_2 DURING EXERCISE

Exercise Speed/Grade	Mean CBF (ml/min/g)		O_2 Extraction (ml O_2/ml blood)		MVO_2 (ml O_2/min/g)	
(kph)/%	Vehicle	Praz	Vehicle	Praz	Vehicle	Praz
0/0	0.90 ± 0.8	0.91 ± 0.8	0.079 ± 0.021	0.095 ± 0.016	0.085 ± 0.015	0.084 ± 0.015
6.4/0	1.03* ± 0.13	1.06* ± 0.13	0.107* ± 0.015	0.106* ± 0.015	0.122* ± 0.022	0.120* ± 0.019
6.4/4	1.12* ± 0.13	1.16* ± 0.12	0.105 ± 0.017	0.104* ± 0.016	0.129* ± 0.025	0.128* ± 0.021
6.4/8	1.16* ± 0.14	1.31*† ± 0.13	0.111* ± 0.015	0.110* ± 0.014	0.140* ± 0.026	0.154* ± 0.020
6.4/12	1.23* ± 0.13	1.51*† ± 0.16	0.113* ± 0.015	0.113* ± 0.014	0.146* ± 0.024	0.186*† ± 0.025
6.4/16	1.62* ± 0.29	2.16*† ± 0.49	0.115* ± 0.014	0.116* ± 0.014	0.156* ± 0.025	0.208*† ± 0.036

Abbreviations: CBF, left circumflex blood flow; O_2, oxygen; MVO_2, myocardial oxygen consumption.
Values are means ± SE for 6 dogs.
*$P < 0.05$ vs value at 0 kph/0% grade by Student Neuman Keuls post-hoc test.
†$P < 0.05$ vs vehicle at matched exercise by Paired t-test.

exercise. These data indicated that an α_1-adrenergic receptor constrictor tone does indeed increase with exercise intensity. Furthermore, increases in CBF without change in oxygen extraction allow for increase MVO_2 at the two most strenuous exercise levels.

Table 3 shows the effects of intracoronary prazosin on global contractile function in six dogs. Compared to the resting values, LVSP increased at every level of exercise during administration of the vehicle and at all but the lowest level of exercise during administration of prazosin. The increase in LVSP was similar during both exercise tests. LVEDP did not change during exercise during infusion of either prazosin or its vehicle. dP/dt_{max} increased above resting values during every level of exercise both during administration of the vehicle and during administration of prazosin. In addition, dP/dt_{max} at the highest two levels of exercise workload during treatment with prazosin as compared to during vehicle infusion.

Table 4 shows the effects of intracircumflex administration of prazosin on regional left ventricular contractile function in the anterior and posterior regions. Exercise was never associated with a change in end diastolic length compared to resting values in either the anterior or posterior region of the left ventricle. Compared to resting values, exercise was always associated with increases in

contractile function in both the anterior and posterior region as indicated by increases in %SL and in systolic dL/dt_{max}. Compared to vehicle infusion, regional prazosin infusion into the posterior left ventricle was associated with greater increases in contractile function in the posterior region as indicated by significantly increases in systolic dL/dt_{max} of 39±13%, 36±12%, and 42±13% at exercise workloads of 6.4 kph, 8% grade, 6.4 kph, 12% grade, and 6.4 kph, 16% grade, respectively. Percent segment length shortening and EDL in the posterior region were not significantly altered by prazosin. It should also be noted that regional infusion of prazosin into the posterior left ventricle did not alter contractile function of the anterior region of the left ventricle. This suggests that the α_1-adenergic blocking effects of prazosin infused into the left circumflex artery were limited to the left circumflex artery and that no recirculation of prazosin occurred and no systemic reflexes were responsible for the observed changes in circumflex flow and contractile function.

Figures 4, 5, 6, and 7, show percentage change compared to rest in mean CBF, left ventricular dP/dt_{max}, and systolic dL/dt_{max} in the posterior region during exercise. Note in Figure 4 that mean CBF increases at each level of exercise when the vehicle was infused. When the prazosin was infused during exercise, the percentage increase in mean CBF above

resting value with each level of exercise is similar at the lower and moderate levels of exercise. However, infusion of prazosin results in a significantly greater increase in mean CBF compared to vehicle infusion at the higher exercise levels. Furthermore, the increase in percent change of mean CBF associated with prazosin infusion became greater at higher intensities of exercise. Thus, during prazosin infusion the rise in percent change of mean CBF at 6.4 kph, 16% grade was significantly greater than the increase at 6.4 kph, 8% grade. These increases in percent change of mean CBF during prazosin administration were associated with greater increases in percent change of dP/dt_{max} (Figure 5) and in percent change of systolic dL/dt_{max} (Figure 6) of the posterior region at these higher intensity exercise levels compared to vehicle infusion. Figure 7 illustrates that there was no significant difference in the percent change systolic dL/dt_{max} in the anterior region between vehicle and prazosin runs.

Resting myocardial $\dot{M}VO_2$ and CBF were similar before and after α_1-receptor blockade (Figure 8). Myocardial MVO_2 increased 2.4 fold during submaximal exercise with vehicle infusion, and 3.3 fold during submaximal exercise with prazosin infusion. A separate linear regression line was fit to the observations from each dog of percent change CBF from control value (%CBF) versus percent change myocardial $\dot{M}VO_2$

TABLE 3

EFFECTS OF INTRACORONARY PRAZOSIN ON GLOBAL LEFT VENTRICULAR CONTRACTILE FUNCTION DURING EXERCISE

Exercise Speed/Grade	LVSP (mmHg)		LVEDP (mmHg)		dP/dt_{max} (mmHg/sec)	
(kph)/(%)	Vehicle	Praz	Vehicle	Praz	Vehicle	Praz
0/0	101 ± 6	99 ± 5	- 4 ± 1	- 1 ± 2	3551 ± 223	3232 ± 299
4.8/0	114* ± 5	105 ± 7	- 4 ± 2	- 6 ± 3	5084* ± 589	4771* ± 340
6.4/0	114* ± 5	108* ± 9	- 2 ± 1	- 3 ± 3	5449* ± 711	5385* ± 383
6.4/4	122* ± 6	118* ± 9	- 1 ± 3	- 6 ± 3	6305* ± 932	6511* ± 752
6.4/8	125* ± 4	121* ± 6	- 1 ± 1	- 7 ± 3	6580* ± 889	6869* ± 643
6.4/12	128* ± 3	136* ± 7	- 2 ± 2	- 6 ± 3	7599* ± 1283	8212*† ± 1252
6.4/16	139* ± 3	140* ± 12	- 2 ± 2	2 ± 5	8576* ± 1087	8865*† ± 1365

Abbreviations: LVSP, Left ventricular systolic pressure; LVEDP, Left ventricular end-diastolic pressure; dP/dt_{max}, Maximal rate of left ventricular pressure development.
Values are mean ± SE for 6 dogs.
*$P < 0.05$ vs value at 0 kph/0% grade by Student Neuman Keuls post-hoc test.
†$P < 0.05$ vs vehicle at matched exercise level by Paired t-test.

TABLE 4

EFFECTS OF INTRACORONARY PRAZOSIN ON REGIONAL LEFT VENTRICULAR CONTRACTILE FUNCTION DURING EXERCISE

	ANTERIOR						POSTERIOR					
Speed/Gr Exercise	EDL		% SL		dL/dt_{max}		EDL		% SL		dL/dt_{max}	
(kph)/(%)	Veh	Praz	Veh	Praz	Veh	Praz	Veh	Praz	Veh	Praz	Veh	Praz
0/0	15.7 ± 0.8	15.6 ± 0.7	13.6 ± 3.2	12.0 ± 2.6	-27.3 ± 6.8	-26.3 ± 5.1	18.4 ± 0.6	18.4 ± 0.7	9.2 ± 2.2	9.4 ± 1.2	-20.4 ± 3.3	-22.3 ± 3.8
4.8/0	16.0 ± 0.9	15.9 ± 0.9	15.6 ± 3.1	15.0 ± 3.3	-32.3* ± 6.8	-31.3* ± 5.7	18.7 ± 0.8	18.6 ± 0.8	13.2* ± 2.6	13.1* ± 2.7	-27.6* ± 3.6	-30.1* ± 3.4
6.4/0	16.0 ± 0.9	16.0 ± 0.9	16.7* ± 3.0	16.1* ± 3.3	-33.1* ± 6.5	-33.6* ± 5.9	18.8 ± 0.8	18.7 ± 0.8	14.2* ± 2.7	13.8* ± 2.2	-29.9* ± 4.1	-33.9* ± 3.6
6.4/4	16.0 ± 0.8	15.8 ± 1.0	16.4* ± 3.6	17.1* ± 3.3	-36.6* ± 6.6	-36.0* ± 7.1	18.6 ± 0.7	18.7 ± 0.8	14.6* ± 2.8	15.7* ± 3.2	-33.0* ± 3.6	-32.6* ± 4.0
6.4/8	16.0 ± 0.9	15.8 ± 0.9	17.2* ± 3.2	17.2* ± 3.4	-42.7* ± 7.3	-43.1* ± 8.5	19.0 ± 0.8	18.9 ± 0.8	15.1* ± 2.8	15.9* ± 3.3	-32.0* ± 3.8	-42.0*† ±4.4
6.4/12	15.6 ± 1.0	15.9 ± 0.9	18.2* ± 3.3	18.8* ± 3.4	-42.6* ± 8.1	-46.6* ± 8.1	18.2 ± 0.9	18.9 ± 0.9	15.2* ± 3.0	17.0* ± 3.8	-36.0* ± 4.6	-45.3*† ± 4.6*
6.4/16	15.9 ± 0.8	15.7 ± 1.2	17.8* ± 3.9	18.3* ± 3.7	-49.2* ± 8.5	-49.4* ± 10.8	18.9 ± 0.9	18.8 ± 0.9	15.1* ± 2.5	16.7* ± 3.1	-36.7* ± 5.0	-52.2*† ± 6.3

Abbreviations: Anterior, region of left ventricle which was not exposed to vehicle or prazosin; Posterior, region of left ventricle which was exposed to vehicle or prazosin; Gr, grade; Veh, vehicle; EDL, end-diastolic length; % SL, percent shortening of segment length during systole; dL/dtmax, maximal rate of shortening of segment length; kph, kilometers per hour; Praz, prazosin.
Values are mean ± SE for 6 dogs

* $P < 0.5$ vs vehicle at kph/0% grade by Student Neuman Keuls post-hoc test

† $P < 0.5$ vs vehicle at matched exercise level by Paired t-test

Figure 4 - Effect of Intracoronary Prazosin on Mean Coronary Blood Flow During Exercise

Response of mean coronary blood flow (CBF) to different intensities of submaximal exercise during infusion of vehicle or prazosin into the left circumflex artery. All values are given as means ± SE and are expressed as a percent compared to control.
+$\underline{P} < 0.05$ vs control at matched exercise intensity (Paired t-test).
* With prazosin, the increse in CBF vs control is significantly greater than the increase in CBF at 6.4kph, 8% grade ($\underline{P} < 0.05$ by Student Neuman Keuls).

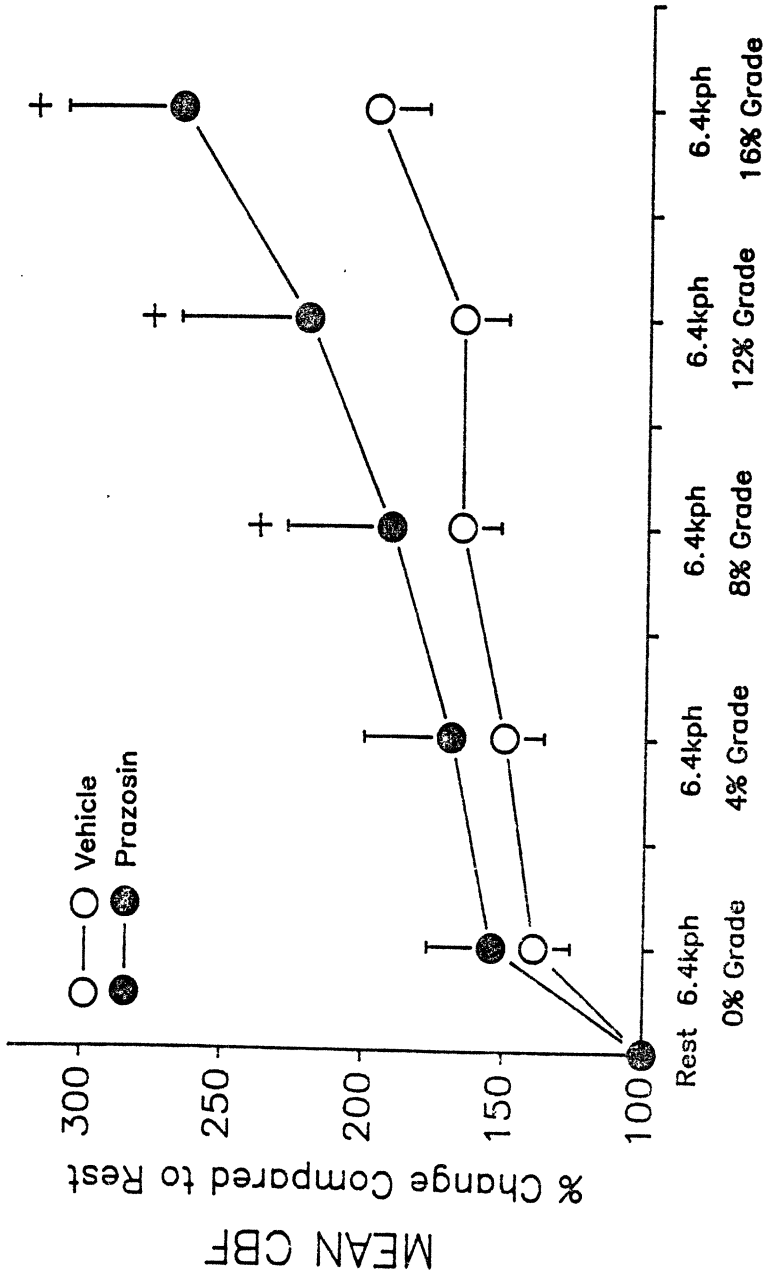

MEAN CBF
% Change Compared to Rest
300
250
200
150
100
Vehicle
Prazosin
Rest
0% Grade
6.4kph
4% Grade
6.4kph
8% Grade
6.4kph
12% Grade
6.4kph
16% Grade
EXERCISE LEVEL

Figure 5 - Effect of Intracoronary Prazosin on dP/dtmax During Exercise

Response of maximal rate of left ventricular pressure development (dP/dt_{max}) to different intensities of submaximal exercise during infusion of vehicle or prazosin into the left circumflex artery. All values are given as means $\pm$ SE and are expressed as a percent compared to control.

+$P < 0.05$ vs vehicle at matched exercise level by Paired t-test.

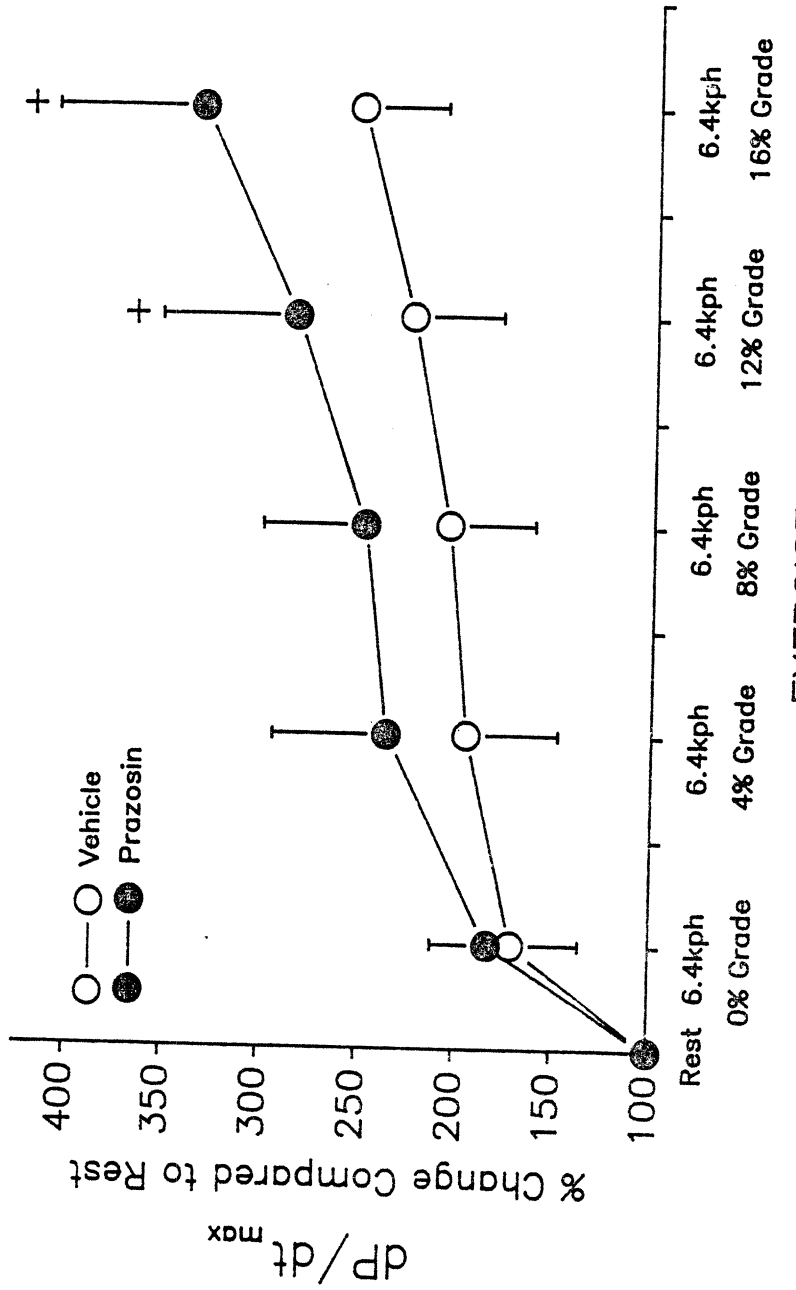

dP/dt max
% Change Compared to Rest
400
350
300
250
200
150
100
Vehicle
Prazosin
Rest
0% Grade
6.4kph
4% Grade
6.4kph
8% Grade
6.4kph
12% Grade
6.4kph
16% Grade
EXERCISE LEVEL

Figure 6 - Effect of Intracoronary Prazosin on Posterior dL/dtmax during Exercise

Response of maximal rate of segment length shortening (dL/dt_{max}) in the posterior perfusion territory to different intensities of submaximal exercise during infusion of vehicle or prazosin into the left circumflex artery. All values are given as means $\pm$ SE and are expressed as a percent compared to control.
+$P < 0.05$ vs vehicle at matched exercise level by Paired t-test.

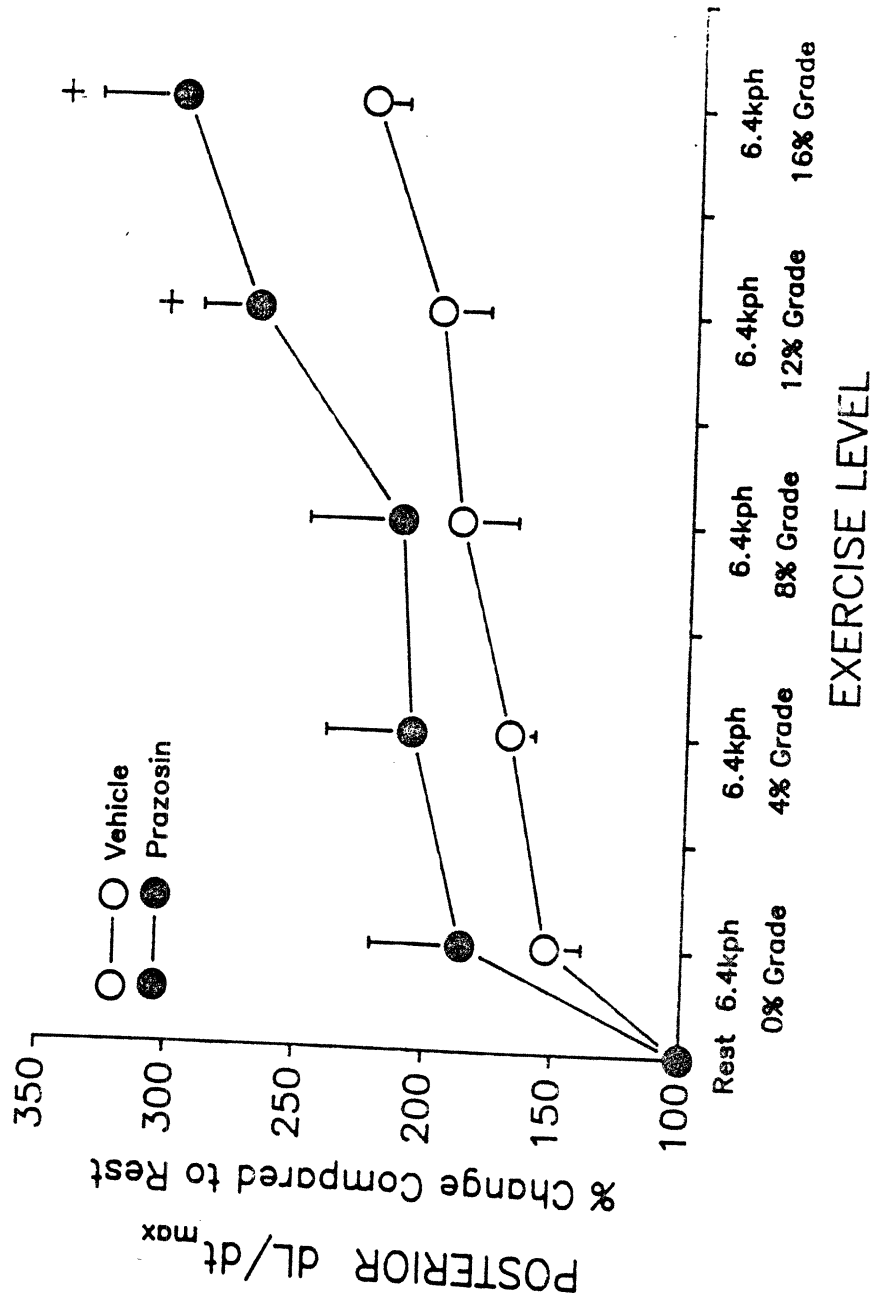
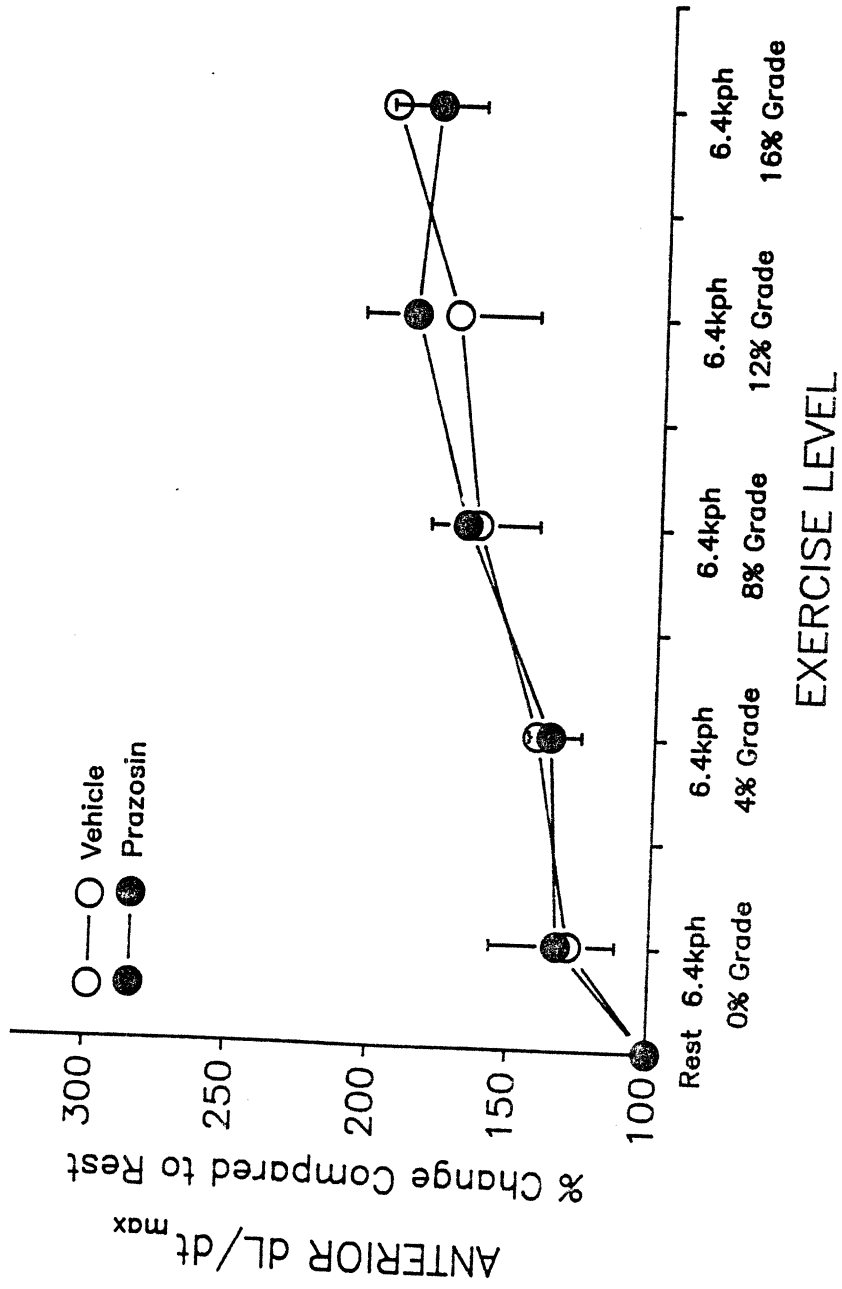

POSTERIOR dL/dt max
% Change Compared to Rest
350
300
250
200
150
100
Vehicle
Prazosin
Rest
0% Grade
6.4kph
4% Grade
6.4kph
8% Grade
6.4kph
12% Grade
6.4kph
16% Grade
EXERCISE LEVEL

Figure 7 - Effect of Intracoronary Prazosin on Anterior dL/dtmax During Exercise

Response of maximal rate of segment length shortening (dL/dt_{max}) in the anterior perfusion territory to different intensities of submaximal exercise during infusion of vehicle or prazosin into the left circumflex artery. All values are given as means $\pm$ SE and are expressed as a percent compared to control.


ANTERIOR dL/dt max
% Change Compared to Rest
300
250
200
150
100
Vehicle
Prazosin
Rest
0% Grade
6.4kph
4% Grade
6.4kph
8% Grade
6.4kph
12% Grade
6.4kph
16% Grade
EXERCISE LEVEL

from control value (%$\dot{M}VO_2$). From this relationship, %CBF was determined at equivalent values of %$\dot{M}VO_2$ between 0% and 200%. This allowed comparison of %CBF and equivalent values of %MVO_2 for each dog during administration of vehicle and during administration of prazosin. The comparison was limited by the highest %$\dot{M}VO_2$ of the dogs exercising during the vehicle infusion. At the upper range of oxygen demand (when %MVO_2 was 250% resting value or greater), %CBF was significantly greater during prazosin infusion vs vehicle infusion ($P<0.05$).

STUDY 2: Influence of α-Adrenergic Constrictor Tone Upon Subendocardial and Subepicardial Ventricular Function During Stellate Stimulation. This study examined the influence of isolated sympathetic adrenergic neural stimulation on subepicardial contractile function, subendocardial contractile function, and transmural blood flow in the left ventricle.

Figure 9 is a representative tracing from a Group I dog demonstrating the effect of stellate stimulation alone (first arrow) and after i.c. prazosin administration (second arrow). Stellate stimulation caused increases in global left ventricular function (LVP, dP/dt_{max}, AP), regional function (posterior subendocardial and subepicardial %SL and dL/dt_{max}), and CFV. Intracoronary prazosin administration

Figure 8 - Effect of Intracoronary Prazosin on the Relationship Between % Change Mean Coronary Blood Flow and % Change $M\dot{V}O_2$ During Exercise

The relationship between CBF and $M\dot{V}O_2$ during vehicle infusion and during prazosin infusion, expressed as percent change compared to control of each variable. Each value represents mean ± SE.
+P <0.05 vs vehicle at matched exercise level by Paired t-test.

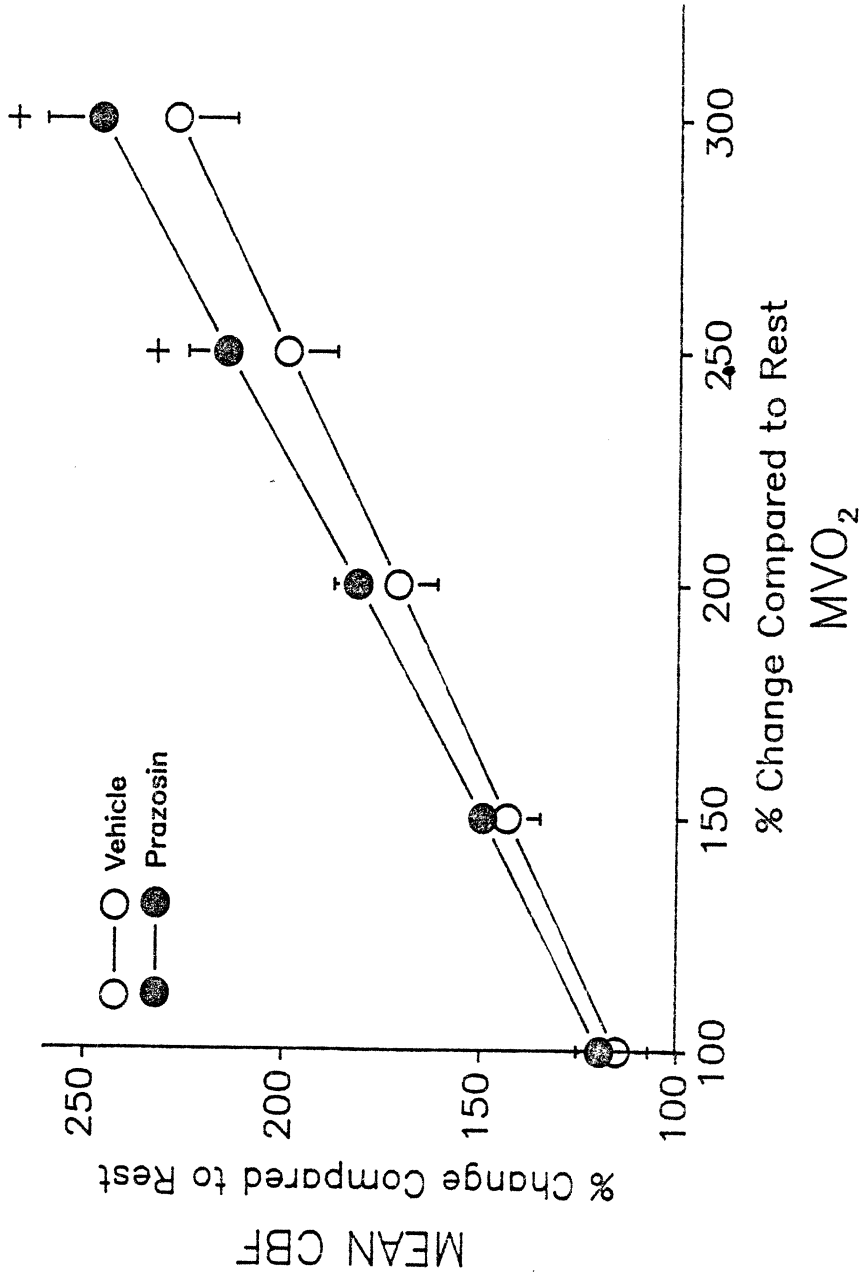
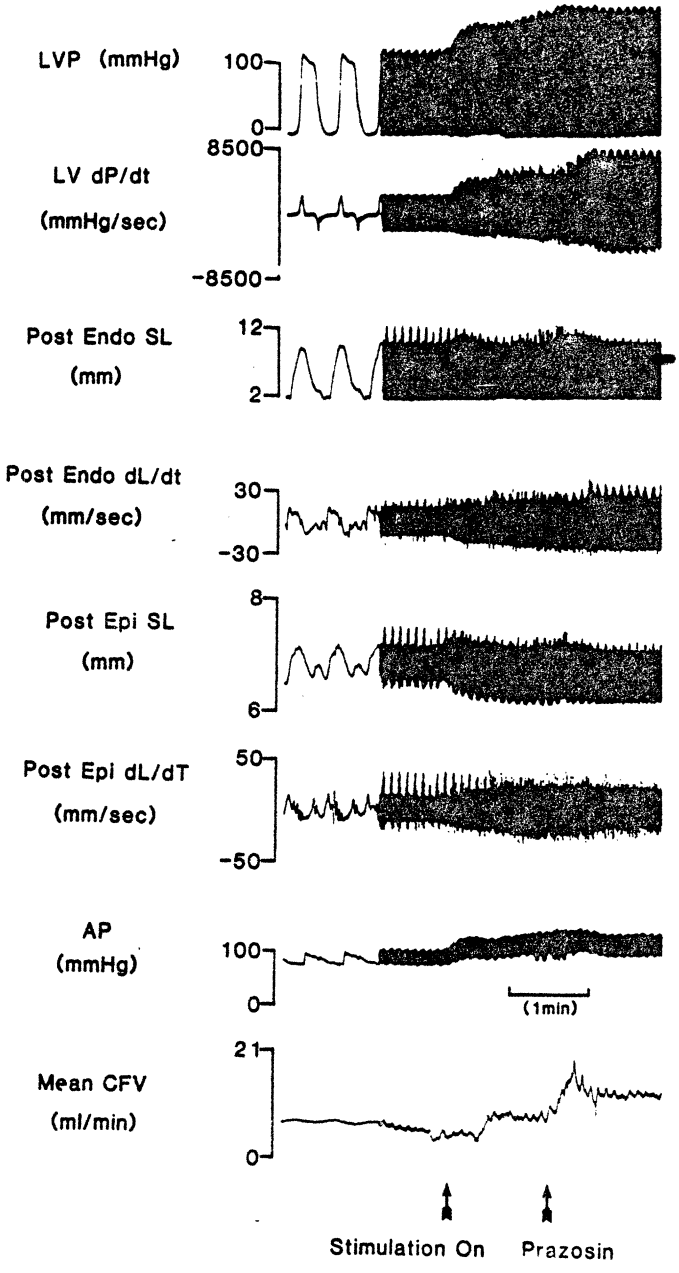

MEAN CBF
% Change Compared to Rest
250
200
150
100
Vehicle
Prazosin
100
150
200
250
300
% Change Compared to Rest
MVO_2

Figure 9 - Effect of Intracoronary Prazosin on Cardiovascular Hemodynamics During Left Stellate Ganglion Stimulation

Tracings from a representative experiment in a Group 1 dog showing left ventricular pressure (LVP), rate of change of left ventricular pressure (dP/dt), posterior subendocardial segment length (SL) and rate of change of posterior segment length (dL/dt), posterior subepicardial SL and dL/dt, and arterial blood pressure. Stellate ganglion stimulation is indicated by the first arrow at the bottom of the tracings. Intracircumflex injection of prazosin during stellate stimulation is indicated by the second arrow. Note that α_1-adrenergic blockade with prazosin elicited a clear increase in posterior subendocardial shortening and dL/dt, which was accompanied by an increase in dP/dt.


LVP (mmHg)
100
0
8500
LV dP/dt
(mmHg/sec)
-8500
Post Endo SL
(mm)
12
2
Post Endo dL/dt
(mm/sec)
30
-30
Post Epi SL
(mm)
8
6
Post Epi dL/dT
(mm/sec)
50
-50
AP
(mmHg)
100
0
(1min)
Mean CFV
(ml/min)
21
0
Stimulation On
Prazosin

during stellate stimulation had no additional effect upon LVP, or AP. However, when compared to stellate stimulation alone, prazosin resulted in further increases in regional CFV. This was followed shortly by increases in both global contractile function (dP/dt_{max}) and in regional contractile function of the subendocardial layer of the posterior perfusion territory (posterior subendocardial dL/dt_{max}). Prazosin was not associated with a change in contractile function of either the posterior subepicardial region (posterior subepicardial dL/dt_{max} and %SL) or of the anterior subendocardial region (not shown).

Results similar to those in Figure 9 were consistently observed in all Group I dogs. The mean data are presented in Tables 5 and 6. Note in Table 5 that intracoronary prazosin during stellate ganglion stimulation resulted in a statistically significant increase in global left ventricular function, as indicated by an increase in dP/dt_{max}. This increase in global function was solely due to an increased subendocardial function in the posterior region. Prazosin caused no substantial changes in LVSP, HR, mean AP. Similarly, prazosin did not cause changes in contractile function in the posterior subepicardial region or in the anterior subendocardial region.

Table 6 demonstrates the effect of the various interventions upon regional and transmural flow distribution

as measured by tracer microsphere studies in Group 2 dogs. Values are given as mean ± SE. Stellate stimulation results in an increase in blood flow to both layers of the anterior and posterior regions of the left ventricle. Within a given region, the increase in flow to each layer was similar so that the subendocardial to subepicardial flow ratio was no different from control values in either region following left stellate ganglion stimulation. Following intracoronary administration of prazosin, significant increases in coronary inflow compared to left stellate ganglion stimulation alone were noted in the posterior region of the left ventricle. Within this region, the increase in flow was similar in both layers, so that the subendocardial to subepicardial flow ratio was not significantly different from values during left stellate ganglion stimulation alone. Administration of prazosin to the vasculature perfusing the posterior region of the left ventricle did not alter flow to either layer of the anterior region of the left ventricle. This suggests that the regionally administered prazosin did not recirculate into the anterior region or cause in changes in coronary inflow as a result of effects upon the systemic vasculature.

Figure 10 displays changes in key variables during stellate stimulation and following α_1-adrenergic blockade during stellate stimulation. All values are expressed as a

TABLE 5

EFFECTS OF INTRACORONARY PRAZOSIN ON GLOBAL AND REGIONAL LEFT VENTRICULAR CONTRACTILE FUNCTION DURING LEFT STELLATE GANGLION STIMULATION

	CONTROL STIMULATION	STELLATE STIMULATION	STELLATE & PRAZOSIN
Global Function			
LVSP (mmHg)	125 ± 6	168 ± 10*	173 ± 2 *
LVEDP (mmHg)	1 ± 1	0 ± 1	0 ± 1
dP/dt_{max} (mmHg/sec)	2935 ± 248	5724 ± 576*	6897 ± 852 *†
HR (bpm)	169 ± 9	189 ± 7*	190 ± 10 *
MAP (mmHg)	112 ± 9	143 ± 6*	134 ± 11 *
Post Subendo Function			
EDL (mm)	10.8 ± 0.3	10.9 ± 0.7	10.9 ± 0.3
% SL	8.7 ± 1.3	10.7 ± 1.7*	11.6 ± 1.6
dL/dt_{max} (mm/sec)	19.0 ± 1.9	32.7 ± 3.3*	38.5 ± 3.5 *†
Post Subepi Function			
EDL (mm)	8.8 ± 0.7	8.9 ± 0.7	8.8 ± 0.7
% SL	9.2 ± 1.6	12.4 ± 2.1*	11.4 ± 1.9 *
dL/dt_{max} (mm/sec)	17.2 ± 3.1	26.8 ± 3.4*	28.6 ± 3.7 *
Ant Subendo Function			
EDL (mm)	9.6 ± 1.9	9.6 ± 1.9	9.7 ± 2.1
% SL	8.7 ± 0.8	10.6 ± 1.9*	10.5 ± 1.5 *
dL/dt_{max} (mm/sec)	31.8 ± 8.7	37.9 ± 1.9*	35.6 ± 7.5 *

Abbreviations: LVSP, peak left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt_{max}, maximal rate of left ventricular pressure change; HR, heart rate; MAP mean arterial pressure; Post Subendo, posterior subendocardial region; EDL, end-diastolic length; %SL, percent segmental shortening; dL/dt_{max}, maximum rate of segment length shortening; Post Subepi, posterior subepicardial region; Ant Subendo, anterior subendocardial region.
Values are mean ± SE for 11 dogs.
*P<0.01, vs control by Duncan's Post-hoc Test.
†P < 0.01, vs stellate stimulation by Duncan's Post-hoc Test.

TABLE 6

EFFECTS OF INTRACORONARY PRAZOSIN ON TRANSMURAL MYOCARDIAL BLOOD FLOW DURING LEFT STELLATE GANGLION STIMULATION			
	CONTROL	STELLATE STIMULATION	STELLATE & PRAZOSIN
Posterior Regional Flow			
Subendo (ml/min/g)	1.11 ± 0.18	1.34 ± 0.13*	1.79 ± 0.27*†
Subepi (ml/min/g)	1.03 ± 0.12	1.36 ± 0.24*	1.65 ± 0.21*†
Subendo/Subepi	1.15 ± 0.11	1.14 ± 0.07	1.16 ± 0.11
Anterior Regional Flow			
Subendo (ml/min/g)	1.15 ± 0.14	1.53 ± 0.19*	1.48 ± 0.20*
Subepi (ml/min/g)	1.05 ± 0.12	1.30 ± 0.24*	1.40 ± 0.26*
Subendo/Subepi	1.20 ± 0.20	1.20 ± 0.20	1.20 ± 0.10

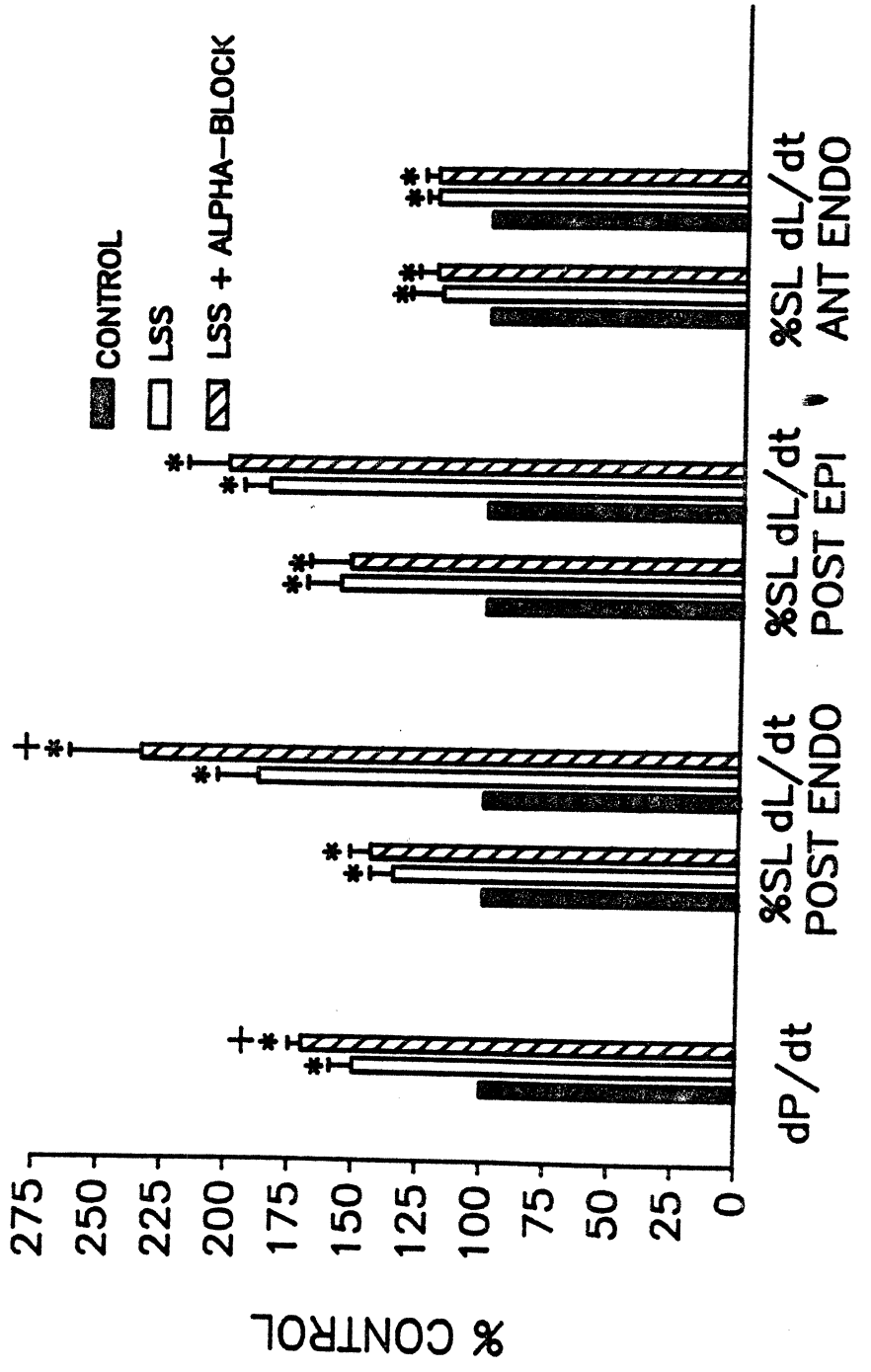
Abbreviations: subendo, subendocardial flow; subepi, subepicardial flow; Subendo/Subepi, left ventricular subendocardial/subepicardial flow ratio.
Values are mean ± SE for 5 dogs.
*P <0.02, vs. control by Duncan's Post-hoc Test.
†P <0.02, vs. stellate stimulation by Duncan's Post-hoc Test.

percent compared to the control value. Stellate stimulation resulted in a significant increase in left ventricular global function as demonstrated by a 50% increase in dP/dt_{max}. This index of global contractile function increased an additional 18% (from 5724 ± 576 mmHg/sec to 6897 ± 852 mmHg) after i.c. administration of prazosin during stellate stimulation. Similarly, stellate stimulation resulted in significant increase in regional contractile function. Thus, %SL and dL/dt_{max} were increased by left stellate ganglion stimulation in the posterior subendocardial, posterior subepicardial, and anterior subendocardial regions. However, prazosin resulted in a further increase in contractile function only in the posterior subendocardial region, where dL/dt_{max} increased an additional 20%. In contrast, prazosin administration did not significantly alter contractile function above stellate stimulation levels in either the anterior subendocardial region or the posterior subepicardial region.

Figure 11 graphically illustrates the effects of intracoronary administration of prazosin on left ventricular oxygen extraction ratio and left ventricular lactate extraction ratio during left stellate ganglion stimulation. Stellate stimulation increased left ventricular oxygen extraction ratio by 15±3% (from 57+4 to 68±2%) and decreased left ventricular lactate extraction ratio by 25±5% (from

Figure 10 - Effect of Intracoronary Prazosin on Global and Transmural Left Ventricular Contractile Function During Left Stellate Ganglion Stimulation

Effect of left stellate ganglion stimulation and α_1-adrenergic blockade on global and regional contractile function. All values are mean $\pm$ SE and are expressed as a percent change compared to control in 11 dogs. Abbreviations: dP/dt, maximal rate of left ventricular pressure generation; % SL, percent segmental shortening; dL/dt, maximal rate of segment length shortening; Post Endo, posterior subendocardial region; Post Epi, posterior subepicardial region; Ant Endo, anterior subendocardial region; LSS, left stellate ganglion stimulation.
*$P < 0.05$ vs control by Duncan's Post-hoc test.
+$P < 0.05$ vs LSS by Duncan's Post-hoc test.


CONTROL
LSS
LSS + ALPHA-BLOCK
% CONTROL
275
250
225
200
175
150
125
100
75
50
25
0
dP/dt
%SL dL/dt POST ENDO
%SL dL/dt POST EPI
%SL dL/dt ANT ENDO

32±11 to 24±4%). At the same time, left stellate ganglion stimulation increased mean CFV by 14±4% (not shown). These data indicate that some region of the left ventricle became more dependent upon anaerobic metabolism during stellate ganglion stimulation. Following intracoronary prazosin injection during stellate ganglion stimulation, oxygen extraction ratio decreased to 60±4% (a value not different from control value), and lactate extraction ratio increased to 34±9% (a value not different from control value). At the same time, intracoronary prazosin injection during left stellate ganglion stimulation caused a further and significant increase in mean CFV of 16±4% (not shown). These responses to intracoronary prazosin during stellate stimulation indicate that removing the α_1-adrenergic vasoconstrictor tone improved oxygen perfusion of the posterior left ventricle.

Figure 11 - Effect of Intracoronary Prazosin on Left Ventricular Oxygen Extraction and Left Ventricular Lactate Extraction During Left Stellate Ganglion Stimulation

Effect of left stellate ganglion stimulation and α_1-adrenergic blockade on percent oxygen extraction and percent lactate extraction. All values are mean $\pm$ SE and are expressed as a percent change compared to control in 11 dogs. Abbreviation: LSS, left stellate ganglion stimulation.
*$\underline{P} < 0.05$ vs control by Duncan's Post-hoc test.
+$\underline{P} < 0.05$ vs LSS by Duncan's Post-hoc test.

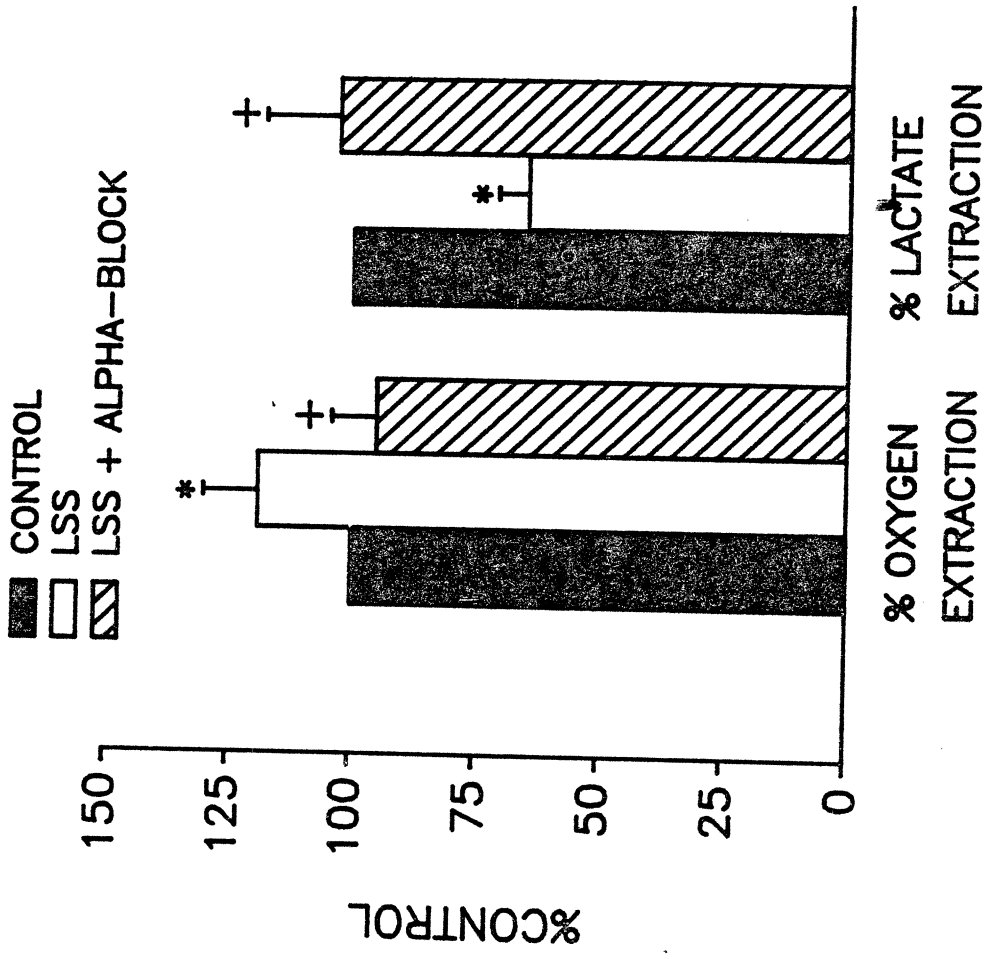
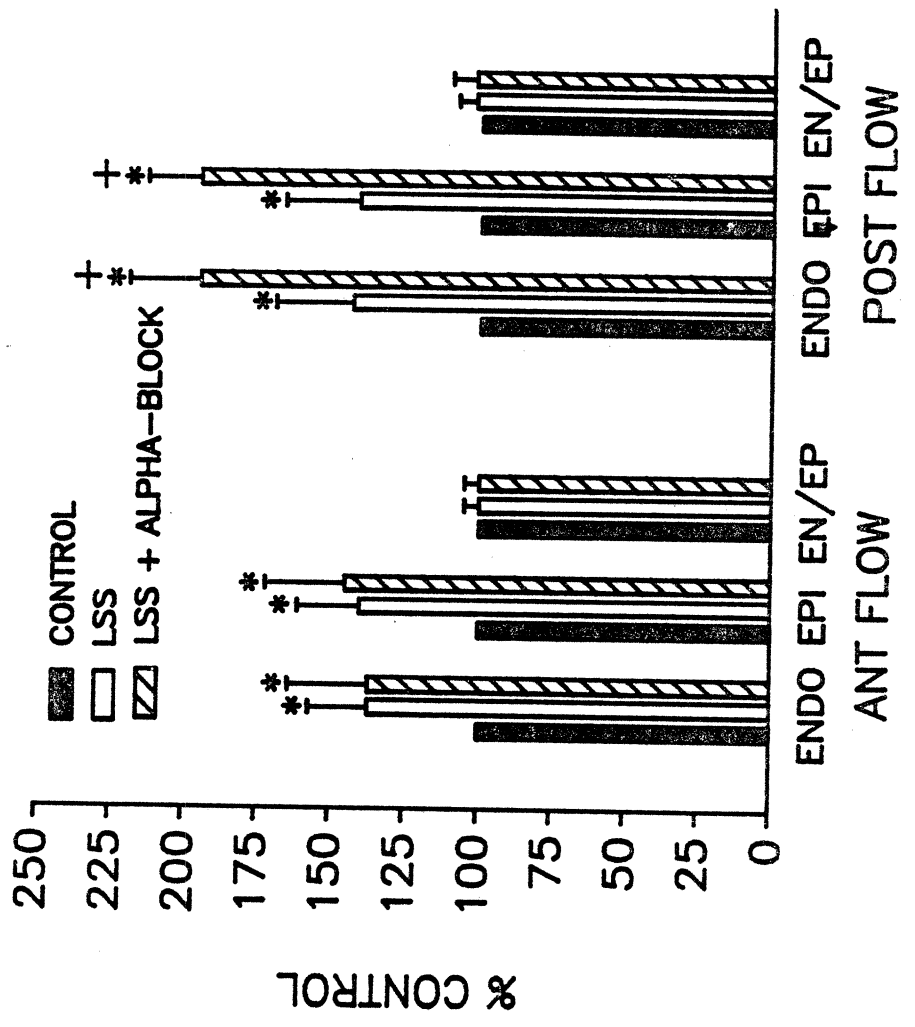

CONTROL
LSS
LSS + ALPHA–BLOCK
%CONTROL
150
125
100
75
50
25
0
*
+
% OXYGEN EXTRACTION
% LACTATE EXTRACTION

Figure 12 graphically illustrates the changes in regional and transmural blood flow during stellate stimulation and during α_1-adrenergic blockade during stellate stimulation in Group 2 dogs. Values are given as mean $\pm$ SE. In the anterior perfusion territory, left stellate ganglion stimulation significantly increased perfusion above control levels in both the subendocardial and the subepicardial layers equally such that the subendocardial-to-subepicardial blood flow ratio was not altered. Administration of prazosin into the circumflex artery did not alter perfusion in either the subepicardial or subendocardial layers of the anterior perfusion territory. Thus, the subendocardial-to-subepicardial flow ratio in this region was unchanged.

STUDY 3: Effect of Direct Coronary Vasodilation on Myocardial Function Contractile During Exercise. This study evaluated whether the change in contractile function following α_1-adrenergic blockade was due to an increase in myocardial perfusion or to some other action of the α_1-adrenergic receptor antagonist. For this purpose, adenosine was infused into the circumflex artery to increase circumflex flow to levels equal to the flow increases produced by removing the coronary α_1-adrenergic receptor constrictor tone using prazosin.

Figure 12 Effect of Intracoronary Prazosin on Transmural Blood Flow in the Left Ventricle During Left Stellate Ganglion Stimulation

Effect of left stellate ganglion stimulation and α_1-adrenergic blockade on transmural left ventricular blood flow distribution. All values are means ± SE and are expressed as percent compared to control in 5 dogs. Abbreviations: Endo, subendocardium; Epi, subepicardium; En/Ep, subendocardial-to-subepicardial flow ratio; LSS, left stellate ganglion stimulation.
*$P < 0.05$ vs control by Duncan's Post-hoc test.
+$P < 0.05$ vs LSS by Duncan's Post-hoc test.


% CONTROL
250
225
200
175
150
125
100
75
50
25
0
CONTROL
LSS
LSS + ALPHA-BLOCK
ENDO EPI EN/EP
ANT FLOW
ENDO EPI EN/EP
POST FLOW

Injection or infusion of the saline vehicle alone during exercise never caused a response in any of the measured variables. Therefore, any response to either prazosin or adenosine during exercise can be attributed to the agent administered.

Representative tracings during rest, during exercise, and during exercise with the intracoronary administration of 0.5 mg prazosin are shown in Figure 13. Note the changes in all parameters during exercise at 6.4 kph at a 16% incline when compared to rest, indicating global increases in cardiac contractile function as well as increased coronary blood supply. These changes were consistent with exercise induced sympathetic stimulation. After administration of prazosin (indicated by an arrow), CBF increased 22%. This was followed by a 10% increase in posterior %SL, and a 23% increase in posterior dL/dt_{max} (arrow). Finally, a 26% increase in dP/dt_{max} occurred (arrow), indicating an increase in global ventricular function. There were no observed changes in HR, AP, or anterior regional function. Representative tracings during rest, exercise and during intracoronary infusion of adenosine during exercise are shown in Figure 14. Similar to Figure 13, exercise at 6.4 kph at a 16% incline produced changes in the parameters consistent with sympathetic stimulation. As described in the protocol, adenosine was infused in a sufficient dose to

increase CBF by an amount comparable to that observed after prazosin. Similar to the responses observed after α_1-adrenergic blockade, adenosine infusion resulted in a 24% increase in posterior %SL, a 40% increase in posterior dL/dt_{max} and a 28% increase in dP/dt_{max}. It is important to note that the changes in cardiac function observed after the increase in CBF were limited to the posterior region of the heart (the circumflex perfusion territory) and that LVP, anterior SL, anterior dL/dt, AP, and HR remained relatively unaffected. The noted changes in cardiac function were regional, occurred without alterations in systemic parameters, and were not seen when the vehicle alone was administered during exercise, which suggests these changes result from local manipulation of a function-limiting variable and are not due to the method of administration or to systemic exposure to the agents. These results typify the response seen in all dogs after intracoronary adenosine infusion while exercising.

Mean values for all 6 dogs at rest and during exercise before and after intracoronary administration of either prazosin or adenosine are shown in Table 7. The two treatment groups showed similar resting values, and significant increases of comparable degree during exercise in LVSP, dP/dt_{max}, HR, %SL, and dL/dt_{max} in both anterior and posterior regions. During peak exercise, CBF also showed

Figure 13 - Effect of Intracoronary Prazosin on Cardiovascular Hemodynamics During Exercise

Representative tracings from a dog showing the responses of systemic hemodynamics and regional myocardial contractile function to rest and submaximal exercise. During exercise, 0.5 mg prazosin was injected into the circumflex artery. Arrows indicate time of injection and the onset of changes in CBF, posterior dL/dt_{max}, and dP/dt_{max}.

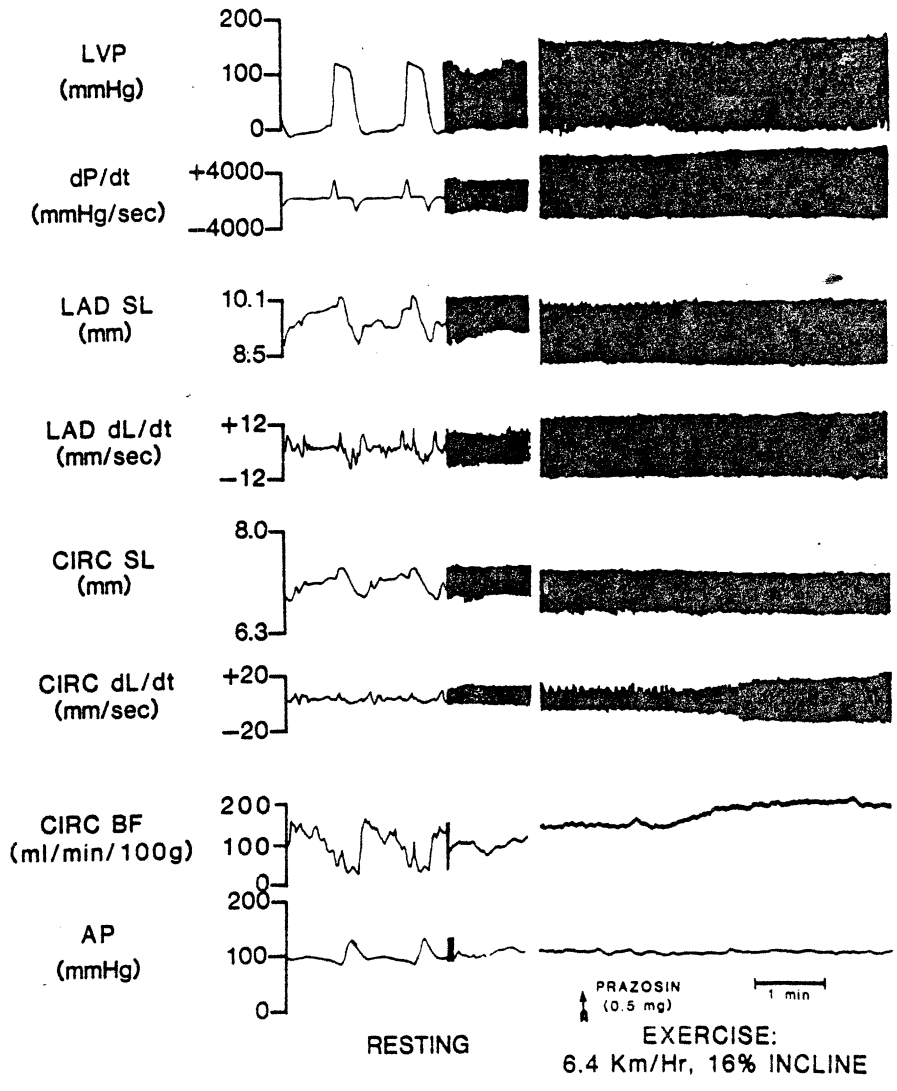
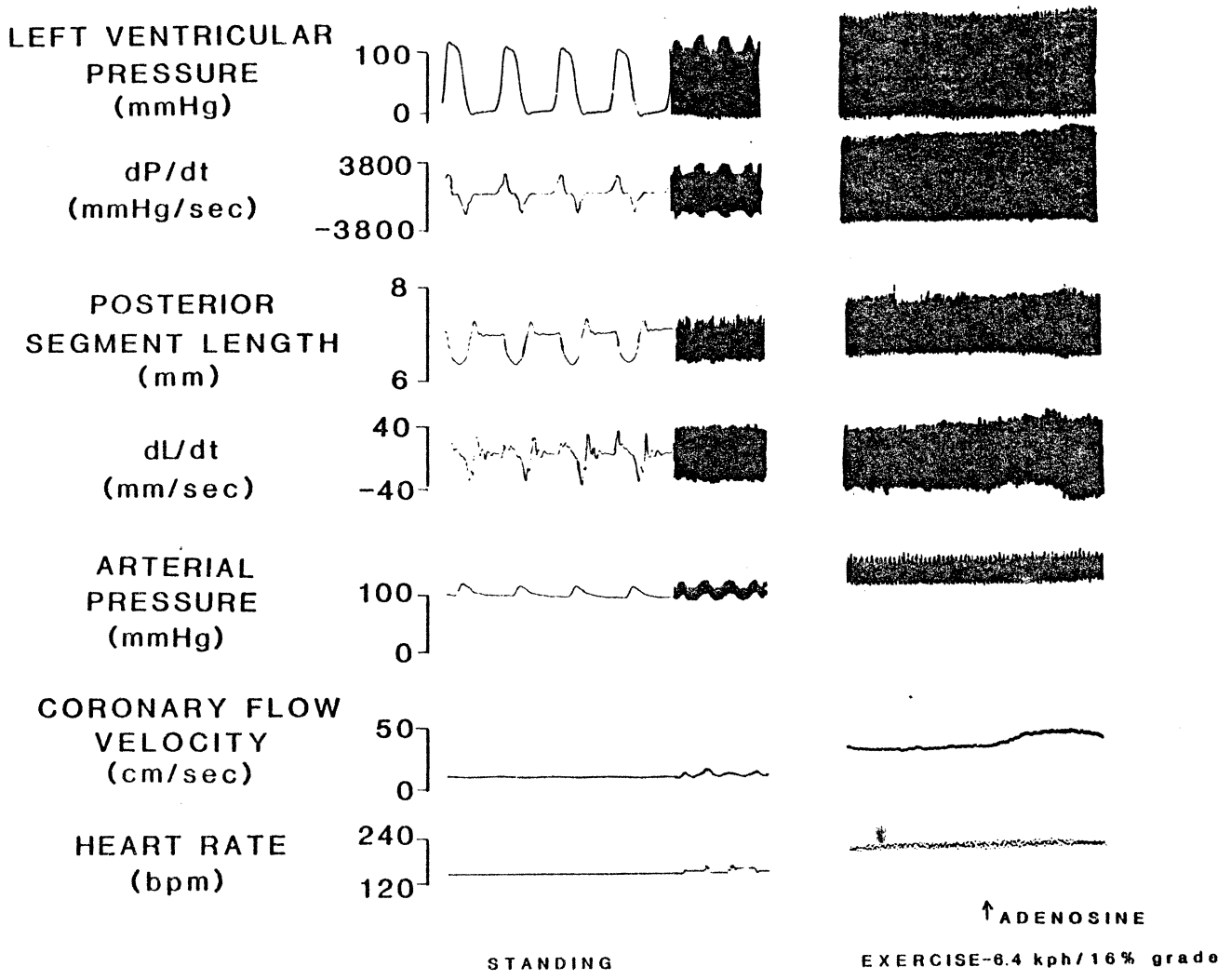

LVP
(mmHg)
200
100
0
dP/dt
(mmHg/sec)
+4000
−4000
LAD SL
(mm)
10.1
8.5
LAD dL/dt
(mm/sec)
+12
−12
CIRC SL
(mm)
8.0
6.3
CIRC dL/dt
(mm/sec)
+20
−20
CIRC BF
(ml/min/100g)
200
100
0
AP
(mmHg)
200
100
0
PRAZOSIN
(0.5 mg)
1 min
RESTING
EXERCISE:
6.4 Km/Hr, 16% INCLINE

Figure 14 - Effect of Intracoronary Adenosine Infusion on Cardiovascular Hemodynamics During Exercise

Representative tracings from a dog showing the responses to rest and exercise. During exercise, adenosine was infused into the circumflex artery in a dose sufficient to increase CBF by 25%. Arrows indicate beginning of adenosine infusion, and the onset of changes in CBF, posterior dL/dt_{max}, and dP/dt_{max}.


LEFT VENTRICULAR PRESSURE (mmHg)
100
0
dP/dt (mmHg/sec)
3800
-3800
POSTERIOR SEGMENT LENGTH (mm)
8
6
dL/dt (mm/sec)
40
-40
ARTERIAL PRESSURE (mmHg)
100
0
CORONARY FLOW VELOCITY (cm/sec)
50
0
HEART RATE (bpm)
240
120
↑ADENOSINE
STANDING
EXERCISE-6.4 kph/16% grade

similar increases in the two treatment groups. Circumflex injection of prazosin during exercise resulted in a 22±5% increase in CBF, which was associated with significant increases in posterior dL/dt_{max} (30±8%) and dP/dt_{max} (17±2%). By comparison, adenosine infusion produced a 26±8% increase in CBF, which was comparable to the increase in flow observed after prazosin. Like the myocardial contractile function changes observed after prazosin, infusion of adenosine was associated with significant increases in posterior dL/dt_{max} (27±6%) and in dP/dt_{max} (15±2). Neither prazosin nor adenosine precipitated a change in anterior segment dL/dt_{max}, %SL, LVSP, LVEDP, AP or HR, indicating that the changes in posterior left ventricular myocardial function were a regional effect and not due to significant recirculation or to systemic reflex affects.

TABLE 7

RESPONSES OF MYOCARDIAL FUNCTION AFTER AUGMENTING CORONARY BLOOD FLOW DURING SUBMAXIMAL EXERCISE

	REST	EXERCISE	EXERCISE & PRAZOSIN	REST	EXERCISE	EXERCISE & ADENOSINE
LVSP (mmHg)	127 ± 5	169 * ± 12	171 * ± 12	121 ± 9	159 * ± 14	159 * ± 13
LVEDP (mmHg)	5 ± 2	6 ± 2	6 ± 2	4 ± 3	6 ± 3	6 ± 2
dP/dt_{max} (mmHg/sec)	3914 ± 437	7239 * ± 762	8459*† ± 854	3700 ± 348	7825 * ± 924	8957*† ± 1141
MAP (mmHg)	113 ± 7	130 * ± 8	132 * ± 9	102 ± 5	120 * ± 6	120 * ± 6
HR (beats/min)	120 ± 11	229 * ± 5	229 * ± 7	139 ± 7	228 * ± 9	229 * ± 8
CBF (ml/min/100g)	103 ± 26	165 * ± 38	197*† ± 43	97 ± 23	163 * ± 41	206*† ± 45
POSTERIOR REGIONAL FUNCTION						
EDL (mm)	12.3 ± 1.3	12.0 ± 1.4	12.0 ± 1.5	11.8 ± 1.0	11.8 ± 1.0	11.9 ± 1.1
% SL	12.3 ± 3.0	16.0 * ± 3.1	17.6 * ± 3.1	12.1 ± 1.5	15.0 * ± 2.7	15.2 * ± 2.6
dL/dt_{max} (mm/sec)	24.6 ±10.1	32.4 * ± 8.9	42.5*† ± 9.7	22.3 ± 8.4	29.8 * ± 9.5	36.6*† ± 10.4
ANTERIOR REGIONAL FUNCTION						
EDL (mm)	10.7 ± 1.7	10.8 ± 1.9	10.9 ± 1.9	11.7 ± 2.6	12.3 ± 3.0	12.4 ± 3.0
%SL	12.9 ± 1.3	15.7 * ± 1.1	16.6 * ± 1.2	12.3 ± 1.7	16.3 * ± 3.1	17.0 * ± 3.4
dL/dt_{max} (mm/sec)	22.0 ± 6.2	35.0 * ± 10.3	37.0 * ± 7.6	18.1 ± 3.3	30.9 * ± 8.6	32.4 * ± 9.0

Abbreviations: LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt_{max}, maximum rate of change of left ventricular pressure; MAP, mean arterial pressure; HR, heart rate; CBF, mean circumflex blood flow; EDL, end-diastolic length; %SL, percent segment shortening; dL/dt_{max}, maximum rate of segment length shortening. Values are mean ±SE for 6 dogs.

*P < 0.05, exercise vs rest by Duncan's Post-hoc Test.

†P < 0.05, alpha-block or adenosine vs exercise by Duncan's Post-hoc test.

CHAPTER V

DISCUSSION

Previous studies have suggested that an α-adrenergic constrictor tone is present in the coronary vasculature and limits the extent of coronary dilation and oxygen delivery during exercise (Murray and Vatner, 1979; Heyndrickx et al, 1982; Gwirtz and Stone, 1981; Heyndrickx et al, 1984; Gwirtz et al, 1986; Chilian et al, 1988; Strader et al, 1988; Dai et al, 1989). Few of these studies examined the effects of a sympathetic coronary constriction on actual myocardial oxygenation and contractile function. It is apparent that even during conditions in which myocardial oxygenation is flow-limited and maximal coronary dilation is expected (such as high metabolic demand or coronary obstruction), the presence of a coronary constrictor tone impedes oxygen delivery and myocardial contractile function. For example, Heyndrickx et al (1982) reported that systemic α-adrenergic blockade with phentolamine in dogs subjected to strenuous exercise increased both coronary blood flow and myocardial oxygen consumption. In addition, studies by Gwirtz and coworkers (Gwirtz et al, 1986; Strader et al, 1988) showed that intracoronary administration of phentolamine or prazosin increased coronary blood flow 26% during

strenuous exercise in dogs. Associated with the increase in flow was a significant increase in oxygen delivery as well as regional and global myocardial contractile function. These studies implied that a sympathetic coronary α-adrenergic constrictor tone opposed vasodilatory mechanisms to the extent that myocardial oxygenation and, as a result, contractile function was restricted in its ability to increase. In previous studies, the degree of flow-limitation during exercise by the α-adrenergic constrictor tone varied. Thus, coronary blood flow after α-adrenergic blockade was found to be greater than in the unblocked state by 30% (Gwirtz et al, 1986), 14% (Heyndrickx et al, 1982) 21% (Strader et al, 1988), and 6% (Huang and Feigl, 1988). These differences may be related to differences in experimental protocol or exercise intensity. In other words, the magnitude of the coronary constrictor tone may vary with the intensity of exercise, but no study had directly examined this possibility. Therefore, the aim of Study 1 was to evaluate the presence of an α-adrenergic coronary constrictor tone at various levels of submaximal exercise. Additionally, it was desired to determine whether the constrictor tone affects myocardial contractile performance in either a beneficial or deleterious manner. A beneficial effect of this coronary constriction on myocardial performance could be expected if the vascular tone acted to

distribute blood to the regions of the myocardium most prone to ischemic compromise. In contrast, a deleterious effect of this coronary constriction on myocardial performance could be expected if the vascular constrictor tone restricted perfusion of the myocardium to such a degree that the exercise-related increase in myocardial contractile performance was blunted.

The most important finding of Study 1 was that an α_1-adrenergic coronary constrictor tone during dynamic exercise was not associated with improved left ventricular contractile function at the exercise levels evaluated. Rather, removal of the α_1-constrictor tone increased contractile function due to an increase in left coronary artery blood flow. During mild levels of exercise, a coronary constrictor tone did not appear to impede the increase in coronary blood flow accompanying exercise. As exercise levels became more strenuous, the coronary constrictor tone significantly limited the increase in coronary blood flow, and this attenuated rise in coronary blood flow became associated with a blunting of the increases in ventricular contractile function and in myocardial oxygen consumption. The results of this study indicate that a sympathetic adrenergic coronary constrictor tone mediated by the α_1-adrenergic receptors imposed a limitation on coronary blood flow, and that this constrictor

tone did vary with intensity of exercise. Thus, the coronary constrictor tone was present at mild levels of exercise, but the increase in coronary blood flow after α_1-adrenergic receptor blockade was statistically insignificant. However, the coronary constrictor tone became significant at higher levels of exercise and imposed a limitation on coronary functional hyperemia of 15±7%, 24±9%, and 35±10% at exercise workloads of 6.4 kph and 8%, 12%, and 16% incline, respectively. Furthermore, the limitation to functional hyperemia at 6.4 kph, 16% grade was significantly greater than the limitation at 6.4 kph, 8% grade. As a result of this limitation on coronary blood flow, the increase in regional left ventricular contractile function was also apparently restricted, especially at the two highest workloads, by 36±12% and 42±13%.

Recent evidence also indicates that an α_1-adrenergic coronary vasoconstriction opposes metabolic coronary dilation and, as a result, minimizes the increases in ventricular contractile function associated with higher levels of exercise. Thus, Gwirtz and coworkers (Gwirtz et al, 1986; Strader and Gwirtz, 1988) showed that intracoronary prazosin administered during exercise at 6.8 kph, 16% grade, results in an increase in coronary blood flow which is followed shortly by an increase in ventricular contractile function. However, these studies did not

evaluate the effects of coronary α_1-adrenergic receptor blockade at less strenuous levels of exercise. Also, Huang and Feigl (Huang and Feigl, 1988) recently proposed that this α_1-adrenergic coronary constrictor tone results in a redistribution of blood flow distribution across the ventricular wall such that blood tends to be shunted toward the subendocardium as exercise intensity increase. These investigators produced regional blockade of coronary α-adrenergic receptors by intracoronary administration of the nonspecific α-blocking agent phenoxybenzamine. They used radioisotope-labeled microspheres to measure regional blood flow and compared blood flow in the blocked region of the ventricle to the unblocked region in running dogs during systemic β-adrenergic blockade (propranolol). Their findings showed that, although mean coronary blood flow during exercise was less in the unblocked region than in the blocked region, the ratio of subendocardial-to-subepicardial blood flow was more equitably maintained in the α-blocked region than in the unblocked region. These investigators concluded that these data provide evidence that an α_1-adrenergic receptor mediated coronary constrictor tone maintained blood flow to the subendocardial layers in spite of limiting mean transmural coronary blood flow. Huang and Feigl (1988) refer to this as a "reverse steal" phenomenon. The combined effects of a greater degree of shortening and

tension development by the subendocardial myocytes compared to the subepicardial myocytes (Streeter et al, 1970), a smaller vasodilator reserve of in the subendocardium (Braunwald and Sobel, 1980), and a shorter duration of perfusion during each cardiac cycle in the subendocardium vs the subepicardium (Braunwald and Sobel, 1980) make the subendocardial myocytes more prone to ischemia than are the subepicardial myocytes. In this regard, a "reverse steal" effect of this coronary constrictor tone would theoretically be associated with improved ventricular contractile function by perfusing the myocardial layers most prone ischemia-related compromise. However, these investigators did not evaluate ventricular contractile function. In addition, the study by Huang and Feigl (1988) does not easily explain the increase in ventricular contractile function found after α_1-adrenergic blockade of the coronary circulation seen by Gwirtz and coworkers (Gwirtz et al, 1986; Strader and Gwirtz, 1988).

Both the findings of Huang and Feigl (Huang and Feigl, 1988) and those of Gwirtz and coworkers (Gwirtz et al, 1986; Strader and Gwirtz, 1988) would be consistent with a mechanism whereby the transmural redistribution of blood flow associated with α_1-adrenergic constrictor tone during exercise had an effect on ventricular contractile function which changed with exercise intensity. That is, the

transmural redistribution of ventricular blood flow mediated by α_1-adrenergic receptor stimulation during exercise would improve ventricular function at low levels of exercise by maintaining blood flow to the potentially ischemic subendocardial layers. As exercise becomes more strenuous, the decrease in mean coronary blood flow which accompanies this "reverse steal" phenomenon would begin to counterbalance the benefit of flow redistribution. At higher levels of exercise, the restriction to coronary blood flow becomes so great that myocardial oxygen delivery is impaired, especially to the subendocardium, and, as a result, ventricular contractile function would be attenuated. However, the findings of the present study do not seem to indicate that an α_1-adrenergic coronary constrictor tone actually improves subendocardial or global left ventricular contractile function at any exercise level evaluated. Although regional myocardial blood flow was not measured in the present study, and the possibility of a transmural redistribution of ventricular perfusion was not evaluated, it is apparent that any change in coronary blood flow associated with coronary vascular α_1-adrenergic receptor activation during exercise does not benefit ventricular contractile function at the exercise levels studied here. In view of these findings, it is likely that the maintained uniformity of transmural blood flow

distribution associated with α-adrenergic receptor blockade of the coronary vasculature in exercising dogs seen by Huang and Feigl (1988) was not a reflection of increased absolute flow to the subendocardial layers. Rather, this maintained transmural flow ratio was probably due to a blunting of the degree to which a coronary constrictor tone restricted flow increases in the subendocardial layer compared to the degree this tone limited flow increases to the subepicardial layers. This interpretation of the results of the study by Huang and Feigl (1988) implies that α-adrenergic blockade in the coronary arteries can increase blood flow to all layers of the ventricle, including the subendocardial layers. In this regard, removing a coronary constrictor tone by α-adrenergic blockade would augment oxygen delivery to the myocardium during exercise and allow for improved contractile function compared to the unblocked state. Because no absolute values of region blood flow were presented by Huang and Feigl (1988), this latter interpretation of their results must be considered. The findings of Study 2 also support this interpretation. In Study 2, it was found that intracoronary administration of the α_1-adrenergic blocking agent prazosin during left stellate ganglion stimulation resulted in an increase in mean coronary blood flow to the ventricle. Furthermore, this increase in mean coronary blood flow was distributed equally

across the subepicardial and subendocardial layers of the ventricle.

The results of Study 1 imply that the increase in ventricular oxygen utilization during exercise after α_1-adrenergic blockade was due to a greater increase in blood flow without changes in oxygen extraction or arterial hemoglobin content. This is graphically displayed in Figure 9, illustrating the relationship between increases in MVO_2 and coronary blood flow. Note that, during prazosin infusion as compared to vehicle infusion, the increase in mean coronary blood flow is greater for any increase in MVO_2. This relationship becomes significant at the higher levels of exercise, and can be interpreted to indicate that the increase in maximal achieved MVO_2 associated with prazosin is the result of greater coronary blood after α_1-adrenergic blockade. The oxygen consumed by the myocardium is supplied by oxygen-saturated hemoglobin arriving to the myocardium at a given rate. Assuming that the perfusate does not alter its hemoglobin concentration or degree of saturation of the hemoglobin, the supply of oxygen to the myocardium can be adjusted by altering the rate at which the hemoglobin arrives at the myocardium (i.e., by altering coronary flow velocity) and/or by altering the amount of oxygen removed by the myocardium from each molecule of hemoglobin (i.e., oxygen extraction). In the present study, arterial oxygen

saturation remained constant during exercise. This is consistent with previous findings by others (Rowell, 1986). Likewise, hemoglobin content did not change. Although it has been reported that release of stored red blood cells by splenic contraction can cause hemoglobin concentration to increase above resting values with exercise (Vatner et al, 1974), others have found that this does not occur if the "resting" dog is anticipating exercise (Ordway et al, 1984). α_1-Adrenergic blockade of the coronary vasculature did not alter percent oxygen extraction during exercise. Therefore, the increase in oxygen supplied to the heart which permitted the improved contractile function and increase oxygen consumption after α_1-adrenergic blockade resulted solely from an increase in coronary blood flow. These data could be interpreted to indicate that an α_1-adrenergic receptor-mediated constrictor tone limits coronary vasodilation during submaximal exercise and that removal of this restriction to vasodilation allows ventricular contractile function to approach its potential.

The findings of Study 1 suggest that the normal myocardium, especially the subendocardial layer where regional contractile function was measured, has the potential to become underperfused at more strenuous levels of exercise, and that this underperfusion may impose a limitation on left ventricular contractile performance. The

factors affecting ventricular contractile performance are preload, afterload, contractility, and heart rate (Parmley and Tyberg, 1988). These same factors, in addition to basal oxygen consumption, determine myocardial oxygen demand (Parmley and Tyberg, 1988). During α_1-adrenergic blockade of the circumflex artery, the response of each of these factors to submaximal exercise was similar compared to exercise without α_1-adrenergic blockade except that ventricular contractility was significantly increased above values obtained at the same workloads in the unblocked condition. Thus, the increase in myocardial oxygen consumption was accompanied by an increased rate of regional myocardial segmental shortening and left ventricular pressure generation. Compared to values obtained during administration of the vehicle, there was no significant difference in heart rate. Prazosin administration also did not alter ventricular preload, as indicated by a lack of significant change in either left ventricular end diastolic pressure, end diastolic segment length the normally perfused anterior region of the left ventricle, or end diastolic length of the posterior region of the left ventricle after α_1-adrenergic receptor blockade. Similarly, ventricular afterload was comparable during exercise with prazosin administration vs vehicle administration, since mean arterial pressure and left ventricular systolic pressure

were similar during administration of either the vehicle or prazosin. Finally, the lack of a difference in resting oxygen consumption following α_1-adrenergic blockade is evidence that prazosin did not alter baseline oxygen requirements.

Sympathetic adrenergic stimulation was accomplished using a modification of the submaximal treadmill test described by Tipton et al (1974). This submaximal exercise test has been shown to result in systemic sympathetic activation, as evidenced by decreases in splanchnic flow at stresses near 30% maximal oxygen consumption (Musch et al, 1987). Such levels of intensity are met in running dogs when their heart rates reach approximately 180 beats/min (Musch et al, 1986). The untrained dogs in our Study 1 achieved this heart rate at an exercise workload of 6.4 kph, 4% incline. Similar findings have been reported by others (Stone,1980; Ordway et al, 1984; Musch et al, 1985). It is interesting to note that, compared to unblocked dogs, α_1-adrenergic blockade significantly increased mean coronary blood flow in dogs at equivalent levels of exercise more strenuous than 6.4 kph, 4% grade. As exercise intensity increased, the degree of α_1-adrenergic receptor constrictor tone increased (the percentage increase in mean coronary blood flow after α_1-adrenergic receptor blockade as workload increased). This increase in coronary blood flow was

accompanied by increases in regional contractile function (systolic dL/dt_{max}), global contractile function (dP/dt_{max}), and myocardial $\dot{M}VO_2$. Administration of prazosin did not alter oxygen extraction. Altogether, this evidence suggests that the restrictive effects of coronary α_1-adrenergic mediated vasoconstriction upon coronary blood flow and ventricular function do not become significant until physical stress has raised total body $\dot{M}VO_2$ above 30% of $\dot{M}VO_{2max}$. Since the increased myocardial $\dot{M}VO_2$ associated with this prazosin-related improved left ventricular contractile function was achieved by increasing circumflex blood flow without changing myocardial oxygen extraction, it is attractive to speculate that the limitations to left ventricular contractile function associated with increasing levels of exercise occur because the heart can extract no more oxygen from the blood. Therefore, any increases in oxygen delivery to the myocardium must be provided by increased coronary blood flow, and these increases in coronary blood flow are restricted by α_1-adrenergic tone on the coronary arteries.

In Study 1, the left ventricle did not augment oxygen availability by increasing myocardial oxygen extraction as exercise workload increased even though increased oxygen delivery to the myocardium accompanied an increase in myocardial contractile function at increased intensities of

intensities of exercise. There are three possible explanations for these results:

(1) Since submaximal levels of exercise were used in these animals, myocardial extraction did not increase with exercise intensity because the body receives adequate perfusion at these exercise intensities. Therefore, the increased left ventricular contractile function (and the presumed associated increased cardiac output) permitted by the rise in myocardial oxygen availability associated with α_1-adrenergic receptor blockade under these conditions was not needed by the body. It could be argued that if the heart were more exhaustively or maximally taxed, metabolic coronary vasodilatory forces would be stronger. This would completely override the α-adrenergic coronary constrictor forces, allowing sufficient coronary blood flow to support maximal ventricular contractile function. However, the results of Study 2 imply that this reasoning is probably incorrect. In Study 2, α_1-adrenergic receptor blockade of the coronary circulation increased myocardial perfusion and improved contractile performance in normal, healthy hearts under conditions of maximal sympathetic stimulation. This suggests that a_1-adrenergic receptor mediated coronary constriction can restrict coronary blood flow and, as a result, limit cardiac contractile performance even when demands for cardiac output are high (to be discussed below).

In this regard, cardiac output can be blunted by sympathetic coronary vasoconstriction such that systemic demands for cardiac output are not satisfied.

(2) The observed increase in myocardial oxygen utilization could be the result of, rather than the cause of, improved ventricular contractile performance. In this regard, intracoronary prazosin administration could increase ventricular contractile performance by some direct mechanism. Subsequent to this increased ventricular performance, metabolic vasodilation would allow flow to increase and make more oxygen available to the heart for utilization. This mechanism associating coronary α_1-adrenergic receptor blockade with both increased coronary flow and increased ventricular contractile performance is unlikely for a number of reasons. First, the changes in contractile function have been noted to follow, rather than precede, the increase in coronary blood flow (Gwirtz et al, 1986). Secondly, stimulation of myocardial α_1-adrenergic receptors is associated with increased inotropy (Langer et al, 1985). Therefore, a direct effect of blockade of α_1-receptors would be expected to be a decrease in ventricular contractile performance rather than an increase. Thirdly, the findings of Study 3 indicate that ventricular contractile performance during exercise can be increased when coronary blood flow is increased by direct coronary

dilation with adenosine (to be discussed below). This is seen despite a direct negative inotropic of adenosine on the myocardium (Linden et al, 1985).

(3) Oxygen availability to the ventricle is limited by the α_1-adrenergic mediated coronary constrictor tone during exercise even though this flow restriction may be "detrimental" to ventricular contractile performance and this phenomenon has no apparent teleologic benefit. This possibility is intrinsically difficult to accept. Most reflexes which limit the body's ability to function become harmful only after the stimulus for the reflex becomes persistent or excessive. Initially, the reflex allows the body to perform more efficiently in a given situation. Only after the stimulus for the reflex is raised to inappropriately high levels do the effects of this reflex compromise the body's ability to perform. Thus, even the fluid retention associated with heart failure becomes deleterious only after it has improved cardiac output by increasing end-diastolic length and improving Starling forces in the heart.

Few studies have examined the transmural nature of the coronary α-adrenergic constrictor tone and its actual influence on myocardial contractile function. The results from Study 1 indicate that, during exercise, a coronary α_1-adenergic constriction not only limits myocardial perfusion

and oxygen delivery, but as a result may also limit myocardial contractile function. The study showed that there is little likelihood that this constrictor tone permits the ventricular contractile performance to achieve levels greater than those achievable in its absence. The study used piezoelectric crystals implanted only in the left ventricular subendocardium to measure regional contractile function. No analysis of regional contractility in the more superficial layers was made. Similarly, no conclusion could be drawn regarding the transmural nature of an α_1-adrenergic vasoconstrictor tone during this experiment. Therefore, the aims of Study 2 were to more closely examine a possible differential effect of α_1-adrenergic constrictor tone on left ventricular subendocardial versus subepicardial myocardial perfusion and contractile function during a high degree of cardiac sympathetic stimulation. For this purpose, the left stellate ganglion was stimulated in anesthetized, open-chest dogs, and the effects of selective α_1-adrenergic blockade on contractile function in both deep and shallow muscle layers was measured. As described previously, selective α_1-adrenergic receptor blockade was employed because previous work has indicated that under a variety of conditions leading to increased sympathetic stimulation of the healthy heart, the adrenergic coronary constriction is entirely attributable to activation of the α_1-receptor

subtype (Jones et al, 1986; Gwirtz et al, 1986; Bache et al, 1987). To determine if any differential effects of α_1-vasoconstriction on contractile function in deep and shallow layers was associated with a nonuniform influence on perfusion across the ventricular wall, transmural coronary blood flow was measured using tracer microspheres during stellate stimulation and α_1-adrenergic blockade.

The four important findings of Study 2 were: 1)During left stellate stimulation, intracoronary α_1-adrenergic blockade improved global myocardial contractile function. 2)This improved global contractile function reflects a change in the contractile function of the subendocardial layer only. 3)Under conditions of direct stellate ganglion stimulation, coronary blood flow was limited by an α_1-adrenergic tone. 4)The distribution of this α_1-adrenergic limitation in flow was equal transmurally and did not alter the normal ratio of subendocardial-to-subepicardial blood flow. Altogether, these findings suggest that a flow-related restriction to contractile function affecting mainly the deeper layers of the heart exists during conditions of intense sympathetic stimulation.

The observed increase in subendocardial contractile function without a change in subepicardial function following α_1-adrenergic receptor blockade can feasibly be explained by two mechanism. First, it is possible that an

adrenergic constrictor tone is uniformly distributed across the ventricular wall, and abolition of this tone causes an equal increase in subepicardial and subendocardial perfusion. In such a case, the subsequent increase in contractile function only in subendocardial layer could be attributed to a flow-dependent limitation to function existing in these layers only. Second, the increase in contractile function solely in the subendocardial layers could be due to a nonuniform sympathetic constrictor tone across the ventricular wall such that the constrictor influence was greater in the subendocardial layers.

The uniform transmural nature of α_1-adrenergic tone reported here is in agreement with findings by others. Thus, Johannsen et al (1987) used 9 μm microspheres to determine that intracoronary administration of phenylephrine does not rectify the diminished subendocardial-to-subepicardial flow ratio resulting from systemic infusion of adenosine. Similarly, Chen et al. (1988) noted no transmural redistribution of blood flow (radioactive microspheres) after left circumflex artery perfusion of either phenylephrine of BHT 933. Likewise, Williams et al (1988) showed that the α-adrenergic receptor mediated coronary vasoconstrictor reflex caused by acute systemic hypoxia is uniformly distributed across the left ventricular free wall. Furthermore, Rinkema et al (1982) observed no change in

transmural distribution of flow during stellate ganglion stimulation, while Holtz et al (1977) reported no change in transmural distribution following chemical sympathectomy with 6-hydroxydopamine.

However, as pointed out above, other investigators (Ross and Mulder, 1969; Giudicelli et al, 1980; Nathan and Feigl, 1986; Chilian and Ackell, 1988; Huang and Feigl, 1988) have favored either a subepicardial or a subendocardial predominance of an α-adrenergic coronary constriction. Huang and Feigl (1988) proposed that the adrenergic coronary constriction is not uniform across the ventricular wall but may be more intense in the subepicardial muscle layers such that it diverts blood flow to the more vulnerable deeper layers during exercise. These investigators tested this hypothesis in dogs by selectively blocking α-receptors in one myocardial region, using the nonspecific antagonist phenoxybenzamine, and measuring regional myocardial perfusion during graded treadmill exercise. They observed that average transmural flow was less in the unblocked region than in the blocked region, as would be expected from the results of other investigators. However, they also observed that the ratio of subendocardial-to-subepicardial perfusion was better maintained in the region in which α-receptors were intact. A similar nonuniform adrenergic constriction across the

ventricular was has also been reported by Chilian and Ackell (1988) in exercising dogs subjected to coronary stenosis. It was proposed by Huang and Feigl (1988) that this redistribution of blood flow in the ventricle would allow the heart to maintain contractile performance under conditions of intense sympathetic stimulation. As stated previously, though, the results of Study 1 indicate that this α_1-adrenergic vasoconstriction is never associated with augmented global regional contractile performance compared to the unblocked state at equivalent levels of submaximal exercise. The results of Study 2 suggest that, even if the heart is stimulated by electrical stimulation of the left stellate ganglion to cause maximal degrees of sympathetic activation, global and regional contractile performance are still less in the absence of coronary α_1-adrenergic blockade compared to in its presence. It should also be noted that there is no evidence in the literature for a nonuniform distribution of α_1-adrenergic receptors in left ventricular coronary vessels. In this regard, no difference in the density of α_1-adrenergic receptors between subepicardial and subendocardial coronary arterioles can be detected using receptor binding techniques (Saffitz, 1989). Huang and Feigl (1988) proposed the possibility that the endogenous vasodilator adenosine is released from myocardial myocytes preferentially in the deeper layers, where contractile

function may be more flow-limited. Since adenosine is known to attenuate release of norepinphrine from sympathetic nerve terminals, it is then feasible that neuronal release of norepinphrine during exercise is less in subendocardial layers than in subepicardial layers. However, such a mechanism depends on a greater degree of ischemia in the subendocardium compared to the subepicardium, or requires a greater sensitivity to ischemia in the subendocardium compared to the subepicardium, for adenosine to be released in greater quantities by the subendocardium than by the subepicardium. In either of these cases, subendocardial ischemia stimulates the release of adenosine. As such, a greater degree of subendocardial vasodilation compared to subepicardial vasodilation would never occur because the stimulus for preferential subendocardial release of adenosine would be removed when blood flow to the subendocardium became greater than blood flow to the subepicardium. The results of Study 2 indicate that the prazosin-related increase in contractile function only in the deeper layers may be due to the fact that, even though an adrenergic vasoconstriction was uniform across the entire ventricular wall, only in the deeper, more ischemia-prone layers did this vasoconstriction limit contractile function. In regard to transmural distribution of an α-adrenergic vasoconstriction, it should also be recalled that Laxson et

al (1989) saw an 87% increase in subendocardial perfusion following specific α_1-adrenergic blockade in exercising dogs following partial coronary stenosis. The basis of the differences between the results of these investigations is at present unclear. While the proposal of Huang and Feigl (1988) is attractive and provides a logical physiological basis for an adrenergic coronary constriction in exercise and other conditions, this hypothesis requires further exploration.

Evaluating the distinction between neural and hormonal stimulation of coronary adrenergic receptors may help clarify the apparently diverse findings of the investigations described above. Autonomic nerves are closely associated with both the larger conduit vessels and with the smaller resistance vessels of the coronary vasculature (Dolezel, 1978). These nerves terminate on the adventitial (vs luminal) side of the vessel (Dolezel, 1978; Denn and Stone, 1976). Neural stimulation results in release of the neurotransmitter norepinephrine which interacts with α-adrenergic receptors on coronary smooth muscle cells along the adventitial surface of the blood vessel media. In contrast, hormonal adrenergic stimulation by circulating substances (eg. epinephrine) results from activation of α-adrenergic receptors on smooth muscle cells along the media-intima border. Thus, the transmural distribution of

adrenergic receptors stimulated by neurally released norepinephrine may not parallel that of α-adrenergic receptors stimulated by circulating catecholamines in the coronary vasculature.

Stellate ganglion stimulation may theoretically activate neural adrenergic receptors of the heart and its vasculature without precipitating release of catecholamines from the adrenal medulla. However, the effect of stellate ganglion stimulation is not uniform. Thus, Rinkema et al (1982) found that the coronary vascular effects of stellate ganglion stimulation vary not only with which ganglion is stimulated (left vs right) but also with the location of the vessel being evaluated. However, the regional blood flow results of Study 2 may not accurately reflect the regional blood flow distribution occurring in the exercising dog. Rinkema et al (1982) demonstrated using 20 um microspheres that most, but not all, of the vasoconstrictive effects of neural sympathetic stimulation result from left stellate ganglion activation. Additionally, the transmural distribution of this vasoconstrictive effect from stimulation of either the right or left stellate ganglion varies among the two ventricles of the heart and throughout the different regions within each ventricle. This regional nonuniformity in the effects of stellate ganglion stimulation upon transmural blood distribution in the

ventricle is a reflection of regional variations in the degree of subepicardial vasoconstriction during left ganglionic stimulation. Similar findings have been reported by others (Haws et al, 1987; Schwartz and Stone, 1977; Johannsen et al, 1982). Therefore, maximal degrees of subendocardial vasoconstriction from neurally-released adrenergic receptor stimulation should be accomplished in our model of a coronary sympathetic activation.

In the Study 2, sympathetic stimulation of the heart was associated with an increase in oxygen extraction by the heart with a decrease in lactate extraction. Though lactate extraction remained positive under all interventions, these observations imply that oxygen supply to the myocardium was less effective in meeting oxygen demand during stellate stimulation. Consequently, a transition toward anaerobic metabolism developed in at least some areas of the heart (Griggs et al, 1966; Jones and Bethea, 1975; Knansnow and Gorlin, 1963). Interestingly, the increases in subendocardial contractile function and coronary inflow following α_1-adrenergic receptor blockade during sympathetic stimulation was paralleled by a return of oxygen extraction and lactate extraction towards their normal resting values. Such an association of increased contractility and increased coronary inflow with decreased extraction of both oxygen and lactate suggest that the observed contractility changes

resulted from a released inhibition of oxygen supply following α_1-adrenergic blockade during sympathetic stimulation. Although these changes in oxygen and lactate extraction following α_1-adrenergic blockade do not precisely indicate regional or transmural effects on myocardial oxygenation and metabolism, it is attractive to speculate that the increase in subendocardial contractile function was secondary to an improved oxygen delivery this region.

The possibility that increased flow induces increased function by a "Gregg phenomenon" must be considered (Gregg, 1957; Feigl, 1983). As such, the strength of myocardial contraction and the degree of myocardial oxygen consumption can be augmented by increases in coronary blood flow. This cause-effect relationship is the inverse of the mechanism in which the degree of coronary perfusion is determined by the metabolic demands of the heart. The "Gregg phenomenon" can occur in well perfused hearts with intact coronary autoregulation (Gregg, 1957; Abel and Reis, 1970; Feigl, 1983), and the mechanism behind it is unclear. (A detailed discussion of the Gregg Phenomenon is presented in Appendix A). The possibility that this phenomenon is expressed to varying degrees in the different layers of the heart has not previously been examined. Thus, equal increases in flow to all layers of the heart could result in greater improvements in subendocardial function if this layer were more sensitive

to the Gregg effect than the more superficial layers. It should be noted that in the present experiments, α_1-adrenergic blockade during stellate stimulation caused an equal increase in perfusion of subepicardial and subendocardial muscle. However, an increase in contractile function was seen only in the deeper layers. This observation suggests that the increased mechanical function could only be due to the Gregg effect if there were a differential sensitivity to this effect in the different layers of the myocardium such that equal increases in blood flow to all layers would result in a preferential augmentation of contractile function in the subendocardial layers. A transmural distribution of the Gregg effect has never been explored. The results of Study 2 are compatible with the proposal that, although the increased perfusion was transmural, oxygen delivery and contractile function only in the deeper layers were flow-limited during stellate stimulation. In this regard, it is important to recognize that the coronary vasodilatory reserve in left ventricular subendocardium is generally felt to be less than in subepicardium (Berne and Rubio, 1978).

The results of Study 2 imply that during stellate ganglion stimulation, the increases in contractile function of left ventricular subendocardial muscle is limited by an α_1-adrenergic constriction which opposes the metabolic

vasodilation. These results from open-chest, anesthetized dogs are compatible with the results observed in conscious animals or humans during more physiological sympathetic stimulation of the heart. Thus, Gwirtz et al (1986) observed that prazosin injected intracoronarily during treadmill exercise in instrumented dogs was associated with a significant increase in coronary inflow in conjunction with increases in left ventricular subendocardial and global ventricular mechanical function. Subepicardial contractile function was not measured in these previous studies. Likewise, Barnard et al (1973) have reported that when humans were subjected to strenuous exercise without previous warm-up, there was a consistent electrocardiographic indication of left ventricular ischemia. Results similar to those of Barnard et al (1973) have been observed by others (Rose et al, 1972; Gibbons et al, 1977). While it is not generally established that moderate to severe exercise causes myocardial ischemia, the results of Study 2 in conjunction with the experiments outlined above imply that the sympathetic stimulation of the heart and coronary circulation associated with exercise may indeed be associated with myocardial ischemia, especially in the deeper muscle layers. These studies also suggest that this ischemia during exercise is in large part due to an α_1-adrenergic constriction of the coronary vasculature.

One possible critique of this Study 2 is that a question may exist regarding the stimulation parameters required to accomplish maximal sympathetic response. It has been suggested that a stimulation frequency of 20 Hz permits maximal cardiac sympathetic effects (Heusch and Deussen, 1983). In Study 2, a frequency of 10 Hz was used since this frequency produced effects qualitatively similar to, though minimally weaker than those produced at 20 Hz. Other laboratories reported similar findings (Heusch and Deussen, 1983). However, the lower frequency was required in Study 2 because pilot studies showed that the cardiac effects of stimulation with higher frequencies would actually deteriorate before enough time elapsed to allow for completion of data acquisition. Chilian et al (1989) were able to measure transmural blood flow while maintaining stellate stimulation at 20 Hz in cats. However, their protocol was different in that a shorter reference sampling period was used (1.5 min vs 2.0 min) and systemic β-adrenergic blockade attenuated the work requirements of the heart. This combination may have prevented any significant deterioration in degree of sympathetic stimulation during their collection period.

It should also be recognized that an attenuation of sympathetic outflow to the heart and a blunting of sympathetic reflexes are intrinsic to the anesthetized model

(Manders and Vatner, 1976). More specifically, pentobarbital has been shown to inhibit α-adrenergic receptor coronary constriction (Rosendorff et al, 1981; Gwirtz and Stone, 1982). Therefore, the results presented here may actually represent an underestimation of the influence of neurally-mediated α_1-adrenergic receptor coronary constriction upon coronary flow and ventricular function. Because pentobarbital reduces the maximal degree of coronary constriction which can be produced with sympathetic stimulation, the flow increase associated with administration of α_1-adrenergic receptor blocking agent would also be reduced. Additionally, any benefit to ventricular function due to removal of this flow-limiting coronary constriction would also be reduced because pentobarbital would minimize the maximal degree of coronary constriction produced.

In Study 1 and Study 2, it was also observed that accompanying the increase in coronary blood flow after α_1-adrenergic blockade was a substantial increase in myocardial contractile function, suggesting that an α-adrenergic constrictor tone limits myocardial perfusion in the exercising dog and, as a result, imposes a limitation on myocardial contractile function. However, from these previous studies it cannot be ascertained whether the increase in cardiac function was indeed due to the increase

in coronary perfusion or to some other affect of the α-adrenergic antagonist. Study 3 was performed in order to explore the possibility that the increase in left ventricular contractile function after α_1-adrenergic receptor blockade was due to some action of the α-adrenergic receptor antagonist other than an increase in perfusion of the myocardium. In Study 3, the effects of prazosin on myocardial flow and left ventricular contractile function during exercise were compared with those observed after direct coronary vasodilation with adenosine. It was reasoned that if the increase in left ventricular contractile function after prazosin were secondary to an increase in coronary blood flow, similar increases in left ventricular contractile function should be obtained when the potent vasodilator adenosine is infused intracoronarily at a rate sufficient to increase coronary blood flow to the same degree as observed after α_1-adrenergic receptor blockade.

In the Study 3, increasing coronary blood flow approximately 26% by intracoronary adenosine infusion during exercise was associated with a 27% increase in regional rate of segmental shortening and a 15% increase in rate of pressure generation by the left ventricle. These changes were comparable to those resulting from a 22% increase in coronary blood flow by α_1-adrenergic blockade with prazosin. Such similar results using two entirely different means of

increasing coronary blood flow and the observation that in all cases the increase in cardiac function followed the increase in flow suggests that the observed changes in myocardial contractile function were secondary to the increase in perfusion.

In Study 3, both prazosin and adenosine were administered directly into the circumflex coronary artery in order to minimize any systemic affects of the agents. The dose of intracoronary prazosin used in this study has been shown in previous studies to affect only the circumflex perfusion territory (Gwirtz, 1986; Gwirtz et al, 1986; Strader et al, 1988). The rate of adenosine infusion was determined not by absolute dose of adenosine, but by the degree of coronary flow increase, i.e. to match the increase in flow caused by 0.5 mg prazosin. The lack of systemic effects of adenosine is suggested by the observed stability of systemic variables (arterial pressure and heart rate) and of variables measured in the anterior region of the left ventricle (%SL, dL/dt_{max}). Thus, it is likely that the effects observed with prazosin and adenosine were not attributable to reflex actions associated with systemic effects of the agents. Likewise, the increase in myocardial function associated with either prazosin or adenosine is not likely due to a direct effect of these agents on the myocardium. Indeed, any direct effect of prazosin or

adenosine on the myocardium would likely have minimized the increase in inotropy observed. Thus, it has been reported that activation of myocardial α-receptors elicits an increase in myocardial contractility (Ledda et al, 1975; Osnes et al, 1978; Strader et al, 1988) and that blockade of these receptors with either phentolamine or prazosin elicits a negative inotropic effect (Rabinowitz et al, 1975; Otani and Das, 1988). Similarly, in isolated myocardial tissue, adenosine has been reported to exert a negative inotropic effect (Urthaler et al, 1981; Linden et al, 1985). Finally, it should be considered that the increase in myocardial contractile function associated with coronary vasodilation by either prazosin or adenosine is probably not due to Gregg's phenomenon ("garden-hose" effect) (Feigl, 1983). Gregg's phenomenon suggests that an increase in coronary perfusion results in an increase in myocardial oxygen consumption (which is the inverse of the more commonly accepted idea that coronary flow is determined by myocardial oxygen demand via local mechanisms). According to this hypothesis, coronary vasodilation, and the resulting coronary distention, leads to an increase in myocardial sarcomere length resulting in an increased cardiac performance by the Starling effect. However, in the present experiments neither posterior ventricular end-diastolic length nor left ventricular end-diastolic pressure were

affected by either prazosin or adenosine, indicating the absence of any myocardial segmental stretch.

The results of Study 3 imply that left ventricular myocardial function may indeed be flow-limited during submaximal exercise and that an increase in perfusion, either by coronary α_1-adrenergic blockade or by direct vasodilation, leads to an increase in contractile function. In support of this proposal, the results from Study 2 demonstrated that left ventricular lactate extraction is reduced during sympathetic stimulation in the dog. This reduction in myocardial lactate extraction was reversed by a coronary vasodilation with prazosin, indicating an improved myocardial oxygenation associated with coronary α_1-adrenergic blockade. In this regard, Heyndrickx et al (1982) have also demonstrated an improvement in myocardial oxygenation by α-adrenergic blockade during exercise in dogs. Furthermore, electrocardiographic evidence of myocardial ischemia has been reported during the onset of strenuous exercise in humans suggesting a limitation of myocardial perfusion (Barnard et al, 1973; Gibbons et al, 1977).

The data from Study 3 also suggest that an α_1-adrenergic coronary constrictor tone contributes to the flow limitation of myocardial function during exercise. This proposal is supported by the results of Study 1, Study 2,

and by previous experiments by others (Gwirtz et al, 1986; Strader et al, 1988; Bache et al, 1987; Williams et al, 1988). In this regard, an α_1-adrenergic coronary constriction has also been shown to limit myocardial perfusion, oxygenation, and function during sympathetic stimulation associated with hemorrhagic hypotension (Jones et al, 1983), coronary hypoperfusion (Liang and Jones, 1985; Jones et al, 1986), systemic hypoxemia (Williams et al, 1988) and the carotid chemoreceptor reflex (Murray et al, 1984). It must be emphasized that some investigators have reported that a coronary adrenergic constriction during cardiac stress is solely due to activation of α_2-receptors (Heusch et al, 1985; Heusch et al, 1986) or both α_1- and α_2-receptors (Chen et al, 1988). The basis of this discrepancy in results is unclear.

The transmural nature of the α_1-adrenergic coronary constriction across the left ventricular wall was not directly addressed by Study 3. However, it must be recalled that the sonomicrometer crystals for measurement of regional contractile function in the posterior ventricle were implanted within the subendocardial layer, suggesting that prazosin elicited an increase in perfusion of subendocardium. This proposal is supported by the findings of Study 2, in which prazosin improved perfusion equally to all layers of the left ventricular wall. Similarly, Chen et

al (1988) found no transmural redistribution of myocardial perfusion during α_1- or α_2-receptor stimulation, suggesting a uniform distribution of α-receptors. These results are corroborated by experiments by Williams et al (1988) who demonstrated that during systemic hypoxemia, the carotid chemoreceptor reflex elicited a uniform α_1-adrenergic coronary constriction across the left ventricular free wall in dogs. Buffington et al (1983) additionally found a transmural gradient did not exist during sympathetic constriction. On the other hand, Huang and Feigl (1988) reported that during exercise the adrenergic vasoconstriction was primarily subepicardial since α-adrenergic blockade increased the subepicardial-to-subendocardial flow ratio. Unfortunately, no absolute values of flow were reported by these investigators. Regardless of whether or not a redistribution of flow occurred in the present study, it is striking that in both cases, ie. α_1-adrenergic blockade and adenosine infusion, subendocardial and global function increased.

Studies 1, 2, and 3 were designed to avoid the problems of previous studies. To minimize misinterpretation of results secondary to peripheral circulatory reflexes, the α_1-antagonist was administered directly into the circumflex coronary artery. To accomplish α-adrenergic blockade in these studies, the selective α_1-adrenergic receptor

antagonist prazosin was chosen. Though studies (Heusch et al, 1985; Heusch et al, 1986; Chen et al, 1988) have demonstrated the presence of α_2-adrenergic receptors on the coronary circulation, no evidence exists supporting the presence of an α_2-receptor mediated constrictor tone in exercising dogs without coronary stenosis (Gwirtz et al, 1986; Bache et al, 1987; Strader et al, 1988; Dai et al, 1989). In addition, prazosin does not block presynaptic α_2-adrenergic receptors, which would have resulted in an increase in neuronal release of norepinephrine.

The studies presented here were performed on dogs with normal coronary vasculature. This is an important consideration, as some may disagree with our use of a specific α_1-adrenergic receptor blocker rather than an agent such as phenoxybenzamine which has additional α_2-adrenergic receptor blocking capabilities. An α_2-adrenergic receptor mediated vasoconstriction can be demonstrated in the coronary circulation. As such, an increase in coronary flow results from the intracoronary administration of specific α_2-adrenergic receptor antagonists can be demonstrated in stellate stimulated (Heusch and Deussen, 1983) and in running dogs (Seitelberger et al, 1988). However, in these experiments the adrenergic receptor blocking agent was administered distal to a stenosis known capable of exhausting all coronary dilator reserve. It is possible that

loss of this dilator reserve may effect the local milieu in such a way as to make the vasculature relatively more sensitive to α_2-adrenergic receptor stimulation. In this connection, ischemia (Deussen et al, 1985) and acidosis (McGrath et al, 1982; Flavahan and McGrath, 1982) have been shown to attenuate α_1-adrenergic receptor coronary constriction without altering α_2-adrenergic receptor coronary constriction.

Recent evidence indicates that there are more than one subclass of α_1-adrenergic receptor and that these subclasses may vary in their susceptibility to blockade by prazosin (Flavahan and Vanhoutte, 1986). As such, there may be question as to the effectiveness of our dose of prazosin in preventing activation of all α_1-adrenergic receptor subtypes. In the studies presented here, the dose of prazosin used has been shown to effectively block the vasoconstrictive effects of intracoronary administration of 20 μg phenylephrine.

In summary, the results of Study 1 indicate that coronary blood flow can be increased in exercising dogs by blockade of coronary α_1-adrenergic receptors using prazosin. This prazosin-related increase in coronary blood flow increases in degree with increased exercise intensity, and becomes associated with increased ventricular contractile function as exercise becomes more strenuous. The resultant

increase in myocardial oxygen consumption at matched levels of exercise compared to the unblocked state is supplied by an increase in coronary blood flow without a change in oxygen extraction. The observations from Study 2 indicate that the phenomenon of increased coronary blood flow and ventricular contractile performance following intracoronary administration of prazosin in sympathetically stimulated canine hearts persists even at maximal levels of sympathetic stimulation. Furthermore, this increase in left ventricular perfusion following coronary α_1-adrenergic receptor blockade is distributed equally across the layers of the left ventricular wall during high levels of sympathetic stimulation. However, the associated improvement in global left ventricular contractile function compared to the unblocked state is a result of improved contractile function of the subendocardium only, without change in subepicardial contractile function. Finally, the results of Study 3 indicate that the improved contractile function associated with prazosin-induced coronary vasodilation of the sympathetically stimulated heart is due to an increase in perfusion of the flow-limited myocardium, and not to a direct action of prazosin on the myocardium.

APPENDIX A - FLOW MEASUREMENTS WITH MICROSPHERES

Regional myocardial blood flow distribution was determined with the use of radioactive microspheres. Radioactive microspheres are microspheres whose component atoms have nuclei which are unstable (i.e. radionuclides). A nucleus is the positively charged center of an atom. It is composed of protons and neutrons and is surrounded by electrons circling it in different orbits. A radionuclide is a nucleus which is unstable. The stability of a nucleus is determined by the balance between two types of forces: strong forces and electromagnetic forces. The strong forces act between any pair of components of the nucleus such that no more than one of the components is charged (e.g. neutron-neutron, proton-proton). These are attractive forces acting only when the two components are close together. Electromagnetic forces are repulsive forces between to like-charged components of the nucleus (e.g. proton-proton). Whenever the balance between strong (attractive) and electromagnetic (repulsive) forces is disturbed, the nucleus becomes unstable. These unstable nuclei are called radionuclides. In an attempt to become stable, the radionuclide emits energy as either electromagnetic radiation or as charged particles (a.k.a. radioactive decay).

Charged particle emission (or gain, in the case of electron decay) by the nucleus is classified as either alpha

or beta decay, depending upon the size of the particle emitted. In alpha decay, a particle is emitted which is about 4 times heavier than either a neutron or proton and which carries an electric charge twice that of a proton. In beta decay, the strong charges within the nucleus are changed by either converting a proton to a neutron or by converting a neutron to a proton. In this way, the repulsive forces within the nucleus are either decreased (former case) or increased (latter case). By better equilibrating the electromagnetic and strong forces, this stabilizes the nucleus. In the process, energy is emitted by one of three methods: 1) Beta$^-$ Emission - neutron converted to proton and excess energy release as an electron and an antineutrino (particle which has neither rest mass nor electric charge); 2) Beta$^+$ Emission - proton converted into a neutron and excess energy emitted as positron (an electron with a unit positive charge instead of a unit negative charge) and neutrino (basically the same as an antineutrino); 3) Electron Capture - proton is converted to a neutron by capture of an electron from an extra-nuclear orbit. This leaves a space for an electron from a more distant shell to move closer to the nucleus, and the resultant energy excess is emitted as a neutrino.

When the energy is given off but the charge of the nucleus is not affected, this is known as Gamma Decay. The

energy is dissipated through either of two processes: 1) High Energy Photon Emission - high energy (greater than 100 eV) photon (packet of energy having no rest mass or charge but which is produced by the interaction between charged particles and which behaves according to the laws of electromagnetic forces) emission used to dissipate the excess energy; 2) Internal Conversion - energy is transferred from nucleus to one of the orbiting electrons (extranuclear). This added energy propels the electron to a more distal orbit, thus creating a vacancy in an internal orbit. This vacancy is filled by the dropping of an outer electron into the inner orbit, with associated release of energy. The energy release by this "backfilling" of an inner orbit by an outer electron produces the energy counted during the gamma emission.

A nucleus can be made unstable by exposure to a reactor, by exposure to a cyclotron, or by fission. In reactor production of a radionuclide, the nucleus is bombarded with neutrons of small kinetic energy. Due to the small quantity of kinetic energy and the neutral charge of the neutron, these particles are not repelled by the positive charge of the nucleus. The neutrons and their energy are thus captured by the nucleus. In Cyclotron (a.k.a. Accelerator) production of radionuclides, the nucleus is exposed to charged particles (e.g. protons,

deutrons or "heavy" hydrogen) of high energy (MeV range). The high energy is required to overcome the repulsive forces of the positively charged nucleus. The result is a nucleus of different charge, so that the balance between strong and electromagnetic forces of the nucleus is disrupted. This results in nuclear instability. In Fission production of radionuclide, the nucleus is again bombarded with neutron. If the original nucleus was large, this process results in the splitting of the nucleus into two smaller nuclei (i.e. fission). Each of these smaller nuclei is unstable.

Each radionuclide emits energy of a characteristic quantity or energy level based upon the degree of instability in the nucleus. A group of radionuclides emitting the same energy level is known as a species. Different species emit different energy levels. When measuring on an atomic scale, the unit of energy is known as the electron volt (eV). One electron volt is the energy acquired by an electron accelerated through 1 volt of potential difference.

To the extent that radioactive microspheres flow through the vasculature and distribute themselves in the tissue in a manner similar to the flow of red blood cells, these microspheres can be a tool to study blood flow. That is, blood flow to a tissue can be determined by measuring the quantity of radioactive microspheres trapped in that

tissue. Thus, if the characteristic energy level of a microsphere species is known, the quantity of radioactivity having that emission energy can be measured from a tissue. This quantity can be converted to flow rate by comparing it with the activities of reference sample producing that emission energy and flowing at a known rate. This utilizes the following equation:

$$MBF = (F_{ref})(R_{tis} / R_{ref})$$

where MBF = mean blood flow (ml/min)

F_{ref} = flow rate (ml/min) of the reference blood samples

R_{tis} = radioactivity (cpm) of the tissue sample

R_{ref} = radioactivity (cpm) of the reference blood samples

(Heymann et al, 1977)

Different species of either 10 um or 15 um diameter plastic microspheres in 10% dextran with 0.01% Tween 80 were used. The choice of this size microspheres is an attempt to approximate RBC flow as closely as possible. The specific gravity of plastic microspheres is 1.3 (Heymann et al, 1977). The normal red blood cell is 6-8 um in diameter (Widmann, 1983) with a specific gravity of 1.05 (Heymann et al, 1977). It has been found that microspheres of diameter greater than 15 um fail to distribute themselves throughout

tissues proportional to the volume of perfusion in that tissue (Heymann et al, 1977). Additionally, 9 um microspheres tend to show varying degrees of shunting as perfusion pressure changes, (Crystal et al, 1979), while 15 um microspheres do not present this problem (Yonekura et al, 1988). Since systemic pressure (and, presumably, perfusion pressure) rises with exercise, this may result in an effect on trapping of 9 um microspheres independent of changes in transmural flow distribution. Furthermore, mixing in 10% dextran with 0.01% Tween 80 is an attempt to minimize aggregation of the microspheres (Heymann et al, 1977). It has been shown that Tween 80 injected into the vasculature can both cause systemic hypotension and can change cardiac dimensions independent of an effect upon systemic blood pressure change vasculature resistance (Millard et al, 1977). Either of these changes could theoretically affect coronary flow. These effects are most prominent at levels of Tween 80 near 0.05%. They are much less prominent at levels of 0.01%, the minimal level of Tween 80 required to prevent microsphere aggregation (Millard et al, 1977).

Each dose of microspheres consisted of 1 million units. Assuming a 200 g heart receiving 5% of the 5 liter per minute cardiac output, this dose of microspheres will lodge at least 400 beads in each 2 g sample of myocardium. When this quantity of microspheres is lodged in an organ's most

poorly perfused tissue sample, we can feel sure that the microspheres were adequately mixed and that a sufficient number of microspheres was used to insure representative distribution of the microspheres throughout the arterial system (Heymann et al, 1977). Others have shown that repeat bolus injections of 1 million 15 um microspheres can be given with no adverse effects (Heymann et al, 1977). Each dose of microspheres was diluted with 8-9 ml normal saline, sonicated for no less than 30 min, and vortexed prior to infusion. The dog was heparinized (500 u/kg) prior to administration of the first microsphere dose. The microspheres were injected through the left atrial catheter. It is important that the microspheres be dispersed thoroughly throughout the ejected blood volume in order to assure a distribution in tissues which accurately reflects relative blood flow to the tissues. Though some investigators feel this mixing can be complete if the microspheres are injected into the left ventricle, injection into to the left atrial catheter is more universally accepted (Heymann et al, 1977).

APPENDIX B - GREGG EFFECT

Gregg reported that alterations in coronary perfusion result in changes in cardiac oxygen consumption (MVO_2) (Gregg, 1957). Since then, the observation of a direct correlation between changes in coronary perfusion and changes in both cardiac oxygen consumption and contractile strength has come to be known as the "Gregg Phenomenon" (Feigl, 1983). In Gregg's study, there were simultaneous changes in coronary flow, coronary perfusion pressure, and oxygen delivery (Gregg, 1958; Gregg et al, 1957). Thus, controversy developed as to which variable was operative in causing the observed changes in MVO_2 and contractile function. Four possible mechanisms explaining this phenomenon in the working heart are described by Feigl (1983):

1)Hypoperfusion - Increased perfusion results in the Gregg Phenomenon because ischemia exists prior to augmenting flow. As such, Bacaner et al (1971) studied the effects of separately changing oxygen content of perfusate (by either changing oxygen saturation or by changing perfusate hemoglobin content without changing saturation) or of changing coronary blood flow (by changing perfusion pressure) in an isolated canine heart preparation. They found that MVO_2 and left ventricular developed tension each decreased when oxygen delivery was decreased by desaturation unaccompanied by changes in coronary blood flow. Similarly,

they found that left ventricular developed tension increased when initial hemodilution was corrected even though coronary blood flow fell. These findings are consistent with the hypothesis that increasing flow results in improved ventricular performance because the increased perfusion corrects a pre-existing ischemia which is present in the autoregulated state. Opponents of this rationale argue that this would imply that the Gregg Phenomenon occurs only under ischemic conditions and would not be a physiological mechanism of regulating ventricular performance. In fact, some investigators only observe the Gregg Phenomenon at flow levels less than those maintained by autoregulation. Thus, Downey (1976) measured the effect of changing coronary blood flow (with associated changes in perfusion pressure) on contractile force of the ventricular myocardium in open-chest, anesthetized dogs. He found that contractile force was directly related to coronary blood flow as long as blood flow was maintained below autoregulatory levels. When coronary blood flow was raised above autoregulatory levels, this direct relationship was lost. No measure of blood oxygen content is given. These findings agreed with those previously reported by Sarnoff et al (1963) in an isolated heart preparation. Similarly, the studies by Bacaner et al (1971) evaluated conditions in which oxygen delivery was changed from normal to subnormal levels or from subnormal to

normal levels. Though oxygen consumption and ventricular performance varied directly with oxygen delivery under these conditions, the effect of increasing oxygen delivery to levels greater than those maintained by autoregulation were not evaluated. These studies imply that no improvement in myocardial oxygenation results from raising flow above autoregulated levels. Others, though, observe the Gregg Phenomenon when flow is greater than levels set by autoregulation. However, even when the Gregg Phenomenon is observed at flow levels above those of autoregulation, there is no evidence that correction of hypoxia is the mechanism responsible. Thus, in an isolated rat heart preparation, the direct variation of MVO_2 and ventricular performance with coronary blood flow persists when flow is greater than normal levels even though lactate extraction is positive (Abel and Reis, 1970) and venous effluent oxygen content is at levels greater than those felt to inhibit MVO_2 (Bacaner et al, 1971; Opie, 1965). These findings make resolution of hypoxia a less obvious explanation for the Gregg Phenomenon when flow is increased to levels greater than that of autoregulation. However, it should be pointed out that small pockets of ischemia have not been ruled out in these preparations in spite of the apparent lack of global ischemia. Thus, Rubanyi and Kovach (1980) found that when the Gregg Phenomenon was observed at high flow levels, the

further increases in perfusion were not accompanied by continued increases in oxygen saturation of venous effluent. This inability to further lower oxygen extraction in spite of increased total oxygen supply implies a rising oxygen demand. Such a situation could be explained by perfusion of previously ischemic pockets by the increased blood flow. It is also interesting that the experiments evaluating this hypothesis examine the influence of flow on function only in non-working hearts or in resting hearts. Thus, no evaluation has been made of a possible direct variation of contractile function with coronary flow when flow is increased above autoregulated levels in exercising dogs. As such, autoregulation may impose a relative hypoxia in exercising dogs such that increasing flow will increase function by reversing an ischemic condition.

2)Cardiac Compliance - It has been suggested that increased coronary perfusion makes the myocardium more compliant, resulting in increased myocardial fiber length at any given diastolic filling pressure (Cross et al, 1961; Salisbury et al, 1960). Such changes would improve contractile performance by the Starling Effect. This proposed mechanism for the Gregg Phenomenon has been refuted by those who found that increases in coronary perfusion were associated with decreases in left ventricular compliance (Buckley, 1964; Arnold et al, 1968; Abel and Reis, 1970).

The apparent conflict in findings may be due to the influence of myocardial hypoxia in the studies by Salisbury and co-workers (Salisbury et al, 1960; Cross et al, 1961). That is, these studies showed that left ventricular end diastolic pressure decreased as coronary perfusion pressure was lowered from 130 mm Hg to 30 mm Hg. However, there was also a decrease in peak systolic left ventricular pressure as perfusion pressure was lowered. As such, lowering perfusion pressure may have resulted in hypoxia, thereby explaining the decreased ventricular performance under these conditions. This hypoxia, in turn, may have influenced ventricular compliance in these studies.

3) Coronary Distension - Increased distending pressure within the coronary vasculature increases the sarcomere length of the surrounding myocardium. Again, this would improve contractile performance by the Starling Effect. This is often referred to as the "garden-hose" effect. Many findings support this hypothesis. Arnold et al (1968) observed the Gregg Phenomenon when coronary perfusion pressure was increased without a concomitant increase in coronary flow. These investigators separated the influences of flow and of perfusion pressure on left ventricular performance. In isolated guinea pig hearts, coronary perfusion pressure was raised but coronary flow kept constant by adding dextran to the Krebs-Heinsleit solution

perfusing the coronaries. The resultant change in viscosity of the perfusate mandated that increased perfusion pressure be used to maintain the original flow velocity. The oxygen saturation of the perfusate was unchanged. These modifications in perfusion parameters resulted in changes in contractility which were directionally similar to the changes in perfusion pressure. In the same studies, hypoxic vasodilation was used to increase flow without changes in perfusion pressure. Flow doubled as oxygen partial pressure in the perfusate was reduced by about 60%. These modifications in perfusing parameters changed neither MVO_2, nor peak pressure of the left ventricle, nor rate of pressure development by the left ventricle. Others have demonstrated, with electron microscopic evaluation, that raising perfusion pressure in arrested hearts is associated with increased sarcomere length (Poche et al, 1971). Furthermore, Fischer et al (1969) showed that an equal amount of oxygen deficiency is more detrimental to papillary muscle tension development when the oxygen deficiency is produced by interruption of flow than when it is produced by perfusion with oxygen-poor perfusate.

4)Flow and Oxygen Delivery - An increase in flow, even at constant perfusion pressure and in the absence of ischemia prior to the increase in flow, results in Gregg's Phenomenon. This mechanism is distinct from the "garden

hose" hypothesis, which states that it is a change in perfusion pressure which ultimately leads to performance changes. Abel and Reis (1970) found that pharmacologic vasodilation of the coronary arteries results in increased velocity of shortening of the ventricular myocardium. This was observed in spite of a lack of change in perfusion pressure and under conditions of positive lactate extraction by the heart (i.e., no ischemia). When perfusion pressure was lowered at constant flow, no change in ventricular function was noted. In both parts of the study (vasodilation alone and change in perfusion pressure alone), the results were the same when coronary blood flow and perfusion pressure were above normal resting values. Similarly, Zborowska-Sluis et al, (1977) found oxygen consumption was not altered when coronary pressure was changed at constant coronary flow. In these studies using an open-chest canine preparation with a pump-perfused left coronary artery, perfusion pressure was lowered by pharmacologic vasodilation with dipyridamole and was increased by pharmacologic vasoconstriction with angiotensin. Further support can be found in the prior study by Monroe et al (1972). They used an isolated canine heart preparation and raised left ventricular systolic pressure from 5 to 100 mm Hg over 15 sec. By measuring how this change in outflow pressure affected left ventricular circumference and left ventricular

end diastolic pressure, they determined whether changes in perfusion parameters altered the heart's ability to function against an increased afterload. They found that, at constant perfusion pressure and against equal increases in outflow pressure, the left ventricle is able to empty more completely (achieve smaller left ventricular circumference) and have smaller increases in left ventricular end diastolic pressure when coronary flow is greater. No mention of oxygen extraction or of oxygen content of the perfusate was made. Thus, it is unclear whether these changes in function were due to a modification of oxygen supply or due to changes in coronary blood flow. Nonetheless, alterations in perfusion pressure did not appear to be responsible.

To help clarify the mechanism responsible in this case, regional end diastolic length and end diastolic pressure were monitored. The lack of change in either of these parameters make either changes in cardiac compliance or the "garden-hose" effect unlikely explanations. Arterial pressure was not different after the administration of prazosin as compared to before its administration. Thus, changes in perfusion pressure were not a factor.

The possibility that left ventricular contractile function improved secondary to flow increases but not due to resolution of a pre-existing ischemia is more difficult to refute. By this mechanism, the increase in myocardial MVO_2

would then be the result of improved contractile function, not the cause of it. However, the findings of Study 2 make this less likely. In Study 2, maximal left stellate ganglion stimulation resulted in a decreased myocardial lactatae extraction and an increased myocardial oxygen extraction. Following intracoronary prazosin administration, the increase incoronary blood flwo was associated with a return to normal of myocardial lactate extraction and myocardial oxygen extraction as well as improved left ventricular contractile function. It is attractive to speculated that the association of intracoronary prazosin with the return toward resting values of myocardial lactate extraction which decreased and myocardial oxygen extraction which increased with exercise signify the resolution of an ischemic condition.

APPENDIX C - ADENOSINE RECEPTORS

Two classes of adenosine receptors have been described (Van Calker et al, 1978; Londos et al, 1980). A negative inotropic effect of myocardial adenosine (Dobson et al, 1986) is felt to result from stimulation of A_1 receptors, located on the cardiomyocyte. This effect is felt to be mediated through a decrease in cAMP production (Henrich et al, 1988). A_2 receptors, located on the vascular smooth muscle cell (Anand-Srivastava et al, 1982), have a vasodilatory influence felt to be mediated though an increase in cAMP (Anand-Srivastava and Franks, 1985). The dichotomous effect upon intracellular cAMP levels is felt to be a result of differences in the guanine nucleotide regulatory component with which the receptor is associated (Londos et al, 1981). Additionally, cAMP is felt to decrease the release of norepinephrine from electrically stimulated adrenergic nerve endings to vascular smooth muscle (Verhaeghe et al, 1977). Though this reduction in norepinephrine release alters the amount of catecholamine to which the smooth muscle cell is exposed, it is unclear whether this would change the quantity of catecholamine to which the cardiomyocyte is expose. However, any such influence would only decrease the inotropy of the ventricular muscle. As such, any improved inotropy associated with adenosine vasodilation restricted to the

coronary vasculature would be from something other than a direct effect of the agent upon cardiac myocytes.

REFERENCES CITED

1. Abel R.M. and R.L. Reis. Effects of coronary blood flow and perfusion pressure on left ventricular contractility in dogs. Circ. Res. 27:961-971, 1970.

2. Anand-Srivastava M.B., D.J. Franks, M. Cantin, and J. Genest. Presence of "Ra" and "P"-site receptors for adenosine coupled to adenylate in cultured vascular smooth muscle cells. Biochem. Biophys. Res. Comm. 108:213-219, 1982.

3. Anand-Srivastava M.B. and D.J. Franks: Stimulation of adenylate cyclase by adenosine and other agonists in mesenteric artery smooth muscle in culture. Life Sci. 37:857-867, 1985.

4. Arnold G., F. Kosche, E. Miessner, A. Neitzert, and W. Lochner. The importance of the perfusion pressure in the coronary arteries for the contractility and the oxygen consumption of the heart. Pfluegers Arch. 299:339-356, 1968.

5. Bacaner M.B., F. Lioy, and M.B. Visscher. Coronary blood flow, oxygen delivery rate and cardiac performance. J. Physiol. (London). 216:111-127, 1971.

6. Bache R.J., X.Z. Dai, C.A. Herzog, and J.S. Schwartz. Effects of nonselective and selective α-adrenergic blockade on coronary blood flow during exercise. Circ. Res. 61(Suppl. II):36-41, 1987.

7. Barnard, R.J., G.W. Gardner, N.V. Diaco, R.N. MacAlpin, and A.A. Kattus. Cardiovascular responses to sudden strenuous exercise - heart rate, blood pressure, and ECG. J. Appl. Physiol. 34:833-837, 1973.

8. Berne, R.M. The role of adenosine in the regulation of coronary blood flow. Circ. Res. 47:807-813, 1980.

9. Berne, R.M. and R. Rubio. Coronary Circulation. In: Handbook of Coronary Physiology. Section 2, Baltimore:Williams and Wilkins, 1978, p.873-952.

10. Billman, G.E. and D.C. Randall. Mechanisms mediating the coronary vasculature response to behavioral stress in the dog. Circ. Res. 48:214-222, 1981.

11. Birinyi, F., D.B. Hackel, and E. Mikat. Effects of α-adrenergic blockade on coronary blood flow of dogs in hemorrhagic shock. Circ. Shock. 4:297-303, 1977.

12. Brachfeld, N., R.G. Monroe, and R. Gorlin. Effect of pericoronary denervation on coronary hemodynamics. Am. J. Physiol. 199:174-179, 1960.

13. Braunwald E. and B.E. Sobel. Coronary blood flow and myocardial ischemia. In: Heart Disease: A Textbook of Cardiovascular Medicine. 3rd edition. E. Braunwald (ed.), Philadelphia: W.B. Saunders, 1988, p.1191-1221.

14. Buckley, N.M. E.P. Porter, and L.A. Jedeikin. Effects of varying coronary perfusion pressure on ventricular function in isolated dog hearts. Am. J. Physiol. 207:683-690, 1964

15. Buffington C.W., and E.O. Feigl. Effect of coronary artery pressure on transmural distribution of adrenergic coronary vasoconstriction in the dog. Cir. Res. 53:613-621, 1983.

16. Bukoski, R.D. H.V. Sparks, Jr., and L. Mela-Riker. Adenosine release by rat heart mitochondria: site of adenosine production. Fed. Proc. 43:307, 1984 (Abstract).

17. Bunger, R., F.J. Haddy, A. Querengasser, and E. Gerlach. Studies of potassium induced coronary dilation in the isolated guinea pig heart. Pfluegers. Arch. 363:27-31, 1976.

18. Bunger, R. S. Soboll, and B. Permanetter. Effects of norepinephrine on coronary flow, myocardial substrate utilization, and subcellular adenylates. In: Ca^{2+} Entry Blockers, Adenosine and Neurotumors. B.F. Merril and H.R. Weiss (ed.), Munich: Urban and Schwarzenberg, 1983, p.267-279.

19. Carlson, E.L., S.L. Selinger, J. Utley, and J.I.E. Hoffman. Intramyocardial distribution of blood flow in hemorrhagic shock in anesthetized dogs. Am. J. Physiol. 230:41-49, 1976.

20. Chen D.G., X-Z Dai, B.G. Zimmerman, and R.J. Bache. Postsynaptic α_1- and α_2-adrenergic mechanisms in coronary vasoconstriction. J. Cardiovasc. Pharmacol. 11:61-67, 1988.

21. Chilian W.M., R.B. Boatwright, T. Shoji, and D.M. Griggs, Jr. Evidence against a significant resting sympathetic coronary vasoconstrictor tone in the conscious dog. Circ. Res. 49:866-876, 1981.

22. Chilian W.M., and P.H. Ackell. Transmural differences in sympathetic coronary constriction during exercise in the presence of coronary stenosis. Circ. Res. 62:216-225, 1988.

23. Chilian, W.M., S.M. Layne, C.L. Eastham, and M.L. Marcus. Heterogeneous microvascular coronary α-adrenergic vasoconstriction. Circ. Res. 64:376-388, 1989.

24. Coburn, R.F. Oxygen tension sensors in vascular smooth muscle. Adv. Exp. Med. Biol. 78:101-123, 1977.

25. Cross C.E., P.A. Rieben and P.F. Salisbury. Influence of coronary perfusion and myocardial edema on pressure-volume diagram of left ventricle. Am. J. Physiol. 201:102-108, 1961.

26. Crystal, G.J., H.F. Downey, and F.A. Bashour. Small vessel and total coronary blood volume during intracoronary adenosine infusion. Am J. Physiol. 241: H194-H201, 1981.

27. Crystal, G.J., H.F. Downey, and F.A. Bashour. Persistent coronary vasodilation during long-term, supramaximal doses of adenosine. Am. J. Physiol. 247: H869-H873, 1984.

28. Crystal, G.J., R.B. Boatwright, H.F. Downey, and F.A. Bashour. Shunting of microspheres across the canine coronary circulation. Am. J. Physiol. 236:H7-H12, 1979

29. Dai, X-Z, E. Sublett, P. Lindstrom, J.S. Schwartz, D.C. Homans, and R.J. Bache. Coronary flow during exercise after selective α_1- and α_2-adrenergic blockade. Am. J. Physiol. 256 (Heart Circ. Physiol. 25): H1148-H1155, 1989.

30. Denn, M.J. and H.L. Stone. Autonomic innervation of dog coronary arteries. J. Appl. Physiol. 41:30-35, 1976.

31. Deussen, A., G. Heusch, and V. Thamer. α_2-adrenoceptor-mediated coronary vasoconstriction persists after exhaustion of coronary dilator reserve. European J. Pharmacol. 115:147-153, 1985.

32. Dobson, J.G., R.W. Ordway, and R.A. Fenton. Endogenous adenosine inhibits catecholamine contractile responses in normoxic hearts. Am. J. Physiol. 251:H455-562, 1986.

33. Dolezel, S., M. Gerova, J. Jero, T. Sladek, and J. Vasku. Adrenergic innervation of the coronary arteries and the myocardium. Acta Anat. 100:306-316, 1978.

34. Downey, J. Myocardial contractile force as a function of coronary blood flow. Am. J. Phys. 230:1-6, 1976.

35. Drake, A.J., J. Stubbs, and M.I.M. Noble. Dependence of myocardial blood flow and metabolism on cardiac innervation. Cardiovasc. Res. 12:69-80, 1978.

36. Drury, A.N. and A. Szent-Byorgyi. The physiological activity of adenine compounds with special reference to their action upon the mammalian heart. J. Physiol. (London). 68:213-237, 1929.

37. Endoh, M., T. Shimizu, and T. Yanagisawa. Characterization of adrenoceptors mediating positive inotropic responses in the ventricular myocardium of the dog. Br. J. Pharmacol. 64:53-61, 1978.

38. Ely S.W., D.C. Sawyer, D.L. Anderson, and J.B. Scot. Carotid sinus reflex vasoconstriction in right coronary circulation of dog and pig. Am. J. Physiol. 241:H149-H154, 1981.

39. Feigl, E.O. Carotid sinus reflex control of coronary blood flow. Circ. Res. 23:223-237, 1968.

40. Feigl, E.O., 1983. Coronary physiology. Physiol. Rev. 63:1-205, 1983.

41. Fisher V.J., R.A. Martino, R.S. Harris and F. Kavaler. Coronary flow as an independent determinant of myocardial contractile force. Am. J. Physiol. 217:1127-1133, 1969.

42. Flavahan, N.A. and J.C. McGrath. α_1-adrenoceptor activation can increase heart rate directly or decrease it indirectly through parasympathetic activation. Br. J. Pharmac. 77:319-328, 1982.

43. Flavahan, N.A. and P.M. Vanhoutte. α_1-adrenoceptor subclassification in vascular smooth muscle. Trends Pharmacol. Sci. 7:347-349, 1986.

44. Franklin, D.L., W.A. Schlegam, and R.F. Rushmer. Blood flow measure by Doppler frequency shift of back scattered ultrasound. Science. 134:564-565, 1961.

45. Franklin D.E., N.W. Watson, K.E. Pierson, and R.L. Van Citters. Technique for radiotelemetry of blood flow velocity from unrestrained animals. Am. J. Med. Electron. 5:24-28, 1966.

46. Furchgott, R.F. The role of endothelium in the responses of vascular smooth muscle to drugs. Annu. Rev. Pharmacol. Toxicol. 24:175-197, 1984.

47. Gallagher, K.P., J.D. Folts, R.J. Shebuski, J.H.G. Rankin, and G.G. Rowe. Subepicardial vasodilator reserve in the presence of critical coronary stenosis in dogs. Am J. Cardiol. 46:67-73, 1980.

48. Gellai, M., J.M. Norton, and R. Detar. Evidence for direct control of coronary vasculature tone by oxygen. Circ. Res. 32:279-681, 1973.

49. Gibbons, L.W., K.H. Cooper, R.P. Martin, and M.L. Pollok. Medical examinations and electrocardiographic analysis of elite distance runners. Ann. N.Y. Acad. Sci.301:283, 1977.

50. Giudicelli, J.F., A. Berdeaux, F. Tato, and M. Garnier. Left stellate stimulation: regional myocardial flows and ischemic injury in dogs. Am. J. Physiol. 239:H359-H364, 1980.

51. Govier, W.C. Myocardial alpha adrenergic receptors and their role in the production of a positive inotropic effect by sympathomimetic agents. J. Pharmacol. Exp. Ther. 159:82-90, 1968.

52. Graham, R.M. and S.M. Lanier. Identification and characterization of alpha-adrenergic receptors. In: The Heart and Cardiovascular System H.A. Fozzard, R.B. Jennings, E.Haber, A.M. Katz, and H.E. Morgan(ed.), New York:Raven Press, 1986, p.1059-1095.

53. Gregg D.E., C.R Rayford, E.M. Khouri, A.A. Kathis, E.P. McKeever. Effect of alteration of coronary perfusion pressure on oxygen uptake of left myocadium. Circulation. 16:888, 1957, (Abstract).

54. Gregg, D.E. Regulation of the collateral and coronary circulation of the heart. In: Circulation. Proceedings of the Harvey Tercentenary Congress. J. McMichael (ed.). Oxford, U.K.: Blackwell, 1958, p.163-186.

55. Griggs, D.M., J.S. Najano, J.G. Lipana, and P. Novack. Myocardial lactate oxidation in situ and the effect thereon of reduced coronary flow. Am. J. Physiol. 211:335-340, 1966.

56. Gwirtz P.A. Construction and evaluation of a coronary catheter for chronic implantation in dogs. J. Appl. Physiol. 2:720-26, 1986.

57. Gwirtz P.A., H.J. Mass, S.P. Overn, and C.E. Jones. Alpha$_1$-adrenergic contriction limits coronary flow and cardiac function in running dogs. Am. J. Physiol. 150:H117-H1126, 1986.

58. Gwirtz, P.A. and H.L. Stone. Coronary blood flow and myocardial oxygen consumption after alpha-adrenergic blockade during submaximal exercise. J. Pharmacol. Exp. Ther. 217:998, 1981.

59. Gwirtz P.A., and H.L. Stone. Coronary blood flow changes following activation of adrenergic receptors in the conscious dog. Am. J. Physiol. 243:H13-H19, 1982.

60. Harder, D.R. Pressure-dependent membrane depolarization in cat middle cerebral artery. Circ. Res. 55:741-746, 1984.

61. Hartley, C.J., R.M. Lewis, T. Ishida, J.E. Chelly, and M.L. Entman. High frequency pulsed doppler measurements of blood flow and myocardial dimensions in conscious animals. In: Cardiovascular Instrumentation: Proceedings of the Working Conference on Applicability of New Technology to Biobehavioral Research. NIH Publication No. 84-1654, U.S. Dept. of Health and Human Services, 1984, p.95-106.

62. Haws, C.W., L.S. Green, J.M. Burgess, and J.A. Abildskov. Effects of cardiac sympathetic nerve stimulation on regional coronary blood flow. Am. J. Physiol. 252:H269-H274, 1987.

63. Henrich M., H.M. Piper and J. Schrader. Evidence for adenylated cyclase-coupled A_1-adenosine receptors on ventricular cardiomyocytes from adult rat and dog heart. Life Sci. 41:2381-2388, 1987.

64. Herd, J.A. and A.C. Barger. Simplified technique for chronic catheterization of blood vessels. J. Appl. Physiol. 19:791-792, 1964.

65. Heusch G. and A. Deussen. The effects of cardiac sympathetic nerve stimulation on perfusion of stenotic coronary arteries. Circ. Res. 53:8-15, 1983.

66. Heusch G., A. Deussen, J. Schipke, and V. Thamer. α_1- and α_2-adrenoceptor mediated vasoconstriction of large and small canine arteries in vivo. J. Cardiovasc. Pharmacol. 6:961-968, 1984.

67. Heusch, G., A. Deussen, J. Schipke, H. Vogtelsang, and V. Thamer. Role of cardiac sympathetic nerves in the genesis of myocardial ischemia distal to coronary stenoses. J. Cardiovasc. Pharmacol. 7(Suppl 5):S13-S18, 1985.

68. Heusch, G., J. Schipke, and V. Thamer. Sympathetic mechanisms of post-stenotic myocardial ishemia. J. Cardiovasc. Pharmacol. 8(Suppl 3):S33-S40, 1986.

69. Heymann, M.A., B.D. Payne, J.I.E. Hoffman, and A.M. Rudolph. Blood flow measurements with radionuclide-labeled particles. Prog. Cardiovasc. Dis. 20:55-79, 1977.

70. Heyndrickx, G.R., P. Muylaert, and J.L. Pannie. Alpha-adrenergic control of oxygen delivery to myocardium during exercise in conscious dogs. Am. J. Physiol. 242: H805-H809, 1982.

71. Heyndrickx, G.R., J.P. Villaine, D.J. Moerman, and L. Teusen. Role of prejuncitonal α_2-adrenergic receptors in the regulation of myocardial performance during exercise in conscious dogs. Circ. Res. 54:683-693, 1984.

72. Hintze, T.H. and G. Kaley. Prostaglandins and the control of blood flow in the canine myocardium. Cir. Res. 40:313-320, 1977.

73. Holtz, J., E. Mayer, and E. Bassenge. Demonstration of alpha-adrenergic coronary control on different layers of canine myocardium by regional myocardial sympathectomy. Pflugers Arch. 372:187-194, 1977.

74. Huang A.H. and E.O. Feigl. Adrenergic coronary vasoconstriction helps maintain uniform transmural blood flow distribution during exercise. Circ. Res. 62:286-298, 1988.

75. Insel, P.A. Structure and function of alpha-adrenergic receptors. Am. J. Med. 87(Suppl 2A):12S-18S, 1989.

76. Johannsen U.J., A.L. Mark, and M.L. Marcus. Responsiveness to cardiac sympathetic nerve stimulation during maximal coronary dilation produced by adenosine. Circ. Res. 50:510-517, 1982.

77. Jones, C.E., and H.L. Bethea. Myocardial lactate extraction during hemorrhagic hypotension in the dog and the effect of dipyridamole. Circ. Shock. 2:41-47, 1975.

78. Jones C.E., T.A. Farrell, and R. Ator. Evidence that a coronary alpha adrenergic tone limits myocardial blood flow and oxygenation in acute hemorrhagic hypotension. Circ. Shock. 11: 329-340, 1983.

79. Jones C.E., I.Y.S. Liang, and M.M. Maulsby. Effects of prazosin and phenoxybenzamine on coronary blood flow and cardiac function during coronary hypotension. J. Pharmacol. Exp. Ther. 236:204-221, 1986.

80. Jones, C.E., I.Y.S. Liang, and P.A. Gwirtz. Effects of α-adrenergic blockade on coronary autoregulation in the dog. Am. J. Physiol. 253:H365-H372, 1987.

81. Kedem, J., J. Sonn, M. Scheinowitz, and H.R. Weiss. Relationship between local oxygen consumption and local and external cardiac work: effect of tachycardia. Cardiovasc. Res. 23:1043-1052, 1989.

82. Kelley,K.O. and E.O. Feigl. Segmental α-receptor mediated vasoconstriction in the canine coronary circulation. Circ. Res. 43:908-917, 1978.

83. Khouri, E.M., D.E. Gregg, and C.R. Rayford. Effect of exercise on cardiac output, left coronary flow, and myocardial metabolism in the unanesthetized dog. Circ. Res. 17:427-437, 1965.

84. Knansnow, N. and R. Gorlin. Myocardial lactate metabolism in coronary insufficiency. Ann. Intern. Med. 59:781-787, 1963.

85. Langer, S.Z. and N.B. Shepperson. Recent developments in vascular smoothe muscle pharmacology: the post-synaptic α_2-adrenoceptor. Trends Pharmacol. Sci. 3:440-44, 1982.

86. Langer, S.Z., N. Duval, and R. Massingham. Pharmocologic and therapeutic significance of α-adrenoceptor subtypes. J. Cardiovasc. Pharmacol. 7(Suppl.8):S1-S8, 1985.

87. Laxson D.D., X.Z. Dai, D.C. Homans, and R.J. Bache. The role of α_1- and α_2-adrenergic recepptors in mediation of coronary vasoconstriction in hypoperfused ischemic myocardium during exercise. Circ. Res. 65:1688-1697, 1989.

88. Ledda F., P. Marchetti, and A. Mugelli. Studies on the positive inotropic effects of phynylephrine: a comparison with isoprenaline. Br. J. Pharmacol. 54:83-90, 1975.

89. Liang, C.S. and J.M. Lowenstein. Metabolic control of the circulation: effects of acetate and pyrouvate. J. Clin. Invest. 62:1029-1038, 1978.

90. Liang, I.Y.S., and C.E. Jones. α_1-Adrenergic blockade increases coronary blood flow during coronary hypoperfusion. Am. J. Physiol. 249:H1070-1077, 1985.

91. Linden, J. C.E. Hollen, and A. Patel. The mechaninsm by which adenosine and cholinergic agents reduce contractility in rat myocardium. correlation with cyclic adenosine monophosphate and receptor densities. Circ. Res. 56:728-735, 1985.

92. Londos C., D.M.V. Cooper and J. Wolff. Subclasses of external adenosine receptors. Proc. Natl. Acad. Sci. USA 77:2551-2554, 1980.

93. Londos C., J. Wolff, D.M.F. Cooper. Adenosine as a regulator of adenylate cyclase. In: Receptors and Recognition. Series B, Vol 12, G. Burnstock (ed.), London:Chapman and Hall, 287-322, 1981.

94. Manders, W.T. and S.F. Vatner. Effects of sodium pentobarbital anesthesia on left ventricular function and distribution of cardiac output in dogs with particular reference to mechanisms of tachycardia. Circ. Res. 39:512-517, 1976.

95. Marbach E.P., and M.H. Weill. Rapid enzymatic measurements of blood lactate and pyruvate. Clin. Chem. 12:314-325, 1967.

96. Mass H.J., J.T. Gean, and P.A. Gwirtz. Computer analysis of cardiovascular parameters. Comput Biol Med. 17:75-84, 1987.

97. McGrath, J.C., N.A. Flavahan, and C.E. McKean. α_1- and α_2-adrenoceptor-mediated pressor and chronotropic effects in the rat and rabbit. J. Cardiovasc. Pharmacol. 4(Suppl 1):S101-S107, 1982.

98. Millard R.W., H. Baig, and S.F. Vatner. Cardiovascular effects of radioactive microsphere suspensions and tween 80 solutions. Am. J. Physiol. 233 :H331-H334, 1977.

99. Mohrman D.E. and E.O. Feigl. Competition between sympathetic vasoconstriction and metabolic vasodilation in the canine coronary circulation. Circ. Res. 42:79-86, 1978.

100. Molnar, J.I., J.B. Scott, E.D. Frolich, and F.J. Haddy. Local effects of various anions and H^+ on dog limb and coronary vascular resistances. Am. J. Physiol. 203:125-132, 1962.

101. Monroe R.G., W.J. Gamble, C.G. LaFarg, A.E. Kumar, J. Stark, R. Plenge, G.L. Sanders, C. Phornphutkul and M. Davis. The anrep effect reconsidered. J. Clin. Invest. 51:2573-2583, 1972.

102. Murray, P.A., F.L. Belloni, and H.V. Sparks. The role of potassium in the metabolism control of coronary vascular resistance of the dog. Circ. Res. 44:767-780, 1979.

103. Murray P.A. and S.F. Vatner. Alpha-adrenoceptor attenuation of the coronary vascular responses to severe exercise in the conscious dog. Circ. Res. 45:654-660, 1979.

104. Murray, P.A., M. Lavalle, and S.F. Vatner. α-adrenergic-mediated reduction in coronary blood flow secondary to carotid chemoreceptor reflex activation in conscious dogs. Circ. Res. 54:96-106, 1984.

105. Musch, T.I., G.C. Haidet, G.A. Ordway, J.C. Longhurst, and J.H. Mitchell. Dynamic exercise training in foxhounds I. oxygen consumption and hemodynamic responses. J. Appl. Physiol. 59:183-189, 1985.

106. Musch, T.I., D.B. Friedman, G.C. Haidet, J. Stray-Gundersen, T.G. Waldrop, and G.A. Ordway. Arterial blood gases and acid-base status of dogs during graded dynamic exercise. J. Appl. Physiol. 61:1914-1919, 1986.

107. Musch, T.I., D.B. Friedman, K.H. Pitetti, G.C. Hidet, J. Stray-Gundersen, J.H. Mitchell, and G.A. Ordway. Regional distribution of blood flow of dogs during graded dynamic exercise. J. Appl. Physiol. 63:2269-2277, 1987.

108. Nakashima, M., K. Maeda, A. Sekiya, and Y. Hagino. Effect of hypothyroid status on myocardial responses to sympthomimetic drugs. Jpn. J. Pharmacol. 21:819-825, 1971.

109. Nathan, H.J., and E.O. Feigl. Adrenergic vasoconstriction lessens transmural steal during coronary hypoperfusion. Am. J. Physiol. 250:H645-H653, 1986.

110. Needlemand, P., S.L. Key, P.C. Isakson, and P.S. Kulkarni. Relationship between oxygen tension, coronary vasodilation and prostaglandin biosynthesis in the isolated rabbit heart. Prostaglandins. 9:123-134, 1975.

111. Olsson, R.A. Local factors regulatin cardiac and skeletal muscle blood flow. Annu. Rev. Physiol. 43:385-395, 1981.

112. Olsson, R.A. and W.J. Bugni. Coronary circulation. In: The Heart and Cardiovascular System. H.A. Fozzard, R.B. Jennings, E.Haber, A.M. Katz, and H.E. Morgan (ed.), New York:Raven Press, 1986. p.987-1037.

113. Olsson, R.A. C.J. Davis, E.M. Khouri, and R.E. Patterson. Evidence for an adenosine receptor on the surface of dog coronary myocytes. Circ. Res. 39:93-98, 1976.

114. Opie L.H. Coronary flow rate and perfusion pressure as determinants of mechanical function and oxidative metabolism of isolated perfused rat heart. J. Phys. 180:529-541, 1965

115. Ordway, G.A., D.L. Floyd, J.C.Longhurst, and J.H. Mitchell. Oxygen consumption and hemodynamic responses during graded treadmill exercise in the dog. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 57:601-607, 1984.

116. Osnes J.B., H. Refsum, T. Skomedal and I. Oye. Qualitative differences between β-adrenergic and α-adrenergic inotropic effects in rat heart muscle. Acta. Pharmacol. et Toxicol. 42:235-247, 1978.

117. Otani H., H. Otani and D.K. Das. α_1-Adrenoceptor-mediated phosphoinositide breakdown and inotropic response in rat left ventricular papillary muscles. Circ. Res. 62:8-17, 1988.

118. Parmley, W.W., and J.V. Tyberg. Determinants of myocardial oxygen demand. In: Progress in Cardiology. Philadelphia:Lippincott, 1988, p.19-36.

119. Poche R., G. Arnold and D. Gahlen. The influence of coronary perfusion pressure on metabolism and ultrastructure of the myocardium of the arrested aerobically perfused isolated guinea-pig heart. Virchows Arch. B. 8:252-266, 1971.

120. Powel, J.R. and E.O. Feigl. Carotid reflex coronary vasoconstriction during controlled myocardial oxygen metabolism in the dog. Circ. Res. 44:44-51, 1979.

121. Rabinowitz, B., L. Chuck, M. Kligerman, and W.W. Parmley. Positive inotropic effects of methoxamine: evidence for alpha-adrenergic recptors in ventricular myocardium. Am. J. Physiol. 229:582-585, 1975.

122. Rinkema, L.E., J.X. Thomas, Jr, and W.C. Randall. Regional coronary vasoconstriction in response to stimulation of stellate ganglia. Am. J. Physiol. 243:H410-H415, 1982.

123. Rose, K.D. J.A. Ursick, and R.D. Maca. Exercise and serum potassium flux: myocardial metabolic implication. Myocardiology. 1:673-683, 1972.

124. Ross, G. and D.G. Mulder. Effects of right and left cardiosympathetic nerve stimulation on blood flow in major coronary arteries of the anesthetized dog. Cardiovasc. Res. 3:22-29, 1969.

125. Rosendorff, C., J.I.E. Hoffman, E.D. Verrier. J. Rouleau, and L.E. Boerboom. Cholesterol potentiates the coronary artery response to norepinephrine in anesthetized and conscious dogs. Circ. Res. 48:320-329, 1981.

126. Rowell, L.B. Circulatory adjustments to dynamic exercise. In: Human Circulation: Regulation During Physical Stress. New York: Oxford Univ. Press, 1986, p.213-256.

127. Rubanyi G. and A.G.B. Kovach. The role of coronary perfusion pressure in the control of mechanical performance and oxygen consumption in the isolated rat heart. Acta. Physiolgica Academiae Scientiarum Hungaricae 55:189-196, 1980.

128. Saffitz, J. Distribution of α_1-adrenergic receptors in myocytic regions and vasculature of feline myocardium. Am. J. Physiol. 257:H162-H169, 1989.

129. Salisbury P.F., C.E.Cross and P.A. Rieben. Influence of coronary artery pressure upon myocardial elasticity. Circ. Res. 8:794-800, 1960.

130. Sarnoff S.J., J.P. Gilmore, N.S. Skinner, A.G. Wallace and J.H. Mitchell. Relation between coronary blood flow and myocardial oxygen consumption. Circ. Res. 13:514-521, 1963.

131. Schrader, J., S. Nees, and E. Gerlach. Evidence for a cell surface adenosine receptor on coronary myocytes and atrial muscle cells. Pfluegers Arch. 369:251-257,1977.

132. Schumann, H.J., M. Endoh, J. Wagner. Positive inotropic effects of phenylephrine in the isolated rabbit papillary muscle mediated both by α- and β-adrenoceptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 284:133-148, 1974.

133. Schwartz, P.J. and H.L. Stone. Tonic influence of the sympathetic nervous system on myocardial reactive hyperemia and on coronary blood flow distribution in dogs. Circ. Res. 41:51-58, 1977.

134. Scott, J. B., E.D. Frolich, R.A. Hardin, and F.J. Haddy. Na^+, K^+, Ca^{++} and Mg^{++} action on coronary vascular resistance in the dog heart. Am. J. Physiol. 201:1095-1100, 1961.

135. Seitelberger, R., B.D. Guth, G. Heusch, J.D. Lee, K. Katayama, and J. Ross, Jr. Intracoronary α_2-adrenergic receptor blockade attenuates ischemia in conscious dogs during exercise. Circ. Res. 62:436-442, 1988.

136. Shepherd, J.T., and P.M. Vanhoutte, 1985 = Local modulation of adrenergic neural transmission in blood vessels. J. Cardiovasc. Pharmacol. 7:S167-S178, 1985.

137. Soboll, S., and R. Bunger. Comparmentation of adenine nucleotides in the isolated working guinea pig heart stimulated by noradrenaline. Hoppe Seylers Z. Physiol. Chem. 362:125-132, 1981.

138. Stein, P.D., M. Marzilli, H.N. Sabbah, T. Lee. Systolic and diastolic pressure gradients within the left ventricular wall. Am. J. Physiol. 238:H625-H630, 1980.

139. Stone, H.L. Coronary flow, myocardial oxygen consumption, and exercise training in dogs. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49:759-768, 1980.

140. Strader, H.R., P.A. Gwirtz, and C.E. Jones. Comparative effects of α_1- and α_2-adrenoceptors in modulation of coronary flow during exercise. J. Pharmacol. Exper. Ther. 246:772-778, 1988.

141. Streeter, D.D., N. Vaishnav, D.J. Patel, H.M. Spotnitz, J. Ross, and E.H. Sonnenblick. Stress distribution in the canine left ventricle during diastole and systole. Biophys. J. 10:345-363, 1970.

142. Stroebeck, J.E. and E.H. Sonnenblick. Myocardial contractile properties and ventricular performance. IN: The Heart and Cardiovascular System H.A Fozzard, R.B. Jennings, E.Haber, A.M. Katz, and H.E. Morgan (ed.). New York:Raven Press, 1986, p.31-49.

143. Theroux, P., D. Franklin, J. Ross, Jr., and W.S. Kemper. Regional myocardial function during acute coronary artery occlusion and its modification by pharmacologic agents in the dog. Circ. Res. 35:896-908, 1974.

144. Tipton C.M., R.A. Carey, W.C. Easten, and H.H. Erickson. A submaximal test for dogs: evaluation of effects of training, detraining, and cage confinement. J. Appl. Physiol. 37:271-275, 1974.

145. Uchida, Y. and S. Murao. Sustained decrease in coronary blood flow and excitation of cardiac sensory fibers following sympathetic stimulation. Jpn. Heart J. 16:265-279, 1975.

146. Urthaler, F., W.T. Woods, T.N. James, and A.A. Walker. Effects of adenosine on mechanical performance and electrical activity in the canine heart. J. Pharmacol. Exp. Ther. 216:254-260, 1981.

147. Van Calker D., M. Muller, and B. Hamprecht. Adenosine inhibits the accumulation of cyclic AMP in cultured brain cells. Nature. 276;839-841, 1978.

148. Vatner, S.F., D. Franklin, and R.L. Van Clitters. Simultaneous comparison and calibration of the doppler and electromagnetic flow-meters. J. Appl. Physiol. 29:907-910, 1970.

149. Vatner, S.F., C.B. Higgins, R.W. Millard, and D. Franklin. Role of the spleen in the peripheral vascular response to severe exercise in untethered dogs. Circ. Res. 8:276-282, 1974.

150. Vatner, S.F. Alpha-adrenergic regulation of the coronary circulation in the conscious dog. Am. J. Cardiol. 52:15A-21A, 1983.

151. Verhaeghe R.H., P.M. Vanhoutte, J.T. Shepperd. Inhibition of sympathetic neurotransmission in canine blood

vessels by adenosine and adenine nucleotides. Circ. Res. 40:208-215, 1977.

152. Vlahakes, G.J., R.J. Baer, P.N. Uhlig, E.D. Verrier, J.D. Bristow, and J.I.E. Hoffmann. Adrenergic influence on the coronary circulation of conscious dogs during maximal vasodilation with adenosine. Circ. Res. 51:371-384, 1982.

153. Wagner, J., and O.E. Brodde. On the presence and distribution of α-adrenoceptors in the heart of various mammalian species. Naunyn-Schmiedeberg's Arch. Pharmacol. 302:239-254, 1978.

154. Wenzel, D.G., and J.L. Su. Interactions between sympathomimetic amines and blocking agents on the rat ventricle strip. Arch. Int. Pharmacodyn. Ther. 160:379-389, 1966.

155. Widmann, F.K. Clinical interpretation of laboratory tests. 9th Ed. Philadelphia:F.A. Davis, 1984.

156. Williams, A.G., Jr., D.P. Grice, C.E. Jones, and H.F. Downey. Hypoxia-induced, relex-mediated coronary vasoconstriction is uniform across the left ventricular free wall. The FASEB Journal. 2:A1712, 1988, (Abstract).

157. Woodman, O.L. and S.F. Vatner: Coronary vasoconstriction mediated by α_1- and α_2-adrenoceptors in the conscious dog. Am. J. Physiol. 253:H388-H393, 1987.

158. Yonekura, S., W., Noriyasu, and H.F. Downey. Transmural variation in autoregulation of right ventricular blood flow. Circ. Res. 62:776-781, 1988.

159. Young, S.H. and H.L. Stone. A new indwelling catheter or coronary sinus blood sampling in dogs. J. Appl. Physiol. 36:767-768, 1974.

160. Young, M.A., D.E. Vatner, D.R. Knight, R.M. Graham, C.J. Homcy, and S.F. Vatner. α-Adrenergic vasoconstriction and receptor subtypes in large coronary arteries of calves. Am. J. Physiol. 255:H1452-H1459, 1988.

161. Zborowska-Sluis D.T., R.R. Mildenberger, and G.A. Klassen. The role of coronary flow and pressure as determinants of myocardial oxygen consumption in the presence or absence of vasomotor tone. Can. J. Physiol. Pharmacol. 55:471-477, 1977.