AUTONOMIC REFLEXES OF THE HEART DURING ACUTE MYOCARDIAL ISCHEMIA.

DISSERTATION

Presented to the Graduate Council of the

University of North Texas in Partial

Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

André F. Meintjes, BSc.(MED)HONS.

Denton, Texas

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This study investigated whether acute myocardial ischemia of the anterior left ventricular wall induced an increase in cardiac sympathetic efferent nerve activity and thereby affected regional myocardial blood flow and contractile function.

Chronically instrumented, intact, unanesthetized, resting dogs were studied. Global and regional myocardial contractile function were monitored. Regional blood flows were measured using radiolabelled microspheres. Ischemia was induced by occluding the left anterior descending coronary artery and stenosing the circumflex artery. Dogs were studied with and without combined systemic ß-adrenergic and muscarinic receptor blockade. In the unblocked state, no infusion (time control) or intracoronary (i.c.) phentolamine infusion were compared. With double blockade, either saline or phentolamine was infused.

In the unblocked state, responses to ischemia of the time control and phentolamine infusion conditions were similar. Heart rate (HR) and left ventricular end-diastolic pressure (LVEDP) increased and regional contractile function of the anterior wall of the left ventricle decreased. The increase in HR

suggests a reflex increase in cardiac sympathetic efferent nerve activity. Vagal withdrawal may also have occurred.

With double blockade, responses to ischemia were similar to that described above, except for no change in HR and significant decreases in the maximal rate of left ventricular pressure development. LVEDP increased more in the phentolamine infusion condition.

During the occlusion, transmural flows in the ischemic region were lower relative to baseline in both infusion conditions. Anterior:posterior wall transmural flow ratios decreased. There were no differences between the 2 infusions when α-adrenergic receptors were blocked by phentolamine. During saline infusion, subendocardial flow of the posterior wall increased in response to severe regional ischemia of the anterior wall, while subepicardial flow did not. The occlusion had no effect on posterior regional flows when phentolamine was infused.

The data suggest, but does not clearly demonstrate, that cardiac sympathetic efferent nerve activity probably increases in conjunction with vagal withdrawal during acute regional myocardial ischemia. No α-adrenergic coronary constrictor tone could be isolated.

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CHAPTER 1

INTRODUCTION

Sympathetic afferent and efferent nerve activity increases in response to acute myocardial ischemia (Brown, 1967; Malliani et al, 1969; Uchida and Murao, 1974; Lombardi, 1986; Heusch et al, 1985). Brown (1967) reported increases in cardiac sympathetic afferent nerve activity during periods of ischemia lasting 4 - 10 seconds. Increases in both cardiac sympathetic afferent and efferent nerve activity in response to myocardial ischemia have been measured prior to any decrease in mean arterial pressure (MAP) (Brown and Maliiani, 1971). Maliiani et al (1969) proposed a sympathetically mediated cardio-cardiac reflex in response to a coronary artery occlusion while Heusch et al (1985) demonstrated an α_2-adrenergic receptor-mediated coronary constriction during severe regional myocardial ischemia. In anesthetized dogs, postganglionic sympathetic nerve activity increased continuously throughout 20 minutes of severe circumflex artery stenosis and was abolished by spinal anesthesia of segments C7 to T6. Similar coronary vasoconstrictor responses have been observed under conditions of coronary hypotension (Jones et al, 1986), ischemia during exercise (Seitelberger et al, 1988) and ischemia during cardiac pacing in the resting dog (Vallance et al, 1990). Thus, there seems to

be little doubt as to the existence of an ischemia-induced coronary vasoconstrictor tone.

Stimulation of the cardiac nerves have differential effects on regional blood flow, an important consideration for the control of myocardial blood flow distribution under ischemic conditions (Haws et al, 1987). α-adrenergic receptor-mediated coronary constriction has been shown to reduce the extent of transmural steal from subepicardium to the ischemic subendocardium in the anesthetized animal (Nathan and Feigl, 1986; Westby et al, 1992). Indeed, this has also been demonstrated in exercising dogs subjected to ischemia (Chilian and Ackell, 1988). The constrictor tone also facilitates regional flow distribution towards ischemic myocardium (Giudicelli et al, 1980). A potential mechanism for the steal phenomenon was demonstrated by Goto et al (1992). They injected nitroglycerin distal to a coronary stenosis and measured intra-myocardial phasic flow patterns as well as regional blood flows. Retrograde septal artery flow and subepicardial flow increased while subendocardial flow remained unchanged. This reflects the greater vasodilatory reserve of the subepicardium, possibly due to α-adrenergic receptor-mediated constrictor tone.

The "physiological relevance" of ischemia-induced reflex increases in cardiac sympathetic efferent activity in intact animals has been questioned (Felder and Thames, 1980; Malliani, 1980). All studies which address changes in cardiac sympathetic efferent nerve activity due to myocardial ischemia in

"resting" dogs were done in anesthetized, vagotomized and/or sinoaortic denervated and/or spinalectomized animals. Thus, the question of whether such reflex changes in cardiac sympathetic efferent nerve activity can be detected in conscious, intact, unanesthetized animals is a valid one and remains to be answered. The present study addresses this question directly. More specifically, the aim was to investigate whether the ischemia-induced increase in cardiac sympathetic efferent nerve activity is beneficial, detrimental or of no consequence to myocardial blood flow and contractile function in conscious, resting dogs. The null hypotheses tested were:

1. Acute regional myocardial ischemia (2.5 minutes) does not increase heart rate (HR). An increase in HR was used as an indirect index of elevated cardiac sympathetic efferent nerve activity (Gayheart et al, 1991) since it was not possible to measure nerve traffic.
2. Acute regional myocardial ischemia does not result in an increase in α-adrenergic receptor-mediated coronary constrictor tone.
3. Transmural myocardial blood flow is not affected by an increase in α-adrenergic receptor-mediated coronary constrictor tone due to acute regional myocardial ischemia.
4. Distribution of blood flow between ischemic and non-ischemic myocardium is not affected by an increase in α-adrenergic receptor-mediated coronary constrictor tone.

5 Changes in regional blood flow distribution during acute regional myocardial ischemia do not alter regional myocardial contractile function of the ischemic and/or the non-ischemic myocardium.

An unanesthetized, conscious chronically instrumented dog model was used. Instrumentation allowed the measurement of both global and regional myocardial contractile function. Regional blood flow was measured by the radiolabelled microsphere technique (Heymann et al, 1977). Dogs were studied in a rested state when α-adrenergic receptor-mediated coronary constrictor tone is negligible (Chilian et al, 1981; Gwirtz et al, 1986). The left anterior descending coronary artery was occluded and the circumflex artery was stenosed to produce transmural ischemia of the anterior wall of the left ventricle. The presence of an α-adrenergic receptor-mediated coronary constriction was detected by intracoronary infusion of the nonspecific α-adrenergic receptor antagonist, phentolamine.

CHAPTER 2

LITERATURE REVIEW

A. Determinants of Myocardial Blood Flow

A linear relationship exists between coronary blood flow and myocardial oxygen consumption (Feigl,1991). To prevent myocardial ischemia from developing, a fine balance is maintained between myocardial oxygen demand and oxygen supply. Myocardial oxygen demand is determined primarily by three factors: heart rate, myocardial contractility and systolic wall tension (Braunwald and Sobel,1992). Since these factors are interdependent, under normal physiological or pathophysiological conditions it is impossible to change one without affecting the others. For example, sympathetic stimulation of myocardial β_1-adrenergic receptors increases the inotropic state of the heart. This results in a greater force of contraction requiring a greater oxygen supply and hence an increase in coronary blood flow. At the same time heart rate (HR) is increased. An increase in HR results in an increase in myocardial oxygen consumption by increasing the proportion of the cardiac cycle spent in systole, a time during which wall tension is high. HR also influences contractility. Since contractility is increased, the ejection fraction increases, thereby reducing left ventricular end systolic volume. In addition, the higher HR results in reduced filling time during diastole which, coupled to the reduced

end systolic volume results in a reduction in end-diastolic volume. Overall, there is a decrease in heart size. By the Law of Laplace, developed tension is proportional to the radius of the ventricle and the intraventricular pressure but inversely proportional to the thickness of the myocardial wall. Changes in inotropic state of the heart will, therefore, affect wall tension as well and, thereby, influence myocardial oxygen consumption. Such increases in myocardial activity cause increases in oxygen demand which are not met solely by increasing oxygen extraction since at rest myocardial oxygen extraction is approximately 70 % and can only increase by an additional 20 % (Feigl,1983). Therefore, oxygen delivery must increase to attain the all important balance between oxygen supply and demand.

Factors affecting oxygen supply are the oxygen carrying capacity of the blood and the coronary blood flow (CBF). Oxygen carrying capacity is determined by the hemoglobin concentration and percent oxygen saturation. Under normal physiological circumstances these remain relatively constant from moment to moment and will, therefore, not be addressed further.

To balance oxygen supply with oxygen demand, oxygen delivery must, therefore, increase via an increase in CBF. The magnitude of CBF is determined by coronary vascular resistance (CVR) assuming a constant coronary perfusion pressure. CVR is, in turn, regulated by mechanical, humoral, metabolic and neural mechanisms:

1. Mechanical compressive forces: Intramyocardial pressure increases during systole. The contracting myocardium, therefore, compresses the intramyocardial vessels increasing the resistance to flow. During diastole with normal left ventricular end-diastolic pressure, mechanical compression of the coronary vasculature is minimal. Hence, the phasic pattern of CBF - low systolic flow and higher diastolic flow. Systolic intramyocardial pressure in the subendocardium is greater than in the subepicardium which influences the transmural distribution of CBF.

2. Humoral regulation: Humoral modulators of CBF include histamine, serotonin, vasopressin, angiotensin II, bradykinin, prostaglandins and circulating catecholamines (Bassenge and Heusch,1990). Endothelial cells play a significant role in the actions of these humoral agents either by actively producing them (prostaglandins) or by mediating their actions (Ardehal and Ports,1990; Braunwald and Sobel,1992). The actions of bradykinin, histamine, angiotensin II, vasopressin and serotonin are dependent on an intact endothelium and may involve endothelial derived relaxing factor (EDRF). Angiotensin II and vasopressin cause vasoconstriction, while bradykinin, serotonin and histamine cause vasodilation.

Circulating catecholamines (norepinephrine and epinephrine) exert their effects on the coronary vessels through stimulation of α- and $ß_2$-adrenergic receptors. Stimulation of α_1- and α_2-adrenergic receptors results in vasoconstriction, while stimulation of $ß_2$-adrenergic receptors causes

vasodilation. Indirect coronary vasodilation occurs as a result of the circulating catecholamines stimulating myocardial $ß_1$-receptors, thereby increasing myocardial contractile function and inducing a metabolic vasodilation. The net effect of circulating catecholamines is an increase in CBF.

3. Metabolic mechanisms: As myocardial contractile function increases the metabolic rate of the myocytes increases and, thus, more metabolites are produced. This local release of metabolic factors such as adenosine (a byproduct of energy metabolism), potassium, hydrogen ion and carbon dioxide causes coronary vasodilation (Belloni,1979). Reduced oxygen tension may also have a vasodilatory effect on the coronary vessels and particularly on the precapillary sphincters (Deltar and Bohr,1968; Duling,1972). However, myocardial oxygen tension is relatively constant because of the well balanced oxygen supply and demand so the relative importance of oxygen as a direct regulator of CVR is debatable (Feigl,1983).

Adenosine is regarded as the primary metabolic vasodilator (Marcus,1983). Increased adenosine triphosphate utilization results in interstitial adenosine accumulation. By specific receptor binding, adenosine causes coronary vasodilation and hence an increase in CBF. Although adenosine seems to be the all important metabolic regulator of CBF, it is not the sole regulator. Interaction of numerous metabolites, as discussed above, is a more appropriate control mechanism than a single agent.

4. Neural regulation: Neural mechanisms are also involved in controlling CBF. Sympathetic coronary vasoconstriction and metabolic vasodilation oppose each other such that the adrenergic vasoconstriction limits the metabolic vasodilation by 20 to 30% (Mohrman and Feigl,1978). Both parasympathetic and sympathetic branches of the autonomic nervous system innervate the coronary vasculature (Denn and Stone,1976). Afferent and efferent nerve fibers of both the parasympathetic and sympathetic branches of the autonomic nervous system comprise the cardiac nerves. The gross anatomy of the cardiac nerves differs between the left and the right sides (Thomas and Gerdisch,1991). They originate from the left and right vagosympathetic trunks, the middle (caudal) cervical ganglia and the stellate ganglia. Those originating from the stellate ganglia, viz. the left and right stellate cardiac nerves, are comprised of sympathetic fibers with the right stellate cardiac nerve serving both afferent and efferent functions while the left stellate cardiac nerve being primarily afferent in nature (Armour and Randall,1975). Right stellate afferents originate in the atria while left stellate afferents originate in the left atrium and to a lesser extent in the ventricles. The ventrolateral cardiac nerve comprises sympathetic efferents and innervates the left ventricle. All other cardiac nerves are mixed nerves, i.e., they carry both sympathetic and parasympathetic afferent and efferent fibers to a greater or lesser extent and project to localized regions of the myocardium and conduction system (Armour et al,1975; Randall et al,1968).

Parasympathetic stimulation of the coronary vessels induces direct coronary vasodilation in dogs (Feigl,1969). In man, cholinergic control of the coronary vessels has not been clearly defined (Heusch and Guth,1989). Sympathetic stimulation results in both a direct vasoconstriction and an indirect (metabolic) vasodilation in both the canine and human species (Feigl,1967).

α- and ß-adrenergic receptors as well as muscarinic receptors exist in the coronary vasculature. Large coronary artery vasoconstriction is mediated by α_1-adrenergic receptors (Heusch et al,1984) or by both α_1- and α_2-adrenergic receptors (Young et al,1988). Resistance vessel constriction has been shown to be mediated by α_2- (Heusch et al,1984; Holtz et al,1982) or by both α-adrenergic receptor subtypes (Woodman and Vatner,1987). Gwirtz et al (1986) and Bache et al (1987) attributed the coronary constrictor tone in exercising dogs to sympathetic neural stimulation of α_1-adrenergic receptors. Similar debate exists as to the α-adrenergic receptor subtype which mediates coronary vasoconstriction during myocardial ischemia (Jones et al,1986; Heusch et al,1985; Seitelberger et al,1988; Chilian,1991). Although there is uncertainty as to the exact α-adrenergic receptor subtype which mediates coronary constrictor tone, it is clear that stimulation of coronary α-adrenergic receptors causes vasoconstriction.

Both $ß_1$- and $ß_2$-adrenergic receptors cause vasodilation of the coronary vessels. $ß_2$-adrenergic receptors predominate on coronary resistance vessels (Ross,1976), while epicardial vessels contain both subtypes (Young and

Vatner,1986). The importance of coronary vasodilation via ß-adrenergic receptors remains unclear (Mass and Gwirtz,1987; Gutterman et al,1991).

Muscarinic receptors exist on the coronary vessels, the stimulation of which results in vasodilation in the dog (Feigl,1969). If the endothelium is removed this vasodilatory response changes to one of vasoconstriction (Vanhoutte and Cohen,1984). It appears that the vasodilatory response of acetylcholine is mediated via endothelial derived relaxing factor or EDRF (Furchgott and Zawadzki,1980).

B. Physiological and Pathophysiological Coronary Constrictor Tone

No α-adrenergic receptor-mediated coronary constrictor tone is evident in resting, conscious animals (Chilian et al,1981; Gwirtz et al,1986). However, any physiological or pathophysiological condition characterized by elevated sympathetic neural activity will result in an α-adrenergic receptor-mediated coronary constrictor tone. This constrictor tone, during exercise, has been shown to be either neurally (Gwirtz et al,1991) and/or humorally mediated (Chilian et al,1986).

Carotid sinus hypotension (Murray and Vatner,1981), carotid chemoreceptor stimulation (Murray et al,1984) and esophageal distension (Gayheart et al,1991) result in an increased sympathetic efferent activity and thus an increase in coronary α-adrenergic receptor-mediated vasoconstriction. A sympathetic α-adrenergic receptor-mediated coronary constrictor tone is also

present during exercise (Bache et al,1987; Chilian and Ackell,1988; Guth et al,1990; Gwirtz et al,1986), coronary hypoperfusion (Jones et al,1983), coronary hypotension (Jones et al,1986) as well as myocardial ischemia (Buffington and Feigl,1981; Heusch et al,1985; Laxson et al,1992; Vallance et al,1990). It appears that an oxygen supply-demand imbalance, whether at rest or during exercise, produces a change in cardiac sympathetic afferent and efferent activity. Studies in the anesthetized dog have demonstrated that when coronary perfusion pressure is reduced to 50 mmHg and less due to hemorrhagic hypotension, intracoronary injection of an α-adrenergic receptor antagonist causes a larger percentage increase in circumflex blood flow compared to normotensive controls (Jones et al,1983). Associated with the larger percent increase in flow was a greater increase in myocardial oxygen consumption. The arteriovenous oxygen difference did not decrease as occurred under control conditions. Therefore, acute hemorrhagic hypotension induced an α-adrenergic receptor-mediated coronary constrictor tone which contained myocardial oxygen consumption by limiting CBF. It should be noted, however, that peripheral effects of systemic hypotension cannot be ignored in the latter study. In another study, Jones et al (1986) demonstrated an α-adrenergic receptor-mediated coronary constrictor tone in response to coronary hypotension. Following a reduction in coronary perfusion pressure to 50 mmHg, non-selective α- and selective α_1-adrenergic receptor blockade was produced by intracoronary injection of phenoxybenzamine and prazosin

respectively. Phenoxybenzamine resulted in a significantly greater increase in circumflex blood flow and myocardial oxygen consumption than did prazosin. This difference was abolished by ß-adrenergic blockade with propranolol. Thus non-specific α-blockade resulted in an increase in circumflex blood flow by a direct removal of a coronary constrictor tone by blocking α_1-adrenergic receptors and also by metabolic vasodilation due to presynaptic α_2-blockade. Blockade of the presynaptic α_2-adrenergic receptors results in reduced autoinhibition of norepinephrine release from the presynaptic adrenergic nerve terminals. The greater release of norepinephrine from the cardiac sympathetic nerve terminals stimulates myocardial function thereby causing the greater increase in circumflex blood flow. Jones et al (1986) proposed that the coronary constrictor response to coronary hypotension was mediated entirely by α_1-adrenergic receptors. Sympathetic afferents were probably stimulated by hypoperfusion of the myocardium thereby increasing cardiac sympathetic efferent activity and hence the α-adrenergic coronary constrictor tone.

These results are supported by a similar observation in paced hearts (Vallance et al,1990). The hearts of conscious dogs at rest were paced at 210 b/min and a circumflex coronary artery stenosis was produced by inflation of a pneumatic occluder. Segmental shortening in the ischemic myocardium decreased by 55% from the unpaced control state. Under the same conditions of pacing and stenosis, intracoronary infusion of phentolamine resulted in smaller decrements in ischemic segmental shortening ($\pm$15%). Although they

did not measure circumflex blood flow, their data does suggest removal of an α-adrenergic receptor-mediated constrictor tone present during myocardial ischemia.

Exercising dogs may also experience an ischemia-induced coronary vasoconstrictor tone. Studies in this laboratory suggest that a potential ischemia-induced sympathetic reflex with coronary actions may exist during exercise (unpublished). When dogs were exercised at a relatively low work intensity without a stenosis of the circumflex coronary artery, intracoronary injection of prazosin caused only a slight increase in circumflex blood flow (0 - 10 %). This suggests relatively little sympathetic coronary constriction. However, if coronary flow was not permitted to increase during exercise, thereby causing a "high demand" myocardial ischemia, α_1-blockade caused a substantially larger increase in circumflex blood flow (30 %). An ischemia-induced α_2-adrenergic receptor-mediated coronary constriction in exercising dogs has also been demonstrated (Seitelberger et al,1988).

Laxson et al (1992) examined coronary vasodilator reserve in exercising dogs with circumflex flow limited by stenosis of the artery such that coronary perfusion pressure was reduced to 40 mmHg. The results demonstrated a residual coronary flow reserve which they proposed to be due to an adrenergically-mediated coronary constrictor tone. This hypothesis is supported by the studies of Vallance et al (1990), Buffington and Feigl (1981) and Heusch et al (1985). Under flow limited conditions in dogs, Vallance et al

(1990) documented a neurogenic constrictor tone mediated by α-adrenergic receptors. Buffington and Feigl (1981) reported an α-receptor-mediated vasoconstriction which limited oxygen delivery to the myocardium. Heusch et al (1985) demonstrated an α_2-adrenergic receptor-mediated coronary constriction during severe regional myocardial ischemia. Thus, there is little doubt that an ischemia-induced coronary vasoconstrictor tone exists. It is reflexly mediated as myocardial ischemia stimulates cardiac mechanoreceptors and/or cardiac chemoreceptors.

C. Cardiac Reflexes

Two types of atrial mechanoreceptors have been identified (Hainsworth,1991). Located in the subendocardium are Vagal complex unencapsulated nerve endings. Less well defined is the network of unmyelinated fibers within the endocardium, commonly referred to as an end-net. Both Vagal and sympathetic fibers contribute to the formation of the end-net.

Three discharge patterns have been identified for atrial receptors - type A discharge relates to atrial systole, type B discharge corresponds to atrial filling and intermediate discharge corresponds to a pattern of nerve activity with bursts during the atrial 'a' and 'v' waves. Of particular importance to this study are those receptors which respond to atrial stretch as the stimulus for their discharge since myocardial ischemia results in poor pump function and,

hence, elevated left atrial pressures. Thus, type-B receptors would be stimulated. The net result would be an increase in afferent myelinated Vagal activity which in turn would lead to an increase in sympathetic efferent activity to the heart.

Indeed, such a reflex was first documented by Bainbridge (1915), who showed that increases in left atrial pressures induced increases in heart rate. More recently, in anesthetized dogs, Drinkhill et al (1989) showed that distension of the left atrium resulted in an increase in heart rate with a concomitant increase in CBF. However, when heart rate was kept constant by pacing to eliminate metabolic vasodilation of the coronary vessels as a contributing factor, atrial distension resulted in a decrease in CBF. This reduction in CBF was attributed to a cardiac neural reflex since cooling the vagosympathetic trunk and intravenous administration of bretylium tosylate abolished the constrictor response. Thus, atrial stretch results in coronary vasoconstriction with the increase in neural activity originating in the atrial stretch receptors and then traveling via the vagal afferent and sympathetic efferent nerves. A linear relationship between left atrial pressure and atrial nerve fiber activity has indeed been documented (Mary,1987).

Sympathetic afferent nerves also originate in the atria and respond to atrial stretch in a similar manner. Sympathetic afferent nerve activity has been shown to increase in response to a coronary artery occlusion that caused atrial stretch (Malliani et al,1973).

Stimuli for cardiac receptors may be mechanical and/or chemical in nature (Uchida,1979). Chemical stimuli include potassium, bradykinin, prostaglandins and lactic acid. All these substances may be present during a coronary artery occlusion. Buffering lactic acid by intravenous sodium bicarbonate and administration of aspirin to inhibit prostaglandin synthesis reduces the rate of discharge of afferent fibers (Uchida,1979).

Stimulation of ventricular mechanoreceptors and ventricular chemoreceptors with Vagal afferents results in reflex inhibition of the sympathetic nerve activity and hence a reflex bradycardia, vasodilation and sympathoinhibition - a reflex commonly referred to as the Bezold-Jarisch reflex (von Bezold and Hirt,1867; Mark,1983; Thames and Abboud,1979). Vagotomy abolishes this response. Systolic bulging causes stimulation of the mechanoreceptors while changes in H^+, K^+, prostaglandins and bradykinin stimulate the chemoreceptors (Thoren,1972; Hintze,1987). It has been suggested that the primary stimulus of ventricular Vagal afferents is activation of the chemoreceptors since inhibition of prostaglandin synthesis by administration of indomethacin or sodium meclofenamate greatly attenuates the sympathoinhibitory response to myocardial ischemia of the inferoposterior left ventricular wall (Thames and Minisi,1989).

It is well known that ischemia of the inferoposterior left ventricular wall elicits a sympathoinhibitory response while sympathoexcitation occurs in response to ischemia of the anterior wall (Felder and Thames,1979). Indeed,

this has been demonstrated in the human by Perez-Gomet et al (1979) who demonstrated that coronary vasospasm resulting in anterior wall ischemia elicited an increase in heart rate while coronary vasospasm resulting in posterior wall ischemia elicited a decrease in heart rate. In the present study sympathoexcitation was required. Therefore, myocardial ischemia was restricted to the anterior wall of the left ventricle will be made ischemic by totally occluding the left anterior descending coronary artery.

Sympathetic afferent fibers exist side-by-side with the Vagal afferent fibers (Malliani,1982). The major difference between the Vagal and sympathetic afferent nerve fibers is in their firing pattern. While Vagal afferent fibers fire in bursts, the sympathetic afferent fibers fire single impulses. Elevation of right atrial pressure increases and hypovolemia reduces sympathetic afferent activity (Malliani et al,1973). Chemosensitive sympathetic afferent nerve endings exist in both the atria and the ventricles and are stimulated by similar substances to Vagal afferents. During coronary artery occlusion elevated end-diastolic pressures in both the atria and the ventricles as well as systolic bulging occurs, depending on the degree of myocardial dysfunction. Also, accumulation of metabolites in the interstitium occurs. Therefore, both mechanosensitive and chemosensitive sympathetic afferent fibers are excited (Malliani et al,1973; Uchida and Murao,1974).

The question arises: what is the primary reflex response that accompanies an episode of acute transmural ischemia of the anterior left

ventricular wall, assuming no change in arterial pressure? Increases in sympathetic afferent nerve activity in response to acute myocardial ischemia have been measured directly. Using spinally intact anesthetized dogs, Bosnjak et al (1979) measured afferent nerve activity at the distal end of the sectioned second or third white ramus communicans with the sympathetic trunk sectioned at T3. Occlusion of the left anterior descending coronary artery for both 30 and 60 second time periods caused increases in the sympathetic afferent activity (115% above control levels). This increase in nerve activity was associated with an 80% increase in ischemic segment length. Although they made no reference to the different lengths of the occlusions, it appears that a larger increase in sympathetic afferent activity occurs during the longer occlusion.

Gatenberg and Hageman (1991) also reported large increases in cardiac sympathetic efferent nerve activity in response to a 180 minute left anterior descending coronary artery occlusion. They measured nerve activity to the ischemic/infarcted region and to the nonischemic region. Cardiac sympathetic efferent nerve activity increased by 168 ± 22% and by 170 ± 25% respectively. The nerve activity increased continually for the duration of the occlusion. There was no significant decrease in mean arterial pressure. Since HR was paced, no change due to the increase in sympathetic efferent activity would be expected.

By stimulating α_2-adrenergic receptors with B-HT 920 in anesthetized dogs, Deussen et al (1985) showed an increase in coronary resistance under control conditions (no stenosis) as well as after coronary flow reserve had been exhausted by stenosing the circumflex artery such that resting flow was decreased by 50%. No change in cardiac sympathetic nerve activity was associated with this increase in coronary vascular resistance. However, cardiac sympathetic efferent nerve activity was 17 ± 5% higher after 20 minutes of ischemia and increased by an additional 27 ± 7% after 40 seconds of α_2-adrenergic receptor stimulation in the presence of the stenosis. This increase in cardiac sympathetic nerve activity together with a significant drop in myocardial oxygen consumption and coronary venous oxygen content, is indicative of activation of cardiac sympathetic afferents (and thus efferents) as a result of myocardial ischemia.

A sympathetically mediated cardio-cardiac reflex in response to coronary artery occlusion was first proposed by Malliani et al (1969). Measuring the firing rates of preganglionic fibers from the T3 rami communicantes in cats, they showed an increase in nerve activity during 20 - 90 seconds of ischemia in spinalectomized, vagotomized preparations. They confirmed this finding in a later study (Malliani et al,1972).

Reflex increases in sympathoexcitatory responses mediated by cardiac sympathetic afferents were also demonstrated by Minisi and Thames (1991). In an anesthetized dog preparation, cervical vagotomy and sinoaortic

denervation was performed. The spinal cord was left intact. Transmural ischemia of the anterior wall of the left ventricle was produced by simultaneously occluding the left anterior descending coronary artery and stenosing the circumflex coronary artery. A similar method was used in the present study. The circumflex stenosis served to reduce collateral blood flow to the collateral-dependent myocardium in the left anterior descending coronary artery perfusion territory. Renal and cardiac sympathetic nerve activities were measured during a 2 minute period of ischemia. A significant increase in both renal (32 ± 5%) and cardiac sympathetic nerve activity (58 ± 15%) occurred. Mean arterial pressure did not change. The absence of a depressor response to the ischemia was attributed to intense sympathoexcitation which resulted in an increase in total peripheral resistance, thereby compensating for the reduction in cardiac contractile function. Interruption of the sympathetic afferent pathways (bilateral stellectomy, section of the thoracic sympathetic chains caudal to T4, section of the T1 to T4 rami communicantes) abolished the sympathoexcitation. Clearly, transmural myocardial ischemia of the anterior free wall of the left ventricle results in an increase in sympathetic efferent activity to the heart.

Heusch et al (1985) reported a progressive increase in postganglionic sympathetic nerve activity during 20 minutes of severe circumflex artery stenosis in anesthetized dogs. This increase in sympathetic efferent nerve activity resulted in an α_2-adrenergic receptor-mediated circumflex artery

constriction which was abolished by spinal anesthesia of segments C7 to T6. Thus, an ischemia-induced spinally integrated cardio-coronary reflex was proposed.

Of importance is the fact that the latter studies were done in anesthetized, vagotomized, baroreceptor denervated and/or spinalectomized preparations. The dogs used in the present study were unanesthetized and were neurally intact i.e. no baroreceptor denervation, no vagotomy and no spinalectomy. The effect of changes in cardiac sympathetic efferent nerve activity on regional myocardial blood flow and contractile function in conscious, neurally intact animals has as yet not been directly investigated.

How can an increase in cardiac sympathetic efferent activity be quantified without measuring actual changes in nerve activity? During acute myocardial ischemia an increase in HR reflects an increase in cardiac sympathetic efferent nerve activity. Peterson et al (1973) reported a 34 b/min increase in HR during a 1 minute occlusion of the circumflex coronary artery. Bilateral baroreceptor denervation reduced but did not abolish the tachycardia. They attributed this reflex tachycardia to increased sympathetic efferent activity originating in receptors in or near the heart as well as to the arterial baroreceptors responding to a small drop in mean arterial pressure. In the present study an increase in HR during the ischemic period was used as an index of increased sympathetic efferent nerve activity.

D. Role of Increased Cardiac Sympathetic Efferent Nerve Activity

The significance of increases in cardiac sympathetic efferent nerve activity as a result of myocardial ischemia has been debated for the reason that most studies which demonstrated an increase in cardiac efferent nerve activity used anesthetized, vagotomized and/or sinoaortic denervated and/or spinalectomized animals. Based on the latter, Felder and Thames (1980) have questioned the "physiological relevance" of such reflex increases in cardiac sympathetic efferent activity in neurally intact animals. The present study will address this question directly. Indeed, Felder and Thames (1981) have shown that ischemia-induced increases in cardiac sympathetic afferent activity do not alter cardiac sympathetic efferent nerve traffic in anesthetized dogs with intact neuraxes. They suggested that interruption of descending inhibitory (bulbospinal) pathways is required to demonstrate a cardiocardiac sympathetic reflex. However, conscious, chronically instrumented and neurally intact dogs have not been used to investigate the effect of acute myocardial ischemia on cardiac sympathetic efferent nerve activity at rest. In particular, the effect of this elevated sympathetic nerve activity on regional blood flow and myocardial contractile function needs to be addressed.

Since the coronary vessels are innervated by sympathetic nerves (Denn and Stone,1976), it stands to reason that any change in sympathetic efferent activity to the heart will affect the coronary vascular resistance and hence CBF. CBF and myocardial contractile function are closely correlated (Vatner,1980).

Thus, it can be postulated that the changes in cardiac sympathetic efferent activity during myocardial ischemia will affect myocardial blood flow and, as a result, myocardial contractile function. This is one of the main focuses of the present investigation.

The effect of an α-adrenergic vasoconstrictor tone on myocardial regional blood flow distal to a stenosis of a coronary vessel has been investigated. Persistent vasodilatory reserve during myocardial hypoperfusion has been documented. Using anesthetized dogs, Aversano and Becker (1985) reported an 88% increase in circumflex artery blood flow when adenosine was administered intracoronary at low coronary perfusion pressures (35 - 50mmHg). Pantely et al (1985) reported a similar finding in anesthetized swine. Under conditions of ischemia during exercise, maximal vasodilation is also not reached (Laxson et al,1992). A possible explanation for the persistence of vasodilatory reserve during myocardial ischemia is the presence of an α-adrenergic receptor-mediated vasomotor tone. Teleologically, an increase in vasoconstrictor tone may alter transmural blood flow distribution during the ischemic episode. It may reduce subepicardial transmural steal thereby reducing the severity of the prevailing subendocardial ischemia. One would then expect that on removal of this constrictor tone, the subendocardial ischemia would worsen causing further deterioration in function. Indeed, this has been demonstrated by removal of the vasodilator reserve by intracoronary infusion of adenosine (McFalls et al,1990). The left anterior descending

coronary artery was stenosed to reduce systolic wall thickening by 15 - 20%. Once a new steady-state had been reached, adenosine was infused into the same artery and the occluder adjusted to maintain flow at pre-infusion levels. Regional blood flow measurements under the latter circumstances showed an increase in subepicardial flow which was reflected in a significant drop in the subendocardial:subepicardial flow ratio (0.91 $\pm$ 0.26 to 0.59 $\pm$ 0.16). This implies a greater vasomotor tone in the subepicardium versus that in the rest of the myocardium. Together with data showing decreased regional function on removal of the subepicardial vasomotor tone, these data imply that subepicardial vascular tone serves a role in preserving appropriate transmural distribution of flow and, thereby, preserving contractile function. α-adrenergic coronary constrictor tone has been shown to maintain uniform transmural myocardial blood flow in the absence of myocardial ischemia (Huang and Feigl,1988).

A detrimental effect of ischemia-induced coronary constrictor tone has also been reported (Seitelberger et al,1988). The constrictor tone may compromise flow and, hence, oxygen delivery thereby reducing function (Buffington and Feigl,1981). In exercising dogs, Seitelberger et al (1988) stenosed the circumflex artery such that posterior left ventricular wall percent systolic wall thickening was reduced by 75%. A substantial reduction in subendocardial blood flow, more so than the decrease in the mid- and subepicardial layers, resulted. Blockade of α_2-adrenergic receptors within the

ischemic myocardium increased subendocardial flow from 0.17 ml/min/g to 0.45 ml/min/g with no effect on subepicardial flow. The subendocardial:subepicardial flow ratio increased from 0.22 ± 0.09 prior to the blockade to 0.60 ± 0.40 after the blockade. The increase in subendocardial flow was associated with significant improvement in ischemic wall thickening. Nonischemic regional flow and function was unaffected. Therefore, it appears from this study that an ischemia-induced coronary constriction may compromise subendocardial blood flow and, hence, regional contractile function, i.e. the constrictor tone has a detrimental effect on regional contractile flow and function during exercise.

On the other hand, Nathan and Feigl (1986) showed a beneficial effect for the ischemia-induced coronary vasoconstrictor tone. In an open-chest canine preparation, they controlled coronary blood flow to two regions of the left ventricle. α-adrenergic blockade was induced in one region by intracoronary infusion of phenoxybenzamine while the α-receptors were left intact in the other region. Under ischemic flow conditions, subendocardial flow was significantly greater in the unblocked region than in the blocked region. This implicates a subepicardial constrictor tone that serves to divert blood flow towards the more ischemic subendocardium. The heart was not instrumented for evaluation of myocardial function so that the change in transmural blood flow distribution could not be related to any changes in regional function. One

of the strengths of the present study is that both regional flow and contractile function were measured.

Chilian and Ackell (1988) used exercising dogs as a model to investigate the effect of increased sympathetic efferent activity on regional blood flow in ischemic myocardium. Part of the ischemic myocardium was denervated using topical application of phenol leaving nerves innervating the proximal part of the ischemic region intact. This allowed for the comparison of regional blood flows between the intact and the denervated ischemic myocardium of the same dog. With the dog running on a treadmill and in the absence of a stenosis, the subendocardial:subepicardial flow ratio was no different between the intact and the denervated regions (1.48 ± 0.08 vs 1.50 ± 0.05 respectively). Therefore, sympathetic efferent activity to myocardium with normal perfusion has no effect on transmural blood flow distribution, a finding unlike that of Huang and Feigl (1988). This was not the case in the presence of a stenosis. The circumflex artery was stenosed such that circumflex blood pressure was reduced to approximately 45 mmHg. Under such conditions, the subendocardial: subepicardial ratio was significantly lower in the intact region (0.47 ± 0.09 vs 0.37 ± 0.07). The latter is a result of higher subendocardial flows and lower subepicardial flows in the intact ischemic region compared to the sympathectomized ischemic region. Intracoronary injection of phentolamine eliminated this difference by blocking the α-adrenergic receptor-mediated coronary constrictor tone present in the subepicardium and midwall

of the innervated ischemic myocardium. Removal of the constrictor tone prevented shunting of blood to the subendocardium such that flow ratios of the intact and sympathectomized regions were equalized (0.55 ± 0.10 vs 0.52 ± 0.15 respectively).

Instead of exercise, Buffington and Feigl (1983) used norepinephrine infusion to induce α-adrenergic receptor-mediated constrictor tone in anesthetized dogs. They found no difference in the transmural distribution of myocardial blood flow at a coronary perfusion pressure of 50 mmHg. At a lower perfusion pressure of 38 mmHg, no constrictor tone was detected due to maximal metabolic coronary vasodilation. They theorized that since there is greater metabolic vasodilation in the subendocardium compared to that in the subepicardium, and since the subendocardial:subepicardial flow ratios did not change in their study, the constrictor tone may be greater in the subendocardium than in the subepicardium. Indeed, a greater subepicardial constrictor tone would facilitate transmural coronary steal and blood would be diverted to the subendocardium.

The difference between the latter and the former studies is that Buffington and Feigl (1983) used anesthesia which could have influenced their results (Vatner and Braunwald,1975; Vatner et al,1971). In addition, by comparing the two studies, one assumes uniform transmural distribution of neural stimulation, as would be the case for pharmacological stimulation - the only difference, therefore, would be in receptor density and post-receptor

mechanisms. It is interesting to note that Haws et al (1987) have shown that cardiac sympathetic nerves to have selective regional effects on myocardial perfusion.

Transmural heterogeneity of α-adrenergic vasoconstriction during myocardial ischemia was recently documented by Westby et al (1992) in anesthetized cats. With coronary perfusion pressure set at approximately 70% of baseline and under cover of nonspecific ß-adrenergic blockade, both α_1- and α_2-adrenergic receptors were blocked sequentially. α_1-adrenergic receptor blockade had no effect on any of the variables measured. However, α_2-adrenergic receptor blockade resulted in a significant increase in subepicardial blood flow and a decrease in subendocardial flow in the hypoperfused myocardium. Thus the subendocardial:subepicardial flow ratio decreased. Total shunt flow was unchanged even though coronary vascular resistance of the hypoperfused region decreased significantly. Comparison of the hypoperfused and normally perfused regions showed a reduction in subendocardial flow in the hypoperfused tissue but not in the normal tissue. Subepicardial flow was the same. These data support the notion of an anti-transmural steal phenomenon in hypoperfused myocardium, i.e., removal of subepicardial constrictor tone resulted in a reduction in subendocardial blood flow .

Is the vasoconstrictor response confined to the ischemic region alone or does it occur throughout the entire coronary vasculature, i.e. does myocardial

ischemia induce a purely regional coronary vasoconstriction or is it a global effect? Indeed, Neely and Hageman (1990) have demonstrated increased efferent sympathetic activity to nonischemic regions of the left ventricle in response to 30 minutes of occlusion of either the left anterior descending coronary artery or the left circumflex coronary artery. Similarly, a decrease in efferent sympathetic activity was seen in the ischemic region. Since sympathetic efferent activity may not be uniformly distributed throughout the myocardium, it may have different effects on ischemic and nonischemic tissue. Recall that Chilian and Ackell (1988) showed no transmural difference in blood flow in normally perfused myocardium but did find greater subepicardial constrictor tone in the ischemic region and Haws et al (1987) showed cardiac nerves to have different regional effects on myocardial perfusion.

E. Coronary Constrictor Tone and Reperfusion

Little has been published concerning a role for α-adrenergic receptor-mediated constrictor tone on reperfusion. Braunwald and Kloner (1982) have shown that regional myocardial dysfunction resulting from the reduction in coronary blood flow during a coronary artery occlusion may persist despite release of the occlusion which was maintained for an insufficient time period to cause myocardial necrosis. An occlusion lasting less than 20 minutes is not usually associated with myocardial necrosis. This post-ischemic dysfunction has been termed myocardial "stunning" - a period of reduced myocardial

function in the presence of adequate blood flow. Myocardial stunning has also been documented following physical exertion in the presence of coronary artery stenosis (Homans et al,1986; Thaulow et al,1989). Homans et al (1986) showed that exercise in the presence of a coronary stenosis resulted in severe regional myocardial dysfunction and subendocardial hypoperfusion with subepicardial blood flow being only moderately affected. In addition, the myocardial dysfunction and the subendocardial hypoperfusion were present for as long as two hours after the cessation of exercise and alleviation of the stenosis. The degree of the dysfunction may be related to the intensity of the exercise. Since sympathetic outflow increases with increasing exercise intensity one may speculate that α-adrenergic receptor-mediated coronary constriction may have a role to play in the etiology of myocardial stunning.

Kitakaze et al (1991) documented a role for α_1-adrenergic receptors in the recovery of the myocardial contractile function from a 15 minute occlusion of the left anterior descending coronary artery. Blockade of α_1-adrenergic receptors by intracoronary infusion of prazosin (4.0 μg/kg/min) prior to the occlusion significantly aggravated the post-ischemic contractile dysfunction, whereas stimulation of the α-receptors improved post-ischemic recovery. The reactive hyperemia upon release of the occlusion in the prazosin treated condition was significantly lower than the control group. Methoxamine treatment resulted in a significantly greater hyperemic response. Together with the reduced release of adenosine in the prazosin-treated group and the

increase in adenosine release in the methoxamine-treated group, they proposed that the beneficial effects of the α_1-adrenergic receptors was linked to adenosine release. They hypothesized that the metabolic effects of adenosine and not adenosine's vasodilatory effect were responsible for the reduction in myocardial stunning.

The flow data in the study by Kitakaze et al (1991) is only presented for the period of reperfusion and not for the period of occlusion. Based on the literature already mentioned above, it would be safe to propose that the methoxamine may have changed the regional distribution of myocardial blood flow by increasing the coronary constrictor tone. This may have aggravated the ischemia and resulted in the production of a significantly greater amount of adenosine. Prazosin treatment resulted in less adenosine production than control, which would support this hypothesis.

One cannot discount the possibility that the results of the latter study are due to changes in regional blood flow during the period of ischemia. We propose to investigate the role of the α_1-adrenergic receptor-mediated coronary constrictor tone in myocardial stunning, specifically focusing on the early stages of reperfusion.

F. Summary

It has been well documented that myocardial ischemia results in reflex activation of cardiac efferent nerves (Malliani et al,1969; Heusch et al,1985;

Gatenberg and Hageman,1991; Minisi and Thames,1991). However, the "physiological relevance" of this ischemia-induced reflex increase in cardiac sympathetic efferent nerve activity has been questioned (Felder and Thames,1980,1981; Malliani et al,1980). Even so, it is well recognized that an α-adrenergic coronary constrictor tone exists under a variety of conditions including myocardial ischemia (Bache et al,1987; Buffington and Feigl,1981; Jones et al,1986; Vallance et al,1990). The effect of this coronary constrictor tone on regional myocardial blood flow has been investigated in anesthetized animals (Westby et al,1992; Nathan and Feigl,1986; Buffington and Feigl, 1881,1983; Huang and Feigl,1988) as well as in exercising dogs (Seitelberger et al,1988; Chilian and Ackel,1988; Laxson et al,1992). Aside from the studies by Seitelberger et al (1988) and Laxson et al (1992), regional contractile function was not measured in conjunction with regional blood flow. Thus, there are 2 major shortcomings of the studies published to date. First, the vast majority of them were done using anesthetized animals, and in many of these the preparation involved vagotomy, sinoaortic baroreceptor denervation and/or spinalectomy. Therefore, a more physiological preparation is warranted to answer the question of whether an ischemia-induced coronary constrictor tone does indeed have a role in the regulation of regional myocardial blood flow. Second, the all important link between changes in regional flow and, therefore, changes in contractile function was not directly investigated in many of the studies.

The current study examines the effect of acute myocardial ischemia on regional myocardial blood flow and contractile function in a conscious, intact, resting dog model. The effects of anesthesia have therefore been eliminated (Jones et al,1987; Vatner and Braunwald,1975; Vatner et al,1971). Also, regional myocardial blood flow as well as global and regional contractile function were measured with the purpose of determining if the ischemia-induced coronary constrictor tone altered blood flow in such a way as to affect contractile function.

CHAPTER 3

METHODS

A. Rationale of Study

Myocardial ischemia has been shown to alter the autonomic efferent activity to the heart (Malliani et al,1969; Heusch et al,1985; Minisi and Thames,1991). Whether the sympathetic efferent nerve activity increases as a result of a cardiocardiac reflex (Malliani et al,1969; Heusch et al,1985) or as a result of baroreflex and chemoreflex activation is debatable (Felder and Thames,1980; Malliani,1980; Minisi and Thames,1991). Most studies concerning this topic have been conducted in an anesthetized preparation in which a vagotomy and/or a spinalectomy and/or sinoaortic baroreceptor denervation have been performed. Thus, "the responses in intact animals are not yet known" (Hainsworth,1991). In addition, these studies did not address the effects of the increased sympathetic efferent activity on regional myocardial blood flow and function.

In this study, a chronically instrumented dog model was used to examine the effects of changes in sympathetic outflow to the heart during 2.5 minutes of transmural ischemia of the anterior wall of the left ventricle. Effects of the altered cardiac sympathetic efferent nerve activity on regional myocardial blood flow and global and regional myocardial function were investigated. It

was assumed that an increase in cardiac sympathetic efferent nerve activity would cause an increase in heart rate (HR) during the combined left anterior descending coronary artery occlusion (LADO) and circumflex stenosis (CxPO). Nonspecific α-adrenergic receptor blockade by intracoronary (i.c.) infusion of phentolamine was used to remove the apparent ischemia-induced increase in α-adrenergic receptor-mediated coronary constrictor tone and compared to infusion of 0.9% saline, the control condition.

B. Animal Selection

A total of 8 heartworm free, mongrel dogs (22 - 31 kg, mean: 26.8 ± 1.0 kg) of either sex and in good health were used. All dogs were studied in an unanesthetized, conscious state while supine on a table or suspended in a sling. Due to failure of the instrumentation in 1 dog, 7 of the 8 dogs completed both the time control studies and the double blockade studies. Regional blood flows were successfully measured in 5 of the 8 dogs. Due to partial clotting of the circumflex catheter during the experiments, coronary blood pressures and coronary resistances were not accurately measured in 2 dogs during the double blockade with saline infusion, and in 3 dogs during double blockade with phentolamine infusion. Therefore, these data were eliminated from the analyses. Similarly, coronary resistances were calculated in 4 of 8 dogs for the time control with no infusion, and in 3 of 4 dogs during phentolamine infusion without double blockade.

C. Surgical Preparation

Each dog was sedated with Acepromazine (5 - 7 mg, s.c.) prior to induction of anesthesia using Thiamylal Sodium (0.02 mg/kg, i.v.). The trachea was intubated and a surgical level of anesthesia was maintained using gaseous anesthesia (isofluorane:nitrous oxide:oxygen) with the tidal volume set at approximately 15 ml/kg and the respiratory rate at 15 - 20 breaths per minute. A left thoracotomy was performed at the level of the fifth intercostal space, the heart was suspended in a pericardial cradle, and instrumented for measurement of key variables.

For measurement of left ventricular pressure (LVP), a Konigsberg P6.5 solid state pressure transducer (Konigsberg Instruments, Pasedina, CA) and a fluid-filled Tygon catheter (1.25 mm I.D. and 1.27 mm O.D.) were inserted into the left ventricle through a stab wound in the apex. The Konigsberg pressure transducer was calibrated prior to implantation, and routinely checked by simultaneously measuring pressure through the implanted Tygon catheter connected to an external Isotec® pressure transducer. The Isotec® pressure transducer was calibrated using a mercury manometer. Arterial pressure (AP) was measured via a catheter (1.25 mm I.D. and 1.27 mm O.D.) implanted in the proximal descending thoracic aorta. Reference blood samples for the microsphere regional perfusion technique were withdrawn via the aortic catheter.

For withdrawal of venous blood samples and intravenous drug injection, a Silastic catheter (0.12 mm I.D. and 0.6 mm O.D.) was placed in the coronary sinus.

Regional contractile function was measured using 3 pairs of 5 MHz ultrasonic piezoelectric crystals implanted in the subendocardium approximately 1.0 - 1.5 cm apart. Two pairs were implanted in the circumflex perfusion territory and 1 pair in the LAD perfusion territory. During the experiments, the crystals were activated by a Triton ultrasonic dimension system (Model 120, Triton Technology, San Diego, CA).

The circumflex coronary artery was dissected free for a distance of approximately 2 cm, taking care not to damage the vessel adventitia and surrounding nerves (Denn and Stone,1976). The LAD coronary artery was dissected free for a distance of 0.5 - 1 cm, barely enough to place an occluder around the vessel proximal to the first LAD branch without creating a stenosis. Knight et al (1987) and Inou et al (1985) showed that dissection of the circumflex coronary artery, as was done in this study, does not alter the vessel's responses to various stimuli. It should be noted, however, that significant dissection of the LAD coronary artery may alter the myocardial responses to sympathetic stimulation (Heusch et al,1987). Therefore the dissection of the LAD artery was kept to the bare minimum required for placement of the occluder.

For measurement of circumflex flow velocity a 10 MHz Doppler ultrasonic flow transducer (4 mm I.D.) was placed around the proximal portion of the vessel. The pulsed Doppler flow system used (Model 100, Triton Technology, San Diego, CA) has been shown to have a reliable zero reference (Franklin et al,1966).

To occlude the LAD, a handmade pneumatic occluder (Tygon tubing - heat stretched under pressure) was placed around the LAD proximal to the first branch. The LADO was induced by injecting a previously measured volume of air into the occluder. For precise control of circumflex blood flow (CBF) during the LADO and to establish a zero-flow baseline for the Doppler flow probe, a Model OC4A vascular occluder (In Vivo Metric, Healdsburg,CA) was placed around the circumflex artery immediately distal to the flow probe. A commercially available occluder (In Vivo Metric, Healdsburg, CA) was implanted around the circumflex vessel because they are made with a hard backing, thereby limiting the extent of spontaneous occlusion when intraluminal pressure decreases (See Appendix B for a comparison of pneumatic and hydraulic occluders and a discussion of their limitations).

For i.c. drug infusion and coronary blood pressure (CBP) measurement an heparin-filled Silastic catheter (0.12 mm I.D. and 0.6 mm O.D.) was placed in the circumflex artery. Gwirtz (1986) has described the construction and insertion procedures and showed the implanted circumflex catheter to have no effect on myocardial perfusion and coronary collateral development.

A Silastic catheter (1.55 mm I.D. and 3.13 mm O.D.) was placed in the left atrial appendage for the injection of microspheres.

On completion of the instrumentation the pericardial cradle was released, the chest closed, and the pneumothorax evacuated by suction on a chest tube. All wires and tubing from the instrumentation were tunnelled beneath the skin to exit between the scapulae. The catheters were maintained patent by daily flushing with heparinized saline. Antibiotics and analgesics were given as deemed necessary by the Animal Care Facility veterinarian. At least 10 days recovery were allowed before beginning experimentation.

After completion of all experiments, each dog was anesthetized with sodium pentobarbital (30 mg/kg, i.v.). With the pneumatic occluders inflated to prevent retrograde flow of dyes, Evans blue dye was injected into the circumflex artery to delineate the circumflex perfusion territory and India ink was injected into the LAD artery to delineate the LAD perfusion territory. Upon autopsy, the ventricle was examined to verify that the ultrasonic segment length crystals were correctly positioned in the respective circumflex and LAD perfusion territories. The circumference of the circumflex artery was measured and used to calculate its cross-sectional area for conversion of flow velocity to volume flow rate. Blood flow velocity and volume flow rate are linearly related (Vatner et al,1970). This laboratory has also shown a similar linear relationship between flow velocity and volume flow rate (Gwirtz et al,1986).

D. Protocol

On different days, each dog was subjected to the same experimental protocol under the following conditions :

1. no systemic autonomic blockade - time control.
2. double blockade with both propranolol (1 mg/kg i.v.) and atropine (100 μg/kg i.v.).
3. double blockade (as in 2) with microsphere injections for regional blood flow measurements.

In each double blockade protocol, either saline or phentolamine was infused into the circumflex coronary artery. Saline infusion served as the control condition and phentolamine infusion as the experimental condition. The 2 conditions compared in the time control experiments were no infusion versus phentolamine infusion. The effectiveness of ß-adrenergic receptor blockade by propranolol (1 mg/kg i.v.) was tested by intracoronary injections of norepinephrine (0.3 μg) and isoproterenol (0.3 μg). Muscarinic blockade was not tested by injection of acetylcholine. The same dose was used and was shown to effectively antagonize muscarinic receptors by Gayheart et al (1991). In addition, Peterson et al (1973) used the same dose effectively in their study, apparently without testing the blockade. The fact that large increases in HR were observed in every dog after intravenous administration of atropine implies effective blockade of cardiac muscarinic receptors.

The protocols for each of the experimental conditions were as follows:

1. Time Control: The dogs were studied under resting conditions while reclining in a sling or on a cushioned table. The laboratory was darkened for the duration of the experiment to facilitate maintenance of a resting state. Once the dog had attained a sufficiently calm state (HR < 100 b/min) baseline data were collected.

To produce transmural ischemia of the anterior wall of the left ventricle, necessary to stimulate the sympathetic afferent nerves which originate in the subepicardium (Coleridge et al,1978; Barber et al,1984), the LAD was totally occluded and the circumflex artery was stenosed (LADO + CxPO) simultaneously. Total occlusion of the LAD was assured by inflating the pneumatic occluder with a volume of air slightly larger than the predetermined volume of the occluder tubing, using a handheld 5 ml syringe. A severe reduction in anterior percent segment length shortening was observed on the strip chart during the occlusion (see Figures 1 and 2). The occluder tubing was then clamped using a pair of Babcock forceps. The CxPO was induced by gradually inflating the pneumatic occluder placed around the circumflex artery using a handheld 2 ml micrometer syringe (Gilmont Instruments, Inc., Great Neck, N.Y.) such that percent posterior segment length shortening (%SL) remained at control levels. Table 5 in Chapter 4 shows that severe transmural ischemia did indeed result in the anterior wall of the left ventricle. The LADO + CxPO was maintained for 2.5 minutes, the longest time a conscious dog can endure transmural myocardial ischemia without showing any signs of

discomfort (personal observation). Data were recorded after 1 minute, 1.5 minutes, 2.0 minutes and 2.5 minutes of the LADO + CxPO. The LADO + CxPO were then simultaneously released. Data were collected at 1 minute intervals for 7 minutes post release.

2. Double blockade: Dogs were studied under resting conditions as for the time control experiments. Baseline data were collected once the dog was sufficiently calm (HR < 100 b/min).

In order to block the effects of phentolamine-induced release of norepinephrine from presynaptic sympathetic nerve terminals, and to negate the competitive effects of metabolic vasodilation with any ischemia-induced α-adrenergic receptor-mediated coronary constrictor tone (Mohrman and Feigl,1978), propranolol (1 mg/kg in 10 ml of sterile saline) was then injected i.v. via the coronary sinus catheter. Five minutes later data were collected.

To eliminate parasympathetic influences on the heart and the coronary vasculature, atropine (100 µg/kg, i.v.) was then injected. After a 5 minute stabilization period data were once again collected. The double blockade eliminated muscarinic and ß-adrenergic receptor influences on the coronary vasculature, thereby isolating sympathetic vasoconstrictor tone due to α-adrenergic receptor stimulation.

After a 0.5 ml bolus injection (i.c.) of 1 mg phentolamine or 0.9% sterile saline, phentolamine (0.5 - 1 µg/kg/min) or 0.9% sterile saline (vehicle) was infused at a rate of 0.25 ml/min, respectively. The infusion was maintained

throughout the rest of the protocol. After the first 5 minutes of the infusion, data were collected. The phentolamine infusion did not appear to block systemic α-adrenergic receptors since the dose was tested prior to the experiments in each dog. The same pressor response to an 100 μg intravenous bolus of phenylephrine, both before and after 20 minutes of i.c. phentolamine infusion, was obtained. At no stage was the phentolamine infusion maintained for longer than 20 minutes. Further discussion of potential systemic spillover from the coronary circulation is presented in Chapter 5.

A 2.5 minute LADO and CxPO was established and data were recorded after 1 minute, 1.5 minutes, 2.0 minutes and 2.5 minutes of the LADO + CxPO. On release of the LADO + CxPO, data were recorded for 7 minutes at 1 minute intervals.

3. Double blockade protocol for regional blood flow measurement: An additional double blockade protocol was performed for the measurement of regional myocardial blood flow. The aortic catheter was used for reference blood sample withdrawal during the microsphere injections, hence AP was not measured. Separate experiments for regional blood flow measurements simplifies the protocol, thereby reducing the chance of error. Four species of radioactive tracer microspheres (Sc^{46}, Sr^{85}, Nb^{95}, Sn^{113}) were used (Heymann et al,1977). Two regional blood flow measurements were made during each protocol, viz. at rest 5 minutes after the start of the infusion (baseline) and after 1 minute of the LADO + CxPO, each during the i.c. infusion of saline (2

species) and phentolamine (2 species). To minimize the duration of the microspheres being retained within the conscious dog, one experiment was done during the afternoon of one day (2 species injected) and the next experiment was done the following morning (2 species injected). Following the second protocol the dog was euthanized. Since there were approximately 15 hours separating the 2 experiments, loss of microspheres from the tissue could be a potential problem. Levken and Andersen (1980) reported an 11 to 60% loss of 15 μm microspheres during 10 hours of coronary artery occlusion. However, under prolonged periods of ischemia as used in their study, the vascular integrity is compromised (West et al,1978). Thus, microspheres will leak from the vessels. It should also be noted that, in contrast to the findings reported by West et al (1978),White et al (1978) reported no loss of microspheres during a 3 day coronary artery occlusion. Microvascular damage was not anticipated in this study since the occlusion was relatively short (2.5 minutes).

The same protocol as for the double blockade study (described in point 2 above) was followed. A different species of tracer microspheres was injected via the left atrial catheter 5 minutes after the start of the respective infusions and after 1 minute of the LADO + CxPO while the reference blood samples were withdrawn via the aortic catheter at a rate of 5 ml/min using a peristaltic pump. Each dose of microspheres was suspended in 10 ml of 0.9% sterile saline and vortexed prior to injection. The syringe and tubing were flushed

twice with 10 ml of 0.9% saline after the each injection of microspheres. On completion of the second experiment for regional blood flow measurement, the dog was euthanized as previously described.

Assuming coronary blood flow to be 5% of cardiac output and the mean mass of the heart of a 20 to 30 kg dog to be approximately 250 g (personal observation), 1 - 5 million microspheres were injected. For the injection during the ischemic episode, 5 million microspheres labelled with the isotope of highest specific activity available were injected.

The methodology used for the microsphere studies was as follows: Reference blood sample withdrawal from the aortic catheter, was begun 10 seconds prior to injection of the microspheres and continued for 120 seconds at a rate of 5 ml/min using a Minipuls 2 peristaltic pump (Gilson Medical Electronics, Wis.). After sonicating the stock solution for a minimum of 30 minutes, 1 - 5 million microspheres were withdrawn and suspended in 10 ml of 0.9% sterile saline. After extensive vortexing, the spheres were injected into the left atrium via the left atrial catheter followed by two 10 ml 0.9% sterile saline flushes to ensure no microsphere residue in the catheter tubing and syringes. This same procedure was followed for each microsphere injection.

On completion of the second experimental protocol involving the injection of microspheres the dog was euthanized. Prior to euthanasia the LAD and circumflex perfusion territories were dyed using India ink and Evans Blue dye as previously described. This dyeing procedure served to demarcate the

LAD perfusion territory of the left ventricle and the area which was affected by phentolamine (circumflex perfusion territory), respectively. Appropriate placement of the piezoelectric crystals was verified according to the respective regions dyed.

The heart was excised and prepared for sectioning. The atria were removed from the ventricles. A 1 - 2 g tissue sample was cut from each atrium, each placed in a numbered glass test tube and capped ready for counting. Similarly, a sample was taken from the free wall of the right ventricle. The left ventricular free wall and interventricular septum were divided horizontally into four rings of approximately equal size, with the rings numbered consecutively 1 through 4, from base to apex.

Each ring was cut in half with the origin of the circumflex artery as the marker for the first cut. The resulting halves were again bisected, and the process was repeated until each of the 4 rings were divided into eight sectors of approximately equal mass. Each of these eight pieces were then divided into 3 layers (subendocardium, midwall and subepicardium) of approximately equal thickness, placed in appropriately labelled glass test tubes and counted.

In addition to the tissue samples taken from the heart, samples from the right and left lungs were taken to test for shunting of blood and for determining the extent of nonentrapment of the microspheres in the organ capillary networks (Heymann et al,1977). Right and left kidney tissue samples were also taken, the comparison of which was used as an index of adequate

microsphere mixing (the flows measured in each kidney should be approximately equal).

Radioactivity of myocardial tissue samples and blood reference samples was counted using a gamma spectrometer (Canberra Instrument, Downers Grove, Illinois). The four different nuclide labels were separated and blood flow computations were performed using an IBM microcomputer and the following blood flow equation:

$$MBF = (Fref)(Rtis/Rref)$$

where MBF = myocardial blood flow (ml/min)

Fref = flow rate (ml/min) of the reference blood samples

Rtis = radioactivity (cpm) of the tissue sample

Rref = radioactivity (cpm) of the reference blood samples

(Heymann et al,1977). MBF was then expressed per gram wet weight.

E. Data Collection and Analysis

Data was simultaneously recorded using a Coulbourn 8-channel chart recorder and an 8-channel Hewlett-Packard Model 3968A magnetic tape recorder. On-line variables recorded included LVP, segment length shortening (SL) in the circumflex and LAD perfusion territories, AP, CBP and circumflex flow velocity (CxFV). HR was derived from the LVP signal. The LVP and the

SL signals were differentiated to obtain the rate of left ventricular pressure development (+dP/dt) and the rate of segment length shortening (-dL/dt) in each region. This was accomplished by using an active differentiating circuit which converts slope to vertical displacement from the horizontal. The data on tape was analyzed using a PC computer and a software program previously developed in this laboratory. The program samples recorded data at 2 msec intervals over 10 consecutive beats (Mass et al,1987). Values for peak left ventricular systolic (LVSP) and end-diastolic pressures (LVEDP), the maximal rate of left ventricular pressure generation ($+dP/dt_{max}$), HR, mean AP (MAP), mean CBP (MCBP), mean CxFV (MCxFV) and the maximal rate of systolic SL shortening ($-dL/dt_{max}$) over the 10 beat period are provided by the program. SL was calculated from the difference between the length at the beginning of ejection (EJL) and the end-systolic length (ESL) according to the method of Akaishi et al (1986). EJL was the segment length corresponding to the time point when LVP surpassed aortic pressure, i.e. the beginning of the rapid rise in the phasic aortic pressure tracing. ESL was the segment length corresponding to the time point at which the aortic valve shut, i.e. the dicrotic notch. %SL was calculated as follows:

$$\text{ejection \%SL} = \frac{\text{EJL - ESL}}{\text{EJL}} \times 100$$

Circumflex flow velocity was converted to circumflex blood flow (CBF, volume flow rate) using the cross-sectional area of the vessel within the flow probe as determined by postmortem measurement. The equation is:

$$CBF = [(\pi)(D^2)(CxFV)(60\ sec/min)] / 4$$

where CBF = circumflex blood flow in ml/min,

D = vessel diameter in cm,

CxFV = circumflex flow velocity in cm/sec,

$(\pi)(D^2)/4 = (\pi)(r^2)$ = cross sectional area of vessel (Hartley et al,1984).

In addition, the peak reactive hyperemia upon release of the LADO + CxPO was calculated.

Regional blood flow data were analyzed with the purpose of detecting significant effects of sympathetic efferent activity on myocardial blood flow distribution. In particular, the effect on transmural distribution (endo:epi flow ratio) and on the heterogeneity of flow (anterior:posterior transmural blood flow ratio) were analyzed. The 2 adjoining sectors with the lowest flows represented the severely ischemic region of the anterior region of the left ventricle. To be included, flows in these sectors had to be less than 0.2 ml/min/g. The flows of 2 adjoining sectors within the posterior wall (circumflex perfusion territory - dyed by Evans Blue) were compared to the flows of the

anterior region. The sectors within the posterior wall tended to be located diametrically opposite the severely ischemic sectors of the anterior wall.

For statistical analyses, each dog served as its own control. Control data included data collected during the i.c. infusion of the vehicle, 0.9% sterile saline for the double blockade protocols. For the time control protocol, the condition with no i.c. infusion served as the control. Experimental data included data collected during the i.c. infusion of phentolamine for both the double blockade and the time control protocols. Six conditions were analyzed. The time control with no infusion of either saline or phentolamine served to demonstrate that the experimental protocol induced typical responses to acute myocardial ischemia viz. increases in HR and LVEDP with decreases in $+dP/dt_{max}$ and, %SL and $-dL/dt_{max}$ in the ischemic region (Kaspar et al,1975). Data collected during this protocol was compared to data collected during a similar protocol in which phentolamine was infused into the circumflex artery. This comparison served to detect any potential effects that the drug might have in the unblocked animal. If the time control condition with no i.c. infusion was not different compared to when phentolamine was infused i.c., then the osmolarity of the drug solution infused was probably of negligible importance to the outcome of the study.

Data collected during the double blockade protocols served to isolate the α-adrenergic receptor-mediated coronary constrictor tone which may otherwise be masked by autonomic neural influences on the myocardium, e.g.

positive inotropism causing metabolic vasodilation. Thus, of primary importance is the statistical comparison of the control (i.c. saline infusion) and experimental (i.c. phentolamine infusion) data. The same analyses were performed on data collected during the double blockade protocol during which regional myocardial blood flows were measured.

Prior to performing any significance testing, all data were tested for normality and homoscedasticity. Since criteria for parametric analyses were not met, data was analyzed using nonparametric statistics viz. parametric analyses on ranked data (Zar,1984).

Each variable was subjected to a 2-way ANOVA with repeated measures, the main effects being time (the time course of the protocol) and drug (saline or phentolamine). It is important to understand that data was recorded from each dog at various time intervals within each experiment. Also, data was collected from each dog in the presence of the drug (phentolamine) and in its absence. When significance was reached using the ANOVA, the Duncan's multiple range test was used to isolate the significantly different time points. Since there were only 2 drugs (saline vs phentolamine in the double blockade protocols or no drug vs phentolamine in the time control protocols), no post hoc test was required. When a significant drug effect was shown to exist by the ANOVA, the same analysis was repeated on the data which was collected during the infusions only, i.e. data collected prior to beginning the infusions were eliminated from the analysis. If a drug effect still existed, it was

concluded that it was a consequence of the phentolamine and not due to differences that existed between the 2 conditions prior to beginning the infusions. If statistical significance was still present between the control condition (vehicle infusion) and the experimental condition (phentolamine infusion), it was further investigated by performing a Student's t test comparing each data point of the experimental condition with its respective data point of the control condition. The Student's t test was used in the analysis of the drug effect on LVEDP in the double blockade protocol.

CHAPTER 4

RESULTS

A. Time Control

The purpose of the time control experiments was to demonstrate the response of the intact (i.e. no antagonists) animal to acute myocardial ischemia and thus, validate that the protocol used resulted in appropriate changes in the autonomic nervous system viz. an increase in cardiac sympathetic efferent nerve activity. In addition, the effect of infusing phentolamine in the absence of any systemic blockade was investigated. Data was compared within each condition (time effect) and between conditions (drug effect) with each dog serving as its own control. Table 1 presents the resting hemodynamic values in the animals studied.

Figures 1 and 2 show tracings recorded from the same dog during a time control experiment with no intracoronary (i.c.) infusion (Figure 1) and with an i.c. phentolamine infusion (Figure 2). The beginning and the end of the 2.5 minute period of ischemia are marked by the 2 arrows. In Figure 1, panel A shows the start of the ischemic period. Clearly visible are:

1. the reduction in anterior segment length shortening.

2. the transient compensatory increase in shortening in the posterior region prior to the circumflex stenosis being appropriately adjusted.
3. a reduction in coronary blood pressure (CBP) as the circumflex stenosis was formed.
4. no transient fall in arterial pressure (AP).

Panel B and C, after 1 and 2 minutes of the ischemia, show:

1. severe contractile dysfunction in the anterior region.
2. maintenance of posterior regional contractile function at pre-ischemic levels.
3. a gradual increase in coronary flow velocity (CFV) and CBP, a consequence of manually regulating the stenosis.
4. still no reduction in AP.

Panel D and E show the return to pre-ischemic levels of each variable after release of the LADO and CxPO. It should be noted that a small pressor response during the ischemic period is visible.

During the same protocol but with i.c. phentolamine infusion (Figure 2), a similar response is evident. The apparent reduction in coronary pulse pressure is a result of infusing through the same catheter which was used to record CBP. The infusion was stopped at the predetermined time points when data was recorded. It should be noted that no reduction in AP resulted as a consequence of the i.c. phentolamine infusion. Also, although the posterior

segment length contractile function during the ischemic period appears to be substantially less than the pre-ischemic levels, there is only a 0.9% difference in the posterior percent ejection shortening (P%SL).

Table 1. Control hemodynamics and contractile function for the time control experiment with no infusion, phentolamine infusion without double blockade, saline infusion with double blockade and phentolamine infusion with double blockade.

INFUSION	C	P	S_{DB}	P_{DB}
HR	84±5	78±10	80±6	72±4
$+dP/dt_{max}$	2773±79	2434±188	2471±157	2771±244
LVEDP	4±2	6±1	3±2	5±1
RPP	10137±883	8992±1674	8849±765	7880±473
MAP	93±4	86±8	85±3	80±3
A%SL	13.4±2.6	13.8±4.3	12.6±2.6	14.1±2.9
P%SL	9.8±1.1	10.1±0.5	10.3±0.7	11.6±0.8
MCBF	59.3±10.7	30.1±8.2	38.8±5.9	36.8±5.9
MCR	2.0±0.4	3.9±1.3	2.7±0.5	2.9±0.3

C - time control, no infusion (n = 8); P - phentolamine infusion, no double blockade (n = 4); S_{DB} - saline infusion, double blockade (n = 7); P_{DB} - phentolamine infusion, double blockade (n = 7); HR - heart rate, b/min; $+dP/dt_{max}$ - maximal rate of left ventricular pressure development, mmHg/sec; LVEDP - left ventricular end-diastolic pressure, mmHg; RPP - rate pressure product, b.mmHg/min; MAP - mean arterial pressure, mmHg; A%SL and P%SL - anterior and posterior percent ejection shortening; MCBF - mean circumflex blood flow, ml/min; MCR - mean coronary resistance, mmHg/ml/min. Values are mean ± SE. One dog was eliminated from the MCBF and MCR analysis. There were no significant differences in any measured variable between C, P, S_{DB} and P_{DB} at rest.

Figure 1. Tracing of the response of a dog to 2.5 minutes of acute myocardial ischemia of the anterior wall of the left ventricle. No intracoronary infusion was administered and no systemic autonomic blockade was present. The beginning of the occlusion is marked by the first arrow, and release of the occlusion by the second arrow. Variables shown, from top to bottom, are left ventricular pressure (LVP in mmHg), anterior segment length shortening (Anterior SL in mm), Posterior segment length shortening (Posterior SL in mm), circumflex flow velocity (CFV in KHz), circumflex blood pressure (CBP in mmHg) and arterial pressure (AP in mmHg). A - control,heart rate (HR) = 104 b/min; B - 1 minute of ischemia,HR = 135 b/min; C - 2.5 minutes of ischemia,HR = 140 b/min; D - reperfusion,HR = 116 b/min; E - 7 minutes of reperfusion,HR = 108.

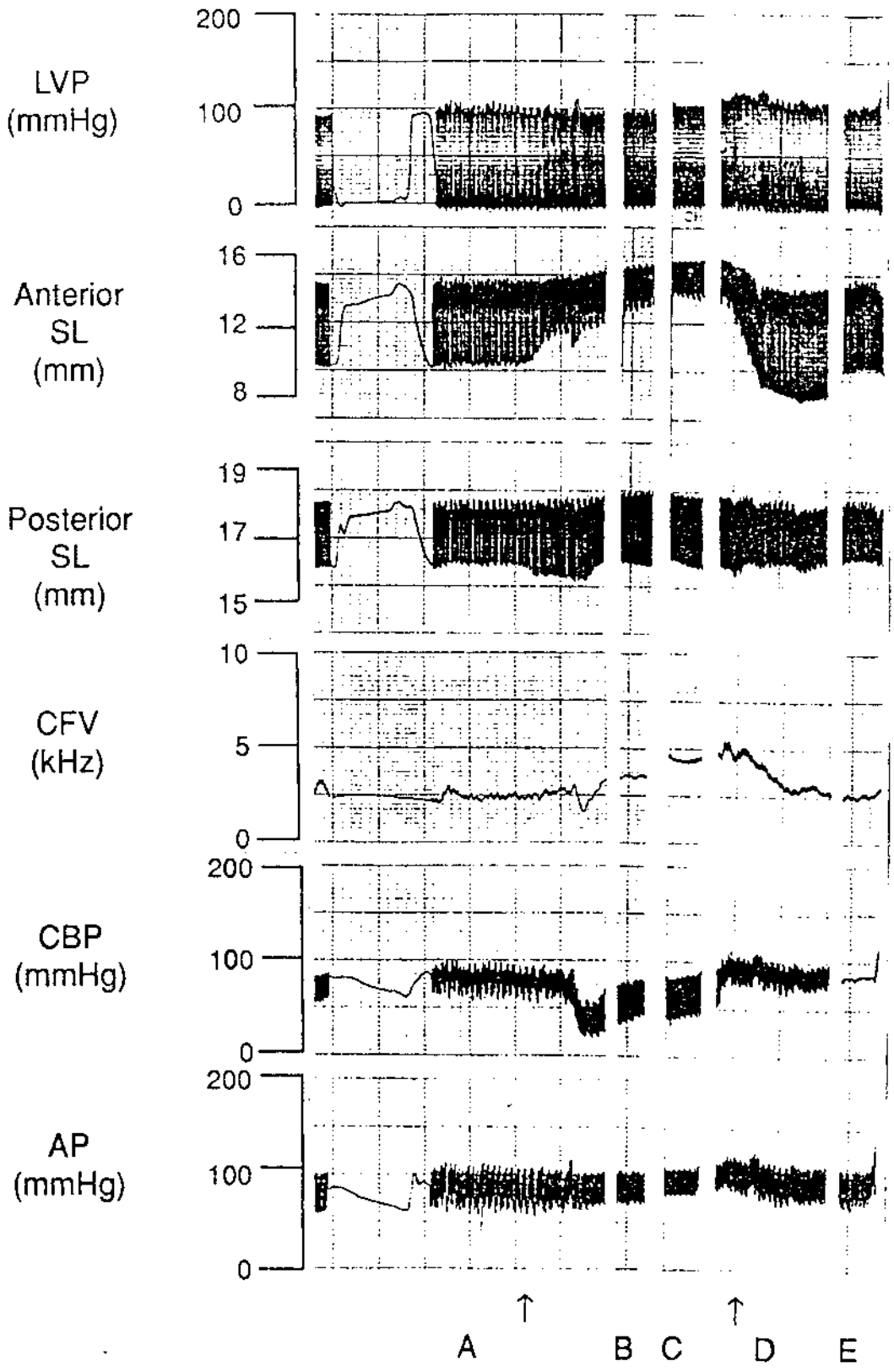
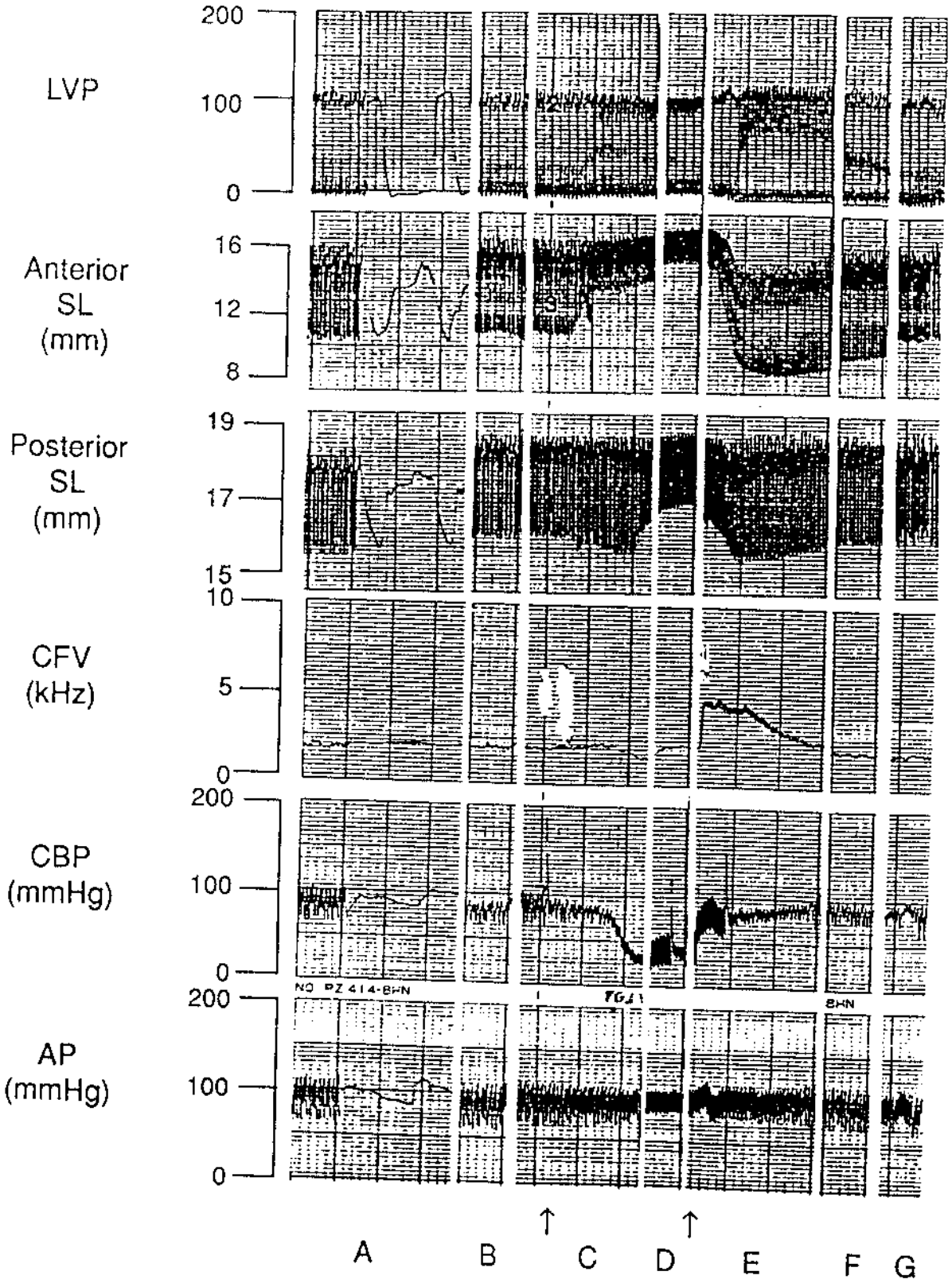

LVP
(mmHg)
200
100
0
Anterior
SL
(mm)
16
12
8
Posterior
SL
(mm)
19
17
15
CFV
(kHz)
10
5
0
CBP
(mmHg)
200
100
0
AP
(mmHg)
200
100
0
A
B
C
D
E

Figure 2. Tracing of the response of the same dog as in Figure 1 to 2.5 minutes of acute myocardial ischemia of the anterior wall of the left ventricle in which phentolamine (0.5 μg/kg/min) was infused into the circumflex artery for the duration of the experiment. No systemic autonomic blockade was present. The beginning of the occlusion is marked by the first arrow, and release of the occlusion by the second arrow. Variables shown, from top to bottom, are left ventricular pressure (LVP in mmHg), anterior segment length shortening (Anterior SL in mm), posterior segment length shortening (Posterior SL in mm), circumflex flow velocity (CFV in KHz), circumflex blood pressure (CBP in mmHg) and arterial pressure (AP in mmHg). A - control prior to starting the infusion,heart rate (HR) = 60 b/min; B - after 5 minutes of phentolamine infusion,HR = 68 b/min; C - start of the occlusion; D - 1 minute of ischemia,HR = 109 b/min; E - beginning of reperfusion,HR = 113 b/min; F - 1 minute of reperfusion,HR = 75 b/min; G - 7 minutes of reperfusion,HR = 68 b/min.


LVP
200
100
0
Anterior SL (mm)
16
12
8
Posterior SL (mm)
19
17
15
CFV (kHz)
10
5
0
CBP (mmHg)
200
100
0
AP (mmHg)
200
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A
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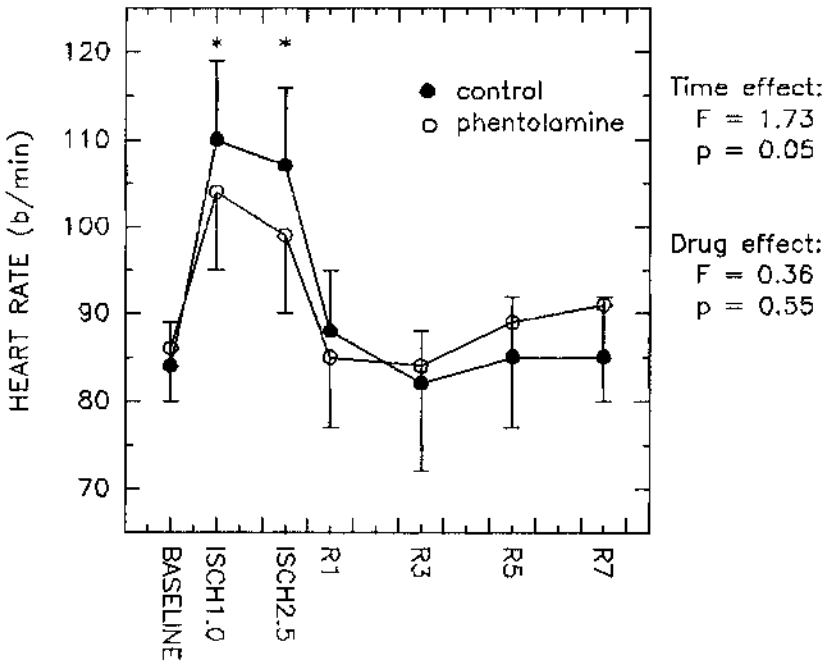


Figure 3. Heart rate response to acute myocardial ischemia during the time control protocol (no systemic blockade) with and without phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 8 for control, n = 4 for phentolamine. * - $p < 0.05$, both groups vs other time points; Duncan's post hoc.

Figures 3 through 6 summarize the responses of global left ventricular contractile function to a 2.5 minute left anterior descending artery occlusion (LADO) combined with a mild stenosis of the circumflex artery (CxPO). A typical response to acute anterior left ventricular wall ischemia is evident.

Heart rate (HR) increased significantly during the period of ischemia in both the control and the infusion conditions (Figure 3). During the control condition (no i.c. infusion), the mean baseline HR was 84 ± 5 b/min. After 1 minute of ischemia HR was 110 ± 9 b/min, and after 2.5 minutes of ischemia HR was 107 ± 9 b/min. During the experimental condition (phentolamine infusion) the respective HRs were 86 ± 6 b/min, 104 ± 9 b/min and 99 ± 9 b/min. These changes in HR were statistically significant (ANOVA:time effect; $F=1.73$, $p = 0.05$). The HR after 5 minutes of phentolamine infusion (labeled baseline in Figure 3) appears to be higher than the control HR recorded prior to beginning the infusion. This difference is not statistically significant. As a result, there may be a tendency to conclude that there was systemic spillover of phentolamine from the coronary vasculature which reduced mean arterial pressure (MAP) and therefore activated the arterial baroreflexes. This does not appear to be the case since MAP did not decrease. The apparent increase in the activity of the sympathetic nervous system is a consequence of one of the dogs becoming more aroused during this data collection time point compared to when the control, pre-infusion data was collected. Indeed, the HR in this dog increased from a resting, pre-infusion value of 60 b/min to 87 b/min after 5 minutes of the infusion. The MAP of the same dog also increased, from 86 mmHg to 99 mmHg. There was a general indication of increased arousal in this dog since all other variables showed a similar trend. In addition, the dog in question was a relatively easily excited animal. The same reasoning can be

applied to the similar discrepency in the rate pressure product (RPP) seen in Figure 6.

Of critical importance to the interpretation of the entire study with respect to the etiology of changes in autonomic activity during the period of ischemia is the absence of a change in MAP. A decrease in MAP would

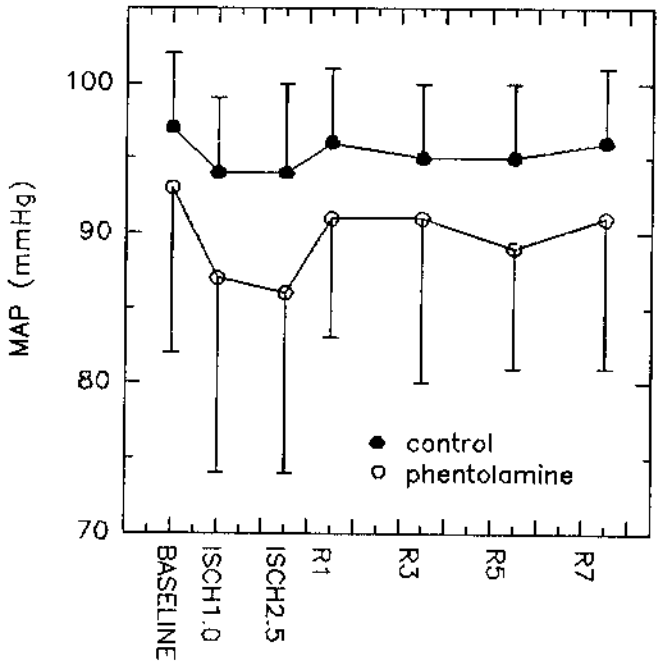


Figure 4. Mean arterial pressure (MAP) response to acute myocardial ischemia during the time control protocol (no systemic blockade) with and without phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 8 for control, n = 4 for phentolamine.

activate the arterial baroreflexes and result in an increase in sympathetic nerve activity while an increase in MAP would suppress baroreflex function, and in so doing, reduce sympathetic nerve activity while increasing parasympathetic nerve activity to the heart. No change in MAP would negate the possibility of the arterial baroreflexes in changing the activity of the autonomic nervous system during the period of ischemia and reperfusion. Indeed, as shown in Figure 4, MAP did not change (ANOVA: time effect; $F = 0.08$, $p = 1.000$). There was no difference in the response of MAP to the 2.5 minutes of ischemia between the control and the experimental conditions (ANOVA: drug effect; $F = 2.11$, $p = 0.149$). MAP ranged from 81 ± 4 mmHg to 98 ± 5 mmHg during the control condition and from 86 ± 12 mmHg to 93 ± 11 mmHg during the experimental condition. Pulse pressure (PP) did not change over time in either group (ANOVA: time effect; $F = 1.31$, $p = 0.21$). The infusion of phentolamine had no effect on the response of PP when compared to the control condition (ANOVA: drug effect; $F = 1.07$, $p = 0.30$). No change in PP would also negate the possibility of the arterial baroreflexes in changing the activity of the autonomic nervous system during the period of ischemia and reperfusion.

As can be seen in Figure 5A, the maximal rate of left ventricular pressure development ($+dP/dt_{max}$) showed a moderate but statistically nonsignificant decrease during the 2.5 minutes of ischemia (ANOVA: time effect; $F = 1.28$, $p = 0.24$). A similar response was observed for the maximal rate of left ventricular pressure decrease ($-dP/dt_{max}$)(Figure 5B).

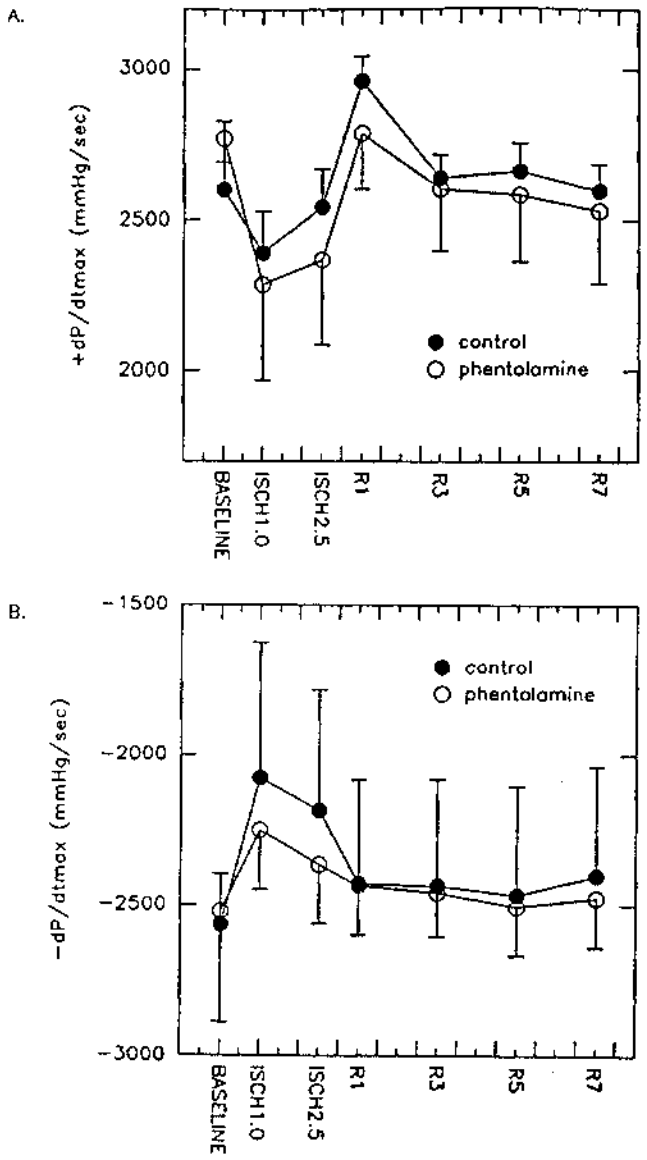


Figure 5. Response of maximal rate of change of left ventricular systolic ($+dP/dt_{max}$) and diastolic ($-dP/dt_{max}$) pressure to acute myocardial ischemia during the time control protocol (no systemic blockade) with and without phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 8 for control, n = 4 for phentolamine.

Figure 6 depicts the response of RPP (an index of myocardial oxygen demand) to the 2.5 minute period of ischemia followed by 7 minutes of reperfusion during the control (no infusion) and the phentolamine infusion

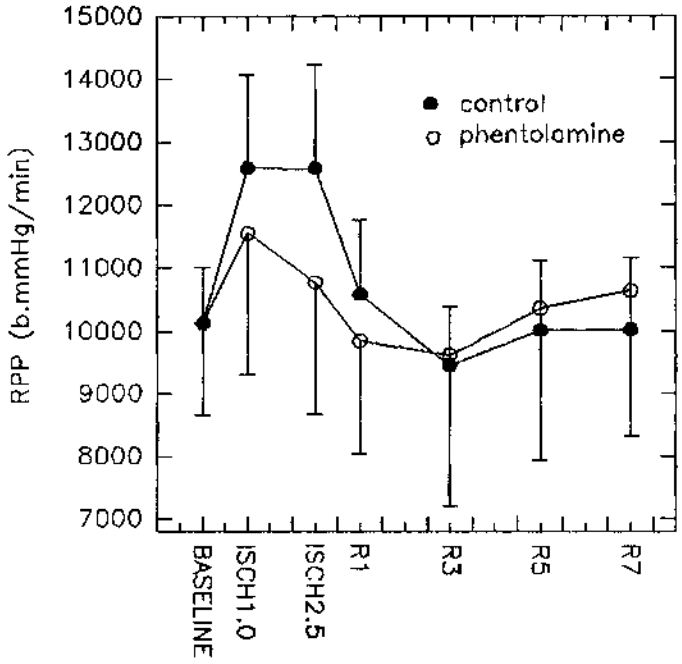


Figure 6. Response of rate pressure product (RPP) to acute myocardial ischemia during the time control protocol (no systemic blockade) with and without phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 8 for control, n = 4 for phentolamine.

conditions. There were no significant changes. Neither HR, $+dP/dt_{max}$, $-dP/dt_{max}$ or RPP were affected by the infusion of phentolamine (ANOVA: drug effect; $F = 0.22$, $p = 0.64$; $F = 2.72$, $p = 0.10$; $F = 0.97$, $p = 0.33$ and $F = 1.42$, $p = 0.23$).

Figure 7 depicts the response of left ventricular end-diastolic pressure (LVEDP) to the 2.5 minutes of ischemia during the control (no infusion) and

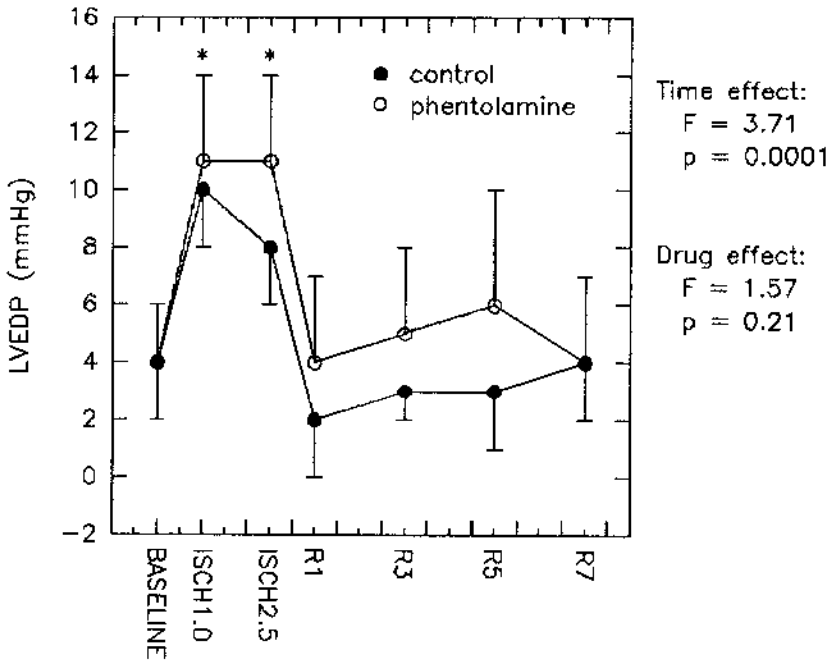


Figure 7. Response of left ventricular end-diastolic pressure (LVEDP) to acute myocardial ischemia during the time control protocol (no systemic blockade) with and without phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 8 for control, n = 4 for phentolamine. * - $p < 0.05$, both groups vs other time points; Duncan's post hoc.

the phentolamine infusion conditions. LVEDP was significantly elevated during the 2.5 minute LADO and CxPO compared to the pre-ischemic baseline value (ANOVA: time effect; $F = 3.71$, $p = 0.0001$; Duncan's post hoc test, $p<0.05$). Under control conditions, LVEDP increased from a mean of 4 ± 2 mmHg to 10

± 2 mmHg after 1 minute of the LADO and CxPO and remained elevated after 2.5 minutes of the ischemia at 8 ±2 mmHg. During the infusion of phentolamine, LVEDP increased from a baseline of 4 ± 2 mmHg to 11 ± 3 mmHg after 1 minute and remained at 11 ± 3 mmHg for the duration of the occlusion. Immediately on reperfusion, LVEDP was still significantly elevated (Duncan's post hoc: $p < 0.05$) but was no different from pre-ischemic values after 1 minute of reperfusion. The response of LVEDP to the occlusion between the control and experimental conditions was no different (ANOVA: drug effect; $F=1.57$, $p = 0.12$).

Figures 8 and 9 depict the changes in regional contractile function in the severely ischemic anterior wall, and the posterior wall of the left ventricle in response to 2.5 minutes of myocardial ischemia during the i.c. infusion of saline and phentolamine. As expected, contractile function deteriorated significantly in the ischemic anterior wall of the left ventricle during the LADO and CxPO (Figure 8). Anterior wall percent ejection shortening (A%SL) of the anterior wall was significantly less than baseline during the period of ischemia as well as during the reactive hyperemia (ANOVA: time effect; $F = 11.10$, $p = 0.0001$; Duncan's post hoc, $p < 0.05$). There were no differences between the control and experimental conditions (ANOVA: drug effect; $F = 0.74$, $p = 0.39$). Baseline A%SL during the control condition (saline infusion) was 13.4 ± 2.6%, while during the infusion of phentolamine it was 13.6 ± 3.8%. After 1 minute of ischemia, A%SL had decreased significantly to 2.4 ± 0.9% and 3.6 ± 1.2%

in the control and experimental conditions, respectively (Duncan's post hoc, $p < 0.05$). One dog in the control condition, but no dogs in the experimental condition, showed paradoxical lengthening. A%SL remained suppressed in both conditions for the duration of the LADO and CxPO (2.5 minutes of ischemia: 3.3 ± 1.1% and 3.6 ± 1.0%). It immediately returned towards pre-ischemic levels on reperfusion, but was still significantly different from baseline (7.9 ± 3.6% and 4.4 ± 0.9% for the control and infusion groups, respectively) during the reactive hyperemic phase of reperfusion (Duncan's post hoc, $p < 0.05$). Pre-ischemic values were reached in both conditions within one minute of reperfusion, indicating little to no post-ischemic myocardial stunning. Similarly, the maximal rate of segment length of shortening ($-dL/dt_{max}$) was attenuated at 1 and 2.5 minutes of ischemia and returned to baseline by one minute of reperfusion (ANOVA: time effect; $F = 2.88$, $p = 0.002$; Duncan's post hoc, $p < 0.05$). No drug effect was evident (ANOVA: drug effect; $F = 0.03$, $p = 0.86$).

There were no significant changes in posterior percent ejection shortening (P%SL) (ANOVA: time effect; $F = 0.44$, $p = 0.95$) or $-dL/dt_{max}$ (ANOVA: time effect; $F = 0.17$, $p = 1.000$) during either the control or the experimental conditions (Figure 9). Baseline P%SL during the control condition and during phentolamine infusion were 9.8 ± 1.1% and 9.3 ± 0.4%, respectively. After 1 minute of ischemia, P%SL for each condition was 9.7 ± 1.3% and 10.2 ± 0.7%, respectively, and 9.7 ± 1.2% and 9.7 ± 0.9%,

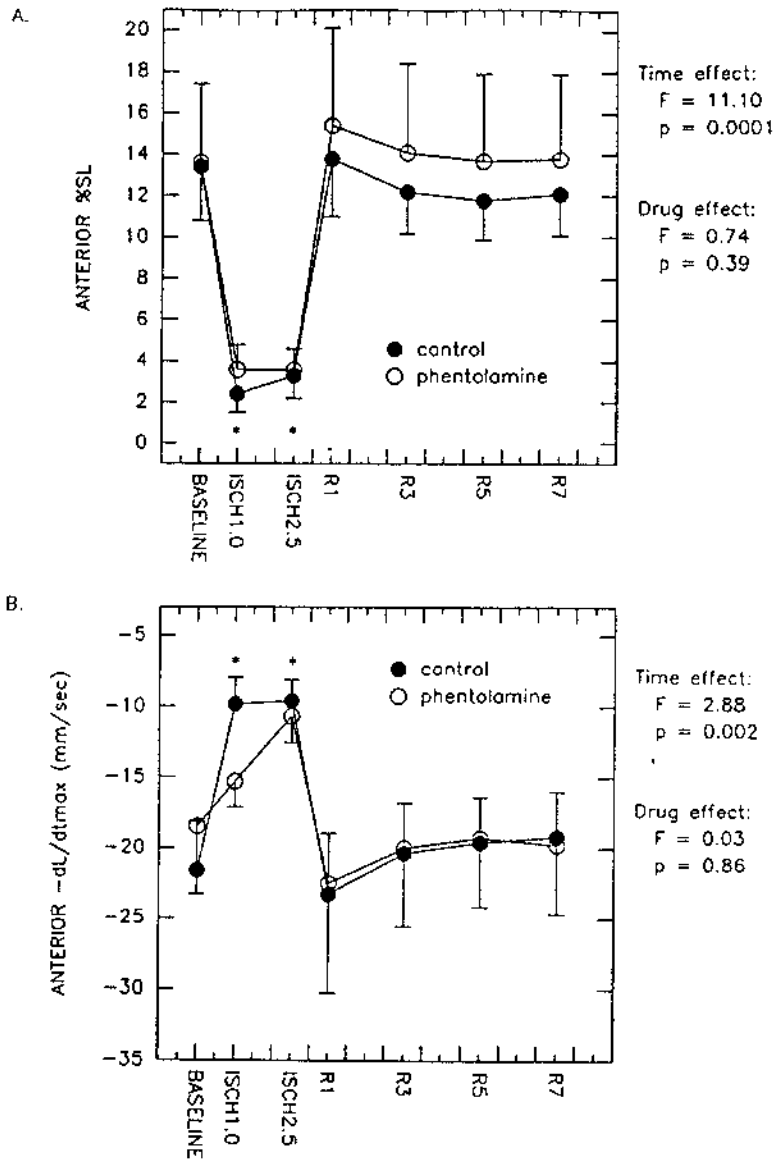


Figure 8. Response of percent ejection shortening (%SL) and maximal rate of shortening ($-dL/dt_{max}$), in the anterior wall of the left ventricle, to acute myocardial ischemia during the time control protocol (no systemic blockade) with and without phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 8 for control, n = 4 for phentolamine. * - $p < 0.05$, both groups vs other time points; Duncan's post hoc.

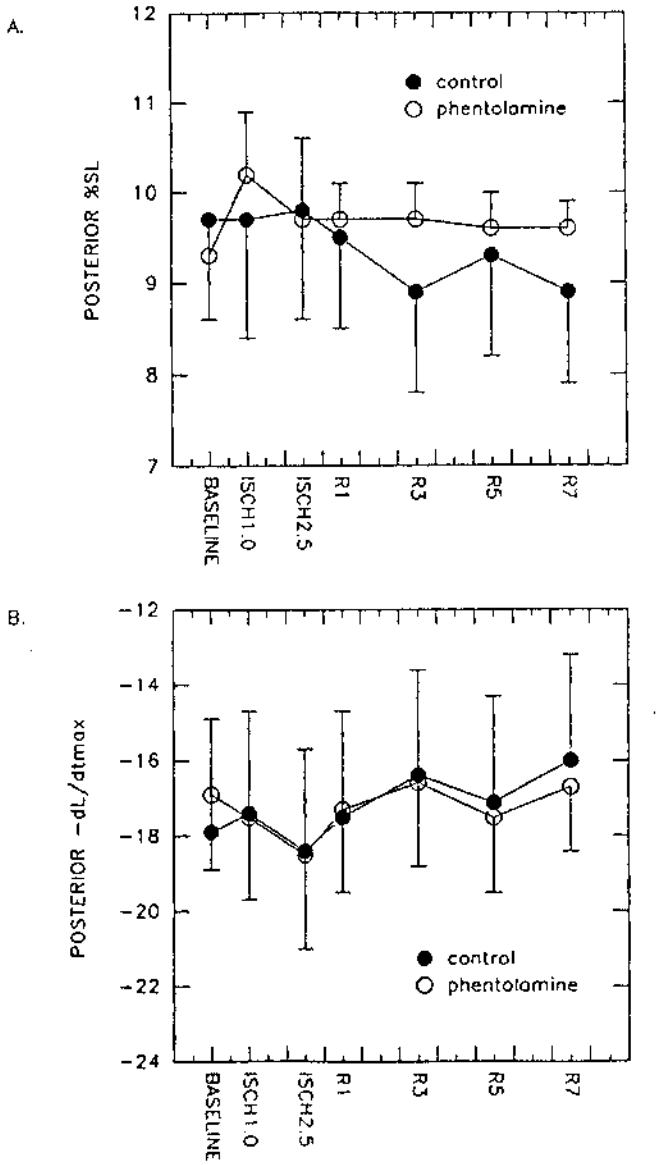


Figure 9. Response of percent ejection shortening (%SL) and maximal rate of shortening ($-dL/dt_{max}$), in the posterior wall of the left ventricle, to acute myocardial ischemia during the time control protocol (no systemic blockade) with and without phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 8 for control, n = 4 for phentolamine.

respectively, after 2.5 minutes of the LADO and CxPO. Thus, the degree of stenosis of the circumflex artery during the LADO was sufficient to eliminate the compensatory increase in regional contractile function (Matre et al,1987) but was not too severe so as to reduce function below baseline levels.

Figure 10 shows the changes in mean Doppler circumflex blood flow (MCBF) and mean coronary blood pressure (MCBP) over time for the control and experimental conditions. One dog was eliminated from the coronary blood flow analyses due to a malfunctioning Doppler flow probe. MCBF was unchanged (ANOVA: time effect; $F = 0.66$, $p = 0.79$). Although a drug effect was evident from the statistical analysis, the infusion of phentolamine, per se, had no effect. This can be concluded from a comparison of the MCBF measured prior to beginning the phentolamine infusion with that measured during the infusion of the drug. Pre-infusion MCBF was 30.1 $\pm$ 8.2 ml/min while after 5 minutes of the phentolamine infusion, MCBF was 30.9 $\pm$ 8.5 ml/min and no further change occurred. It appears that the MCBF measurements in the control condition were erroneously high (see Chapter 5 for further details).

Coronary blood pressure could not be measured in 3 dogs in the control condition. Thus the small sample size limited the power of the statistics. MCBP decreased significantly as a result of the LADO and CxPO (ANOVA: time effect; $F = 5.29$, $p = 0.0001$). In the control condition, the MCBP decreased from a baseline value of 88 $\pm$ 4 mmHg to 59 $\pm$ 8 mmHg

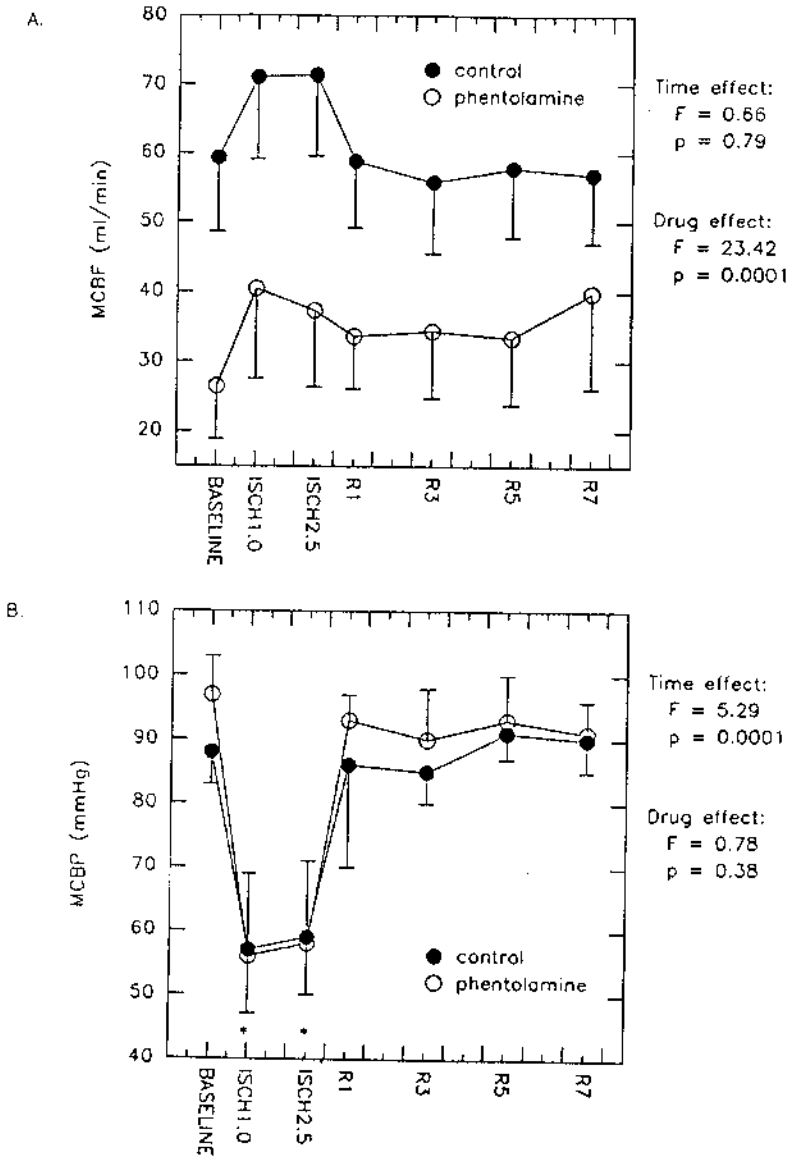


Figure 10. Response of mean circumflex blood flow (MCBF) and mean circumflex blood pressure (MCBP) to acute myocardial ischemia during the time control protocol (no systemic blockade) with and without phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 7 for control, n = 3 for phentolamine. * - $p < 0.05$, both groups vs other time points; Duncan's post hoc.

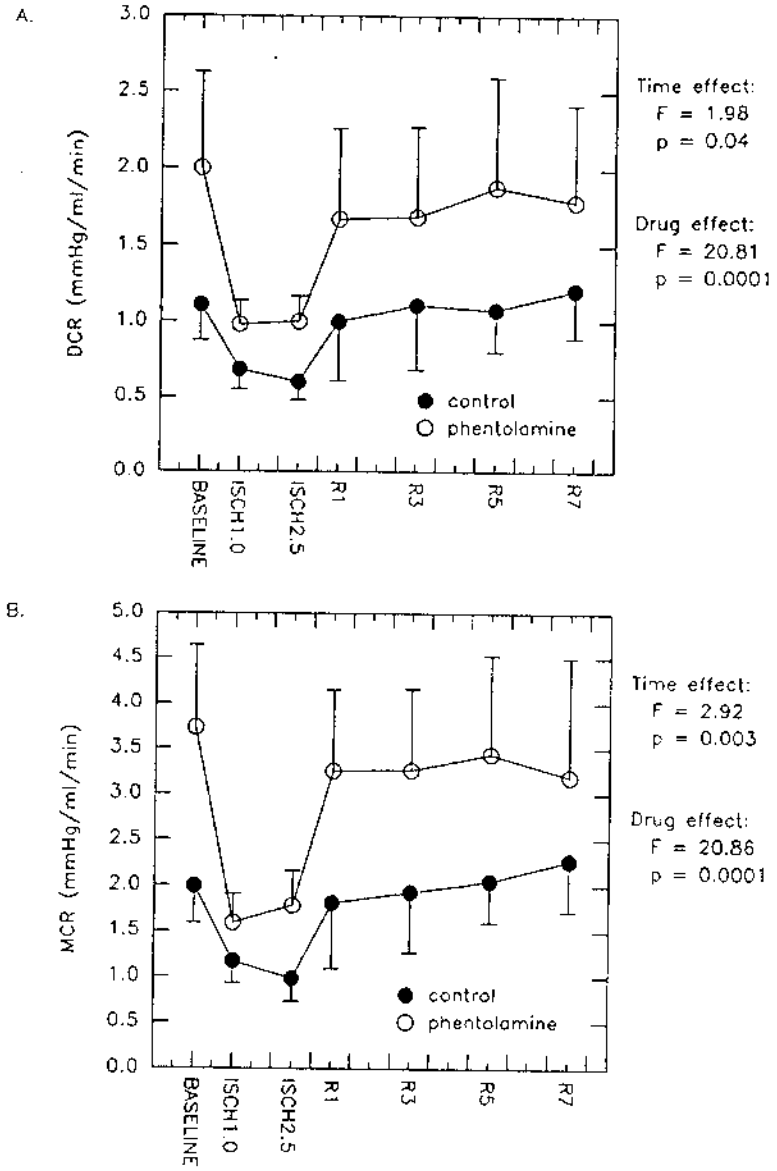


Figure 11 Response of diastolic (DCR) and mean coronary resistance (MCR) to acute myocardial ischemia during the time control protocol (no systemic blockade) with and without phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 4 for control, n = 3 for phentolamine. * - $p < 0.05$, both groups vs other time points; Duncan's post hoc.

after 1 minute of the LADO and CxPO, and to 58 ± 7 mmHg after 2.5 minutes of the LADO and CxPO. Within 1 minute of reperfusion the MCBP had returned to baseline levels. A similar response was observed during the infusion of phentolamine. Baseline MCBP was 88 ± 10 mmHg at baseline, 48 ± 11 mmHg after 1 minute of the LADO and CxPO, and 54 ± 10 mmHg after 2.5 minutes of the LADO and CxPO. Phentolamine infusion had no effect on the changes in systolic, diastolic and MCBP compared to the control condition (ANOVA: drug effects; $F = 0.78$, $p = 0.381$).

Diastolic (DCR) and mean coronary vascular resistance (MCR) decreased significantly during the protocol (ANOVA: time effect; $F = 1.98$, $p = 0.04$ and $F = 2.92$, $p = 0.003$, respectively) (Figure 11). However, due to unequal and small sample sizes as a result of technical problems, the power of the post hoc test was insufficient to separate the differences into statistically distinct groups. As a result of the significantly lower MCBF during the infusion of phentolamine, a statistically significant drug effect on both DCR and MCR was found (ANOVA: drug effect; $F = 20.81$, $p = 0.0001$ and $F = 20.86$, $p = 0.0001$, respectively). As was discussed earlier, the MCBF that was measured during the control condition may be artificially high. Since it was used in the calculation of MCR, the significant difference between the control and experimental group in this case may also be in error.

B. Double Blockade

The double blockade consisted of nonspecific ß-adrenergic blockade with propranolol (1 mg/kg i.v.) and muscarinic blockade with atropine (100 μg/kg i.v.). The combined ß-adrenergic and muscarinic blockade was used to eliminate any sympathetic and parasympathetic influences on the heart and the coronary vasculature. This allowed the α-adrenergic receptor-mediated coronary constrictor tone to be isolated from the direct and the indirect influences of the two branches of the autonomic nervous system. Both drugs were administered prior to beginning the infusions and inducing ischemia. Saline was infused i.c. for the control condition and phentolamine was infused i.c. for the experimental condition.

Figures 12 and 13 show tracings recorded during the double blockade experiments on the same dog as used in Figures 1 and 2. Figure 12 represents the saline infusion condition while Figure 13 represents the phentolamine infusion condition. The beginning and the end of the 2.5 minute period of ischemia are marked by the 2 arrows.

In Figure 12, the saline infusion condition, panel A depicts the recordings made with the dog at rest prior to any drug administration. Panel B shows the recordings made after the dog had been double blocked with i.v. propranolol and atropine. The reduction in segment length shortening is a consequence of an increase in HR due to muscarinic receptor blockade.

Panel C shows the start of the ischemic period. The following should be noted:

1. a reduction in anterior segment length function due to severe ischemia of the anterior wall of the left ventricle.
2. no change in posterior segment length function. Respiratory oscillations can be seen in the posterior segment length tracing.
3. a decrease in CBP. The small coronary pulse pressure is due to infusing through the same catheter via which CBP is being measured.
4. a small drop in AP relative to the pre-infusion level eventhough only 0.9% sterile saline was being infused. The reason for this drop remains unclear. Note that a similar decrease in AP occurred when phentolamine was infused as well (Figure 13). Of particular importance is the fact that AP did not fall as a result of the ischemia but remained unchanged throughout the occlusion and the reperfusion.

Cardiac function remained suppressed until the period of reperfusion when all variables returned to pre-ischemic levels. When phentolamine was infused instead of saline (Figure 13), the same responses to the infusion and the ischemia occurred. Of particular importance, is the fact that no fall in AP can be seen during the ischemic period.

Figure 12. Tracing of the response of the same dog as in the previous tracings to 2.5 minutes of acute myocardial ischemia of the anterior wall of the left ventricle after double blockade with propranolol (1 mg/kg i.v.) and atropine (100μg/kg i.v.) and with the infusion of 0.9 % sterile saline (0.25 ml/min) into the circumflex artery for the duration of the experiment. The beginning of the occlusion is indicated by the first arrow, and release of the occlusion by the second arrow. Variables shown, from top to bottom, are left ventricular pressure (LVP in mmHg), anterior segment length shortening (Anterior SL in mm), Posterior segment length shortening (Posterior SL in mm), circumflex flow velocity (CFV in KHz), circumflex blood pressure (CBP in mmHg) and arterial pressure (AP in mmHg). A - control prior to i.v. administration of drugs and prior to starting the infusion,heart rate (HR) = 68 b/min; B - double blocked but prior to starting the infusion,HR = 182 b/min; C - after 5 minutes of the infusion and beginning the occlusion,HR = 154 b/min; D - 1 minute of ischemia,HR = 161 b/min; E - beginning of reperfusion,HR = 156 b/min; F - 7 minutes of reperfusion,HR = 149 b/min.

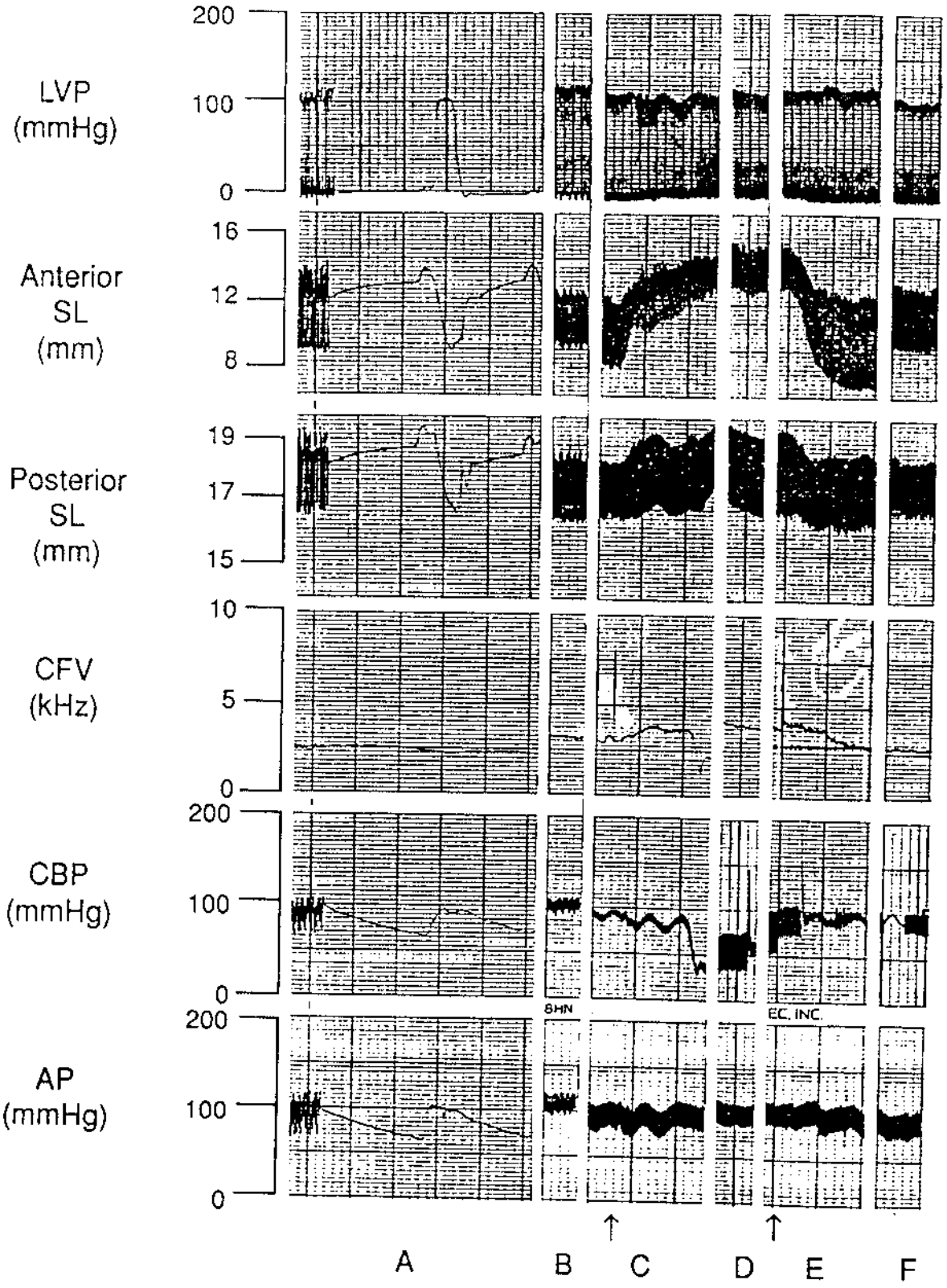
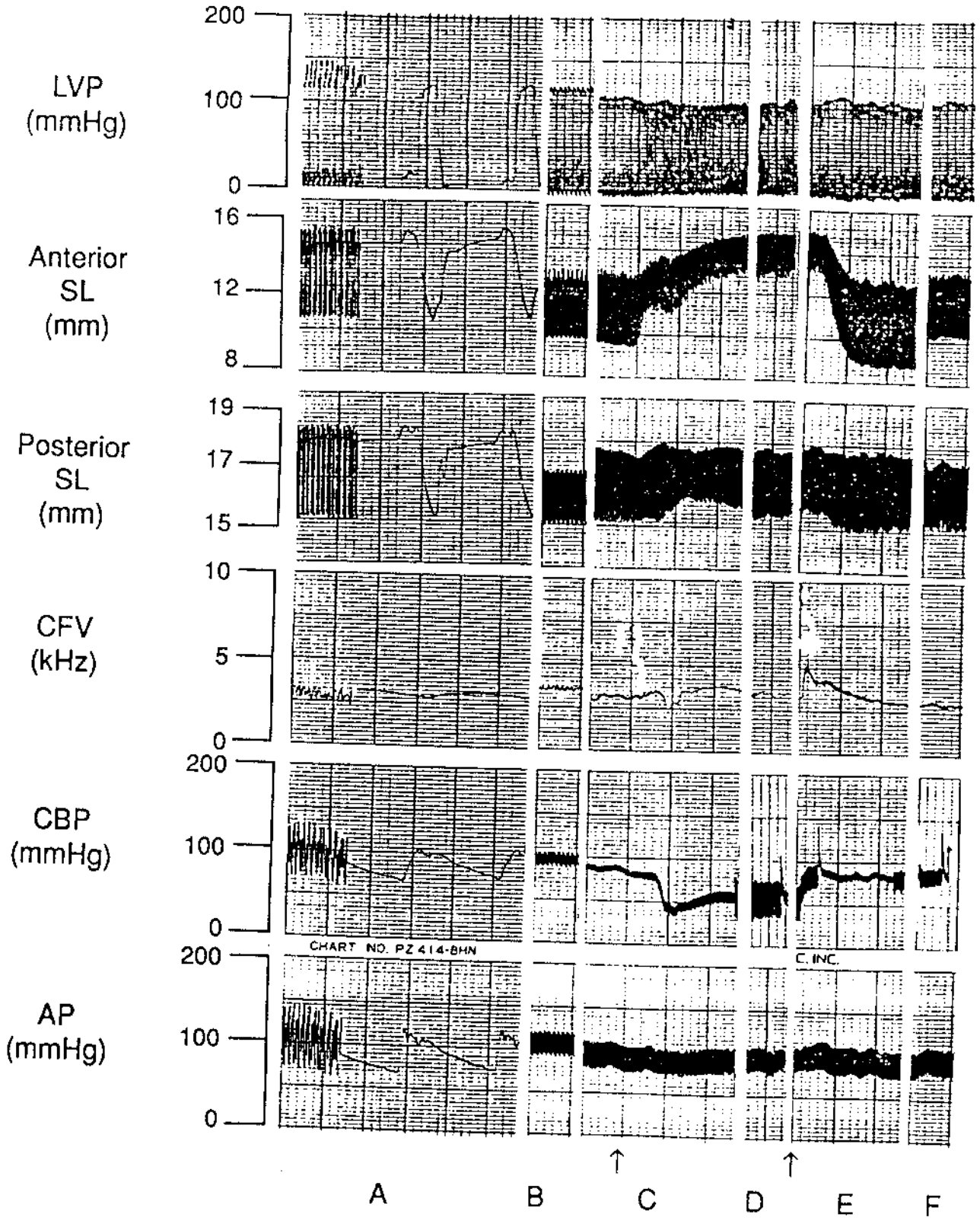

LVP
(mmHg)
200
100
0
Anterior
SL
(mm)
16
12
8
Posterior
SL
(mm)
19
17
15
CFV
(kHz)
10
5
0
CBP
(mmHg)
200
100
0
AP
(mmHg)
200
100
0
8HN
EC, INC.
A
B
C
D
E
F

Figure 13. Tracing of the response of the same dog as in the previous tracings to 2.5 minutes of acute myocardial ischemia of the anterior wall of the left ventricle after double blockade with propranolol (1 mg/kg i.v.) and atropine (100μg/kg i.v.) and with the infusion of phentolamine (0.5 μg/kg/min) into the circumflex artery for the duration of the experiment. The beginning of the occlusion is indicated by the first arrow, and release of the occlusion by the second arrow. Variables shown, from top to bottom, are left ventricular pressure (LVP in mmHg), anterior segment length shortening (Anterior SL in mm), Posterior segment length shortening (Posterior SL in mm), circumflex flow velocity (CFV in KHz), circumflex blood pressure (CBP in mmHg) and arterial pressure (AP in mmHg). A - control prior to i.v. administration of drugs and prior to starting the infusion,heart rate,heart rate (HR) = 61 b/min; B - double blocked but prior to starting the infusion,HR = 167 b/min; C - after 5 minutes of the infusion and beginning the occlusion,HR = 144 b/min; D - 1 minute of ischemia,HR = 147 b/min; E - beginning of reperfusion,HR = 148 b/min; F - 7 minutes of reperfusion,HR = 146 b/min.


LVP
(mmHg)
200
100
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Anterior
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16
12
8
Posterior
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CFV
(kHz)
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5
0
CBP
(mmHg)
200
100
0
CHART NO. PZ 414-8HN
C. INC.
AP
(mmHg)
200
100
0
A
B
C
D
E
F

Figures 14 through 20 depict changes observed in global left ventricular contractile function and in MAP during saline and phentolamine infusion with double blockade.

HR increased significantly as a result of muscarinic blockade (ANOVA: time effect; $F = 6.67$, $p = 0.0001$; Duncan's post hoc, $p < 0.05$). Pre-blockade HR for the saline infusion condition was 80 ± 6 b/min. After double blockade had been achieved, HR was 149 ± 17 b/min. An unexpected but statistically nonsignificant fall in HR occurred after 5 minutes of saline infusion (HR = 125 ± 9 b/min). A similar pattern of change in HR was observed in the experimental condition. Pre-blockade HR was 72 ± 4 b/min. After double blockade, HR increased to 150 ± 13 b/min, a small but statistically nonsignificant decrease in HR resulted after 5 minutes of i.c. phentolamine infusion (HR = 130 ± 4 b/min). No further changes in HR occurred in either the control or the experimental conditions. Indeed, the lack of change in HR during the 2.5 minutes of ischemia and following reperfusion was further substantiated when the same 2-way ANOVA was applied to data collected during the infusions only, i.e. pre-infusion data was eliminated from the analysis. No significant change in HR was evident (ANOVA: time effect; $F = 0.57$, $p = 0.95$). Thus, the changes observed in HR were due to the muscarinic blockade alone (Figure 14). Also, the double blockade successfully blocked the ischemia-induced increase in HR.

MAP was unchanged over time in both the control (saline infusion) and

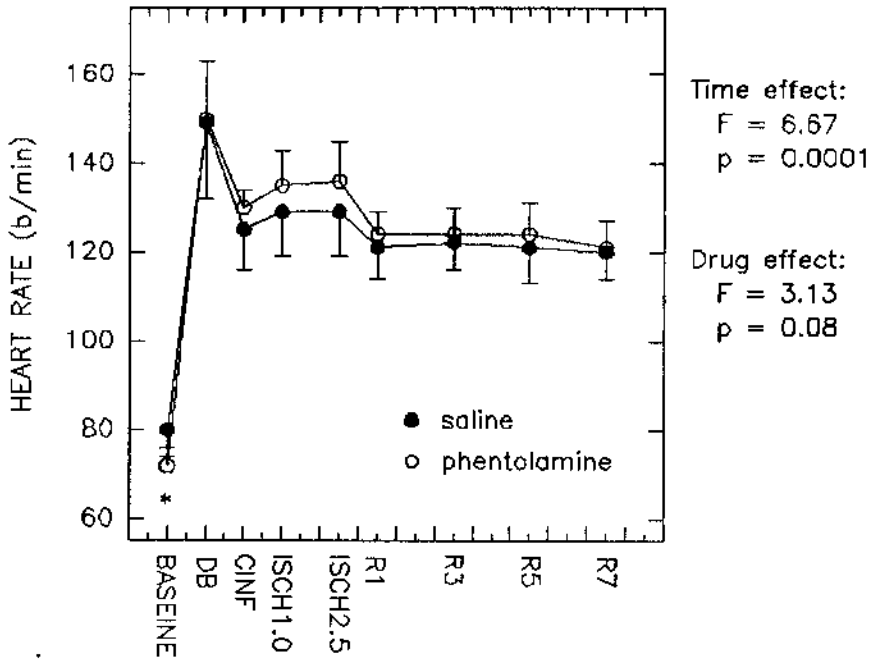

Figure 14. Heart rate response to acute myocardial ischemia during the double blockade protocol with saline and phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 7. * - $p < 0.05$, both groups vs other time points; Duncan's post hoc.

the experimental (phentolamine infusion) conditions (Figure 15). There was also no difference in the MAP response between the control and experimental conditions (ANOVA: $F = 0.77$, $p = 0.80$). MAP prior to double blockade was 85 ± 3 mmHg and 80 ± 3 mmHg in the control and experimental conditions, respectively. After double blockade had been established, MAP was 95 ± 4 mmHg and 91 ± 5 mmHg, respectively. Five minutes of infusing saline or phentolamine had no effect on MAP (90 ± 4 mmHg and 89 ± 5 mmHg,

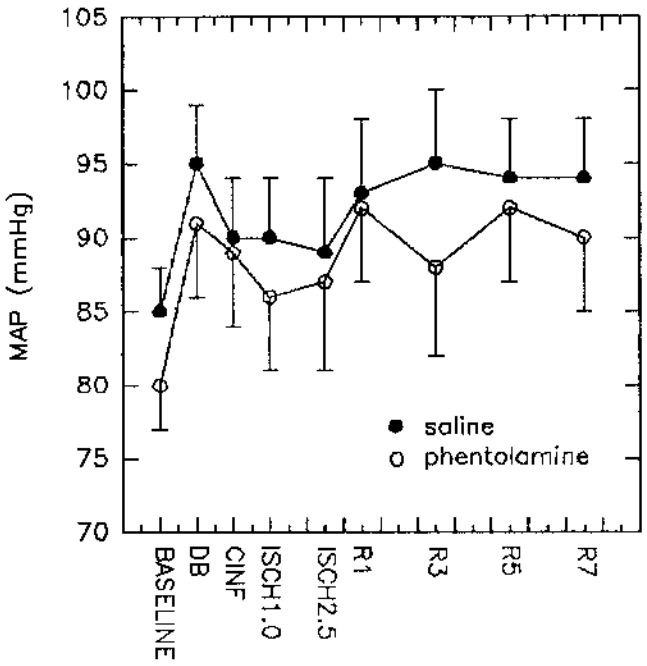

Figure 15. Response of mean arterial pressure (MAP) to acute myocardial ischemia during the double blockade protocol with saline and phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 7.

respectively). In the control condition, MAP ranged between 90 ± 4 mmHg and 95 ± 5 mmHg, while in the experimental condition it ranged between 86 ± 5 mmHg and 92 ± 5 mmHg. There was a significant decrease in PP associated with muscarinic blockade (ANOVA: time effect; $F = 5.53$, $p = 0.0001$;Duncan's post hoc, $p < 0.05$). It is important to not that the response of PP throughout the protocol was not different between the 2 conditions (ANOVA: drug effect; $F = 2.50$, $p = 0.11$).

Figure 16 shows that LVEDP was significantly elevated during the 2.5 minutes of ischemia compared to the LVEDP recorded after 5 minutes of infusion of either saline of phentolamine (ANOVA: time effect; $F = 5.99$, $p = 0.0001$; Duncan's post hoc, $p < 0.05$). For the control condition (saline infusion), LVEDP increased from -0.7 ± 1 mmHg to 5 ± 1 mmHg after 1 minute of ischemia, and to 4 ± 2 mmHg after 2.5 minutes of ischemia. During the infusion of phentolamine, LVEDP increased from -0.6 ± 1 mmHg to 7 ± 2

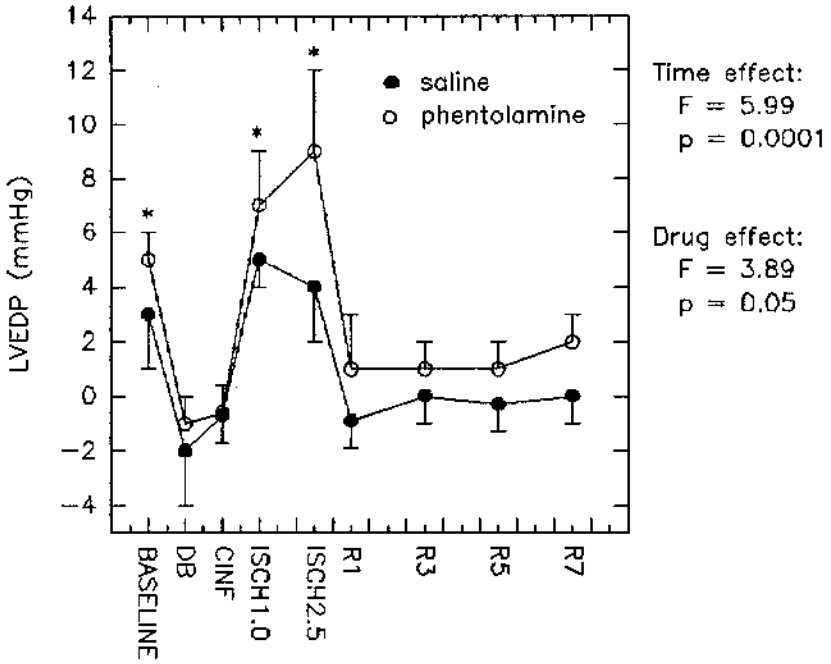

Figure 16. Response of left ventricular end-diastolic pressure (LVEDP) to acute myocardial ischemia during the double blockade protocol with saline and phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. $n = 7$. * - $p < 0.05$, both groups vs other time points; Duncan's post hoc.

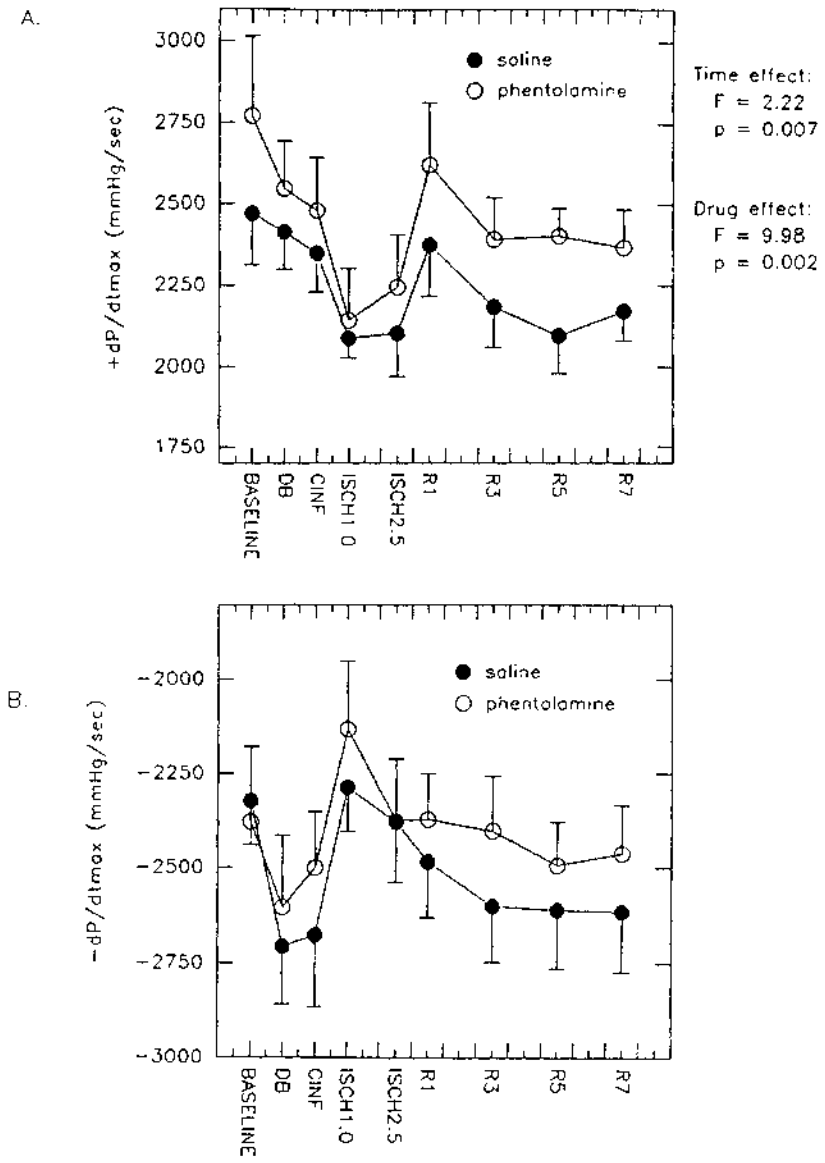

Figure 17. Response of maximal rate of change of left ventricular systolic ($+dP/dt_{max}$) and diastolic ($-dP/dt_{max}$) pressure to acute myocardial ischemia during the double blockade protocol with saline and phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 7. * - $p < 0.05$, both groups vs other time points; Duncan's post hoc.

mmHg after 1 minute of ischemia and to 8 ± 4 mmHg after 2.5 minutes of ischemia. There was a tendency for the increase in LVEDP during the infusion of phentolamine to be greater than the increase during the infusion of saline (ANOVA: drug effect; $F = 2.97$, $p = 0.09$).

Figure 17 depicts the changes in $+dP/dt_{max}$ and $-dP/dt_{max}$ over time for both the saline and the phentolamine infusions. There was a significant decrease in $+dP/dt_{max}$ (ANOVA: time effect; $F = 2.22$, $p = 0.007$), but Duncan's post hoc test was unable to isolate the differences. In addition, an overall drug effect on $+dP/dt_{max}$ was evident, with the phentolamine infusion resulting in significantly higher values (ANOVA: drug effect; $F = 9.98$, $p = 0.002$). However, this was probably a result of higher pre-infusion $+dP/dt_{max}$ values due to daily variation within each dog. Baseline $+dP/dt_{max}$ values during the saline infusion (control) and the phentolamine infusion (experimental) conditions were 2471 ± 157 mmHg/sec and 2771 ± 244 mmHg/sec, respectively. As can be seen in Figure 17, during the 2.5 minutes of ischemia and on reperfusion, $+dP/dt_{max}$ showed a similar response during phentolamine infusion to that observed during saline infusion, but the absolute values were consistently higher. When statistical analysis was restricted to data collected during infusion only, no significant changes in $+dP/dt_{max}$ between the saline and phentolamine infusions were found (ANOVA: $F = 1.19$, $p = 0.26$). Similarly, there were no significant changes in $-dP/dt_{max}$ (ANOVA: $F = 1.48$, $p = 0.47$) (Figure 17).

RPP was used as an index of myocardial oxygen consumption and is shown in Figure 18. There was a significant increase in RPP during the saline and phentolamine infusion conditions (ANOVA: time effect; F = 6.00, p = 0.0001). The higher RPP was associated with the higher HR after muscarinic blockade. There was no difference in the response of RPP when comparing

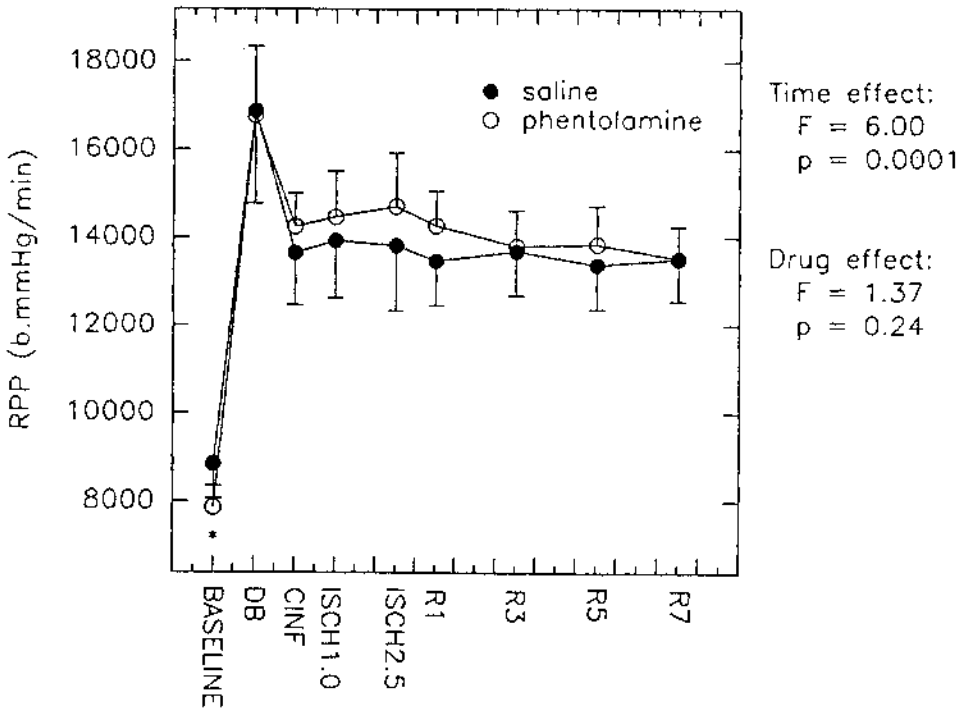

Figure 18. Response of rate pressure product (RPP) to acute myocardial ischemia during the double blockade protocol with saline and phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 7. * - p < 0.05, both groups vs other time points; Duncan's post hoc.

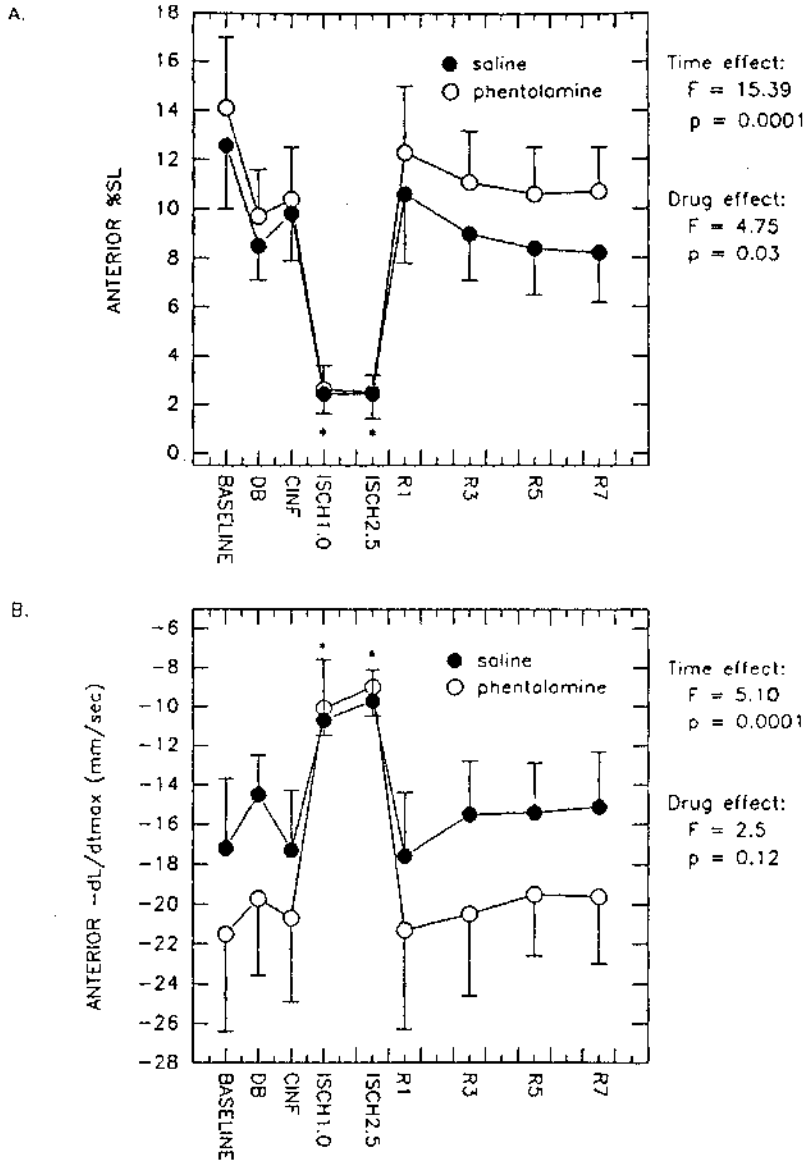

Figure 19. Response of percent ejection shortening (%SL) and maximal rate of shortening (-dL/dtmax), in the anterior wall of the left ventricle, to acute myocardial ischemia during the double blockade protocol with saline and phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 6. * - $p < 0.05$, both groups vs other time points; Duncan's post hoc.

the infusion of saline with that of phentolamine (ANOVA: drug effect; $F = 1.37$, $p = 0.24$).

Figures 19 and 20 show the changes in regional contractile function of the left ventricle during the 2.5 minute LADO and CxPO. Both the control (saline infusion) and the experimental (phentolamine infusion) conditions are represented. One dog was eliminated from both the anterior and posterior regional function analysis due to defective segment length signals. There was a significant decrease in A%SL during the ischemic period for both the control and experimental conditions (ANOVA: time effect; $F = 15.39$, $p = 0.0001$) (Figure 19). A%SL prior to the LADO and CxPO but 5 minutes after commencement of the saline infusion was 9.8 ± 1.9%. This decreased to 2.4 ± 0.8% after 1 minute of ischemia and to 2.4 ± 1.0% after 2.5 minutes of ischemia. Similar changes occurred during the infusion of phentolamine. Prior to the LADO and CxPO and after 5 minutes of phentolamine infusion, A%SL was 10.4 ± 2.1%. After 1 minute of ischemia it was 2.6 ± 1.0% and after 2.5 minutes of ischemia anterior %SL was 2.5 ± 0.7%. After 1 minute of reperfusion, A%SL had returned to baseline in both the control and the experimental conditions. Phentolamine infusion resulted in a significantly smaller overall decrease in anterior %SL than did saline infusion (ANOVA: drug effect; $F = 4.75$, $p = 0.03$). This difference was, however, not great enough to be isolated statistically by paired comparisons of each respective time point in the protocol. Similarly, $-dL/dt_{max}$ was significantly depressed during the

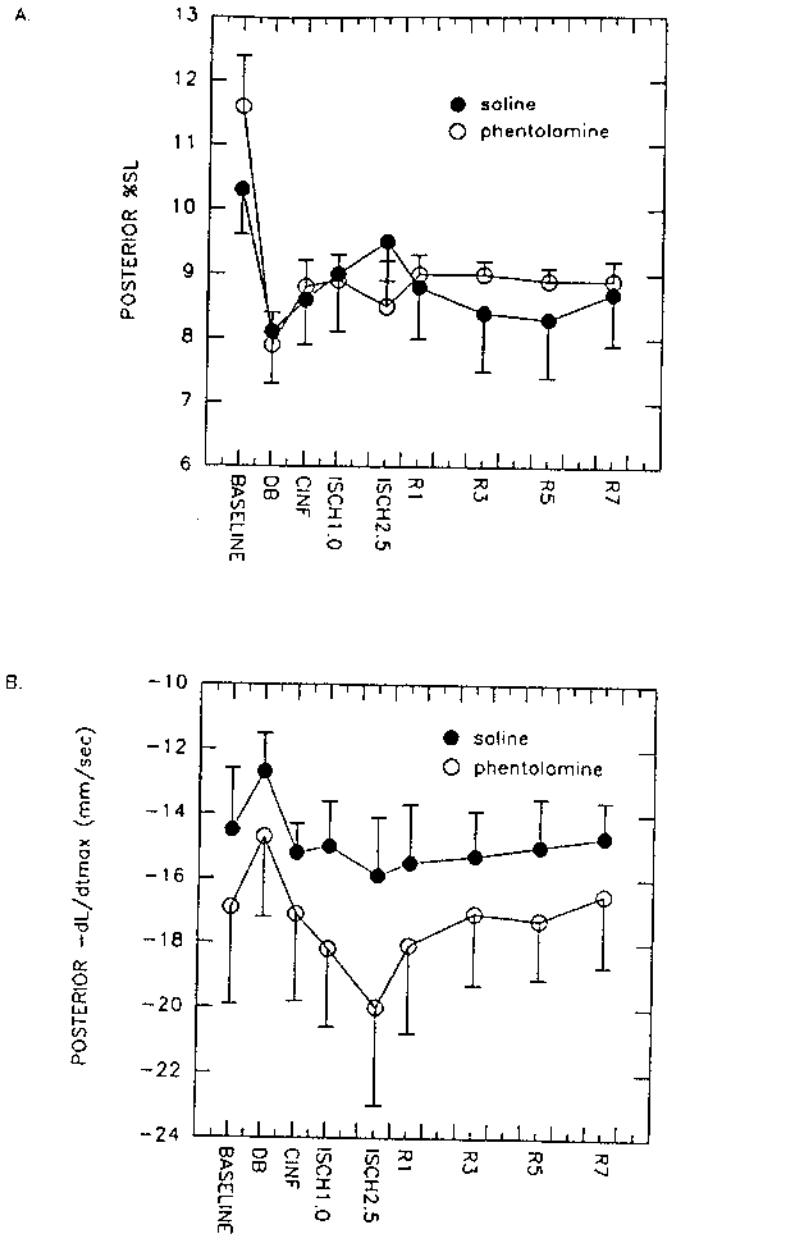

Figure 20. Response of percent ejection shortening (%SL) and maximal rate of shortening (-dL/dtmax), in the posterior wall of the left ventricle, to acute myocardial ischemia during the double blockade protocol with saline and phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 6.

ischemic period in both conditions (ANOVA: time effect;F = 5.10, p = 0.0001). In this case, there was no difference when comparing the response during phentolamine infusion to that during saline infusion (ANOVA: drug effect; F = 2.5, p = 0.12). There were no significant changes in $+dL/dt_{max}$ (ANOVA: F = 0.47, p = 0.98).

There were no significant changes in the regional function of the posterior left ventricular free wall (Figure 20). Pre-infusion, pre-double blockade values of P%SL were 10.3 ± 0.7% and 11.6 ± 0.8% for the saline and the phentolamine infusion conditions, respectively. After double blockade and 5 minutes of the respective i.c. infusions, P%SL was 8.6 ± 0.7% and 8.8 ± 0.4%, respectively. At no stage did P%SL vary by more than 0.8% in either condition throughout the entire protocol. The posterior $-dL/dt_{max}$ and $+dL/dt_{max}$ remained at baseline levels (ANOVA: time effect; F = 0.05, p = 0.98 and F = 0.32, p = 1.000 respectively). The fact that posterior regional function was maintained at baseline levels reflects accurate control of the circumflex stenosis. There were no differences between the control and the experimental conditions.

Figure 21 depicts the changes in MCBF and MCBP, and Figure 22 depicts the changes in DCR and MCR of the circumflex artery during the 2.5 minutes of ischemia and the 7 minutes of reperfusion. Both the control (saline infusion) and the experimental (phentolamine infusion) conditions are represented. One dog was eliminated from the coronary blood flow analyses

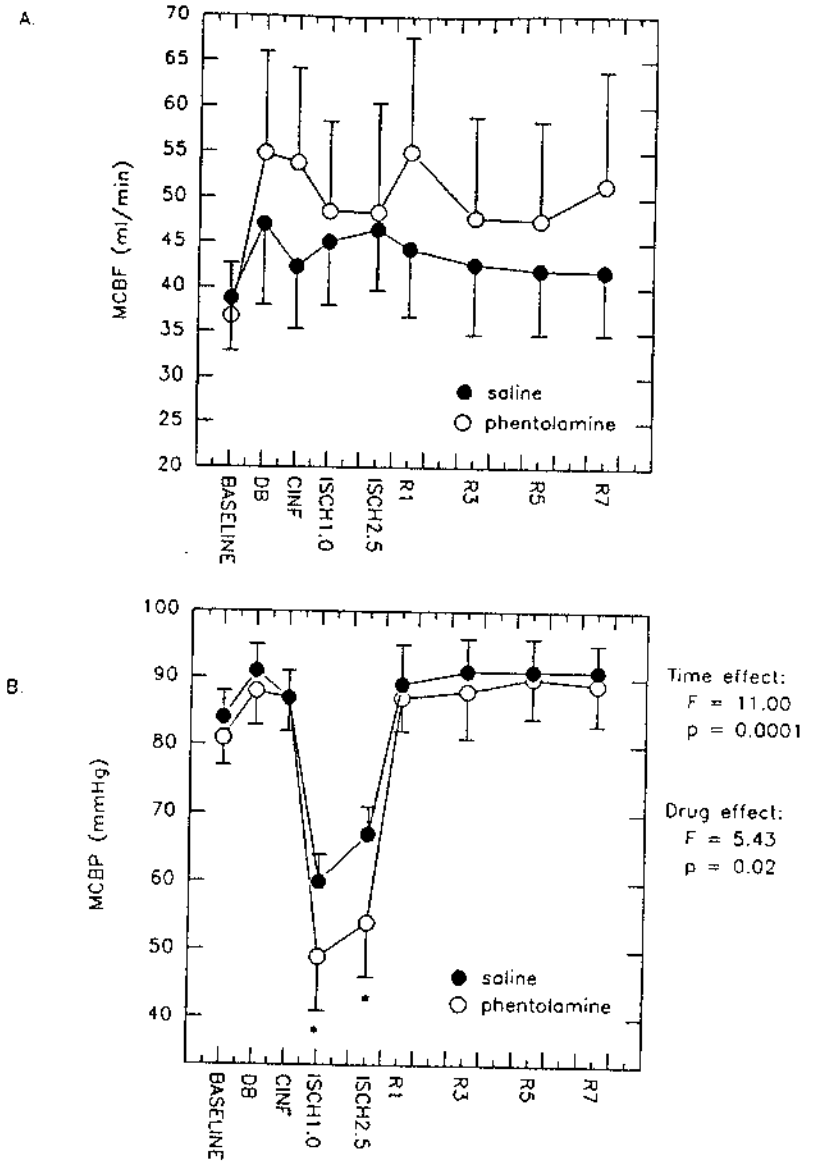

Figure 21. Response of mean circumflex blood flow (MCBF) and mean circumflex blood pressure (MCBP) to acute myocardial ischemia during the double blockade protocol with saline (n = 7 and n = 6, respectively) and phentolamine (n = 7 and n = 5, respectively) infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 6.

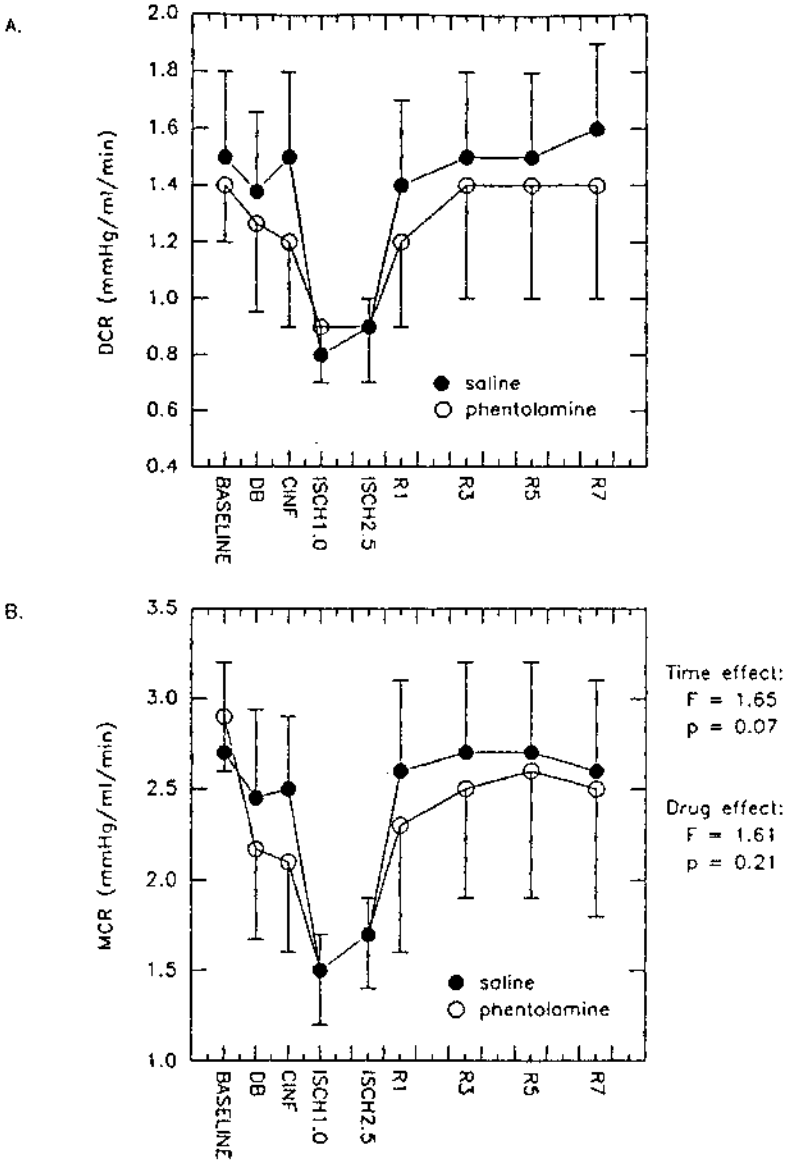

Figure 22. Response of diastolic (DCR) and mean coronary resistance (MCR) to acute myocardial ischemia during the double blockade protocol with saline and phentolamine infusion. ISCH1.0 - 1 minute of ischemia, ISCH2.5 - 2.5 minutes of ischemia, R1 to R7 - 1 to 7 minutes of reperfusion. Values are mean ± SE. n = 6 for control, n = 5 for phentolamine.

due to a malfunctioning Doppler flow probe. MCBF was unaffected by the combined LADO and CxPO (ANOVA: time effect; $F = 0.38$, $p = 0.98$) or by phentolamine infusion (ANOVA: drug effect; $F = 0.39$, $p = 0.53$).

MCBP decreased significantly during the ischemic period as a result of the stenosis (ANOVA: time effect; $F = 11.00$, $p = 0.0001$; Duncan's post hoc, $p < 0.05$). In the control condition, MCBP decreased from a pre-ischemic value of 87 ± 4 mmHg to 60 ± 4 mmHg after 1 minute of the LADO and CxPO, and was 67 ± 4 mmHg after 2.5 minutes of the LADO and CxPO. Within 1 minute of reperfusion MCBP was not significantly different from the pre-ischemic baseline value. A similar response occurred during the infusion of phentolamine. Pre-ischemic MCBP was 87 ± 5 mmHg. After 1 minute of the LADO and CxPO, MCBP decreased to 49 ± 8 mmHg and was 54 ± 8 mmHg after 2.5 minutes of the LADO and CxPO. Intracoronary infusion of phentolamine resulted in a significantly lower MCBP (ANOVA: drug effect; $F = 5.43$, $p = 0.02$).

The decreases in calculated DCR and MCR did not reach statistical significance (ANOVA: time effect; $F = 0.77$, $p = 0.797$ and $F = 0.96$, $p = 0.52$, respectively) (Figure 22). During the infusion of saline, DCR and MCR both decreased from baseline values of 1.6 ± 0.2 mmHg/ml/min and 2.7 ± 0.4 mmHg/ml/min, respectively, to 1.0 ± 0.2 mmHg/ml/min and 1.7 ± 0.3 mmHg/ml/min after 1 minute of the LADO and CxPO. After 2.5 minutes of the combined occlusion and stenosis, DCR and MCR were 1.1 ± 0.2

mmHg/ml/min and 1.9 ± 0.3 mmHg/ml/min, respectively. Similar responses were observed during the infusion of phentolamine. The DCR and MCR values were 1.4 ± 0.3 mmHg/ml/min and 2.5 ± 0,6 mmHg/ml/min, respectively, priorto the LADO and CxPO. After 1 minute of the LADO and CxPO, they had decreased to 0.9 ± 0.2 mmHg/ml/min and 1.7 ± 0.3 mmHg/ml/min, respectively. By 2.5 minutes of the LADO and CxPO, these

Table 2. Hemodynamics and regional myocardial contractile function observed during the experiments in which regional blood flow measurements were made.

EVENT	HR	LVEDP	MAP	A%SL	P%SL	MCBP
SAL C	78±5	8±2	89±8	13.1±3.3	10.3±0.5	87±9
DB	125±12	3±1	97±6	10.0±2.7	8.0±0.5	94±9
B	127±12	0±2	-	10.8±3.5	8.0±0.8	94±12
OCC	126±8	11±2	-	1.9±0.9	9.9±0.6	63±11
PHEN C	80±6	4±3	92±9	13.6±4.0	10.3±0.6	86±10
DB	123±6	0±2	95±11	10.6±3.0	8.0±0.9	88±7
B	118±3	1±2	-	10.2±3.2	7.8±0.8	95±9
OCC	121±4	12±3	-	1.4±1.2	9.5±1.3	75±5

SAL - saline infusion, PHEN - phentolamine infusion, R - resing data prior to any intervention, DB - double blockade prior to respective infusions, B - baseline after 5 minutes of respective infusions, OCC - after 1 minute of ischemia. HR - heart rate (b/min), LVEDP - left ventricular endiastolic pressure (mmHg), MAP - mean arterial pressure (mmHg), A%SL - anterior percent ejection shortening, P%SL - posterior percent ejection shortening, MCBP - mean coronary blood pressure (mmHg). Note: shaded values are the data collected at the same time as the regional blood flow measurements. n = 5.

values were 1.0 ± 0.1 mmHg/ml/min and 1.7 ± 0.2 mmHg/ml/min, respectively.

Hemodynamic and contractile function data recorded during the regional blood flow measurements are represented in Table 2. The changes in hemodynamics and contractile function during the ischemic period were similar to that observed in the double blockade experiments in which regional blood flows were not measured. HR increased significantly after muscarinic blockade had been achieved (ANOVA: Time effect; $F = 4.78$, $p = 0.0001$; Duncans post hoc, $p < 0.05$) but did not change any further during the ischemic period due to ß-adrenergic receptor blockade with propranolol. LVEDP increased significantly during the ischemic period in both the saline infusion and the phentolamine infusion conditions (ANOVA: Time effect; $F = 5.95$, $p = 0.0001$). The post hoc test was unable to separate the various time points into statistically distinct groups. A%SL decreased significantly during the ischemic period (ANOVA: Time effect; $F = 8.83$, $p = 0.0001$; Duncans post hoc, $p < 0.05$). MCBP also decreased significantly but the post hoc test was unable to isolate the statistically different time points (ANOVA:$p = 0.04$). Posterior %SL did not change significantly. The aortic catheter was used for reference blood sample withdrawal. Therefore, no MAP measurements could be made. There were no differences between the saline infusion and the phentolamine infusion groups in the responses of these variables to the ischemia.

C. Regional Blood Flows and Cardiac Output

Regional blood flow measurements were made 5 minutes after i.c. infusion of either saline or phentolamine but prior to the LADO and CxPO as well as after 1 minute of ischemia of the anterior region of the left ventricle. The most ischemic 2 adjoining sectors in the LAD perfusion territory (see Chapter 3) were compared with 2 adjoining sectors in the circumflex perfusion territory.

Blood flows in skeletal muscle of each hindlimb, flows in the left and right kidneys as well as the blood flows in the lower lobes of the left and right lung are presented in Table 3. The RK flows of one dog was excluded due to sampling error as was evident by the 100 fold difference between the two duplicate samples. By using duplicate tissue samples for each measurement,

Table 3. Blood flows in the left and right hindlimb skeletal muscle (LHL and RHL), left and right kidneys (LK and RK), as well as in the left and right lower lobe of the lung (LLL and RLL).

	LHL	RHL	LK	RK.	LLL	RLL
B_s	0.06±0.01	0.05±0.01	3.9±0.7	2.4±1.0	0.5±0.1	0.6±0.2
O_s	0.06±0.01	0.06±0.01	4.7±0.7	3.0±1.3	0.6±0.3	0.5±0.1
B_p	0.06±0.02	0.07±0.0	5.0±1.1	3.8±1.9	0.4±0.1	0.4±0.1
O_p	0.05±0.01	0.04±0.01	4.6±0.7	3.1±1.5	0.4±0.1	0.5±0.2

Units are ml/min/g. n = 5 (* - n = 4; see text). B_s - baseline, saline infusion; O_s - after 1 minute of ischemia, saline infusion; B_p - baseline, phentolamine infusion; O_p - after 1 minute of ischemia, phentolamine infusion. Hindlimb flows of one dog were excluded due to being erroneously high (100-fold).

such sampling errors can be detected. The similarity between the flows measured in the organs of the left side with those measured in organs on the right side should be noted. Pancreatic blood flow was measured in 3 dogs. This small sample size limits the value of doing any statistical comparisons, nevertheless, it appears that the mean baseline pancreatic flow during the phentolamine infusion condition is slightly higher than the mean baseline flow during the saline infusion condition (3.0 ± 0.9 ml/min/g vs 1.9 ± 0.7 ml/min/g, respectively). The pancreatic blood flow during the LADO + CxPO when saline was infused was 1.7 ± 0.6 ml/min/g. When phentolamine was infused it was 2.1 ± 0.6 ml/min/g. Thus, it appears that pancreatic blood flows, measured during the i.c. infusion of phentolamine, tended to be higher than when saline was infused, a possible indication of systemic spillover of phentolamine from the coronary circulation.

Table 4 shows the mean subepicardial, subendocardial and transmural blood flows in the circumflex perfusion territory after 5 minutes of i.c. infusion of either saline (control condition) or phentolamine (experimental condition) prior to the LADO and CxPO (baseline), and after 1 minute of the LADO and CxPO (occlusion). During the occlusion, subepicardial flow was not significantly different from the pre-occlusion baseline (ANOVA: time effect; $F = 0.84$, $p = 0.37$) while subendocardial blood flow increased significantly from baseline (ANOVA: time effect; $F = 4.34$, $p = 0.05$). However, the subendocardial:subepicardial flow ratio in the myocardium perfused by the

circumflex vessel did not change significantly from baseline (ANOVA: F = 0.87, p = 0.36). There was a tendency for transmural myocardial blood flow to increase from baseline during the occlusion

Table 4. Regional myocardial blood flows measured in the circumflex perfusion territory after 5 minutes of the respective infusions (baseline) and after 1 minute of ischemia (occlusion) of the anterior free wall of the left ventricle.

INFUSION	REGION	BASELINE	OCCLUSION
0.9% SALINE (0.25 ml/min)	SUBEPICARDIUM	0.93 ± 0.13	1.16 ± 0.15
	MIDWALL	1.15 ± 0.15	1.49 ± 0.23
	SUBENDOCARDIUM	1.12 ± 0.08	1.68 ± 0.17[a]
	TRANSMURAL	1.08 ± 0.09	1.45 ± 0.10[b]
	ENDO:EPI RATIO	1.28 ± 0.17	1.57 ± 0.28
PHENTOLAMINE (0.5 µg/kg/min) (0.25 ml/min)	SUBEPICARDIUM	0.95 ± 0.11	0.96 ± 0.13
	MIDWALL	1.19 ± 0.11	1.15 ± 0.07
	SUBENDOCARDIUM	1.26 ± 0.20	1.32 ± 0.12[a]
	TRANSMURAL	1.14 ± 0.11	1.15 ± 0.09[b]
	ENDO:EPI RATIO	1.37 ± 0.19	1.51 ± 0.26

Values are mean ± SE. Units are ml/min/g, no units for ratios.
n = 5; a - ANOVA, p = 0.05 occlusion vs baseline; b - ANOVA, p = 0.06 occlusion vs baseline.

(ANOVA: time effect; F = 4.09, p = 0.06). In contrast to when saline was infused, during phentolamine infusion no differences in the regional blood

flows of the posterior wall of the left ventricle were evident between the baseline flows and the flows measured during the occlusion (Table 4).

Table 5 shows the mean subepicardial, subendocardial and transmural blood flows in the LAD perfusion territory after 5 minutes of i.c. infusion of either saline (control condition) or phentolamine (experimental condition) prior to the LADO and CxPO (baseline), and

Table 5. Regional myocardial blood flows measured in the LAD perfusion territory after 5 minutes of the respective infusions (baseline) and after 1 minute of ischemia (occlusion) of the anterior free wall of the left ventricle.

INFUSION	REGION	BASELINE	OCCLUSION
0.9% SALINE (0.25 ml/min)	SUBEPICARDIUM	0.68 ± 0.08	0.12 ± 0.07[a]
	MIDWALL	1.20 ± 0.24	0.15 ± 0.05[a]
	SUBENDOCARDIUM	1.11 ± 0.17	0.05 ± 0.02[a]
	TRANSMURAL	1.00 ± 0.16	0.11 ± 0.04[a]
	ENDO:EPI RATIO	1.61 ± 0.11	0.90 ± 0.51[b]
PHENTOLAMINE (0.5 µg/kg/min) (0.25 ml/min)	SUBEPICARDIUM	0.79 ± 0.08	0.16 ± 0.07[a]
	MIDWALL	1.35 ± 0.21	0.17 ± 0.05[a]
	SUBENDOCARDIUM	1.27 ± 0.15	0.08 ± 0.03[a]
	TRANSMURAL	1.13 ± 0.12	0.14 ± 0.04[a]
	ENDO:EPI RATIO	1.65 ± 0.25	0.73 ± 0.30[b]

Values are mean ± SE. Units are ml/min/g, no units for ratios.
n = 4. a - ANOVA: p = 0.0001 occlusion vs baseline. b - ANOVA: p = 0.03 occlusion vs baseline.

after 1 minute of the LADO and CxPO (occlusion). The LADO and CxPO resulted in significant decreases in subendocardial and subepicardial blood flows (ANOVA: time effect; $F = 92.93$, $p = 0.0001$ and $F = 62.79$, $p = 0.0001$, respectively). As would be expected, transmural blood flow also decreased significantly indicating severe transmural myocardial ischemia (ANOVA: time effect; $F = 82.73$, $p = 0.0001$). The subendocardial:subepicardial flow ratio in the severely ischemic region of the anterior wall of the left ventricle decreased significantly (ANOVA: time effect; $F = 6.26$, $p = 0.03$). This is indicative of a proportionately greater decrease in subendocardial blood flow than subepicardial blood flow. Intracoronary infusion of phentolamine had no effect on the regional blood flows of the severely ischemic myocardium.

Table 6. Anterior:posterior transmural myocardial blood flow ratios measured after 5 minutes of the respective infusions (baseline) and after 1 minute of ischemia (occlusion) of the anterior free wall of the left ventricle.

INFUSION	BASELINE	OCCLUSION
0.9% SALINE (0.25 ml/min)	0.93 ± 0.10	0.08 ± 0.03[a]
PHENTOLAMINE (O.5 μg/kg/min) (0.25 ml/min)	0.98 ± 0.12	0.11 ± 0.03[a]

Values are mean ± SE; n = 4. a - ANOVA: $p = 0.0001$ occlusion vs baseline.

To detect changes in the distribution of myocardial blood flow between the collateral-dependent myocardium and the collateral-independent myocardium, the anterior:posterior transmural blood flow ratio was used. Table 6 presents the transmural blood flow ratio data.

As would be expected from the decrease in the transmural blood flow in the anterior region but not in the posterior region, this ratio decreased significantly as a result of the occlusion (ANOVA: time effect; $F = 121.17$, $p = 0.0001$). No significant effect of the phentolamine infusion is evident when compared to the infusion of saline (ANOVA: drug effect; $F = 0.32$, $p = 0.58$).

Table 7. Cardiac output for each dog in response to 1 minute of regional myocardial ischemia.

DOG	Q_{SB}	Q_{SO}	Q_{PB}	Q_{PO}
35	960	538	888	794
33	952	522	816	734
32	980	362	555	619
34	1187	641	671	1015
36	377	720	687	759
MEAN ± SEM	891 ± 136	557 ± 61	723 ± 58	796 ± 58

Units are ml/min. Q_{SB} = baseline cardiac output, saline infusion; Q_{SO} = cardiac output after 1 minute of ischemia, saline infusion; Q_{PB} = baseline cardiac output, phentolamine infusion; Q_{PO} = cardiac output after 1 minute of ischemia, phentolamine infusion.

By using the dilution technique with the radioactive microspheres as the indicator, cardiac outputs were calculated for each of the 5 dogs. Table 7 depicts the cardiac outputs for each dog after 5 minutes of the respective infusions and after 1 minute of the LADO and CxPO. The means ± SEM are also shown.

Eighteen out of 20 measurements yielded cardiac outputs of less than 1 l/min. Since the mass of the dogs ranged from 22 kg to 31 kg (mean: 26.8 ± 1.0 kg), it appears that these values are extremely low. It should be noted, however, that 1 minute of regional myocardial ischemia tended to reduce cardiac output in 4 out of 5 dogs when saline was infused and in 2 out of 5 dogs when phentolamine was infused. The validity of these cardiac output values are discussed at length in Chapter 5.

CHAPTER 5

DISCUSSION

A sympathetically mediated cardiocardiac reflex in response to coronary artery occlusion was first proposed by Malliani et al (1969). Measuring the firing rates of preganglionic fibers from the T3 rami communicantes in cats, they showed an increase in nerve activity during 20 - 90 seconds of ischemia in spinalectomized, vagotomized cats. Reflex increases in sympathoexcitatory responses mediated by cardiac sympathetic afferents have also been reported in anesthetized, vagotomized, sinoaortic denervated dogs (Minisi and Thames,1991). Both mechanosensitive and chemosensitive sympathetic afferent fibers are excited as a result of the ischemia (Malliani et al,1973; Uchida and Murao,1974).

The goal of the present study was to determine whether such a cardiocardiac excitatory reflex could be demonstrated in conscious resting dogs and, if so, how the increase in cardiac sympathetic efferent nerve activity affects regional myocardial blood flow. Within the confines of investigating a single reflex in an intact animal, and based on the increase in heart rate (HR) observed during the time control experiments in response to acute myocardial ischemia (Peterson et al,1973), it appears that cardiac sympathetic efferent

nerve activity may well have increased as a result of the regional myocardial ischemia in the conscious intact dogs used in the present study.

Increases in cardiac efferent nerve activity have been shown to alter regional myocardial blood flow (Drinkhill et al,1989; Nathan and Feigl,1986; Westby et al,1992). An increase in sympathetic efferent activity to the heart, as suggested by the tachycardia observed in the present study, would be expected to result in an α-adrenergic coronary constrictor tone, which would become evident when α-adrenergic receptors in the coronary vasculature were blocked by the intracoronary infusion of phentolamine. By blocking the α-adrenergic receptors, the constrictor tone would be removed and an increase in myocardial blood flow would be expected.

To isolate the α-adrenergic receptor-mediated coronary vasoconstrictor tone from any other autonomic neural influences, either direct or indirect, the animals were studied after systemic ß-adrenergic and muscarinic receptor blockade, which is referred to as double blockade. This is of particular importance when the level of sympathetic efferent nerve activity is relatively low and may, therefore, not induce a sufficiently strong α-adrenergic coronary constriction to successfully compete with metabolic vasodilation of the coronary vessels. Indeed, many studies concerning coronary α-adrenergic receptor-mediated constrictor tone have been conducted after double blockade (Nathan and Feigl,1986; Heusch et al,1984; Westby et al,1992). Despite these efforts, the effect of the potential increase in cardiac sympathetic

efferent nerve activity on regional myocardial blood flow was not clearly answered by this study.

A. Cardiac Sympathetic Afferent Activation

The present study was based on successful activation of cardiac sympathetic afferent nerve traffic. Without activation of these cardiac afferents, no ischemia-induced neural impulse would travel to the spinal cord or to the brainstem to be integrated, before exiting as an increase in sympathetic efferent nerve activity. Activation of sympathetic efferent nerves as a result of myocardial ischemia has been documented in anesthetized preparations (Heusch et al,1985; Gatenberg and Hageman,1991), in which the sinoaortic baroreceptors were denervated (Minisi and Thames,1991), a vagotomy had been performed (Felder and Thames,1981) or the spinal cord had been cut (Malliani et al,1969; Felder and Thames,1981). Thus, it is imperative that the 2.5 minutes of myocardial ischemia as used in the present study provided appropriate stimuli to activate the afferents. As will be discussed below, such stimuli were present.

Since sympathetic afferent fibers originate in the subepicardium (Barber et al,1984), transmural myocardial ischemia is required to activate them. Also, canine hearts posses an extensive collateral circulation (Schaper,1971). Therefore, to produce transmural ischemia, stenosis of the circumflex vessel had to be used in conjunction with occlusion of the left anterior descending

artery (LAD). The stenosis reduced the pressure distal to the occluder (Figures 8 and 17), thereby decreasing the collateral perfusion pressure. As a result, the pressure gradient between the collateral-dependent (anterior) and collateral-independent territories was reduced resulting in less collateral flow. Minisi and Thames (1991) have shown this technique to be effective in producing a greater degree of ischemia in the collateral dependent myocardium relative to an LAD occlusion alone. In the present study, this technique reduced transmural flow in the anterior wall of the left ventricle to 11% of baseline flow in the control condition (saline infusion) and to 12% of baseline flow in the experimental condition (phentolamine infusion). These flows compare favorably with those measured by Minisi and Thames (1991), who reported flows in the ischemic region that were less than 20% of the nonischemic baseline flows. Clearly, transmural ischemia was induced in the present study and, therefore, the sympathetic afferents were probably activated via stimulation of cardiac chemoreceptors (Uchida and Murao,1974).

The fact that there was a significant drug effect on mean coronary blood pressure (MCBP) during the ischemic period of the double blockade protocol (Figure 17) implies that a greater degree of stenosis of the circumflex vessel was induced in the phentolamine infusion condition. Prior to the combined occlusion and stenosis, mean coronary blood flow (MCBF) appeared to be higher, albeit not statistically so. The latter may be explained by the higher contractility observed in the phentolamine infusion condition (Figure 13) and

the higher left ventricular end-diastolic pressure (LVEDP) during the ischemic period (Figure 12). A higher LVEDP would increase the tension of the myocardium by La Place's law. Since the inotropic state and the tension of the myocardium are determinants of myocardial oxygen demand (Feigl,1983), and coronary blood flow is proportional to myocardial oxygen demand (Feigl,1991), flow would be expected to be a little higher. It should be noted, though, that MCBF in the saline and the phentolamine infusion conditions were not statistically different. It is possible that a greater degree of stenosis was necessary to reduce the compensatory increase in nonischemic regional contractile function to pre-ischemic baseline levels. This increase in regional contractile function could have been potentially greater in the phentolamine infusion condition because of the higher inotropic state to begin with. The fact that the inotropic state of the left ventricle and the MCBF appeared higher prior to beginning the phentolamine infusion suggests that the difference between the phentolamine infusion condition and the saline infusion condition may not be due to the drug itself but a result of different degrees of excitement.

Myocardial ischemia stimulates cardiac afferents via ventricular chemoreceptors and mechanoreceptors (Hainsworth,1991). Cardiac mechanoreceptors were probably activated in this study since LVEDP was elevated during the 2.5 minutes of ischemia in all experimental protocols. Cardiac sympathetic afferent activity increases with an increase in heart size (Brown,1983). No appropriate measurement was made to demonstrate

chemoreceptor stimulation. However, chemoreceptors were probably also stimulated in addition to the mechanoreceptors because myocardial ischemia results in the release of prostaglandins (Berger et al,1976), thromboxanes (Hirsch et al,1981) and bradykinin (Kimura et al,1973), which have been shown to stimulate chemosensitive afferent nerve endings (Uchida,1979).

Assuming appropriate activation of cardiac sympathetic afferent nerve fibers, the impulses will then be integrated in the spinal cord and/or the brainstem and, thereby, influence the sympathetic efferent nerve activity to the heart. Although left atrial pressure (LAP) was not measured, it is feasible that the increase in LVEDP may have resulted in an increase in LAP. This may have stimulated vagal afferent fibers which may also increase cardiac sympathetic efferent nerve activity (Drinkhill et al,1989). Clearly, appropriate stimuli appear to be present to induce an increase in cardiac sympathetic afferent activity by activating both cardiac mechanoreceptors and chemoreceptors.

B. Cardiac Sympathetic Efferent Activation ?

In the absence of combined systemic ß-adrenergic receptor and muscarinic receptor blockade, myocardial ischemia resulted in a mean increase in HR of 25 b/min in the time control condition and an increase of 20 b/min in the phentolamine infusion condition (Figure 1). Similar increases in HR could not have occurred in the double blockade experiments, since

myocardial ß-adrenergic receptors were blocked. However, based on the similar responses of global and regional contractile function to ischemia in both the double blocked and unblocked experiments, the changes in cardiac efferent nerve activity should have been comparable. This increase in HR during the period of myocardial ischemia of the anterior wall of the left ventricle in the time control experiments could be a consequence of either vagal withdrawal or an increase in cardiac sympathetic efferent nerve activity, or both. The increase in sympathetic efferent nerve activity could be due to unloading of arterial baroreceptors due to decreases in mean arterial pressure (MAP) and pulse pressure (PP), as a consequence of an ischemia-induced suppression of cardiac contractile function and an increase in HR, respectively. Activation of ventricular reflexes via activation of low pressure receptors in response to the increase in LVEDP as well as the activation of chemoreceptors located near the coronary vessels can also increase cardiac sympathetic efferent nerve activity (Brown,1983). It should be acknowledged from the outset that the influence of vagal withdrawal on the changes seen in HR cannot be adequately addressed by the data reported in this study but must nevertheless be taken into consideration as a potential facilitatory mechanism. Indeed, Schwartz et al (1973) have documented vagal withdrawal to occur simultaneously with increases in cardiac sympathetic efferent nerve activity in response to stimulation of cardiac sympathetic afferent nerve fibers. Thus, although there may have been some vagal withdrawal due to the ischemia, an

increase in cardiac sympathetic efferent nerve activity during the ischemic period was probably also present. It is now clear that the interaction of the vagal and sympathetic efferent nerves provides an intriguing problem which needs to be overcome when performing studies of this nature in conscious, neurally intact preparations. Clearly, for a more complete answer to the mechanism of a potential cardiocardiac reflex in an intact, conscious preparation, the influence of these 2 branches of the autonomic nervous system need to be separated.

The role of the arterial baroreflexes in increasing cardiac sympathetic efferent nerve activity cannot be ignored. Therefore, the gain of the HR response to changes in MAP was derived from the changes in MAP and HR in response to an intravenous bolus (100 μg) of phenylephrine in each dog. A mean gain of 1.29 ± 0.51 b/min/mmHg was observed and implies that for every 1 mmHg change in MAP, the HR would change by 1.29 b/min. In the time control protocol in which no intracoronary infusion was performed, an increase in HR of 26 b/min was observed after 1 minute of ischemia and 23 b/min after 2.5 minutes of ischemia. Based on the closed loop gain calculation, if the change in HR was entirely due to the baroreflexes, then a decrease in MAP of approximately 20 mmHg and 17 mmHg, respectively, would be expected. In contrast, the actual decrease in MAP was 3 mmHg at both time intervals. The latter calculation suggests that the baroreflexes may

not be the sole mechanism for the change in cardiac sympathetic efferent nerve activity.

In both the time control and the double blockade experiments there were no significant changes in either MAP. The significant reduction in PP in the double blockade experiments was a result of the increase in HR after muscarinic blockade was achieved prior to ischemia being induced. There were no further changes in PP. In addition, as shown in the tracings depicted in Figures 1, 2, 12 and 13, no transient fall in arterial pressure is evident at any stage during the occlusions. Respiratory oscillations are visible in Figure 3. Care should be taken to observe the portion of the tracing immediately prior to the start of the occlusion, indicated by the first arrow at the bottom of the appropriate panel. Therefore, although the role of the baroreflexes in increasing sympathetic efferent nerve activity to the heart cannot be discounted, it appears that other mechanisms are also be involved, possibly acting additively to result in a greater increase in HR than would be expected if only arterial baroreflexes were involved. Since LVEDP increased significantly (Figures 5 and 12) and regional blood flow measurements indicate transmural myocardial ischemia, then activation of cardiac afferent mechanoreceptors and chemoreceptors may also play a role (Brown,1983). Since stimulation of vagal afferents tend to result in bradycardia and hypotension (Brown,1983), it is probably the sympathetic afferent pathway which has been activated in the present study by stretch and ischemia of the myocardium.

It is also important to verify that intracoronary infusion of phentolamine had no systemic effects since this would lower MAP and activate the arterial baroreflexes. Since there was no statistical difference between MAP and PP measured during the intracoronary infusion of phentolamine and that measured during the time control or during the intracoronary saline infusion, systemic effects of phentolamine appear to have been effectively avoided. The possibility of systemic spillover of phentolamine from the coronary circulation was tested for by injecting a bolus of phenylephrine (100 μg) intravenously, prior to starting the intracoronary infusion of phentolamine and after 20 minutes of the infusion. It should be noted, however, that there are limitations to the effectiveness of this test. By using a submaximal dose of phenylephrine, not all the vascular α-adrenergic receptors would be stimulated. Therefore, a small degree of systemic spillover of phentolamine from the coronary circulation would not be detected since the phentolamine would only block a small proportion of the total number of vascular α-adrenergic receptors, leaving sufficient receptors unblocked for phenylephrine to stimulate and, thereby, result in the same increase in MAP as prior to there being any spillover. Nevertheless, the mean increase in MAP in response to the phenylephrine (100 μg i.v.) before the infusion was 28 ± 7 mmHg and the increase in MAP after the infusion was 22 ± 3 mmHg. The fact that the increase in MAP after the 20 minutes of phentolamine infusion was 6 mmHg less than the increase observed before the infusion was started can not be

ignored as a possible indication of systemic spillover. However, it should be noted that MAP was not lower during the phentolamine infusion conditions than in the conditions in which either saline was infused intracoronary or no intracoronary infusion was administered. In addition, the responses of MAP to the ischemia during the phentolamine infusion conditions and the respective control conditions were also not different.

Systemic spillover of phentolamine from the coronary circulation may be evident in the pancreatic blood flows which were measured in 3 dogs. The small sample size limits the value of doing any statistical comparisons; nevertheless, it appears that the mean baseline flow during the phentolamine infusion condition was slightly higher than the mean baseline flow during the saline infusion condition (3.0 ± 0.9 ml/min/g vs 1.9 ± 0.7 ml/min/g, respectively). This may be a result of systemic spillover of phentolamine from the coronary circulation. The phentolamine would block the α-adrenergic receptors in the pancreatic vasculature and, thereby, result in vasodilation due to removal of constrictor tone mediated by sympathetic stimulation of α-adrenergic receptors.

A similar trend (though not statistically significant in the 5 dogs) was evident in the baseline kidney flows (Table 3). However, it should be noted that the flows measured during the ischemic period in the phentolamine infusion condition were of almost the same magnitude as the corresponding flows measured during the saline infusion condition. If the systemic spillover of

phentolamine from the coronary circulation had indeed blocked systemic α-adrenergic receptors, one might expect that the flows during the ischemic period would also be higher than the corresponding flows in the saline infusion condition, since perfusion pressure (as measured by MAP) probably did not change significantly. Therefore, systemic spillover of phentolamine can not be discounted, however, the magnitude of such a spillover appears to be small.

C. Posterior Regional Contractile Function

Regional contractile function in the circumflex perfusion territory was used as an index to gauge the extent of the circumflex stenosis. Ischemia of the anterior region of the left ventricle resulted in a compensatory increase in the contractile function of the remote posterior region (Figure 1, panel A) (Hexeberg et al,1991; Marino et al,1989). As a consequence, myocardial blood flow increases due to metabolic vasodilation (Matre et al,1987). This increase in blood flow serves to meet the increase in myocardial oxygen demand due to the increase in regional work. Posterior regional contractile function was thus reduced as a result of limiting blood flow to the area i.e. as a consequence of ischemia. In the present study, the circumflex artery was stenosed such that the compensatory increase in myocardial contractile function of the posterior wall of the left ventricle was abolished and posterior percent ejection shortening (%SL) was maintained at baseline levels. Since there were no changes in posterior %SL or in posterior $-dL/dt_{max}$ during the

ischemic period in any of the 4 experimental conditions, it appears that the stenosis was well controlled.

Posterior %SL appeared to decrease in the double blockade experiments prior to the ischemic episode. This was probably a consequence of muscarinic blockade which increased HR thereby causing the apparent reduction in posterior %SL. Therefore, the change in posterior %SL in the double blockade experiments was not a consequence of the ischemia. The higher $-dL/dt_{max}$ measured during the phentolamine infusion condition of the double blockade experiments was present prior to starting the infusion and was not changed by the infusion. Therefore, it was not a consequence of the blockade of α-adrenergic receptors in the circumflex perfusion territory. Excluding the significant decrease in posterior %SL prior to the ischemic episode and the higher $-dL/dt_{max}$ of the phentolamine infusion group prior to beginning the infusion, posterior regional contractile function in the double blockade experiments was unchanged.

D. Anterior Regional Contractile Dysfunction

Typical responses to acute myocardial ischemia in this region were observed in all the experimental protocols (Kaspar et al,1975; Vatner,1980; Akaishi et al,1986). Severe regional contractile dysfunction in the LAD perfusion territory was evident in the form of substantial decreases in %SL and $-dL/dt_{max}$. Therefore, it appears that the 2.5 minute LAD occlusion with mild

stenosis of the circumflex artery induced severe regional ischemia of the anterior wall of the left ventricle. Indeed, the regional contractile dysfunction was associated with severe transmural myocardial ischemia (Table 3). Measurement of regional blood flows during the double blockade experiments showed severe ischemia in both the subendocardium and subepicardium during both the saline and the phentolamine infusion conditions. Transmural flow was, therefore, also substantially decreased. Further discussion of regional blood flows appears in section (G) below.

E. Global Contractile Function

Global contractile function was not significantly affected by the ischemia in the absence of systemic autonomic blockade. Although there were decreases in both the maximal rate of left ventricular pressure rise ($+dP/dt_{max}$) and fall ($-dP/dt_{max}$), statistical significance was not reached for either the control (no infusion) or the phentolamine infusion conditions (Figure 2). Thus, left ventricular myocardial contractility was not significantly suppressed by the ischemia. In addition, the rate pressure product (RPP) did not change significantly. This implies that there was no significant change in myocardial oxygen consumption. The increase in HR was apparently insufficient to significantly alter myocardial oxygen consumption. Others have also reported regional contractile dysfunction to have no effect on left ventricular systolic pressure (LVSP) and $+dP/dt_{max}$ (Nagata and Lavallee,1989).

If the ischemic area had been larger in size, global left ventricular contractile dysfunction may have been greater. However, this may have reduced cardiac output to the extent of reducing MAP. A decrease in MAP would activate the arterial baroreceptors. As previously discussed, there was no significant decrease in MAP, with the small change in MAP during the ischemic period probably being insufficient in magnitude to be the only mechanism of increasing cardiac sympathetic efferent nerve activity.

Double blockade of the autonomic nervous system, with intravenous administration of propranolol and atropine, resulted in significant increases in HR and, therefore, in RPP (Figure 10 and 14). The increase in RPP was purely due to the large increase in HR, due to muscarinic receptor blockade (Donald et al,1967). LVSP did not change with autonomic blockade. No further changes in HR were expected since both myocardial ß-adrenergic receptors and muscarinic receptors had been blocked by the propranolol and atropine, respectively. When statistical analysis was restricted to data collected after the systemic autonomic double blockade had been achieved, no significant change in HR was noted ($p = 0.95$). Thus, the ischemia-induced increase in HR, as occurred in the time control experiments, was successfully abolished by the double blockade. By eliminating the ischemia-induced increase in HR, the secondary metabolic vasodilation that occurs as a consequence of increases in cardiac activity was abolished making it easier to isolate a potentially small coronary vasoconstrictor tone.

Left ventricular myocardial contractility, as measured by $+dP/dt_{max}$, was significantly suppressed in both the control and experimental groups during the 2.5 minutes of anterior wall ischemia (Figure 13A). Although $+dP/dt_{max}$ was statistically higher during the infusion of phentolamine compared to the saline infusion condition, the difference appears to be a result of higher baseline values in the phentolamine infusion condition. When analysis was restricted to data collected after double blockade had been achieved, the difference was no longer present. Therefore, the phentolamine, per se', did not appear to have a significant effect on myocardial contractility during the ischemic period and the 7 minutes of reperfusion.

LVEDP decreased significantly from baseline values after double blockade of the autonomic nervous system (Figure 12). This was a result of reduced filling time, a consequence of the higher HR. Decreases in LVEDP with increasing HR were also reported by Sasayama et al (1976).

During the ischemic period, LVEDP increased in all experimental conditions, with and without double autonomic blockade. Similar ischemia-induced increases in LVEDP have been reported by Vatner (1980) who showed a 15.1 ± 5.9 % increase in dogs with a severe stenosis of the LAD artery which completely eliminated contractile function in the ischemic region. Akaishi et al (1986) occluded the flow to the circumflex artery and reported an increase in LVEDP of 7.5 mmHg. The rise in LVEDP may cause stretch of the left ventricle

and thereby stimulate cardiac afferent nerves via ventricular mechanoreceptors (Bosnjak et al,1979; Brown and Malliani,1971; Brown,1983).

F. Time Control With and Without Intracoronary Phentolamine Infusion

There were no significant differences in the responses to acute myocardial ischemia of any variable measured when comparing the time control and the phentolamine infusion conditions, in the absence of systemic double blockade. Although the MCBF during the phentolamine infusion condition was shown to be statistically lower than when no intracoronary infusion was used, this observation is probably an error in the measurement of the control values since the flows measured in the other 3 protocols are of similar magnitude to each other and all are lower than the control MCBF measurements. In addition, regional myocardial blood flows, measured with tracer microspheres, were normal, indicating a normal coronary circulation. Prior to converting the coronary flow velocities to volume flow rate, all the flow velocities for each dog were consistently higher than the corresponding values during the phentolamine infusion condition. Therefore, the error does not appear to be a result of the mathematical conversion of flow velocity to volume flow rate. The difference does also not seem to be due to a greater degree of stenosis during the experiments in which phentolamine was infused. The latter conclusion is based on the relationship between regional contractile function in the nonischemic myocardium and the mean coronary blood pressure (MCBP),

which was measured distal to the circumflex stenosis. No difference in posterior %SL or $-dL/dt_{max}$ existed between the control (no infusion) and the phentolamine infusion conditions. If the MCBF was lower because of a greater degree of stenosis, one would expect the regional function to be less, too. In addition, one would expect the MCBP to be lower during the stenosis. This was not the case (Figure 8). The lack of effect of phentolamine on MCBF is further supported by the fact that pre-infusion MCBF was substantially lower than the control value and was very similar in magnitude to the MCBF values measured during the phentolamine infusion, throughout the entire protocol. Therefore, the reason for the higher MCBF during the control condition is unclear. It appears to be unphysiological. The present study does not provide data to explain the difference.

As a whole, intracoronary infusion of phentolamine appeared to have no significant effect on the cardiac response to acute myocardial ischemia when compared to the control condition in which no intracoronary infusion was administered. If the osmolarity of the phentolamine solution had any effect on the cardiovascular response to ischemia, it would probably have been detected when comparing the time control data with that collected during the infusion of the phentolamine. Since no differences were observed, the effect of the osmolarity of the solution apparently had no influence on the results.

Thus, with the sympathetic and the parasympathetic branches of the autonomic nervous system intact, no α-adrenergic receptor-mediated coronary

constrictor tone could be detected in this study. It may be that the ischemia-induced increase in HR and the mild degree of circumflex artery stenosis resulted in metabolic vasodilation which effectively competed with and masked the α-adrenergic receptor-mediated coronary constrictor tone. On the other hand, the apparent increase in sympathetic efferent nerve activity may not have been of sufficient magnitude to have caused any measurable α-adrenergic receptor coronary constrictor tone. Indeed, when comparing the 20 to 25 b/min rise in HR observed in this study with the 60 b/min increase reported in high demand ischemia studies performed in exercising dogs (Seitelberger et al,1988; Chilian and Ackell,1988), any ischemia-induced increase in cardiac sympathetic efferent nerve activity which may have occurred in the present study appears relatively small. Therefore, it was imperative to eliminate competitive metabolic vasodilatory effects (Mohrman and Feigl,1978), thus allowing the α-adrenergic constrictor tone to be isolated from the metabolic vasodilation. This was the rationale for the double blockade studies.

In summary, in the autonomically unblocked state, no differences between the time control (no infusion) and the phentolamine infusion conditions were evident. Therefore, an ischemia-induced α-adrenergic receptor-mediated coronary constrictor tone did not appear to be present. The α-adrenergic receptor-mediated coronary constrictor tone may have been concealed by competing metabolic vasodilation of the coronary vasculature.

G. Double Blockade - Saline and Phentolamine Infusions

In the presence of combined double blockade, the response of global left ventricular contractile function to the ischemia was unaffected by the intracoronary blockade of α-adrenergic receptors except for LVEDP. During the infusion of phentolamine in the double blockade protocol in which no regional blood flows were measured, LVEDP was significantly higher than during the infusion of saline (Figure 12). It should be noted, however, that the significant drug effect observed barely reached significance ($p = 0.05$). However, LVEDP did not differ between the saline and phentolamine infusion conditions of the double blockade protocol in which regional blood flows were measured.

When statistical analysis was restricted to data collected during the infusions, the statistical significance between the two groups in the double blockade protocol without regional blood flow measurements was lost ($p = 0.09$). Using paired comparisons of the data at each time point of the protocol (Student's t-test on ranked data), no statistical differences could be isolated. The difference in LVEDP may be explained by differences in the degree of myocardial ischemia. More severe ischemia would result in greater contractile dysfunction and hence a higher LVEDP. This does not appear to be the case in the present study since regional contractile function decreased to the same extent in both conditions, suggesting similar severity of the ischemia.

Another possible explanation for the difference between the 2 conditions is that a type I statistical error could exist due to the small sample sizes. The fact that post hoc tests did not differentiate between the 2 experimental conditions may support this explanation. Thus, it appears doubtful that the increase in LVEDP during the ischemic period was of any physiological significance. Indeed, data collected in the present study presents no physiological explanation.

Could the changes in regional blood flow affect LVEDP? Subendocardial blood flow in the posterior wall of the left ventricle increased during the occlusion in the control (saline infusion) condition but did not change when phentolamine was infused into the circumflex artery. In addition, transmural blood flow during the occlusion in the saline infusion condition showed a tendency to increase ($p = 0.06$) despite no change in subepicardial flow. Thus, the increase in transmural flow was a consequence of the change in subendocardial flow. No changes in regional contractile function were associated with these increases in regional blood flow, nor were there any differences in the regional contractile function between the saline infusion and the phentolamine infusion conditions. Therefore, it is doubtful whether an increase in LVEDP can be explained by the changes observed in subendocardial blood flow of the posterior wall of the left ventricle.

Could blockade of α_1-adrenergic receptors on the myocytes be an explanation? Myocardial α_1-adrenergic receptors play a significant role in the

inotropic state of the heart (Landzberg et al,1991; Shen et al,1989). However, the importance of α_1-adrenergic receptors in regulating myocardial contractility is species specific (Shen et al,1989; Endoh et al,1991). Ventricular α_1-adrenergic receptors in the dog heart have been shown to play a minor role in the positive inotropic response to various α-adrenergic agonists (Endoh et al,1991, 1978). Therefore, blockade of myocardial α_1-adrenergic receptors does not appear to be a suitable explanation.

Clearly, the data presented here does not provide a sufficient explanation for the significant drug effect observed in one set of double blockade experiments and not the other. The fact that no physiological explanation can be derived from this data and, that the post hoc tests were unable to differentiate between the saline and the phentolamine infusion conditions, and that significance was only barely reached with the ANOVA suggests that the differences seen in LVEDP are somewhat tenuous and warrant further investigation.

H. Cardiac Outputs and Regional Blood Flows

The cardiac output measurements, based on indirect calculations obtained during the injection of tracer microspheres, appear to be low. Gayheart et al (1989) measured the cardiac outputs of anesthetized dogs weighing 18.9 ± 1.0 (SD) kg and reported baseline values of 1.64 ± 0.21 l/min. Recent unpublished cardiac output measurements from this laboratory

in 4 conscious, resting dogs weighing 28.0 ± 4.9 kg, using a T101 ultrasonic blood flow meter (Transonic Systems, Inc., Ithaca, New York) with the transit time flow probe implanted around the ascending aorta, yielded a mean value of 2.8 ± 0.1 l/min.

The dogs used in the present study were considerably larger than those used by Gayheart et al (1989), the mean mass being 26.8 ± 1.0 kg. One would therefore expect a larger cardiac output since cardiac output increases with body size (Guyton,1986). Clearly, the cardiac output measurements in the present study were erroneously low when compared to those reported by Gayheart et al (1989). In addition, when compared to measurements made in dogs of similar size, there appears to be an even greater discrepancy.

Reasons for these low cardiac output measurements could possibly a result of inaccuracies in the handling of the radioactivity, i.e. the total amount of radioactivity injected may have been more than was calculated based on the number of counts per bead injected and/or the number of beads injected. The reference withdrawal rate was accurately calibrated prior to each experiment using a graduated 10 ml measuring cylinder. Due to the necessity of collecting data quietly and as quickly as possible, to avoid disturbing the conscious dog, withdrawal rate was not recalibrated between the 2 successive withdrawals. Nevertheless, the withdrawal rate remained constant and is, therefore, not likely to be a major source of error.

The total amount of radioactivity injected may have been more than was calculated. The spheres were withdrawn from the stock solution with either a 1 ml or a 3 ml syringe. Since the volumes in these syringes are more than likely not completely accurate, a greater number of beads may have been withdrawn from the stock solution and injected into the dogs. This could result in higher counts appearing in the reference blood samples. Thus, the ratio of total radioactivity injected to radioactivity in the reference blood samples may have been reduced, thereby resulting in the low cardiac output values reported.

To calculate the total radioactivity injected, the amount of radioactivity per bead was determined by accurately withdrawing a number of beads in a small volume from the stock solution, counting the volume and expressing the total counts per bead. If the number of beads were overestimated or if the number of counts were underestimated, the counts per bead would be low. This would further underestimate the calculated total radioactivity that was injected.

Since there appears to be substantial error in these measurements, the validity of the changes observed is questionable. Indeed, 4 out of 10 changes observed in response to the regional myocardial ischemia were increases and not decreases as might be expected, assuming sufficiently severe ischemia. In the saline infusion condition, 4 out of 5 measurements showed large decreases in cardiac output ranging from 44% to 63%. This magnitude of decrease is unlikely since global left ventricular function, as measured by $+dP/dt_{max}$ and

RPP, barely decreased during the ischemic period. Although no measurement of the ischemic area was made, occlusion of the LAD artery proximal to its first branch probably does not result in sufficiently severe regional ischemia to reduce contractile function to such an extent that decreases in cardiac output of this magnitude result. In addition, with decreases in cardiac output of this magnitude, a reduction in MAP might be expected. This does not appear to be the case. Therefore, the cardiac outputs reported in Table 7 are of questionable value to the interpretation of this study.

Flows to tissues other than the myocardium were measured in order to obtain an indication of adequate mixing of the microspheres as well as an index of the extent of first pass entrapment of the spheres. From Table 3, the right kidney (RK) flows appear lower than the left kidney (LK) flows. This is due to RK flows of one dog being lower than the LK flows of that same animal (B_s - 0.9 vs 2.1 ml/min/g; O_s - 0.3 vs 3.1 ml/min/g; B_p - 0.5 vs 2.0 ml/min/g; O_p - 0.3 vs 3.7 ml/min/g). The discrepancy between the kidney flows implies inadequate mixing of the microspheres in this one dog. However, it should be noted that flow to the skeletal muscle of the left hindlimb (LHL) and the right hindlimb (RHL) of the dog were very similar (3.9 vs 4.0 ml/min/g; 4.3 vs 4.5 ml/min/g; 2.5 vs 2.6 ml/min/g and 4.3 vs 5.0 ml/min/g). Thus, it appears that adequate mixing did indeed occur in this dog and that the discrepancy in the kidney flows may have been a result of tissue sampling error, tissue geometry during counting and/or due to infarction of kidney tissue as a consequence of

daily flushing of the aortic or left ventricular catheters. However, no regions of infarction were observed when the kidneys were cut longitudinally. Cortical flows have been shown to be higher than medullary flows (Pilkington et al,1965). Therefore, if the RK samples contained medullary tissue in error, the flows would be lower. In addition, if the tissue sample geometry within the counting well was different, i.e. the RK sample was located at a higher level than the LK sample in the counting well, the counter efficiency would be less resulting in lower flows being reported (Katz and Blantz,1972). It should be noted that the RK and LK flows of all the other dogs were similar.

Aside from the possible error in this one dog, and based on the blood flows measured in the skeletal muscle of each hindlimb as well as the two kidneys in the other dogs, it appears that adequate mixing of the spheres was indeed achieved. This implies that the concentration of microspheres in all arteries was similar and therefore the distribution of the spheres would approximate the distribution of the blood flows (Heymann et al,1977).

Also of importance is the fact that low flows were measured in the lungs (Table 3). This demonstrates successful first pass entrapment of microspheres (Heymann et al,1977). Based on the flows measured in the kidneys, the lungs and the skeletal muscle, the distribution of the radioactive microspheres in the myocardium is probably representative of the regional blood flow distribution.

Diastolic (DCR) and mean (MCR) coronary resistance were no different between the saline and the phentolamine infusion conditions during the double

blockade experiments (Figure 18). Since differences in regional flow between these conditions were so subtle (subendocardial flow only), the concomitant change in resistance was possibly too small to be detected by the calculated DCR and MCR variables. Resistances were calculated from total circumflex blood flows and circumflex blood pressures, using Ohms Law.

The highly significant depression of contractile function in the ischemic region (Figure 15) was associated with a severe reduction in subepicardial, subendocardial and transmural blood flow (Table 3). Transmural myocardial blood flow was reduced by 89% in the saline infusion condition and by 88% in the phentolamine infusion condition. Infusion of phentolamine into the circumflex artery to remove an potential ischemia-induced α-adrenergic receptor-mediated constrictor tone had no significant effect on the regional blood flow in the ischemic region. This is also reflected in the transmural myocardial blood flow ratios (Table 4). As expected, the ischemic:nonischemic transmural flow ratios decreased as a result of the occlusion, with the decrease being the same in the saline and the phentolamine infusion conditions. Therefore, the regional blood flow distribution between the anterior and posterior myocardium does not seem to be altered by blockade of α-adrenergic receptors in the circumflex perfusion territory. Chiariello et al (1977) demonstrated a "reverse coronary steal" phenomenon in anesthetized dogs in the presence of regional myocardial ischemia. Stimulation of α-adrenergic receptors in the nonischemic region caused redistribution of flow

between the ischemic and the nonischemic myocardium. Flow decreased in the nonischemic region and increased in the ischemic region. The data of the present study does not support the "reverse coronary steal" phenomenon. The major difference between the present study and that of Chiariello et al (1977) is the duration of the occlusion. The ischemic period in the present study lasted 2.5 minutes while in the Chiariello et al (1977) study it was 30 minutes in length. Therefore, the difference in the results may be attributed to there being fewer patent collaterals as a consequence of the shorter duration of the regional ischemia. Collateral blood flow was not directly measured in this study, and therefore, it is unknown if sufficient collateral vessels between the ischemic and the nonischemic myocardium were present to allow such a phenomenon to have been observed. In addition, a circumflex stenosis was imposed simultaneously to reduce collateral flow to the ischemic region and this may have limited the possibility of showing any significant redistribution between the collateral-dependent myocardium and the collateral-independent region.

Subendocardial flow increased significantly during the occlusion relative to baseline whereas subepicardial flow did not. Clearly, referring to Table 2, there appears to be a greater increase in flow in the subendocardium than in the subepicardium in the saline infusion condition during ischemia. In contrast, when phentolamine was infused into the circumflex artery to block any α-adrenergic constrictor tone, regional flows did not increase. It is

possible, therefore, that an α-constrictor tone might exist, and that it may have a differential effect across the ventricular wall. There is, however, a major inconsistency with this hypothesis.

In the normal, healthy, resting dog, no significant resting sympathetic coronary vasoconstrictor tone exists (Chilian et al,1981; Gwirtz et al,1986). In the present study, assuming that the ischemia induced an increase in cardiac sympathetic efferent nerve activity, an α-adrenergic coronary constrictor tone in a resting dog could still not be definitively demonstrated. By blocking the α-adrenergic receptors with phentolamine in the coronary circulation, a constrictor tone would be removed and a higher flow would be expected compared to the flow measured in the same region in the unblocked condition. Subendocardial flow did not increase in the present study when the α-adrenergic receptors were blocked. Therefore, if the sympathetic efferent nerve activity did indeed increase during the ischemic period, it did not appear to induce an α-adrenergic coronary constrictor tone.

Westby et al (1992) hypoperfused the left main coronary artery of anesthetized open-chest cats and blocked α-adrenergic receptors by intravenous injection of doxazosin (α_1-adrenergic receptor antagonist) followed by SK&F 104078 (α_2-adrenergic receptor antagonist). Subepicardial flow increased in the hypoperfused region while subendocardial flow decreased, thereby reducing the subendocardial:subepicardial flow ratio. Cardiac function decreased significantly when the α-adrenergic receptor-mediated constrictor

tone was blocked. Similar changes in regional blood flows were expected in the present study but clearly did not occur.

The present study failed to demonstrate a concurrent change in subepicardial flow of the posterior wall of the left ventricle when a change in subendocardial flow of the same region occurred. Recall that the subendocardial flow increased significantly during the occlusion in the saline infusion condition but appeared not to change during the occlusion when phentolamine was infused. Posterior wall subepicardial blood flow was unchanged during the occlusion in both the saline and phentolamine conditions. The fact that the subendocardial:subepicardial flow ratio was unchanged during the occlusion, relative to baseline, in the saline and the phentolamine infusion conditions suggests that no significant transmural blood flow redistribution occurred with blockade of α-adrenergic receptors in the circumflex perfusion territory. Several reasons for the difference between this study and that of Westby et al (1992) are possible. Anesthetized, open-chest preparations have greater sympathetic tone as a result of the surgical stress (Vatner and Brawnwald,1975). Therefore, in the study by Westby et al (1992), the α-adrenergic coronary constrictor tone would have been greater than in the present study in which conscious resting dogs were used. In addition, the region of ischemia in the Westby et al (1992) study was greater. Thus, a stronger stimulation of cardiac afferents may have occurred resulting in the cardiac efferent sympathetic nerve activity being greater thereby inducing a

greater degree of α-adrenergic coronary constrictor tone. Finally, if the dogs used in the present study had a more developed collateral circulation than the cats used by Westby et al(1992), then the degree of the ischemic insult would be further reduced and less activation of cardiac afferents may have resulted.

Nathan and Feigl (1986) documented a similar finding to that of Westby et al (1992). In anesthetized dogs, both the LAD and the circumflex perfusion territories were hypoperfused, and α-adrenergic blockade was restricted to the circumflex vasculature only. Comparison of the regional blood flows in the two regions during the ischemia revealed higher subendocardial flow in the LAD region where α-adrenergic receptors were intact. Chilian and Ackell (1988) demonstrated a similar effect of the α-adrenergic coronary constrictor tone on regional blood flow in exercising dogs who were subjected to a flow-limiting circumflex artery stenosis. Thus, although the present study failed to demonstrate an α-adrenergic receptor-mediated coronary constrictor tone, such a tone has previously been shown to exist in anesthetized preparations. Since the present study has several limitations which may have inadvertently prevented the uncovering of an α-constrictor tone, further studies need to be conducted to address this phenomenon in conscious animals.

There may, however, be an alternative explanation for the difference in flow in the subendocardium between the saline infusion and the phentolamine infusion conditions. Since MAP was not recorded during the regional blood flow measurements, the effect of a reduction in the perfusion pressure, i.e.

MAP, during the occlusion in the phentolamine infusion condition cannot be ignored. If MAP in the phentolamine infusion condition was lower than in the saline infusion condition after 1 minute of ischemia, the compensatory increase in flow, as occurred in the saline infusion condition, would have been less.

The increase in LVEDP during the period of ischemia would influence myocardial blood flow distribution, particularly subendocardial blood flow (Feigl,1983). A higher LVEDP during the ischemic period in the phentolamine infusion condition could explain the difference in the response of subendocardial blood flow between the 2 infusion conditions. This was not the case (Table 2). Therefore, a difference in the LVEDP response to the ischemia does not appear to explain the drug effect on regional blood flows. It appears that the data reported by the present study is insufficient to provide a suitable explanation for the apparent drug effect.

Myocardial ischemia causes NE release from the adrenergic nerve terminals in the myocardium, ultimately depleting the nerve terminals of neurotransmitter (Schomig et al,1984). This myocardial NE depletion in response to regional ischemia has been shown to occur throughout the entire myocardium (Mathes et al,1971) or only in the ischemic tissue (Holmgen et al,1981; Muntz et al,1984). If NE was indeed released from nerve terminals throughout the entire myocardium, then an elevated constrictor tone throughout the entire coronary vasculature would have existed. Similarly, if the NE release was more regional in nature (i.e. from the ischemic myocardium

only), one might expect an elevated constrictor tone in the ischemic region only. In the present study, blockade of α-adrenergic receptors in the coronary vasculature failed to result in an increase in regional blood flow. Therefore, an α-adrenergic receptor-mediated coronary constrictor tone due to the local release of NE from sympathetic nerve terminals does not appear to have occured in the present study.

In summary, the present study was unable to document the presence of an α-adrenergic coronary constrictor tone. Blockade of α-adrenergic receptors by phentolamine infused into the circumflex artery did not result in the anticipated increase in regional myocardial blood flows. Instead, there appeared to be less change in the subendocardial blood flow in the posterior wall in response to the LAD occlusion. This effect of the phentolamine could possibly be explained by a reduction in perfusion pressure which was unfortunately not measured during the regional blood flow measurements. Differences in LVEDP and hence intramyocardial pressure, do not seem to be a suitable explanation.

I. Specific Findings

This study was designed to address the controversy of whether or not myocardial ischemia in resting, conscious dogs results in an increase in cardiac sympathetic efferent nerve activity, and if an ischemia-induced increase in cardiac sympathetic efferent nerve activity was present, what effect did it

have on regional myocardial blood flow? Finally, how did the α-adrenergic receptor-mediated coronary constrictor tone affect contractile function? The basis for these questions stems from the fact that all previous studies related to this phenomenon were conducted in anesthetized preparations (Malliani et al,1969; Felder and Thames,1981; Gatenberg and Hageman,1991; Heusch et al,1985; Nathan and Feigl,1986; Westby et al,1992). Furthermore, in most of the anesthetized preparations, sinoaortic baroreceptor denervation and vagotomy (Minisi and Thames,1991), spinalectomy (Malliani et al,1969) or all three surgical interventions (Felder and Thames,1981) were performed. Clearly, such preparations are far from physiological.

It has been suggested that descending bulbospinal inhibitory pathways diminish cardiocardiac reflex activity by inhibiting the ischemia-induced increases in cardiac sympathetic afferent nerve activity (Felder and Thames,1981). Increases in sympathetic efferent nerve activity are, therefore, not seen. Since no nerve activities were measured in the present study, changes in sympathetic efferent nerve activity to the heart was indirectly assessed by changes in HR (Gayheart et al,1991). However, the contribution of vagal withdrawal must not be ignored (Schwartz et al,1973).

In the absence of any systemic ß-adrenergic or muscarinic blockade, a significant increase in HR occurred during the period of acute regional myocardial ischemia. Therefore, an increase in cardiac sympathetic efferent nerve activity accompanied by vagal withdrawal may have, indeed, resulted.

These changes in cardiac sympathetic and vagal efferent nerve activity were probably due to the regional ischemia activating cardiac sympathetic afferents. The cardiac afferent nerve activity may, therefore, not have been inhibited by descending bulbospinal pathways at the spinal cord level, as proposed by Felder and Thames (1981).

Since all nerves were intact in the present study, it is most likely that the myocardial ischemia activated more than just cardiac sympathetic afferent nerves. Vagal afferents may also have been activated (Hainsworth,1991). In this regard, Lombardi et al (1984) demonstrated that both vagal and sympathetic afferents are stimulated by a 35 second period of "global" ischemia induced by occlusion of the left main coronary artery, as well as by 65 to 70 seconds of "regional" ischemia induced by occlusion of the distal portion of the LAD coronary artery. Stimulation of vagal afferent nerve activity results in a depressor response (Malliani,1982). At the same time, vagal afferents inhibit sympathetic efferent nerve activity (Lombardi et al,1984). Therefore, it is possible that the regional myocardial ischemia in the present study might have resulted in a greater α-adrenergic constrictor tone had the vagi been sectioned to remove their inhibitory influence on sympathetic efferent nerve activity. Thus, in the neurally intact animal, cardiac afferent nerve activity may well be modulated by descending bulbospinal pathways. However, the effect of the descending inhibitory pathways do not appear to abolish cardiac efferent nerve activity. The present study cannot discount the

influence of activation of vagal afferents. Hence, the vagal efferent nerve activity may have also limited the magnitude of the increase in cardiac sympathetic efferent nerve activity.

J. Limitations and Advantages

Using conscious neurally intact dogs to investigate a single reflex which forms part of a more complex cardiac reflex system, makes interpretation of data very difficult. Activation of a single neural reflex cannot be distinguished from activation of a multitude of other reflexes. Therefore, based on indirect indexes and previously published literature, changes in reflex activity could only be assumed to have occurred. It is ,therefore, unfortunate that cardiac afferent and efferent nerve activities were not directly measured. To date, chronic long-term recordings of nerve activity in conscious animals have not been obtained due to the technical difficulty of performing chronic nerve recordings.

When examining a sympathetic reflex, any form of movement or excitation of the dog would alter the response. Performing multiple studies on conscious, resting dogs can be troublesome. All the dogs used in this study were well trained to lie still on a customized, cushioned table, and care was taken to restrict the number of personnel in the laboratory to a minimum. However, even after having taken such precautions, many experiments had to be repeated due to the dog moving at inappropriate times. Having to

periodically repeat studies in which the dog moved increases the chance for deterioration of the chronically implanted instrumentation. Piezoelectric crystal function tended to be the most troublesome. One dog had total malfunction of the anterior segment length crystals.

Intracoronary infusion of phentolamine poses a potential problem since there is a possibility of spillover into the systemic circulation may have occurred. This was discussed at length earlier.

Despite these limitations, the advantages of using a chronically instrumented, conscious dog model lie in the fact that each dog can serve as its own control, thereby reducing variability. No complications of sedation or anesthesia are present, and all nerves and regulatory reflexes are intact. The chronically instrumented, conscious dog is, therefore, a more physiological model than an anesthetized, open-chest preparation in which various denervations have been performed.

K. Clinical Relevance

Vasospastic myocardial ischemia has been documented in humans (Maseri et al,1978). In addition, stimulation of α-adrenergic receptors by the sympathetic nervous system has been shown to increase the potential for dysrhythmias (Sheridan et al,1980). Redistribution of myocardial blood flow towards the subendocardium may limit the potential for developing life threatening dysrhythmias.

L. Future Directions

Of prime importance is the chronic measurement of cardiac nerve activity. Measurement of both the cardiac afferent and the efferent nerve activities would greatly enhance the clarity of studies which are based on changes in nerve activities in response to myocardial ischemia. Thus, a technique needs to be developed to measure chronic nerve activities.

M. Conclusions

Within the limitations of investigating neural reflexes in a conscious animal, the first null hypothesis can only be tentatively rejected since no direct measurements of sympathetic efferent nerve activity was made in the present study. Acute regional myocardial ischemia did result in increases HR during the period of ischemia but the relative roles of vagal withdrawal and increases in cardiac sympathetic efferent nerve activity are unknown. Based on reports by others, one could speculate that both vagal withdrawal and an increase in cardiac sympathetic efferent nerve activity occurred as a result of the ischemia.

Assuming that cardiac sympathetic efferent nerve activity did increase, the data failed to support the rest of the null hypotheses. No ischemia-induced α-adrenergic receptor-mediated coronary constrictor tone could be demonstrated. No single explanation can be provided for the changes seen in subendocardial blood flow in the nonischemic myocardium.

The distribution of blood flow between the ischemic and the non-ischemic myocardium was not affected by the presence or the absence of an α-adrenergic receptor-mediated coronary constrictor tone. The transmural distribution was also not affected. In addition, the changes in regional blood flow distribution during the ischemic period did not alter regional myocardial contractile function of the ischemic or the non-ischemic myocardium.

Thus, the phenomenon of an ischemia-induced increase in cardiac sympathetic efferent nerve activity via activation of a cardiocardiac reflex cannot be fully addressed by the present study. Further investigation in conscious animals with closer attention to isolating the various afferent and efferent nerve activities is necessary to further explain this phenomenon.

APPENDIX A

PRELIMINARY STUDY

INTRODUCTION

During experiments using conscious, resting and exercising dogs, an increase in heart rate (HR) after an intracoronary (i.c.) injection of prazosin (0.5 mg) was occasionally observed (Meintjes, personal observation). Gwirtz et al (1986) reported no significant change in HR after i.c. injection of prazosin in exercising dogs. Pre-prazosin HR during exercise was 213 ± 8 b/min (mean ± SE) and post-prazosin HR was 220 ± 7 b/min, a 4 % difference. Interpretation of the circumflex blood flow (CBF) and contractile function data by Gwirtz et al (1986) was discussed at length by Heusch et al (1991) who proposed that the increase in CBF was due to metabolic vasodilation, secondary to an increase in contractile function and may not be a consequence of the removal of a flow-limiting α_1-adrenergic receptor-mediated coronary constrictor tone. They proposed that the change in contractile function was due to a prazosin-induced increase in presynaptic release of norepinepine (NE).

Dai et al (1989) reported no significant difference in the reponse of HR to submaximal exercise in dogs before and after i.c. α_1- or α_2-adrenergic receptor blockade. However, the HR appeared to be consistantly higher than control in the dogs subjected to i.c. infusion of prazosin, albeit not significantly so. It may be that the prazosin has a small effect on the release of norepinepine from presynaptic sympathetic nerve terminals resulting in an increase in norepinepine release, and that the magnitude of this effect is too

small to alter cardiac flow and contractile function. However, Heyndrickx et al (1984) showed no significant effect of prazosin on NE difference across the heart. Intravenous administration of prazosin during exercise in dogs did not affect MCBF or left ventricular contractile function.

In the resting unanesthetized dog no resting sympathetic tone exists (Chilian et al, 1981). Therefore, i.c. α_1-adrenergic blockade should have no effect on coronary hemodynamics or myocardial contractile function. If prazosin does increase the release of norepinepine from the nerve terminals, then a change in CBF and myocardial contractile function may occur. This assumes that the "prazosin-induced" release of NE is large enough to provide sufficient stimulation of myocardial β_1-adrenergic receptors to increase cardiac contractile function and, thereby, cause metabolic coronary vasodilation.

Based on the above observations and on the proposal of Heusch et al (1991) of a presynaptic α_1-adrenergic receptor, this supplemental study was designed to investigate the effect of an i.c. bolus of prazosin (0.5mg) on NE spillover across the heart in the resting and the exercising dog. The null hypothesis tested was that i.c. prazosin does not increase NE spillover across the heart.

METHODS

A. Surgical Preparation

The same 8 dogs, instumented for the primary study, were used for this study. The surgical preparation was therefore the same and is described in detail in Chapter 3.

B. Experimental Protocol

It is possible that α_1-adrenergic receptors are indeed located on the presynaptic nerve terminal and thus play a role in the autoinhibition of NE release from the sympathetic nerve terminals. In addition, it is possible that the α_1-adrenergic receptor antagonists also block presynaptic α_2-adrenergic receptors because antagonists are only relatively selective. Either mechanism could reduce the autoinhibition of NE release resulting in an increase in the myocardial NE spillover. Experiments were designed to examine the changes in myocardial NE spillover in response to i.c. injection of a specific α_1-adrenergic receptor antagonist (prazosin) in both resting and exercising chronically instrumented dogs.

1. Resting studies

Eight dogs were used for the resting studies. Catecholamine analyses were successful in 5 out of these 8 dogs. To ensure resting conditions, the dog was studied in a quiet, darkened laboratory while either suspended in a sling or lying on a cushioned table. The same position was used for each dog

in all experiments. A dog with a HR of less than 100 b/min was considered to be in rested state (Vallance et al,1990). When the dog was sufficiently calm, control data were recorded, and arterial and venous blood samples withdrawn (6 ml each) from the aortic and coronary sinus catheters respectively. Sterile water (0.5 ml followed by a 1.5 - 2.0 ml heparinized saline flush) was then injected i.c.. Data were collected and blood samples withdrawn 1 minute after the sterile water injection. Prazosin (0.5 mg in 0.5 ml of sterile water followed by a 1.5 - 2.0 ml flush with heparinized saline) was then injected into the circumflex coronary artery. One minute post-prazosin injection, data were recorded and blood samples withdrawn as described previously. Thus, a total of 36 ml of blood was withdrawn during this protocol.

The same protocol was followed to test the effect of the nonspecific α-adrenergic receptor antagonist phentolamine on cardiac NE spillover. Instead of sterile water being used as the vehicle, 0.9% sterile saline was injected i.c.. Each dog was studied under resting conditions as described previously (HR < 100 b/min). Data were recorded and blood samples withdrawn for control, 1 minute after the i.c. injection of 0.5 ml 0.9% sterile saline (phentolamine vehicle) as well as 1 minute after i.c. injection of phentolamine (1.0 mg in 0.5 ml 0.9% sterile saline followed by a 1.5 - 2.0 ml flush with heparinized saline). A total of 36 ml of blood was withdrawn. A total of 3 dogs were studied. Only descriptive statistics are reported for these dogs.

2. Exercise studies

For the exercise studies, 5 dogs were used. Each dog was initially studied in a sling or on a cushioned table in a darkened laboratory to ensure resting conditions. When the dog appeared sufficiently rested (HR < 100 b/min), data were collected and blood samples withdrawn as previously described. In the lighted laboratory, the dog was then moved to the treadmill. Data were recorded and blood samples withdrawn with the dog in the standing position on a Quinton Model 18-60 motor-driven treadmill. The dog was then subjected to a continuous incremental submaximal treadmill protocol comprising 4 workloads. The workloads included 4.8 km/h at 0% grade for 3 minutes, 6.4 km/h at 0% grade for 3 minutes, 6.4 km/h at 4% grade for 3 minutes and 6.4 km/h at 16% grade for 5 minutes. After 2.5 minutes at 6.4 km/h,16% grade data were recorded and a third set of blood samples (3 ml each) was withdrawn. After 3.5 minutes at 6.4 km/h,16% grade, prazosin (0.5 mg) was injected i.c., and 1 minute later data were recorded and a fourth set of blood samples were withdrawn (3 ml each). Thus, a total of 36 ml of blood was withdrawn during this protocol.

The same protocol was used to test the effect of i.c. injection of the nonspecific α-adrenergic receptor antagonist phentolamine on NE spillover during exercise. Data were collected and blood samples withdrawn at rest, during exercise and during exercise 1 minute post i.c. phentolamine (1 mg). A total of 36 ml of blood was withdrawn. A total of 3 dogs were studied using

i.c. phentolamine. Due to the small sample size, only descriptive statistics are reported for these dogs.

Since less plasma is required for analysis when catecholamine levels are elevated (i.e., during exercise), all blood samples withdrawn when the dogs were at rest were 6 ml in volume while the samples withdrawn during exercise were 3 ml in volume. On completion of withdrawal, each blood sample was kept in a test tube on ice containing preservative to prevent reduction and degradation of catecholamines. Test tubes for the 6 ml blood samples contained 100 μl of preservative (60 mg/ml glutathione, 90 mg/ml EGTA) while test tubes for the 3 ml blood samples contained 50 μl of preservative. The blood samples were analysed for NE by Reverse Phase High Performance Liquid Chromatography (Barron and Hexam,1985).

To prevent any potential problems associated with blood loss, these studies were separated by 1 - 2 days.

For quality control of the catecholamine analysis, multiple analyses (4) were done on a venous and an arterial blood sample withdrawn from the same dog. Figure 23 shows the mean values obtained for this analysis. As can be seen from the small standard deviation, reproducible results were obtain from the HPLC analyses of the blood samples indicating consistent catecholamine analysis.

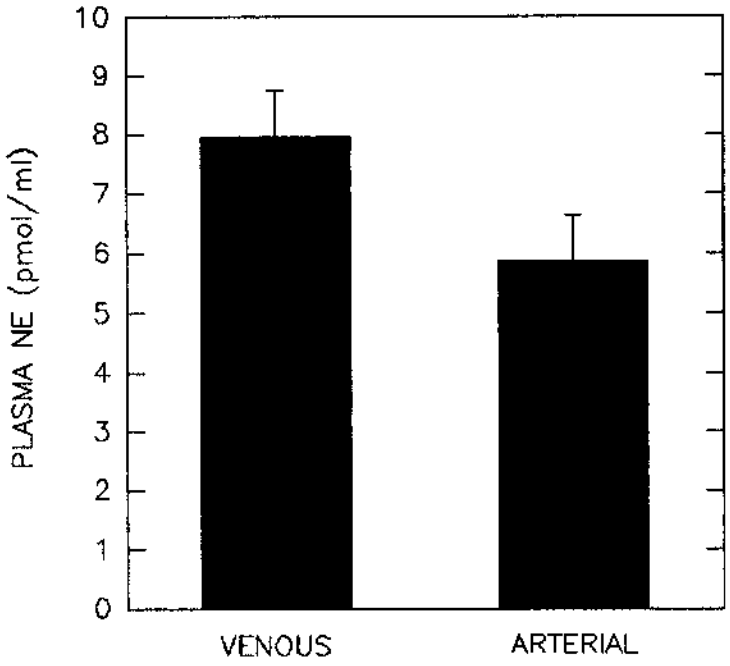

Figure 23. Plasma norepinephrine (NE) concentation from multiple analyses (n = 4) of a single venous and a single arterial blood sample from the same dog. Values are mean ± SD.

C. Data Collection and Analysis

NE data was expressed as myocardial NE spillover and not as arterio-venous NE difference because with an increase in NE release from presynaptic nerve terminals, CBF may also increase thereby underestimating any changes in NE arteriovenous difference. NE spillover was calculated as follows:

NE spillover = NE arteriovenous difference x CBF

Since data were not normally distributed, nonparametric statistical analyses were used. A 1-way ANOVA on ranked data with the main effect being condition (control, vehicle and drug) was used to detect any differences in key variables measured. Significant differences were isolated by using the Student-Newman-Keuls post hoc test (SNK). A probability of less than 0.05 was accepted as significant. Results are expressed as mean ± SEM. No statistical analysis was done on the NE spillover values obtained from the 3 dogs in which phentolamine was injected. Such a small sample size does not lend itself to accurate statistical analysis. Therefore, descriptive statistics are reported.

RESULTS

A. Resting Studies

Figure 24 shows the NE spillover in resting dogs under control conditions, 1 minute after i.c. administration of the vehicle (sterile water) and 1 minute after i.c. prazosin injection. Intracoronary injection of 0.5 mg of prazosin in the resting dog significantly increased cardiac NE spillover while the injection of the vehicle (sterile water) had no significant effect. Baseline NE spillover was -3.944 ± 5.025 pmol/min. After injecting a 0.5 ml bolus of sterile water, the NE spillover was -9.565 ± 6.885 pmol/min. Intracoronary injection of 0.5 mg of prazosin significantly increased NE spillover to 15.155 ± 4.612 pmol/min (ANOVA: $F = 12.75$, $p = 0.0004$; $p<0.05$, SNK).

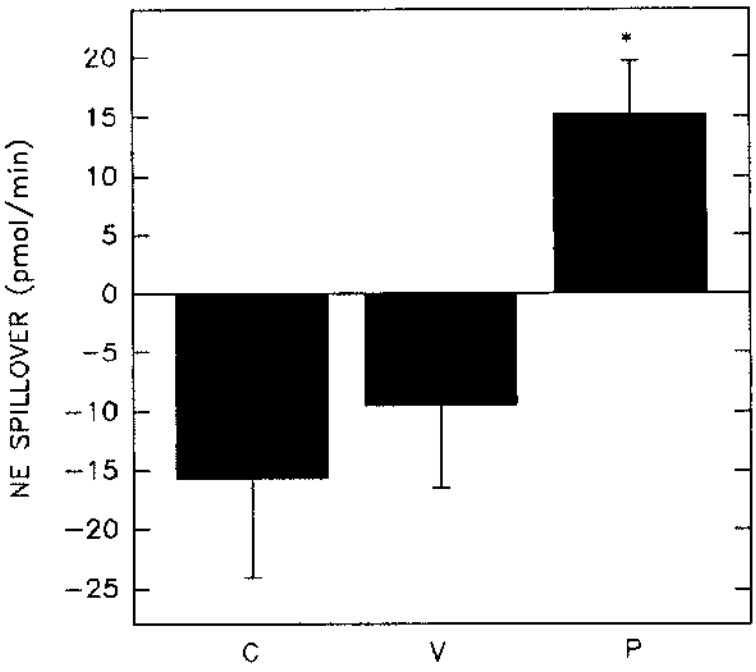

Figure 24. Norepinephrine (NE) spillover in resting dogs under control conditions (C), 1 minute after i.c. administration of the vehicle (sterile water) (V) and 1 minute after i.c. prazosin injection (P). Mean ± SE, n=5.

B. Exercising Studies

Figure 25 shows the NE spillover in dog at rest while supine on a table, while standing on the treadmill, during exercise prior to the i.c. injection of prazosin and 1 minute after the injection of the prazosin at the same exercise intensity.

Intracoronary injection of 0.5 mg of prazosin in the exercising dog significantly increased cardiac NE spillover. With the dogs resting supine on a table, NE spillover was 7.6 ± 5.9 pmol/min. Standing on the treadmill prior to

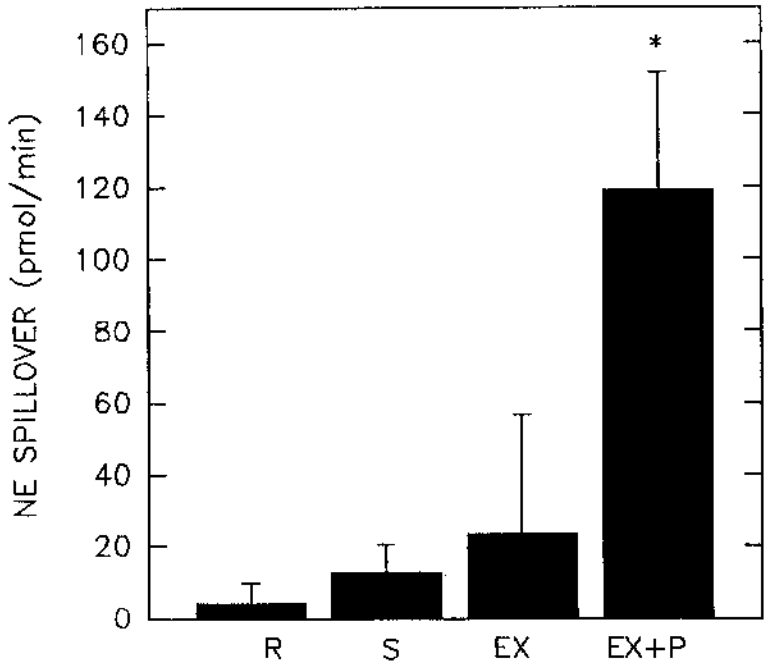

Figure 25. Norepinephrine (NE) spillover of 5 dogs while at rest, supine on a table (R), while standing on the treadmill prior to exercising (S), during the exercise but prior to the i.c. injection of prazosin (EX) and, 1 minute after the injection of the prazosin at the same exercise intensity (EX+P). Values are mean ± SE. * - $p < 0.05$ vs R, S and EX.

exercising, the NE spillover was 9.1 ± 10.3 pmol/min. During the exercise but prior to injecting the prazosin, NE spillover was 23.3 ± 30.3 pmol/min. There were no significant differences between these values. Intracoronary injection of 0.5 mg of prazosin, while the dogs were exercising at the same intensity, significantly increased NE spillover to 103.7 ± 25.2 pmol/min (ANOVA: $F = 4.08$, $p = 0.03$; $p<0.05$, SNK).

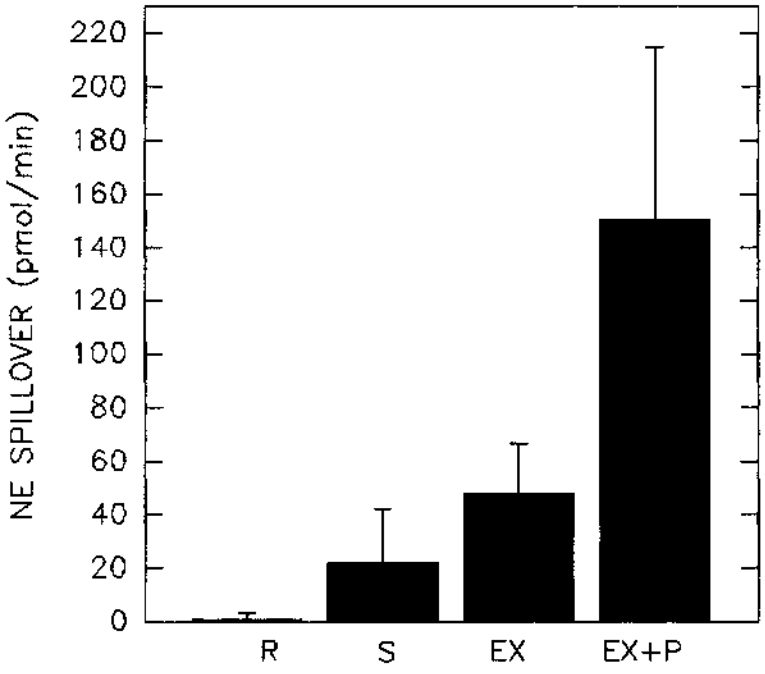

Figure 26. Norepinephrine (NE) spillover during the exercise protocol in which i.c. phentolamine was administered instead of prazosin. n = 2.

C. Phentolamine Studies

Figure 26 shows the mean changes in NE spillover of 2 dogs subjected to the exercise protocol during which i.c. phentolamine was administered instead of prazosin. At rest while supine on a table, NE spillover was 0.62 ± 2.41 pmol/min. With the dogs standing on the treadmill, NE spillover increased to 21.8 ± 20.5 pmol/min. During the exercise immediately prior to the i.c. injection of phentolamine, NE spillover was 47.7 ± 18.9 pmol/min. One

minute after the injection of phentolamine, with the dogs still exercising at the same intensity, NE spillover increased to 150.4 ± 64.2 pmol/min.

DISCUSION

α- and ß-adrenergic receptors have been subdivided into α_1, α_2, $ß_1$, $ß_2$ and $ß_3$ receptor subtypes with further subclassification of the α_1- and α_2-adrenergic receptors into 3 α_1-adrenergic receptor subtypes (α_{1A}, α_{1B} and α_{1C}) and 3 α_2-adrenergic receptor subtypes (α_{2A}, α_{2B} and α_{2C}) (Bylund, 1992). With the complexity of adrenergic receptor classification as it is at present, it is no longer feasable to divide adrenergic receptors into subtypes purely according to their location, as was initially done by Langer (1974). Thus, the concept of α_1-adrenergic receptors being purely postsynaptic has been challenged. Indeed, presynaptic α_1-autoreceptors have been documented (Cavero et al,1979; Constantine et al,1978; Kobinger and Pichler,1980). From a functional aspect, Guth et al (1990) demonstrated a 246% increase in plasma NE (blood drawn from the coronary sinus) when prazosin (80 µg/kg) was injected systemically in exercising dogs. The authors suggested that both presynaptic α_1- and α_2-autoreceptors exist to control the cardiac sympathetic neuronal release of NE. In contrast, Heyndrickx et al (1984) showed no increase in NE release after i.c. prazosin in exercising dogs.

Gwirtz et al (1992) have also refuted the existance of a presynaptic α_1-adrenergic receptor by demonstrating no change in the arteriovenous NE

difference across the myocardium in response to i.c. prazosin (0.5 mg) during left stellate ganglion stimulation in an anesthetized dog preparation. The fact that the latter study used an anesthetized preparation may be of significance in explaining the difference between their results and the results of the present study. Neural control of the coronary vasculature has been shown to be altered by sodium pentobarbital anesthesia (Vatner and Braunwald,1975). Also, Gwirtz et al (1992) did not report changes in NE as NE spillover since they did not measure coronary blood flow. Heyndrickx et al (1984) administered prazosin (0.5 mg/kg) intravenously (i.v.) and did not show a significant increase in NE difference across the heart. They administered phentolamine (1 mg/kg i.v.), and showed a large increase in NE difference. The increase in NE difference also occurred when yohimbine (0.3 mg/kg i.v.) was injected. An i.v. dose of 0.5 mg/kg of prazosin may not have been high enough to block presynaptic α_2-adrenergic receptors while by administering 0.5 mg i.c., the presynaptic α_2-adrenergic receptors may have been blocked.

Based on the results of this study, presynaptic α_1-adrenergic receptors may exist. They would serve as autoreceptors in the negative feedback inhibition of NE release from presynaptic nerve terminals. However, based on the similarity of the response of NE spillover to i.c. prazosin and phentolamine, it appears that the prazosin may be blocking presynaptic α_2-adrenergic autoreceptors. Since prazosin is only relatively selective for α_1-adrenergic receptors, and α_{2B}- and α_{2C}-adrenergic receptors have relatively high affinities

for prazosin compared to α_{2A}-receptors, this is probably the case. Weinshank et al (1990) reported the relative potencies of prazosin and yohimbine for displacement of [H^3]rauwolscine binding to α_2-adrenergic receptors. They reported K_i prazosin:yohimbine ratios for α_{2A}, α_{2B} and α_{2C} receptors of 622, 4.5 and 57 respectively. In addition, Turner et al (1984) showed that both prazosin and yohimbine stimulate the release of NE from the rat submandibular gland, with only a 5-fold difference in potency. Therefore, the presynaptic autoreceptor which is blocked by prazosin in this study is probably of the α_{2B} or α_{2C} subtype, but more likely of the α_{2B} subtype.

In conclusion, this study was designed to investigate the effect of i.c. administration of 0.5 mg of prazosin on NE spillover from the heart. This dose of prazosin does increase the NE spillover from the heart, probably by blocking presynaptic α_2-adrenergic autoreceptors. Therefore, to obviate any complicating effects of increased NE release causing enhanced myocardial stimulation, propranolol was administered systemically to block ß-adrenergic receptor effects.

APPENDIX B

COMPARISON OF HYDRAULIC AND PNEUMATIC OCCLUDERS

INTRODUCTION

The aim of this study was to determine whether an air-filled (pneumatic) and/or a water-filled (hydraulic) occluder spontaneously increases the degree of stenosis when the distal intraluminal pressure decreases. An isolated vessel preparation, using whole blood as the perfusate, was used to simulate coronary vasodilation distal to a pre-existing stenosis.

METHODS

A 28 kg mongrel dog was bled prior to being sacrificed so that the blood could be used as the perfusate. Both femoral arteries were removed and cannulated at each end (Tygon tubing: I.D. = 3.1mm, O.D. = 4.7mm). A T101 ultrasonic blood flow meter (Transonic Systems, Inc., Ithaca, New York) was placed around the vessel to measure flow (F) in ml/min. The occluder (either pneumatic or hydraulic) was placed around the vessel distal to the flow probe. Pressures were measured immediately proximal to (P_1) and immediately distal to the vessel (P_2), using Isotec pressure transducers which were calibrated with a mercury baumanometer prior to starting the experiment. P_1 was established by elevating a reservoire of blood coupled to the proximal cannula. P_2 was regulated by stenosing the distal cannula with a pair of forceps. This method reliably produced the desired distal pressures which ranged from 53 ± 0.9 mmHg to 90 ± 5 mmHg (mean ± SE).

With P_2 set at 100 mmHg and P_1 at 115 mmHg the occluder was inflated to produce a partial stenosis of the vessel such that flow was decreased by 50 - 60%. The stenosis was then maintained by clamping the occluder tubing with a pair of forceps. After recording data (P_1, P_2 and F), P_2 was reduced to approximately 50 mmHg. P_1 was maintained at between 90 and 110 mmHg. F was then measured again. The same procedure was followed for both the pneumatic and the hydraulic occluders. Both occluders were tested on each vessel.

RESULTS

Table 8 shows the mean ± SE for P_1, P_2 and F measured in 2 isolated vessels for the pneumatic and the hydraulic occluders. A total of 5

Table 8. Pressures, flows and calculated occluder resistances for pneumatic and hydraulic occluders on an isolated femoral artery.

		P_1 (mmHg)	P_2 (mmHg)	F (ml/min)	R_{occ} (U)
AIR	$P_2\uparrow$	110 ± 6	101 ± 3	52 ± 31	0.3 ± 0.3
	$P_2\downarrow$	89 ± 18	53 ± 2	115 ± 88	1.4 ± 2.0
WATER	$P_2\uparrow$	110 ± 4	101 ± 3	48 ± 11	0.2 ± 0.2
	$P_2\downarrow$	90 ± 12	54 ± 5	97 ± 37	0.5 ± 0.3

Abbreviations: P_1 - pressure proximal to stenosis; P_2 - pressure distal to stenosis; F - flow; R_{occ} - calculated resistance at site of occluder, U - mmHg/ml/min.

measurements for each variable were made, 2 in the first vessel and 3 in the second vessel. The resistance at the site of the occluder (R_{occ}) was calculated using Ohm's Law:

$$R_{occ} = \frac{P_1 - P_2}{F}$$

The large SE for F and R_{occ} is a result of the larger size of the second vessel.

1) Pneumatic occluder: When P_2 was decreased from 101 ± 3 mmHg to 53 ± 0.9 mmHg, F increase from 52 ± 14 ml/min to 115 ± 39 ml/min, an increase of 121%. The R_{occ} increased by 370%, from 0.304 ± 0.145 mmHg.min/ml to 1.429 ± 0.879 mmHg.min/ml.

2) Hydraulic occluder: When P_2 was decreased from 101 ± 3 mmHg to 54 ± 2 mmHg, F increase from 48 ± 5 ml/min to 97 ± 17 ml/min, an increase of 102%. The R_{occ} increased by 112%, from 0.220 ± 0.074 mmHg.min/ml to 0.466 ± 0.167 mmHg.min/ml.

DISCUSSION

The data indicate that both the pneumatic and the hydraulic occluders spontaneously increase the degree of stenosis when the distal pressure is decreased by approximately 50 mmHg. The drop in the distal pressure

reduces the intraluminal pressure at the site of the occluder. Since the occluder is not sufficiently rigid to prevent the balloon from inflating more spontaneously, the stenosis is increased even though no change in the pressure within the occluder has occured. This finding is of particular importance for studies which involve pharmacological vasodilation distal to a stenosis established by inflation of either a pneumatic or an hydraulic occluder. If the distal pressure drops substantially, the flow would not increase proportionally due to the occluder spontaneously increasing the stenosis of the vessel, thereby hindering the increase in flow.

The hydraulic occluder increased the resistance a third less than did the pneumatic occluder (112% vs 370%). This was due to water being less expandable than air. If an experimental design is such that large changes in distal pressure are expected across a stenosis formed by inflating an occluder, it would be preferable to use an hydraulic occluder rather than a pneumatic occluder so as to minimize the effect of the occluder on any changes in flow.

It is important to note, however, that these data have minimal bearing on the interpretation of the results of the primary study for the following reasons:

1) The drug that may cause a mild degree of vasodilation distal to the stenosis was infused throughout the protocol starting before the stenosis was induced by inflation of the occluder. Since no drug was injected to cause a significant decrease in the distal pressure, it is unlikely that the occluder had any role in preventing increases in CBF.

2) No increase in cirumflex blood flow was expected during infusion of phentolamine during the protocol. Any changes in flow due to the drug can only be found in comparing the data collected during the infusion of phentolamine with that collected during infusion of 0.9% saline, the vehicle. This experimental design should not be confused with one in which pharmacological vasodilation is induced by injecting a vasodiltor distal to a stenosis after the stenosis has been established.

CONCLUSION

A decrease in the pressure distal to a stenosis established by inflation of a pneumatic or an hydraulic occluder will reduce the intraluminal pressure within the vessel passing through the occluder. The occluder will then expand spontaneously to aggravate the stenosis. Since such circumstances do not exist in the primary study, it is believed that the pneumatic occluders used did not prevent any increases in CBF.

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