

Reflex vascular responses from aortic, carotid and coronary baroreceptors

by

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The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others.

ABSTRACT

1. Many studies which have examined reflexes from the left ventricle have failed adequately to localise the pressure stimulus to that area, and have caused simultaneous changes in coronary pressure. Recent studies demonstrate that localized changes in pressure, over a physiological range, to the coronary arteries results in reflex vascular responses. The coronary arteries thus function as baroreceptors. However, there has been no study to compare the characteristics of coronary baroreceptors with those of aortic arch and carotid sinus receptors. This study was therefore designed to compare and contrast these three baroreceptor regions.

2. The experiments described in this thesis were all performed in anaesthetized dogs attached to perfusion circuits. This allowed independent control of pressures perfusing the coronary arteries, aortic arch and carotid sinuses. A cardiopulmonary bypass and an oxygenator incorporated into some of the circuits allowed control of the pulsatility of the pressures applied to the coronary arteries. Reflex responses were assessed from changes in perfusion pressure to the systemic and hind limb circulations and from changes in sympathetic efferent nerve discharge recorded from renal and lumbar nerves.

3. The time courses of the vascular responses to loading and unloading coronary baroreceptors were investigated and compared with responses from aortic arch and carotid sinus baroreceptors. All three baroreceptor groups when stimulated initiated rapid reflex vasodilatation. Coronary baroreceptor unloading resulted in vasoconstriction that was significantly slower than that from either aortic arch or carotid sinus baroreceptors. This effect was not altered by increasing the duration at high coronary pressure from 30 s to 8 minutes or by making the stimulus pressure non-pulsatile. The time course of the responses from aortic and carotid baroreceptors were rapid and not different from each other.

4. A further series of experiments examined the mechanism of the slow coronary induced vasoconstriction. Electrophysiological recordings of sympathetic efferent nerves showed that they responded rapidly to increases in coronary pressure but slowly to decreases, thus mirroring the vascular response. Sympathetic efferent nerve responses to changes in carotid and aortic pressure were rapid.

5. Further experiments compared the responses of each baroreceptor group to non-pulsatile and pulsatile stimuli and their operating ranges. There was no difference between the response curves to non-pulsatile and pulsatile stimulation of coronary baroreceptors. Stimulation of carotid receptors with pulsatile pressures shifted the response curve to lower carotid pressures. Pulsatile aortic pressures induced greater vasodilatation over the mid-range of the baroreceptor response curve compared to non-pulsatile pressures. The threshold for responses from coronary baroreceptors was extremely low with responses obtained at the lowest pressures tested. The operating range of the coronary baroreceptors was over significantly lower pressures than for aortic or carotid baroreceptors, which both had similar operating ranges.

6. These results demonstrate that coronary baroreceptors operate mainly in hypotensive situations initiating a slow vasoconstriction to unloading and were active at extremely low pressures. They are insensitive to changes in pulse pressure whereas both aortic and carotid baroreceptors were sensitive.

LIST OF PUBLICATIONS

Full Papers:

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CHAPTER 1.

**REVIEW OF REFLEXES FROM THE LEFT VENTRICLE, CORONARY
ARTERIES AND SYSTEMIC ARTERIAL BARORECEPTORS.**

Historical introduction on reflexes from the heart and systemic arterial baroreceptors

The presence of receptors in the heart that affect the cardiovascular system has been known for more than one hundred years. Von Bezold & Hirt (1867) were the first to observe a decrease in blood pressure and a bradycardia when *Veratrum* alkaloids were injected intravenously. These responses were shown to be reflex in origin as they were abolished by bilateral cervical vagotomy. These findings were later confirmed by Jarisch & Richter (1939). They concluded that the sensory endings subserving the reflex were mainly located in the ventricles and not in the great vessels. The reflex depressor response to intravenous and intracardiac injections of *Veratrum* alkaloids and other chemicals was named the Bezold-Jarisch reflex.

Dawes (1947) attempted to locate the precise location of the sensory receptors responsible for the Bezold-Jarisch reflex. In cats and dogs he injected small doses of veratridine into the left anterior descending and left circumflex coronary arteries and lungs and noted that this produced the responses characteristic of the Bezold-Jarisch reflex. These responses were not elicited by injecting veratridine in the right coronary artery. This reflex response to chemical stimulation of coronary or ventricular receptors by veratridine and other chemicals has been termed the “coronary chemoreflex” (Dawes & Comroe, 1954).

Several other investigations have concluded that there are sensory receptors in the left side of the heart which are capable of initiating a depressor reflex. Such a reflex triggered by an increase in pressure applied to the heart was first described by Daly & Verney (1927). They utilised an innervated heart-lung preparation, where aortic pressure was kept constant and the left side of the heart was distended by use

of a cardiometer. Ventricular distension produced bradycardia and hypotension which were abolished by vagotomy. In this study it was not possible to determine the location of the receptors subserving this reflex response.

Since these pioneering studies there have been many attempts to characterise reflexes originating from the left ventricle and the coronary circulation and to define the location of the receptors responsible (see Hainsworth, 1991).

Receptors sensitive to changes in arterial pressure are primarily located in two regions in the systemic circulation: the aortic arch and the origins of its major branches, and the carotid sinuses. Discovery of the regulatory cardiovascular influences from the aortic arch and carotid sinus regions are credited to Cyon & Ludwig, (1866) and Hering, (1927) respectively. The reflex responses from these two distinct baroreceptor populations have been extensively reviewed (Heymans & Neil, 1958; Kirchheim, 1976; Persson & Kirchheim, 1991).

Afferent innervation of the left ventricle and the coronary circulation

The heart is innervated by myelinated and non-myelinated vagal and sympathetic nerves. Fibres from both systems mix to form the cardiac plexus from which fibres project as a fine network under the epicardium and beneath the endocardium of all the heart chambers (Woollard, 1926). More specifically the coronary vessels and the surrounding myocardium receive extensive sensory innervation from both sympathetic and vagal fibres (Nettleship, 1936; Hirsch & Borghard-Erdle, 1961; Abrahám, 1962). In fact Woollard (1926) concluded that no other vessel was as richly innervated as the coronary arteries. This innervation terminates as nerve endings located in the adventitia and outer media of the left coronary artery and its main branches (Abrahám, 1962).

Okinaka, Ikeda, Hashiba, Fujii, Kuramoto, Terasawa, Ozawa, Kaneko & Murata, (1963) were the first to report a depressor reflex originating from distension of the left coronary arteries and to perform a histological examination of nervous endings in and around the left coronary artery. They observed a network of fine nerve fibres in the media and larger diameters fibres in the adventitia and media of the proximal portions of the left coronary artery. The larger diameter fibres transposed into thinner fibres which terminated as free endings. The network of fine and larger fibres was absent in branches distal to the bifurcation of this artery. These histological findings confirmed earlier findings from the same group (Kaneko, 1960). In this study a histological examination of the coronary arteries was performed in dogs. The author concluded that the free endings were similar to structures found in the sinus nerve and aortic nerve. These endings were localised to the connective tissue in the media and

not to the muscle and it was suggested that they might represent baroreceptor endings subserved by poorly myelinated or unmyelinated vagal fibres.

This rich innervation of the coronary arteries and its surrounding myocardium subserves both sensory and motor functions (Feigl, 1983; Mary, 1992). Hirsch & Orme (1947) described the distribution of myelinated fibres to the coronary circulation in the human heart. They reported that these fibres terminated in the walls of the coronary arteries.

Sympathetic afferents with their sensory endings within the heart are not discussed further, as it has been demonstrated that stimulation of these receptors by pressure increases results in excitatory reflexes producing pressor responses and are therefore, unlikely to contribute to the reflex vascular responses reported (see Malliani, 1982).

Electrophysiology of left ventricular and coronary receptors

Since the early observations of depressor reflexes originating from the left ventricle and the coronary circulation numerous studies have examined the electrical discharge patterns of the receptors thought to be responsible for initiating this reflex.

The use of electrophysiological techniques has enabled investigators to characterise and localise the cardiac receptors attached to myelinated and non-myelinated vagal and sympathetic afferents, thus greatly furthering our understanding of cardiogenic reflexes.

Afferent activity in myelinated vagal fibres

In contrast to the large numbers of unmyelinated fibres innervating the left ventricle and coronary arteries, there are very few myelinated fibres (Paintal, 1955; Coleridge & Coleridge, 1980). In the cat approximately 75 % of all the cardiac afferent vagal fibres have been shown to be non-myelinated (Agostoni, Chinnock, Daly & Murray, 1957). The vast majority of myelinated fibres are located in the walls of the atria and the veno-atrial junction (Coleridge, Hemingway, Holmes & Linden, 1957; Paintal, 1973).

The first recordings from myelinated fibres attached to receptors in the ventricles were made in the anaesthetized cat by Paintal (1955). Subsequent recordings from myelinated ventricular fibres were performed in frogs (Kolatat, Krammer & Mühl, 1957) and in dogs (Coleridge *et al.*, 1964; Sleight & Widdicombe, 1965).

Paintal (1955) reported an increase in discharge from these receptors in response to ventricular distension produced by balloon inflation in the ventricle. He

therefore claimed that these receptors were pressure receptors as they responded to changes in myocardial tension or intraventricular pressure during ventricular contraction.

Other studies have also reported that these fibres are sensitive to ventricular distension (Coleridge *et al.*, 1964; Coleridge, Coleridge, Dangel, Kidd, Luck & Sleight, 1973; Gupta & Thames, 1983). However, the study by Kolat *et al.*, (1957) on the activity of myelinated vagal afferents in the frog showed that the discharge was directly related to the rise of ventricular pressure and that some of the receptors did not respond to distension.

Brown, (1965) examined the discharge of vagal afferent fibres in the anaesthetized cat which he suggested were attached to mechanosensitive endings in or near the left coronary artery. Out of the 32 fibres studied, 29 were found in the left vagus and 3 in the right vagus. The left coronary arteries were directly perfused with blood from the right carotid artery. Coronary pressure was increased by rapid infusion of arterial blood and decreased by briefly (2 - 6 seconds) occluding the perfusion circuit. Increasing coronary pressure by 1.3 - 2.7 kPa above control immediately increased fibre discharge. Occluding the coronary perfusion decreased receptor discharge and increased systemic pressure with no effect on heart rate. Many of the fibres remained active even at extremely low levels and were not affected by the coronary pressure pulse. He suggested that the pressure pulse was not important in producing the pulsatile discharge which appeared synchronous with ventricular contraction. By probing, he localised the receptor endings to the superficial left coronary artery and its main branches. Of the nine that were localised, eight had their receptive fields closely associated with the left coronary artery and its main branches

and the other was detected on the posterior aspect of the heart, over the left circumflex artery.

These observations, combined with those from a subsequent reflex study by Brown (1966), led him to conclude that these were mechanoreceptors, located in or near the coronary arteries. Paintal (1972, 1973) dismissed Brown's conclusions, arguing that these were not coronary baroreceptors but merely ventricular receptors located in the proximity of the coronary arteries. His reasons were the lack of correlation between the discharge frequency and the coronary pressure pulse and the fact that some fibres exhibited a lag between changes in coronary pressure and activity. A possible explanation for this lag is that the coronary vascular bed would take time to empty or fill in response to the intervention.

Gupta & Thames (1983) examined the responses of myelinated and non-myelinated vagal afferents in cats. Ten out of 16 myelinated fibres displayed discharge synchronous with the cardiac cycle. Discharge in myelinated fibres was not increased during an increase in left ventricular systolic pressure unless it was accompanied by increases in both end-diastolic pressure and in left atrial pressure. They reported that these endings were localised to the left ventricle, close to the atrioventricular groove. However, the close localisation of these receptors to the left atrium and the fact that discharge only increased when left atrial pressure increased, suggests that these are actually myelinated left atrial receptors

Drinkhill, Moore & Hainsworth, (1993) recorded from vagal afferent fibres in the anaesthetized dog which responded to changes in coronary and left ventricular systolic pressures. They used perfusion techniques that allowed pressures to be applied to discrete barosensitive regions whilst recording nervous activity from vagal afferents. Pressures were applied independently to the aortic root which would

include the coronary arteries or to the left ventricle. Pressures were controlled to the aortic arch, carotid sinus and other cardiac chambers. They reported the activity from a total of twenty-one vagal afferents, ten of these were classified as myelinated from their conduction velocities. The discharge in the resting state was pulsatile. None of the myelinated afferents responded to veratridine injected into the aortic root. All myelinated fibres increased their discharge in response to an increase in aortic root pressure which increased coronary arterial pressure and left ventricular systolic pressure at constant end-diastolic pressure. They were much more sensitive to changes in coronary pressure than to changes in ventricular systolic pressure. Furthermore, the receptive fields of the myelinated fibres were mainly localised to the main left coronary artery and the bifurcation of the left anterior descending and circumflex coronary arteries. The authors concluded that the reflex responses were due to stimulation of coronary arterial mechanoreceptors attached to vagal myelinated afferent fibres.

Afferent activity in non-myelinated vagal fibres

Jarisch & Zotterman (1948) were the first to describe the activity from non-myelinated vagal fibres in the cat. The fibres responded to injections of veratrum alkaloids and to pinching the ventricle. They concluded that these fibres constituted the afferent limb of the Bezold-Jarisch reflex.

Since this initial study many further electrophysiological investigations on the discharge characteristics of cardiac non-myelinated afferents at rest and during various interventions have been reported (see Thorén, 1979). Coleridge, Coleridge & Kidd (1964) and Sleight & Widdicombe (1965) recorded the discharge from these fibres in the dog and concluded from their conduction velocities that they were C fibres.

The majority of ventricular C fibres are distributed to the left ventricle (Thorén, 1977; Baker, Coleridge & Coleridge, 1979), with few fibres originating from the right ventricle (Muers & Sleight, 1972*a*; Thorén, 1980). Non-myelinated afferent endings from the left ventricle have been localised superficially to the epicardium (Coleridge *et al.*, 1964; Sleight & Widdicombe, 1965; Gupta & Thames, 1983) and some more deeply in the myocardium (Öberg & Thorén, 1972*a*; Baker *et al.*, 1979).

It is generally agreed that at normal arterial blood pressures although the majority of these fibres have spontaneous irregular discharge many are silent (e.g. Sleight & Widdicombe, 1965; Öberg & Thorén, 1972*a*). This lack of activity has been attributed to the reduction in cardiac volume that has been shown to occur in open chested preparations (Rushmer, Finlayson & Nash, 1954). However, recordings of activity from left ventricular non-myelinated vagal fibres in cats with intact chests have reported that four out of five fibres were silent at rest (Thames, Donald & Shepherd, 1977).

The discharge characteristics of ventricular C fibres to chemical and mechanical stimuli have been examined using a variety of experimental preparations and interventions in an attempt to define the precise stimulus to these receptors.

Activity in ventricular C fibres is increased by exposure to a variety of chemical substances, including veratridine, nicotine, capsaicin, and prostaglandins (Coleridge *et al.*, 1964; Öberg & Thorén, 1972*a*; Baker *et al.*, 1979). Coleridge *et al.*, (1964) and Sleight & Widdicombe, (1965) examined the discharge from these receptors during changes in inspired oxygen and carbon dioxide concentrations and concluded that activity was not affected.

The most commonly employed mechanical intervention to augment activity in C fibre endings has been occlusion of the aorta leading to increases in cardiac volumes and pressures. This method has been used in the anaesthetised dog (Coleridge *et al.*, 1964; Sleight & Widdicombe, 1965; Baker *et al.*, 1979) and cat (Öberg & Thorén, 1972*a*; Öberg & Thorén, 1973; Thorén, 1977; Thames *et al.*, 1977; Gupta & Thames, 1983). Distension of the left ventricle by this method consistently results in systemic vasodilatation and a decrease in heart rate.

The exact nature of the stimulus to these receptors has not yet been fully defined. Several reports have suggested that the effective stimulus to these receptors is an increase in left ventricular end-diastolic pressure which results in an increased fibre discharge (Öberg & Thorén, 1972*a,b*; Thorén, 1977; Thames *et al.*, 1977; Gupta & Thames, 1983). These investigators also reported that discharge was largely unchanged by increases in left ventricular systolic pressures alone. Large increases in ventricular systolic pressure have been necessary to increase receptor discharge. In a study by Thorén (1977), he illustrates an example of a typical receptor discharge during graded aortic occlusion. The activity was unaffected by increasing left ventricular systolic pressure from 16 kPa to 29 kPa at constant end-diastolic pressure. However, when systolic pressure was further increased to 32 kPa with an accompanying increase in end-diastolic pressure, receptor discharge increased. He therefore concluded that there was no discernible relationship between receptor discharge and left ventricular systolic pressure and furthermore discharge frequency correlated well with end-diastolic pressure. Drinkhill *et al.*, (1993) recorded resting discharge from eleven non-myelinated afferent fibres having a sparse and irregular pattern. The response of these fibres to ventricular distension was examined by occluding aortic outflow and this did not increase the activity in the fibres until mean

aortic root pressure had exceeded 20 kPa and ventricular systolic pressure exceeded 30 kPa.

Summary

The use of electrophysiological techniques has greatly advanced the understanding of cardiac vagal afferents and the foregoing sections have described the vagal afferent innervation of the left ventricle and coronary arteries and described some of the discharge properties of these fibres during chemical and mechanical stimulation. Studies using electrophysiological techniques must be combined with controlled assessment of reflex responses to physiological stimuli applied to discrete reflexogenic regions to be of value in determining the physiological function of a specific receptor group.

These electrophysiological studies combined with histological studies have demonstrated that cardiac sensory endings are attached to vagal non-myelinated and myelinated fibres, with the majority being non-myelinated. Discharge from non-myelinated fibres is irregular and sparse over the physiological pressure range, with many receptors inactive at rest. They respond to chemical and mechanical interventions by increasing their discharge. The increases in discharge best correlate to increases in left ventricular end-diastolic pressure. Unphysiological increases in pressure in the left ventricle and the ascending aorta are required to activate these endings.

In contrast to the large numbers of non-myelinated vagal fibres, myelinated innervation of the left ventricle and coronary arteries is sparse. In studies of afferent activity it is mainly pulsatile and regular at normal pressures, and these are the only cardiac receptors that respond over the range of arterial pressures. Some of these studies have shown that the discharge in myelinated fibres has a close correlation to reflex vascular responses occurring to pressure changes localised to the left coronary

arteries. From these studies it is reasonable to infer that these coronary arterial baroreceptors are responsible for the reflex responses produced by physiological increases in pressure described in the next section.

Reflex responses from the Left Ventricle and Coronary Circulation

Reflex responses from Left Ventricular Mechanoreceptors

Daly & Verney (1927) were the first investigators to describe a depressor response from the ventricles. In this study the ventricles were distended by encircling them within a cardiometer bell and applying a negative pressure to the interior of the bell. Since this report, the past seventy years have seen many further studies refining mechanical stimulation of the ventricles in an attempt to apply a physiological stimulus to a defined area.

Distension of the left ventricle has been commonly achieved by obstructing ventricular outflow, by either occluding the aorta with a snare or clamp or by inflating a balloon in the region of the aortic valve (Mark, Abboud, Schmid & Heistad, 1973; Öberg & Thoren, 1973; Fox, Gerasch & Leonard, 1977; Pelletier, 1979). This non-specific intervention would grossly distend the left ventricle as well as altering pressures and volumes in several reflexogenic regions including the coronary and pulmonary circulations, both atria, the right ventricle, carotid sinuses and aortic arch. These studies have reported reflex vasodilatation and small variable heart responses. However, with this type of intervention it is not possible to precisely define the origin of the responses.

The response to left ventricular distension has also been examined in preparations where the left ventricle has been bypassed (Aviado & Schmidt, 1959; Salisbury, Cross & Rieben, 1960; Ross, Frahm & Braunwald, 1961; Chevalier, Weber, Lyons, Nicoloff & Fox, 1974; Zelis, Lloyd, 1977; Lotysh, Brais, Peng, Hurley & Mason, 1977; Kostreva, Hopp, Zuperku & Kampine, 1979). The purpose of the ventricular bypass was to enable the investigators to apply a localised stimulus to the

ventricle. These studies have also reported decreases in vascular resistance and variable changes in heart rate.

Left ventricular reflexes have also been examined in conscious, chronically instrumented dogs (Holmberg & Zucker, 1986; Zucker, Niebauer & Cornish, 1986). Holmberg & Zucker, (1986) reported no change in mean arterial blood pressure or heart rate during increased left ventricular pressure produced by aortic occlusion. This finding confirms those reported in an earlier study by the same group (Zucker *et al.*, 1986). However, when the buffering capacity of the arterial baroreceptors were removed by sinoaortic denervation, control heart rate was significantly elevated and bradycardia occurred in response to large increases in left ventricular pressure.

In these studies and those discussed earlier from anaesthetized dogs, changes in pressure applied to the left ventricle were generally not confined to that area, causing pressure and volume changes in other potential reflexogenic areas. As well as being poorly localised, many of these studies used unphysiological interventions to mechanically distend the ventricle which are difficult to relate quantitatively to physiological events. They also neglected the input from coronary arterial baroreceptors which are likely to be stimulated by distension of the left ventricle, and particularly in experiments involving occlusion of the aorta (Drinkhill *et al.*, 1993).

It has also been proposed that changes in the inotropic state of the heart stimulates left ventricular receptors and produces reflex responses (Fox *et al.*, 1977; Emery, Estrin, Wahler & Fox, 1983; Emery, Estrin, Wahler, Booth, Swayze & Fox, 1986). Increases in cardiac contractility brought about by injection of catecholamines or electrical stimulation of the stellate ganglia have been reported to activate more vagal afferent C-fibres than aortic occlusion (Muers & Sleight, 1972*b*).

Electrical stimulation of cardiac efferent sympathetic nerves and catecholamine infusion into the isolated coronary circulation have been used in several reflex studies in anaesthetised dogs to increase cardiac inotropic state (Fox *et al.*, 1977; Emery *et al.*, 1983; Emery *et al.*, 1986). These studies reported vasodilatation in the perfused systemic circulation in response to increases in inotropic state indicated by increases in dP/dt max. The authors therefore attributed the reflex vascular depression to increases in cardiac contractility stimulating left ventricular mechanoreceptors. However, when dP/dt max increased it was associated with an increase in left ventricular systolic pressure and in some cases a tachycardia. As these variables were not controlled it is difficult to determine the nature of the stimulus. Furthermore, it is likely that this procedure would also increase the stimulus to the coronary arterial baroreceptors.

The effect of changing inotropic state on reflex vascular responses has been further examined in anaesthetized dogs attached to a perfusion circuit which enabled the investigators to control aortic root and ventricular pressures independently of pressure to other reflexogenic regions (Tutt, Al-Timman & Hainsworth, 1988a; Al-Timman & Hainsworth, 1992). In the study by Tutt *et al.*, (1988a) vascular resistance responses were determined to increases in left ventricular systolic pressure during infusion of dobutamine or propranolol. They observed no significant change in the overall vascular response to changes in left ventricular systolic pressure during administration of these inotropic agents. Thus altering inotropic state did not contribute noticeably to the vascular response. This conclusion was supported by the findings of Al-Timman & Hainsworth, (1992) who utilised the same perfusion technique as Tutt *et al.*, (1988a) and electrically stimulated the left cardiac sympathetic nerve to induce changes in inotropic state. Al-Timman & Hainsworth, (1992) related vascular resistance to changes in either aortic root pressure or to left

ventricular systolic pressure during sympathetic stimulation. They observed that increased inotropic state altered the relationship between left ventricular pressure and vascular resistance, but had no effect on the aortic root pressure-vascular resistance relationship. Changing the level of left ventricular filling also altered the left ventricular pressure-vascular resistance relationship but not that of aortic root pressure. These findings led the authors to suggest that changes in coronary pressure was the more potent stimulus to the receptors responsible for this response.

The lack of an association between inotropic state and cardiac depressor response has also been demonstrated in conscious dogs (Zucker *et al.*, 1986). In this study inotropic state was increased by intracoronary injections of catecholamines which significantly increased cardiac contractility with no change in mean arterial pressure or heart rate.

Therefore it is clear from conscious and anaesthetized dogs that there is no firm evidence supporting the claim that increases in inotropic state contribute to the vascular responses originating from the left ventricle or its associated coronary circulation.

Hainsworth and co-workers have devised a perfusion technique whereby an extracorporeal perfusion circuit consisting of pumps and reservoir is connected by perfusion tubing to the animal, allowing improved control of flows and pressures to well defined regions. With this experimental procedure it has been possible to investigate the effects of localised left ventricular distension with physiological stimuli without provoking confounding reflexes from other reflexogenic regions which are separately perfused with the animal's own blood. The main focus of these studies has been the evaluation of reflexes from receptor areas in the coronary arteries and left ventricle.

Investigations over the past ten years in anaesthetized dogs have consistently reported decreases in vascular resistance in the systemic vascular bed and in isolated limbs. However, the heart rate responses to combined increases in coronary arterial pressure and left ventricular systolic pressure at constant left ventricular end-diastolic pressure have been inconsistent (Challenger, McGregor & Hainsworth, 1987; Tutt *et al.*, 1988*a*; Tutt, McGregor & Hainsworth, 1988*b*; Vukasovic, Tutt, Crisp & Hainsworth, 1989; Al-Timman, Drinkhill & Hainsworth, 1993; McMahon, Drinkhill & Hainsworth, 1996*a,b*). These responses were from preparations where left atrial pressure and pressure distending aortic and carotid baroreceptors were controlled. Cooling or cutting the vagus nerves abolished the vascular responses to distension, therefore confirming its reflex nature (Challenger *et al.*, 1987). The efferent pathway of this reflex involves sympathetic nerves (Challenger *et al.*, 1987; Drinkhill, McMahon & Hainsworth, 1996). Crisp, Tutt, McGregor & Hainsworth, (1989) have also provided evidence that increases in coronary and left ventricular systolic pressures consistently depressed respiratory activity, assessed from phrenic nerve discharge, whereas in the same experiments, increasing carotid sinus distending pressure had no effect.

The studies by Al-Timman *et al.*, (1993) and Drinkhill *et al.*, (1993) confirmed that the coronary circulation was the likely site of the receptors responsible for the vascular responses originally attributed to left ventricular mechanoreceptors. These studies are discussed in the later section: Responses from Coronary Arterial Baroreceptors.

Responses to Occlusion of the Coronary Circulation

Occlusion of the coronary arteries and obstruction of the venous outflow from the coronary sinus are two techniques which have been widely used to reproduce myocardial ischemia and the associated cardiovascular responses (Thames, Dibern-Dunlap & Minisi, 1993).

Obstruction of the coronary sinus with a balloon cannula has been used in the anaesthetized dog and it produces systemic hypotension and bradycardia (Szentiványi & Juhász-Nagy, 1962; Carson & Lazzara, 1970; Muers & Sleight 1972*a,b*). The investigators did not report values of coronary arterial pressures during this intervention, and as Gregg & Dewald (1938) have demonstrated in the dog, coronary sinus obstruction, increases coronary venous pressure and raises pressure in the coronary arterial bed. Thus, the stimulus is poorly localised and the observed depressor responses may well be due to stimulation of coronary arterial baroreceptors.

Szentiványi & Juhász-Nagy, (1962) used a modified balloon cannula and an arterio-venous shunt between a femoral artery and the great coronary vein to distend the coronary sinus without obstructing venous outflow during balloon inflation and observed a depressor response which was abolished by vagotomy. However, Muers & Sleight (1972*c*) attempted to reproduce these results in a similar experimental model. They assessed the effects of coronary sinus distension on blood pressure, heart rate, and sympathetic efferent activity recorded from the ventral ansae subclaviae and were unable to determine a depressor reflex to sinus distension without venous obstruction. Therefore they concluded that the responses observed by Szentiványi & Juhász-Nagy, (1962) were not reflex in origin.

Interruption of the coronary arterial circulation by ligating selected arteries is another technique that has been shown to elicit reflex responses. This intervention has been used in anaesthetized (e.g. Pelletier, 1979; Foreman & Ohata, 1980) and conscious animals (e.g. Bishop & Peterson, 1978; Maseri, Chierchia, Davies & Glazier, 1985). Occlusion of arterial flow is achieved by snaring or clamping specific arteries. It has been suggested that afferent nerve fibres from the ventricles which course with the coronary vessels could easily be damaged during this procedure (Thoren, 1973)

The reports concerning the reflex vascular responses to coronary artery occlusion have been conflicting. In one study the renal vascular bed was reported to not respond (Pelletier, 1979) whereas and in another it was shown to dilate (Hanley *et al.*, 1972). Both constrictor and dilator responses have been found in the limb vascular bed (Constantin, 1963; Hanley *et al.*, 1971; Peterson & Bishop, 1974; Pelletier, 1979) and in skeletal muscle vasculature during coronary occlusion (Hanley *et al.*, 1971; Thoren, 1972; Pelletier, 1979). This variation of responses led Hanley *et al.*, (1971) to conclude that coronary occlusion led to non-uniform reflex adjustments.

The likely cause of these differential responses is differences in the methodological procedures employed. The position of the occlusion is known to produce contrasting effects. This has been demonstrated in anaesthetized dogs by Thames, Klopfenstein, Abboud, Mark & Walker, (1978) who compared the effects on heart rate, mean systemic pressure and perfusion pressure in the gracilis muscle during occlusion of the left circumflex artery and the left anterior descending artery. Occluding the circumflex artery produced bradycardia, systemic hypotension and no change in gracilis perfusion pressure, whereas during anterior descending occlusion heart rate was unchanged, systemic hypotension was reduced and gracilis perfusion

pressure was increased. They concluded that the receptors responsible for initiating the depressor response were preferentially distributed to the inferoposterior wall of the heart. This finding is supported by other experimental evidence where occlusion of the left circumflex artery produced cardiovascular depression (Constantin, 1963; Cosin, Gimeno, Alegre, Breto & Perez-Gomez, 1984; Rutlen & Underwood, 1984). However, in another study, Hanley *et al.*, (1971) did not observe a depressor response to circumflex occlusion but this may well have been due to presence of intact arterial baroreflexes which would have buffered against any changes in arterial pressure.

Interruption of coronary blood supply is known to activate both vagal (Thorén, 1972, 1976) and sympathetic afferents (Brown, 1967; Uchida & Murao, 1974). It is proposed that vagal afferents attached to mechanoreceptors are activated during coronary occlusion by bulging of the ischaemic myocardium during systole (Constantin, 1963; Thorén, 1972; Cosin *et al.*, 1984). Alternatively the response may originate from vagal chemosensitive afferents activated during myocardial ischaemia through the direct actions of locally released substances, including prostaglandins and bradykinins (Coleridge & Coleridge, 1980; Clozel, Pissari, Coleridge & Coleridge, 1990).

Obstruction of the coronary blood flow either by occlusion of an artery or distension of the coronary sinus are both unphysiological stimuli. It is possible therefore that resulting responses to these stimuli may only be concerned with pathological conditions.

Responses from Coronary Arterial Baroreceptors

A great volume of literature is dedicated to depressor reflexes from receptors stimulated by pressure changes in the left ventricle. However, as stated in the section on Left Ventricular Reflexes, many of the interventions used to mechanically distend the left ventricle would have also changed pressures in the coronary circulation. The possibility of receptors stimulated by changes in coronary arterial pressure had been overlooked by many investigators and even dismissed by others (Paintal, 1973).

The review by Aviado & Schmidt, (1955) was one of the first manuscripts to discuss reflexes from coronary vessels. Most of their discussion was based on evidence from studies where reflexes were initiated by occlusion or embolization of a coronary artery. However, they do cite unpublished data from J. W. West where he failed to elicit any reflexes responses to increased perfusion pressure in branches of the left coronary artery and in the right coronary circulation. The reason for this is unclear, though the intervention used may have excluded coronary baroreceptors which are mainly located in the proximal parts of the left coronary artery.

The first available report of a cardiovascular reflex arising from the left coronary artery to a localised increase in pressure was in anaesthetized dogs in which the left coronary artery was directly cannulated (Ozawa, 1959). Brief increases in coronary perfusion pressure were produced by injecting 10-15 ml of blood into the perfusion circuit, which decreased systemic pressure in 62 of 106 trials. This induced a depressor response which was abolished by vagotomy, confirming its reflex origin. The response was still present after denervating aortic and carotid baroreceptors. Ozawa, (1959) reported no consistent changes in heart rate to this stimulus. In an attempt to determine the course of the afferent fibres he stimulated the receptors by increasing coronary pressure, after cutting either the anterior ventricular vagal branch

or the posterior ventricular vagal branch. Severing the anterior branch abolished the depressor response in 12 of 16 trials and in only 1 case of 7 after cutting the posterior branch. The anterior ventricular nerves arise from both vagi with those on the right side originating from the first group of the right cardiac nerve and the branches on the left side arising from the left recurrent laryngeal nerve and a nerve described as the left depressor nerve and both right and left branches innervate the anterior surface of the left ventricle and part of the right ventricle (Kurihara, 1964).

Szentiványi & Juhász-Nagy, (1962), using anaesthetized dogs, reported a depressor response from the left coronary circulation which had similar characteristics to classical arterial baroreceptor responses. They distinguished this depressor response from the depressor response from coronary sinus distension and concluded that the receptors responsible for the former reflex were located not in the coronary sinus but elsewhere in the left coronary circulation. This response was abolished by vagotomy like that described by Ozawa, (1959). They attempted further to define the afferent pathways. They examined a nerve described as “a branch of the left thoracic vagus which runs through the left pulmonary radix to the coronary sinus” and observed that severing this nerve attenuated the depressor response in three experiments and abolished it in two. The considerable variation in the nomenclature of cardiac nerves unfortunately does not assist comparison of the effects of denervation reported by Szentiványi & Juhász-Nagy, (1962) with those from Ozawa, (1959) and Kurihara, (1964).

The integration of the coronary depressor reflex with central locations has been investigated in anaesthetized dogs (Juhász-Nagy, Szentiványi, Horkay & Vámosi, 1965). The depressor response was initiated in the same manner as in their earlier study (Szentiványi & Juhász-Nagy, 1962). This procedure was repeated after

making lesions at specific central sites. When a section was made between the pontobulbar junction and the upper third of the mesencephalon the response was still present. The response was abolished by passing the section through the bulbar vasomotor centre. This suggests that the coronary depressor reflex functions independently from inputs from higher centres.

Okinaka *et al.*, (1963) utilized the same left coronary perfusion technique as used by Ozawa, (1959). It appears from close inspection of their manuscript that they report the same results as previously published by Ozawa, (1959). In addition they provide further evidence on the location of the receptors responsible for the depressor response and describe the histological appearance of afferent nervous endings in the same region. Increases in left coronary pressure, generally in the range of 20 to 27 kPa and in some cases pressures as low as 16 kPa, initiated a decrease in vascular resistance. It would appear that usually high pressures are required to initiate the response. However, pressure increases were briefly applied over 3 to 6 seconds, the reflex response was therefore unfortunately not titrated against the change in pressure. Had pressure been increased in a controlled manner and maintained long enough to achieve a steady state, it is conceivable that lower pressures could have triggered the response. In an attempt to define the reflexogenic area responsible for the responses, a pouch was created consisting of the left coronary artery and its bifurcation, which when distended produced the depressor response. No response was observed when the vessels distal to the bifurcation were distended with similar pressures. This location was confirmed in a later study (Kurihara, 1964). Right and left atrial pressures were not reported, but it is stated that these were unaltered during and immediately after the stimulus period. This is an important observation as mechanical distension of the atria produces reflex circulatory responses (Hainsworth, 1991).

Brown, (1966) described the existence of mechanoreceptors in or near the left coronary artery in the anaesthetized cat which responded to changes in pressure. The left coronary arteries were directly perfused with blood from the right carotid artery via a cannula tied into the coronary ostia. Coronary pressure was increased by rapid infusion of arterial blood into the perfusion circuit and decreased by briefly (2 - 6 seconds) occluding the perfusion circuit. The responses to changes in coronary pressure were examined in sympathetic efferent nerves to the kidney and heart, and in systemic pressure and heart rate. Ten cats anaesthetized with pentobarbital exhibited a variety of sensitivities to pressure changes. None responded to decreases in pressure of up to 6.7 kPa below the control pressure. In no animal was a bradycardia observed to coronary pressure increases of up to 20 kPa above the control level, although this reduced sympathetic efferent activity in all animals. Blood pressure responses were only observed in six cases. In three of them an increase in coronary pressure of only 1.3 - 2.7 kPa was sufficient to provoke a reflex fall in blood pressure. In the other three animals a much greater increase in coronary pressure (6.7 - 13.3 kPa) was required to initiate the response.

In the same study responses to changes in coronary pressure were examined in five chloralose anaesthetized cats. During increases in coronary pressure none of these exhibited a significant vascular response. Bradycardia was obtained in three animals, all of which had heart rates of 90 beats min^{-1} or less. This bradycardia was not accompanied by changes in inferior cardiac nerve activity. Bradycardia was not observed in two animals in which the heart rate was more than 150 beats min^{-1} . However, despite the absence of a reflex vascular response, sympathetic efferent discharge decreased when coronary pressure was increased more than 20 kPa above control.

The lack of a response of systemic vascular resistance in the majority of animals may have been due to the buffering effects exerted by aortic arch and carotid sinus baroreceptors which were not denervated. Another possibility is the state of anaesthesia or the influence of the surgical procedure on the levels of circulating vasoactive agents could contribute to the inconsistent responses (Millar & Morris, 1961).

Interest in reflexes from coronary mechanoreceptors was recently revived by Al-Timman & Hainsworth, (1993) and in the accompanying paper detailing an electrophysiological examination of coronary baroreceptors (Drinkhill *et al.*, 1993).

Al-Timman & Hainsworth, (1993) used perfusion techniques in anaesthetized dogs which enabled them to examine steady state vascular and heart rate responses to physiological pressure changes applied to either the coronary arteries or the left ventricle. Either coronary pressure (aortic root) or left ventricular systolic pressure was increased while maintaining a constant mean pressure in the other region and the buffering influences of arterial baroreceptors were excluded by perfusing them at constant pressures. Increasing coronary pressure alone induced a vascular response only slightly smaller than the combined response. An increase in left ventricular systolic pressure at constant mean coronary pressure resulted in vascular responses which were only about 30 % of those to the combined stimulus. When left ventricular systolic pressure alone was changed although mean coronary pressure was maintained constant it was not possible to prevent an increase in the aortic root pulse pressure. This raises the possibility that the response to left ventricular tests were initiated by the increase in pulse pressure perfusing the coronary arteries or by the mechanical distortion of the coronary circulation by distending the ventricle, rather than by stimulation of receptors within the ventricle itself.

Summary

There is wide agreement on the existence of reflexes originating from mechanical distortion of the left ventricle and associated coronary vessels. Many of the studies examining these reflexes have used unphysiological stimuli which have been poorly localised. Precise localisation of a mechanical stimulus is essential in reflex studies so as to avoid secondarily stimulating receptors in other reflexogenic areas with confounding results. The use of direct cannulation techniques indicated that reflexes could be provoked from changes in left coronary arterial pressure. The early evidence supporting the existence of mechanoreceptors in the coronary circulation was dismissed and ignored by many, until the recent publications by Hainsworth and colleagues. For the first time they have been able to distend discretely either the coronary circulation or the left ventricle. The conclusive evidence from these studies confirms the existence of coronary mechanoreceptors. Furthermore, these receptors may well be responsible for many of the responses previously attributed to left ventricular receptors. As coronary baroreceptors respond to physiological changes in blood pressure they can be classified as a third major group of arterial baroreceptors alongside the classical aortic and carotid baroreceptor groupings.

Arterial Baroreflexes

The concept of control of the cardiovascular system by a neural reflexes dates back to the work of Cyon & Ludwig, (1866). They stimulated the cephalic end of the aortic depressor nerve in the rabbit and noted that it caused bradycardia and systemic hypotension. Their work established a role for the aortic arch region in cardiovascular control. It was not until Hering's work, 60 years later in 1927 that the role of the carotid sinus region in circulatory regulation was conceived. These exceptional observations opened up a new field of physiology into the neural control of cardiovascular function. The last 130 years has seen a wealth of publications dedicated to this subject, of which the various aspects have been extensively reviewed (Heymans & Neil, 1958; Kirchheim, 1976; Persson & Kirchheim, 1991).

The following sections will deal briefly with certain aspects of the carotid sinus baroreflex and the aortic arch baroreflex and the methods used to examine them.

Aortic Arch Baroreceptors

Cyon & Ludwig, (1866) reported that stimulation of the central end of the aortic nerve gave rise to a depressor response. Köster & Tchernak (1902) performed a series of degeneration studies on the aortic nerve in dogs, tracing the terminal endings of this nerve to the aortic arch and the origins of its major branches. The aortic arch and the origins of its major branches receive both myelinated and non-myelinated vagal and sympathetic afferent innervation (Douglas, Ritchie & Schauman, 1956; Paintal, 1972; Uchida, 1975). The vagal afferent fibres terminate centrally in the medial nucleus tractus solitarius (Donoghue, Fox, Kidd & McWilliam, 1981).

The aortic nerve is a mixed nerve, composed of barosensory and chemosensory fibres. Coleridge *et al.*, (1973) described vagal myelinated afferent fibres attached to chemo- and mechosensitive endings in the aortic arch in dogs and cats. There are also mechano- and chemosensitive receptors in the aortic arch region with non-myelinated vagal afferents (Brown, Saum & Tuley, 1976).

Aortic chemoreceptors are found mainly in two areas of the aortic arch: located ventral to the aortic arch and pulmonary artery bifurcation and between the aorta and pulmonary artery (Coleridge, Coleridge & Howe, 1970). Coleridge *et al.*, (1970) also described aortic chemoreceptors in two other locations: at the root of the right subclavian artery and near the left coronary artery.

Comroe, (1939) performed an extensive investigation on aortic chemoreceptors in both the cat and dog. In the cat he demonstrated that a group of aortic chemoreceptors located near the coronary orifices received their blood supply from the coronary arteries, whereas he was unable to demonstrate a coronary blood supply to aortic chemoreceptors in the dog. Howe, (1956) also studied aortic chemoreceptors in the cat and described a group of receptors located between the ascending aorta and the pulmonary artery which were supplied with blood from a small branch of the right coronary artery. This group of receptors were classified as group 4 aortic bodies. A blood supply from the left coronary artery to group 4 aortic bodies has been demonstrated in both newborn (Nonidez, 1937) and adult dogs (Coleridge *et al.*, 1970; Eckstein, Shintani, Rowen, Shimomuro & Ohya, 1971).

Aortic baroreceptors are grouped mainly in three locations; area 1, around the aortic arch and the origin of the left subclavian artery, area 2, at the bifurcation of the right subclavian and innominate arteries and area 3, at the origin of the aorta (Coleridge *et al.*, 1973). All the afferent fibres traced to area 1 were found in the left

vagus and the fibres from areas 2 and 3 run in the right vagus. There were reported to be less than 5 % of the total baroreceptors located in area 3. These findings are in agreement with those presented by Nonidez, (1935) who described the distribution of the aortic nerves and their endings in a variety of newborn and adult species. His histological examination of these endings, localised them mainly to the adventitia and between the adventitia and the media.

In comparison to the large number of published *in vivo* investigations concerned with the control of the circulation by the carotid baroreflex, there are relatively few concerned with the control exerted by the aortic baroreflex. This is mainly due to the extensive surgery required to isolate a pressure stimulus to the aortic baroreceptors without interrupting aortic blood flow and altering pressures in other barosensitive regions. To avoid these complicated procedures investigators have used a variety of interventions to stimulate this baroreceptor group and have met with differing levels of success. Many studies have examined the responses to cutting the aortic nerve and stimulating the exposed central end (Neil, Redwood & Schweitzer, 1949; Douglas & Schaumann, 1956; Douglas *et al.*, 1956; Kunze, 1986). These studies have reported a wide range of responses depending on the species, type of anaesthetic used and stimulus characteristics. Aortic nerves are comprised both of baroreceptor and chemoreceptor fibres, which complicates interpretation of the responses from stimulating this mixed nerve.

Investigators have also examined the afferent discharge from pressure sensitive receptors in the aortic region during changes in blood pressure. Changes in arterial pressure have been produced by infusion of vasoactive drugs (Bloor, 1963; Irisawa & Ninomiya, 1967), by withdrawal and rapid reinfusion of blood (Aars, 1967) and by controlling aortic pressure indirectly from a pressurized reservoir (Pelletier,

Clement & Shepherd, 1972). Recordings of whole nerve activity revealed that the discharge increases during systole and decreases during diastole and activity was still present at arterial pressures as low as 4 kPa (Aars, 1967; Irisawa & Ninomiya, 1967). As these recordings include discharge from chemoreceptors as well as baroreceptors it is not possible to ascribe discharge characteristics to a specific receptor type. Few or single fibre electrophysiological recordings made by Bloor, (1963) and Pelletier *et al.*, (1972) allow the receptor subserved by the afferent fibre to be defined and eliminate efferent activity from the recording. These studies have shown the discharge from aortic baroreceptors to be synchronous with the arterial pressure pulse and that activity in these fibres is far from abolished at low arterial pressures.

Daly & Daly, (1959) were the first investigators to attempt to localise a pressure stimulus directly to an isolated aortic arch preparation *in vivo*. In this and later studies by the same group the heart was bypassed and the carotid sinuses, lungs and systemic and cerebral circulations were independently perfused without altering pressures elsewhere in the systemic circulation (Daly, Hazzledine & Howe, 1965; Angell-James & Daly, 1970, 1971; Angell-James, 1971*a,b*). They showed that increasing pressure in the aortic arch decreased systemic vascular resistance. Aortic outflow was interrupted in these studies and it is not clear whether the isolated region also included the coronary arteries.

Levy, Ng & Zieske, (1966) in a novel approach inserted a cannula into the aortic arch which had the aorta tied onto it in two places, distal to the origin of the aorta and distal to the origin of the left subclavian artery, thus creating a pouch of the aorta around the cannula containing the baroreceptors. The heart was bypassed and the coronary circulation was perfused through the lumen of the cannula. The aortic pouch was perfused via the left subclavian artery. Increasing pressure in the pouch

produced bradycardia and depressed respiratory movements. The change in respiratory movements was greatest over the first pressure increase from 0 to 13.3 kPa. At very low aortic pressures, the input from aortic chemoreceptors would have been high and the response to the initial step in pressure was probably largely due to decreasing the chemoreceptor stimulation. Therefore this respiratory response cannot be attributed to baroreceptor stimulation alone.

The technique of aortic cannulation was modified by Hainsworth, Ledsome & Carswell, (1970) so that aortic outflow passed through the lumen of the cannula to a pressurized reservoir from where it was distributed to perfuse the isolated aortic arch, isolated carotid sinuses, an isolated hind limb and the remainder of the systemic circulation. This modified technique avoided the complicated procedure of bypassing the heart. These experiments demonstrated a bradycardia and decreased vascular resistance to an increase in aortic arch pressure, results which were confirmed in a later study (Hainsworth, Karim & Stoker, 1975).

Donald & Edis (1971) used a similar technique to stimulate aortic baroreceptors and obtained the same responses of bradycardia and decreased vascular resistance to step increases in aortic arch pressure. Both this study and that by Hainsworth *et al.*, (1970) compared responses from aortic baroreceptors with those from carotid baroreceptors and concluded that the aortic baroreceptors required higher distending pressures to elicit a response. These findings led to the suggestion that the aortic baroreceptors buffer primarily against hypertension whereas the carotid sinus baroreceptors are mainly concerned with preventing hypotension (Donald & Edis, 1971; Edis, 1971; Pelletier, *et al.*, 1972, Pelletier & Shepherd, 1973).

Mechanical and chemical stimulation of the sympathetic afferent fibres originating from the aortic arch produces an increase in systemic blood pressure and

heart rate. It is uncertain, what if any is the physiological stimulus to the sympathetic afferent nerves. The excitatory response only becomes apparent after vagotomy and the response is abolished by stellectomy (Uchida, 1975; Malliani & Pagani, 1976). This suggests that the excitatory reflex does not dominate in normal circulatory conditions and is likely to function mainly in pathological states.

Carotid Sinus Baroreceptors

The role of the carotid baroreceptors in cardiovascular control has received more interest than any other cardiovascular control system, in the seventy years since Hering's, (1927) initial observations. This is due partly to the relative ease of surgically isolating a pressure stimulus to the sinuses.

Sensory information from carotid sinus baroreceptors and carotid body chemoreceptors is relayed to the central nervous system via the carotid sinus nerve which joins the glossopharyngeal nerve. Anatomical and electrophysiological studies have shown that the carotid sinus nerve consists of both myelinated and non-myelinated fibres (Sato, Fidone & Eyzaguirre, 1968; Fidone & Sato, 1969).

Sympathetic efferent fibres have also been located to the carotid sinus region (Rees, 1967*a,b*; Reis & Fuxe, 1968). Stimulating these efferent fibres modifies the carotid baroreceptor responses to distension (Peveler, Bergel, Gupta, Sleight & Worley, 1980). The full role of these efferents in carotid baroreflex function to physiological stimuli has yet to be determined.

Carotid chemoreceptors are located in the carotid body organ (Comroe, 1964) and respond primarily to hypoxia, hypercapnia and increased $[H^+]$ of the arterial blood (Eyzaguirre & Lewin, 1961). However, they do also become stimulated at low levels

of perfusion pressure, particularly less than approximately 8 kPa (Biscoe, Bradley & Purves, 1970).

Pressure sensitive receptors are located primarily in the carotid sinus, a bulge found at the origin of the internal carotid arteries. At least in cats pressure sensitive endings have also been described at the junction of the common carotid and superior thyroid arteries and along the common carotid artery including the junction with the subclavian artery (Boss & Green, 1956).

The development of the vascularly isolated innervated sinus preparation by Moissejeff, (1926), greatly advanced investigation of the carotid sinus baroreflex. With this relatively simple technique pressure distending the carotid sinus could be altered over a physiological range independently from systemic arterial pressure in a more controlled manner than was previously achievable with the widely used carotid clipping technique.

Bronk & Stella, (1932 & 1935) were the first investigators to record discharge from carotid baroreceptors in response to imposed pressure changes. These classical studies defined accepted ways of characterising baroreceptor afferent activity.

Carotid baroreceptors are attached to both myelinated and non-myelinated fibres, with myelinated fibres responding to increases and decreases in pressure from the set point, whereas non-myelinated fibres signal changes in arterial pressure above the set point (Coleridge, Coleridge & Schulz, 1987).

The activity of carotid baroreceptor afferents is synchronous with the arterial pressure pulse, limited to the period of systole and early diastole (Bronk & Stella, 1932 & 1935; Ead, Green & Neil, 1952). By incorporating a damping chamber into the common carotid artery the arterial pulse could be damped and the afferent discharge compared during pulsatile and non-pulsatile perfusion at the same mean

pressure. In animals in which the buffering effects exerted by the aortic and vagus nerves had been abolished, converting the carotid perfusion from a pulsatile flow to a non-pulsatile flow caused a significant systemic vasoconstriction which was reversed by reverting back to pulsatile perfusion (Ead, *et al.*, 1952). This finding that the carotid baroreflex is displaced when perfusing with non-pulsatile pressures has been firmly established (Scher & Young, 1963; Kezdi & Geller, 1968; Koushanpour & McGee, 1969; Angell-James & Daly, 1970 & 1971; Schmidt, Kumada & Sagawa, 1972; Chapleau & Abboud, 1987). This effect is dependent on the rate of pulsation, as Gero & Gerová, (1962) demonstrated that the pulse frequency had to be at least 1.8 Hz for a significant reflex vascular effect.

Summary

The foregoing sections have briefly covered some of the considerable literature dedicated to the cardiovascular reflexes originating from baroreceptors located in the aortic arch and carotid sinus regions. There are some similarities in the responses from these two groups of baroreceptors in that they both act to counter changes in arterial blood pressure above the resting level through reflex effects in vagal and sympathetic efferent fibres. Carotid sinus baroreceptor also counter against decreases in arterial pressure. Reports from reflex studies generally conclude that aortic arch baroreceptors do not buffer against hypotensive situations, even though studies of afferent discharge have shown that aortic baroreceptor afferents are clearly active at normal arterial blood pressures. A role for aortic receptors in buffering hypotensive episodes has yet to be clearly defined.

OBJECTIVES

The main objective of the research presented in this thesis is the characterization of the cardiovascular reflexes arising from baroreceptors located in the coronary arteries. These responses are compared with those from arterial baroreceptors located in the aortic arch and carotid sinuses and are presented in three chapters.

Chapter 1: The objectives of the research in this chapter was to compare the magnitude of the vascular response to stimulation of the three baroreceptor groups and to determine the time course of the vascular responses. The effect of stimulating coronary baroreceptors with non-pulsatile pressures in the fibrillated heart on the rate of vascular resistance changes was also examined.

Chapter 2: In this chapter the mechanism responsible for the delayed vascular response presented in chapter 1 was examined in recordings of renal and lumbar sympathetic efferent activity following loading and unloading of coronary baroreceptors. These results were compared to carotid and aortic baroreceptor responses.

Chapter 3: The experiments in this chapter were designed to examine and compare the operating ranges of the three baroreceptor groups. Also comparisons were made of the baroreceptor-response curves obtained during non-pulsatile and pulsatile pressures. To examine coronary responses to this a fibrillated heart preparation was used.

CHAPTER 2.

GENERAL METHODS

The experiments reported in this thesis are performed in dogs of mixed sex.

Induction and maintenance of anaesthesia

A local anaesthetic (lignocaine hydrochloride, Phoenix Pharmaceuticals, Gloucester, UK) was injected subcutaneously under the skin overlying a lateral saphenous vein. A cannula was inserted into the exposed vein until its tip reached the inferior vena cava. A sedative dose of barbiturate (0.1 ml kg^{-1}) was first infused. Anaesthesia was induced by a 1% solution of α -chloralose (100 mg kg^{-1} ; Vickers Laboratories Ltd, UK) dissolved in saline which was infused through this cannula. After induction of anaesthesia the animal received benzyl penicillin ($500,000 \text{ i.u.}$; Crystapen, Glaxo Laboratories Ltd., Greenford, UK) and a dextrose solution (5 ml kg^{-1}) which was prepared by dissolving 25 g of dextrin (Sigma Chemical Co., St Louis, MO, USA) and 30 g of glucose (Sigma Chemical Co.) in 500 ml of saline.

Anaesthesia was maintained throughout the experiment by a continuous infusion of α -chloralose ($0.5\text{-}1.0 \text{ mg kg}^{-1} \text{ min}^{-1}$).

Artificial Ventilation

Once anaesthetised a midline incision was made in the neck, the trachea exposed and cannulated with a brass cannula of similar diameter. The animal was then artificially ventilated through this cannula with oxygen enriched air (approximately 40%) using a Starling "Ideal" pump. When the pleura was opened an end-expiratory resistance was applied to prevent the lungs from collapsing by placing the expiratory outlet from the pump under 3 cm of water.

Measurement and maintenance of arterial blood gases and pH

Frequent arterial blood samples were drawn into a heparinised syringe from a cannulated femoral artery. The arterial P_{O_2} , P_{CO_2} , and pH of this sample were measured using a blood/gas analyser (IL 1610, Instrumentation Laboratory (UK) Ltd, Warrington, UK). Arterial P_{O_2} was maintained within normal limits by increasing the concentration of oxygen added to the inspired gas. Arterial P_{CO_2} was maintained between 5.0 and 5.6 kPa by adjustments of the stroke of the respiratory pump. The pH was maintained within normal limits by continuous infusion of molar sodium bicarbonate solution and by bolus injections as necessary. Blood haematocrit was also determined.

Body temperature

Body temperature was measured via a thermister probe (Yellow Springs Instruments, Ohio, USA) inserted into the thoracic oesophagus. The temperature was maintained at 37-39°C by heating the operating table and incorporation of heat exchangers in the perfusion circuit.

Surgical procedure

Left and right femoral arteries and veins were isolated and cannulated for measurement of systemic perfusion pressure, infusion of drugs, sampling sites and for attachment of the temporary arterial bypass.

The carotid sinuses were exposed and vascularly isolated by ligating all vessels originating from the bifurcation with the exception of the lingual arteries and external carotid arteries. The presence of innervation was determined from the reflex systemic pressure response to bilateral carotid occlusion. The carotid arteries were carefully freed from the left and right branches of the vagus nerve to avoid damaging the vagus nerve during subsequent cannulations of the carotid arteries.

The left side of the chest was widely opened by dividing between the 4th and 5th ribs across the sternum to the right side of the chest. These ribs along with the sternum were divided and retracted giving a wide exposure. Bleeding from the bones was minimised by cauterizing the exposed marrow.

The descending aorta was mobilized by ligating and dividing the upper six pairs of intercostal arteries. This frees the aorta from connective tissue facilitating subsequent cannulations and reducing collateral circulation between cephalic and systemic circulations.

The left subclavian artery was exposed and dissected free of attachments and a snare was placed around the brachiocephalic artery near to its origin. The pericardium was opened and a snare was passed around the aorta 0.5-1 cm from its origin, just distal to the coronary ostia. In all cases care was taken to avoid any nerves.

Perfusion circuit

The design of the perfusion circuit allowed independent constant flow perfusion of the systemic vasculature and cerebral vasculature. Pressures perfusing the aortic arch, carotid sinuses and the aortic root, including the coronary circulation, were controlled and altered as required from a constant pressure system. This

constant pressure system consisted of a pressurised air supply which was either non-pulsatile or pulsatile. The pressure was made pulsatile by passing the pressurised air into a pulse generator, which consists of an electronic timer switch and two solenoid valves (Burkert timer unit 1078-2, solenoid unit 311, Bürkert Contromatic Ltd. Stroud, UK) switching between a high and low pressure source as shown in Figure 1. Using the timer switch to alter the period of high and low pressure the amplitude, frequency and shape of the pressure pulse could be controlled. Usually the frequency was set at 2.5 Hz (150 min^{-1}) with one third of the period at the high pressure and two thirds at the low pressure to generate a triangular pressure pulse with a pulse pressure of 5 - 10 kPa. This gave a reasonable approximation of an arterial pressure pulse.

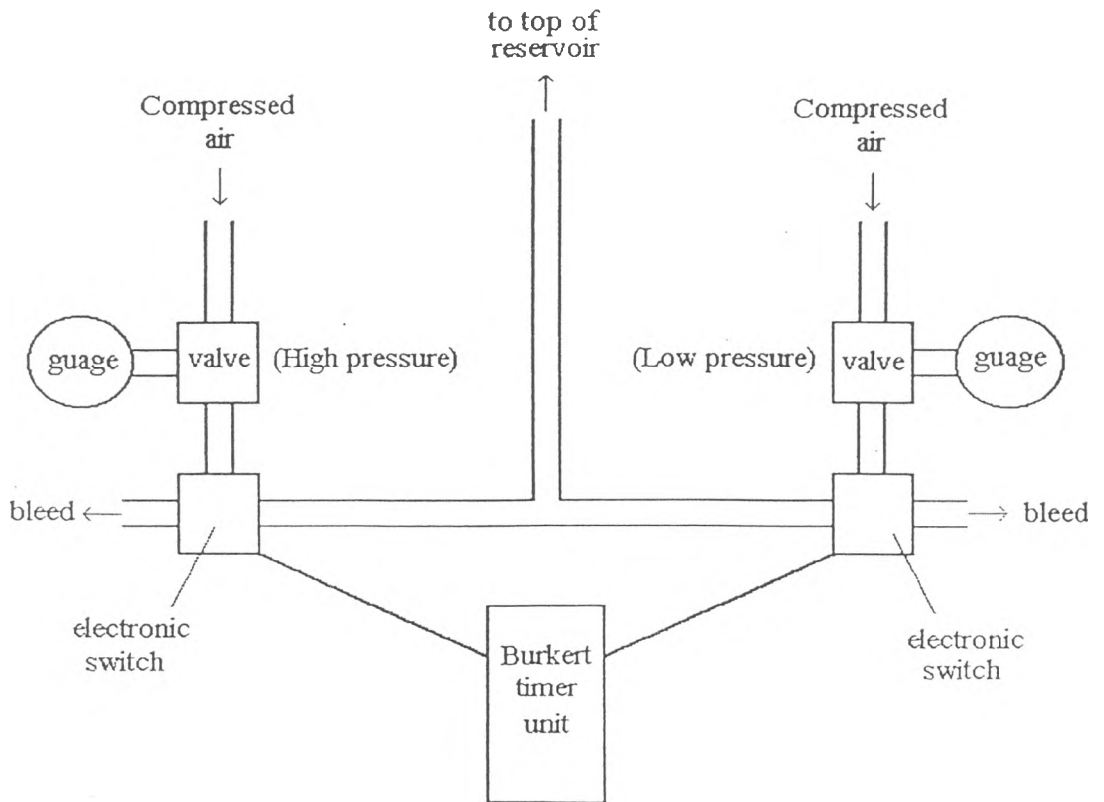


Figure 1. This diagram illustrates the pressure system which was used to generate and apply pulsatile pressures to the reservoirs in the perfusion circuit. The pressure was made pulsatile by passing the pressurised air into a pulse generator, which consists of an electronic timer switch and two solenoid valves switching between a high and low pressure source.

Main reservoir

This was a polyethylene bottle (capacity 2 litres) with three ports and is shown in the perfusion circuit in Figure 2. A port at the base of the bottle conveyed blood into the bottle from the stainless steel cannula in the ascending aorta. A second port in the base provided an outlet for blood distribution to the rest of the circuit. The third port was used as an inlet for blood from the atrial reservoir. The port at the top of the bottle was connected to the constant pressure system which was used to control pressure applied to the aortic root and hence the coronary arteries via the stainless steel cannula. The level in the reservoir was kept between 1/4 - 1/2 full.

Carotid reservoir

This sealed perspex cylinder (capacity 150 ml) had three ports in its base (Figure 2). One acted as an inlet for blood pumped from the main reservoir. The rate of this input was controlled by a float switch which maintains a constant level of blood in the reservoir. The two other ports in the base were connected to cannulae in the carotid arteries through which blood flowed under constant pressure. The constant pressure system was connected to the port in the top of the reservoir and was used to control carotid sinus pressure.

Aortic arch reservoir

This sealed perspex cylinder (capacity 150 ml) had two ports in its base (Figure 2). One acted as an inlet for blood pumped from the main reservoir. The rate of this input was controlled by a float switch which maintained a constant level of blood in the reservoir. The other port was connected to a cannula in the aortic end of the left subclavian artery through which blood flowed under constant pressure. The

constant pressure system was connected to the port in the top of the reservoir and was used to control aortic arch pressure.

Atrial reservoir

This was an open perspex cylinder which received blood drained via a cannula inserted through its appendage into the left atrium (Figure 2). The level of blood in this reservoir was controlled at a constant level by a float controlled switch operating a pump to pump blood to the main reservoir.

Pumps

The systemic and cerebral circulations were perfused at constant flow using variable speed, roller pumps (604U, Watson-Marlow Ltd, Falmouth, UK). In some experiments an isolated hind limb was perfused at constant flow using a smaller variable speed, roller pump (505U, Watson-Marlow Ltd.). Pumps of both type were used to distribute blood to various parts of the perfusion circuit. These pumps had the capacity to be controlled remotely by the float switches.

Heat exchangers

The stainless steel heat exchanger (N.E.P. Heat exchanger, type 2131) incorporated into the circuit had heated water pumped into it as to maintain the temperature of the blood perfusate between 37-39°C.

Washing of the circuit

After each experiment the perfusion circuit was completely dismantled and soaked overnight in Rapidex solution (Rapidex, London, UK). The next day the

circuit was thoroughly rinsed in water. The circuit was filled with a hypertonic saline solution (50 g sodium chloride per litre distilled water) including 250 mg of ampicillin (Penbritin; Beecham Research Labs, UK) the night before the experiment. On the day of the experiment the circuit was rinsed with distilled water and part filled with a 1.5 litre solution consisting of equal parts mammalian Ringer solution and Dextrin in dextrose solution. Heparin (0.1 ml/kg) was added to the perfusate. The solution in the circuit was warmed to 37-39°C before connection of the circuit to the animal.

Connection of the perfusion circuit

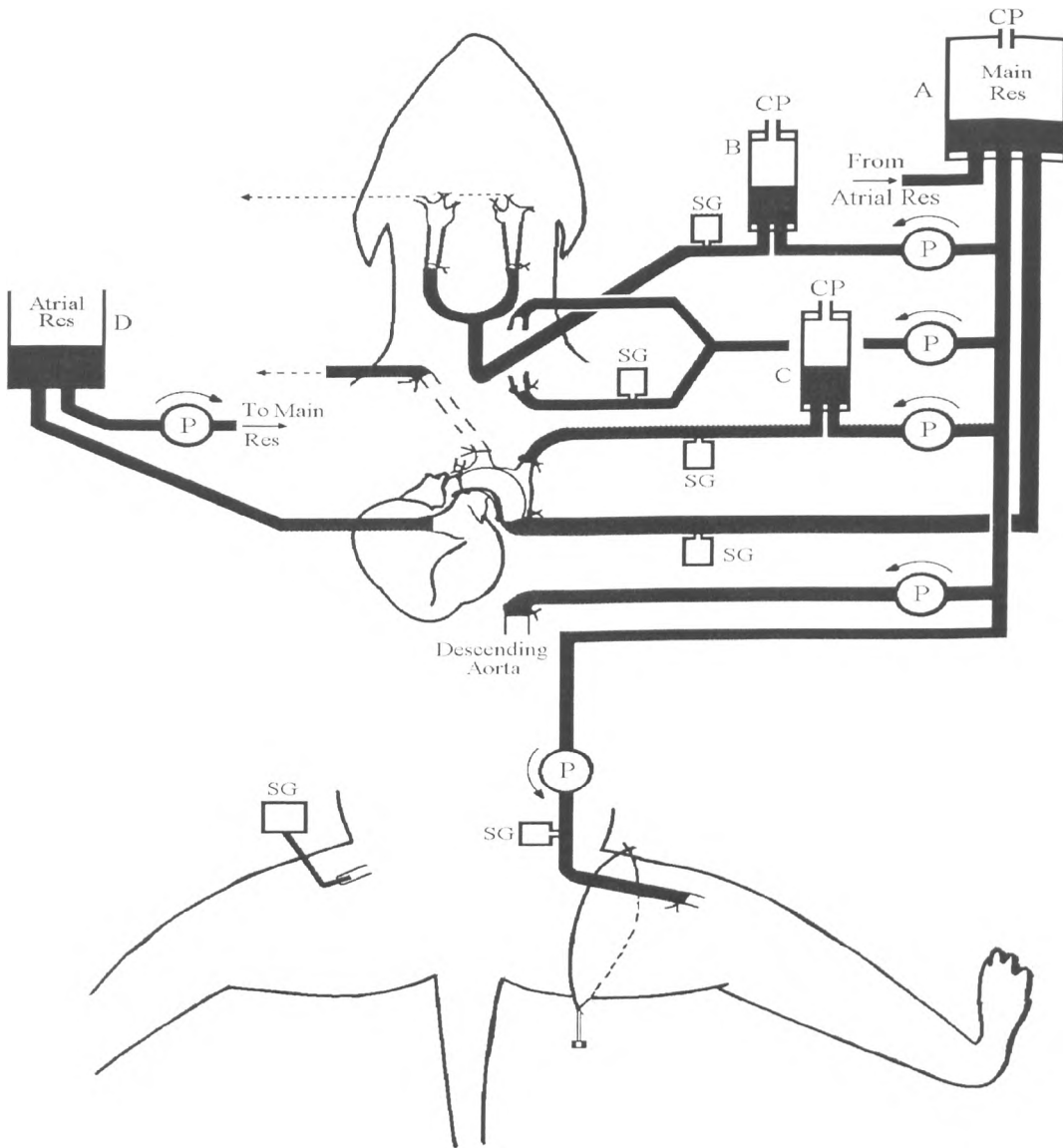
Before cannulation the animal was given heparin (500 i.u. kg⁻¹, I.V.). The perfusion circuit (Figure 2.) was connected to the animal in the following sequence. A curved stainless-steel cannula was inserted into the aorta distal to the origin of the left subclavian artery, and was advanced until it lay just distal to the coronary ostia. This conveyed blood into a pressurized main reservoir from which it was distributed to the various parts of the perfusion circuit. The descending aorta was cannulated and the subdiaphragmatic circulation was perfused at constant flow.

A cannula (7 mm i.d.) was inserted into the left atrium through the left atrial appendage. This drained blood into an open atrial reservoir, from which it was pumped into the main reservoir. This created a partial left-heart bypass to allow control of left ventricular filling.

Both common carotid arteries were cannulated peripherally and perfused with blood from a pressurized reservoir and drained via cannulated lingual arteries into the atrial reservoir. The cephalic region, including the vertebral circulation which was the main blood supply to the brain in these preparations, was perfused at constant flow through cannulae inserted into the peripheral end of the left subclavian artery and the

central end of the left common carotid artery. The aortic arch was perfused from a pressurized reservoir through the central end of the left subclavian artery and drained into the atrial reservoir through a cannula passed down the central end of the right common carotid artery, advanced until its tip reached the aortic arch, and tied close to the origin of the brachiocephalic artery. The snare round the ascending aorta was tied on to the steel cannula, creating a pouch of the aortic arch containing the aortic baroreceptors on the outside of this cannula. The pressure applied to the inside of the steel cannula determined coronary perfusion pressure.

Figure 2. Experimental preparation. A large cannula inserted into the aortic arch, with the aorta tied on to it just distal to the coronary ostia and distal to the left subclavian artery, allows control of aortic root pressure and creates a pouch outside the cannula containing aortic baroreceptors. Blood is drained from the left atrium into reservoir D. Blood from this reservoir is pumped to the main reservoir, A. Blood from A is pumped into pressurised reservoirs B and C to maintain constant levels of blood in these reservoirs which supply the carotid sinuses and the aortic arch. Carotid and aortic arch blood is drained from catheters in the lingual arteries and in the brachiocephalic artery (passed down the right common carotid artery). Blood from reservoir A was pumped into the descending aorta, isolated hind-limb and cephalic circulation at constant flow. Abbreviations: CP, constant pressure; SG, strain gauge transducer; P, pump.



Measurement and recording of cardiovascular variables

Pressures

All blood pressures were measured using strain gauge pressure transducers (P23 Gb; Gould Statham Medical Instruments, USA) attached to nylon catheters. All pressure transducers were calibrated using a mercury manometer over a 0-30 kPa range before each experiment.

The pressures measured were:

1. Systemic arterial perfusion pressure, measured through a catheter inserted into a femoral artery until its tip lay in the abdominal aorta.
2. Cephalic perfusion pressure, measured via a catheter attached directly to the cephalic perfusion cannula.
3. Limb perfusion pressure, measured via a catheter attached directly to the limb perfusion cannula.
4. Aortic arch pressure, measured via a catheter attached directly to the cannula in the aortic end of the left subclavian artery.
5. Carotid sinus pressure, measured via a catheter attached directly to one of the cannulae perfusing the carotid sinuses.

6. Coronary perfusion pressure, measured via a catheter passed through the lumen of the stainless steel cannula to its tip.

The signals from these transducers were passed to amplifiers (EMMA 4000 system, S.E. Laboratories, UK) and recorded on magnetic VHS tape (Racal V-Store, Racal Recorders Ltd, Southampton, UK) and on a direct-writing electrostatic recorder (ES 1000, Gould Electronics, France). Data was also relayed to a data acquisition unit (Fastdaq, Lectromed, Letchworth, UK).

Summary of Animals

A total of 76 animals were used to obtain results for this thesis. Results were accepted from 49 animals. Of the remaining 27 animals, 2 failed whilst being anaesthetized, vessels ruptured during surgery in 7, perfusion circuit failure occurred in 7 and 11 showed no reflex responses to stimulation once on full perfusion circuit.

CHAPTER 3.

**REFLEX VASCULAR RESPONSES TO LOADING AND UNLOADING OF
AORTIC ARCH, CAROTID SINUS AND CORONARY BARORECEPTORS.**

Introduction

Previous studies have reported the presence of receptors located in or near to the coronary arteries that initiate reflex responses during changes in coronary arterial pressure (Al-Timman *et al.*, 1993; Drinkhill *et al.*, 1993).

The reflex responses to changing pressure applied to either aortic or carotid baroreceptors are known to occur rapidly. However, it is not known how rapidly the coronary baroreceptors respond to changes in applied pressure and the extent of the resulting reflex vascular response.

The aim of this series of experiments was to examine the time course and magnitude of the vascular response to loading and unloading of the coronary baroreceptors and to compare these with the responses from aortic and carotid baroreceptors. This investigation is split into three parts:

(A): The purpose of this series of experiments was to determine the maximal vascular response and the stimulus pressure required to produce this. This was achieved by increasing the pressure to one baroreceptor region in steps until no further change in systemic perfusion pressure was observed.

(B): The aim of this series of experiments was to examine the time course of the systemic perfusion pressure response to loading and unloading one baroreceptor region. The effect of loading the baroreceptors for different durations was also examined.

(C): In section (B) aortic and carotid receptors were stimulated with non-pulsatile pressures and coronary receptors were exposed to the cardiac pulsations at the same mean pressure. These experiments aimed to examine whether stimulating the coronary receptors with non-pulsatile pressures influenced the recovery time of systemic pressure.

Methods

Experiments were undertaken in anaesthetized dogs weighing 10-22 Kg. The surgical preparation of the animals is similar to that described in the General Methods section. An incision was made on the left side of the chest between the 4th and 5th ribs and was extended across the sternum, which was divided, to the right side of the chest. This allowed easier access to the inferior vena cava and right atrium for subsequent cannulation.

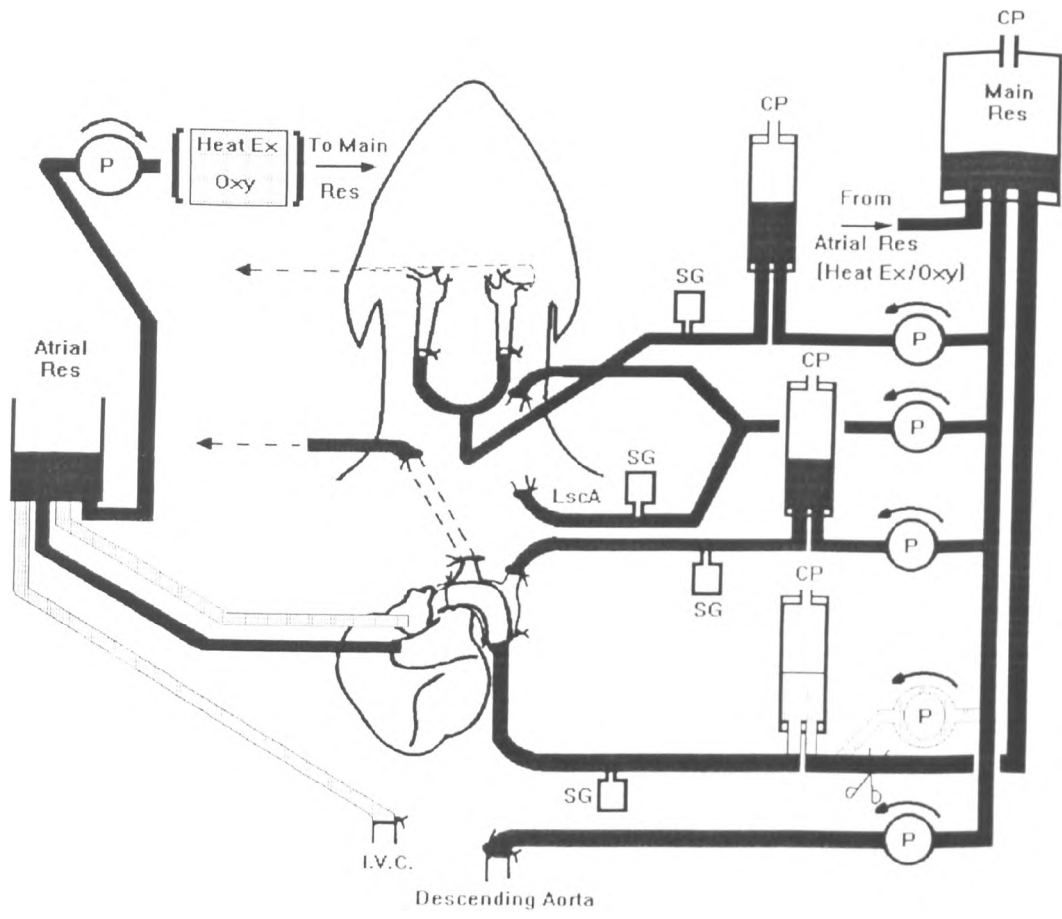
In addition to the basic perfusion circuit (Figure 2), a cardiopulmonary bypass circuit was incorporated into this circuit and the heart fibrillated to prevent the cardiac pulsations from affecting the coronary baroreceptors and allowing a non-pulsatile pressure stimulus to be applied. Cannulae (7 mm i.d.) were inserted into the inferior vena cava and into the right atrium through the right atrial appendage. Blood drained from these, along with that from the left atrium, into an open reservoir. From here the blood was pumped through a HF-5000 Membrane Oxygenator (C.R. Bard Inc. Billerica, MA, USA) to the main reservoir. The heart was made to fibrillate by stimulating at 40 Hz (Bioscience Stimulator 200, C.F. Palmer, BioScience, Sharness, Kent, UK). After setting up the cardiopulmonary bypass the tubing between the small coronary reservoir and the main reservoir was clamped. Controlled set pressures

could then be applied to the small coronary reservoir (150 ml capacity) which was filled at a rate sufficient to maintain a constant level of blood in that reservoir. The cardiopulmonary bypass was connected as required.

The carotid sinuses and aortic arch were both vascularly isolated and perfused at constant pressure and the systemic and cephalic circulations were perfused at constant flow as described in the General Methods section.

The perfusion circuit (Figure 3) was connected in a similar manner to that described in the General Methods. When required, artificial pulsatile pressures were generated by the method described (see General Methods).

Figure 3. Diagram of experimental preparation. Steel cannula tied in the ascending aorta at aortic root distal to coronary arteries and distal to left subclavian artery creates a pouch of aorta outside the cannula and conveys blood to a large pressurized main reservoir. This pressurized main reservoir controls aortic root pressure and hence coronary arterial pressure. Blood from the main reservoir is distributed to the various parts of the circuit. Blood is pumped into: (a) constant pressure chamber and into cannula tied into both common carotid arteries, perfusing the isolated carotid sinuses; (b) constant pressure chamber and into a cannula tied into the central end of the left subclavian artery, perfusing the aortic arch; (c) descending thoracic aorta at constant flow; (d) peripheral end of left subclavian artery and central end of left carotid artery at constant flow to perfuse the cephalic region. A cannula inserted into the left atrium through the left atrial appendage drains blood to the atrial reservoir, from where it is pumped to the main reservoir. Hatched areas indicate parts of the circuit included when heart is fibrillated. Cannulae inserted into the right atrium through the right atrial appendage and inferior vena cava drain blood to the atrial reservoir, from where it is pumped via the heat exchanger/oxygenator to the main reservoir. Coronary arterial pressure is controlled from a pressurised reservoir between the main reservoir and steel cannula. A pump fills this reservoir and the direct line is clamped. Abbreviations: CP, constant pressure; LA Res, left atrial reservoir; L.scA, left subclavian artery; I.V.C., Inferior Vena Cava; Heat Ex/Oxy, heat exchanger and oxygenator; SG, strain gauge transducer; P, pump.



Results

The experiments reported in this chapter were performed in 24 dogs. After connection of the perfusion circuit, pressure to the three baroreceptor regions were held at approximately 8 kPa and the systemic perfusion pressure was set to about 24 kPa by adjusting the rate of the systemic pump. The preparation was allowed to stabilize for 30 minutes during which time blood gases and pH were analysed and corrected as necessary to attain values of: P_{O_2} 19.7 ± 1.7 kPa; P_{CO_2} 5.4 ± 0.1 kPa and pH 7.3 ± 0.01 . Haematocrit was 12.3 ± 0.8 % ($n=24$).

(A) Baroreceptor-response Curves

Experimental Protocol

The baroreceptor-response curves were constructed in the following manner. Pressures perfusing two of the baroreceptor regions were maintained constant at approximately 8 kPa while the pressure applied to the third region was increased from 8 kPa in 4 kPa steps until no further response was obtained. Each pressure increase was maintained for at least 1 minute, at the end of which 30 seconds of recordings were taken. This procedure was repeated for each baroreceptor region. The aortic and carotid baroreceptors were perfused with a non-pulsatile pressure and the coronary baroreceptors were exposed to the natural cardiac pulse pressure with a controlled mean pressure.

The values reported are means \pm S.E.M. and analysis of variance was used to assess statistical significance.

Responses

Baroreceptor-response curves were constructed in all dogs. The data reported are from 8 dogs where response curves were obtained from all three baroreceptor regions. The mean systemic perfusion pressure whilst all baroreceptor pressure were held at 8 kPa was 22.4 ± 0.9 kPa.

The mean baroreceptor-response curves from the three regions are illustrated in Figure 4. An increase in carotid sinus pressure from 8.3 ± 0.1 kPa to 28.2 ± 0.1 kPa decreased systemic perfusion pressure from 21.7 ± 1.6 to 10.5 ± 0.9 kPa, a response of 11.2 ± 1.5 kPa (51.4 ± 5.3 %). When coronary pressure was increased from 8.3 ± 0.2 kPa to 28.5 ± 0.3 kPa systemic perfusion pressure decreased from 22.9 ± 1.9 to 13.6 ± 1.0 kPa, a response of 9.3 ± 1.8 kPa (38.6 ± 5.5 %). An increase in aortic arch pressure from 8.1 ± 0.3 to 28.4 ± 0.2 kPa caused a decrease in systemic perfusion pressure from 22.7 ± 1.0 to 18.4 ± 1.2 kPa, a response of 4.3 ± 0.5 kPa (20.3 ± 2.1 %).

The overall systemic perfusion pressure response in kPa and as a percentage of the initial systemic pressure value from eight dogs is presented in Table 1. The perfusion pressure response to step changes in pressure to aortic baroreceptors was significantly smaller than for either the step changes in carotid ($P < 0.05$) or coronary pressure ($P < 0.05$). The response to stimulation of carotid or coronary baroreceptors were not significantly different from each other.

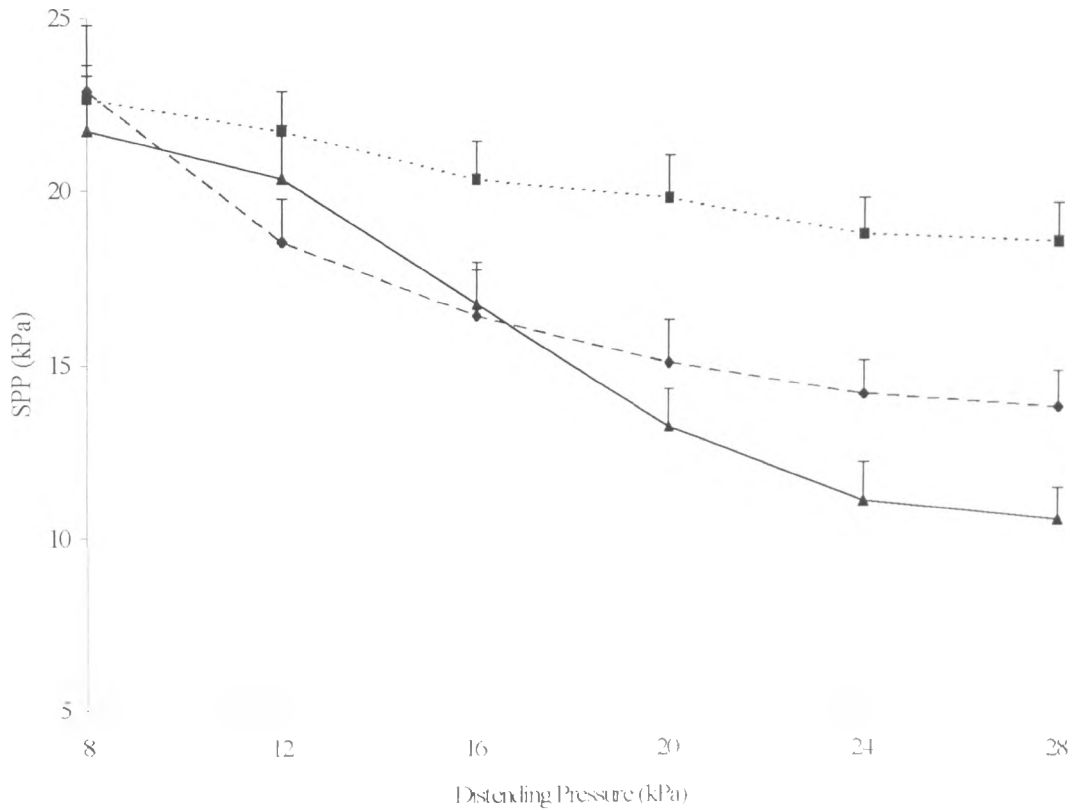


Figure 4. Baroreceptor-response curves from eight dogs. Response of systemic perfusion pressure (SPP) is plotted against distending pressure applied to the baroreceptor region. Responses to increases in carotid pressure (▲), coronary pressure (◆) and aortic pressure (■) are shown. Values are means \pm S.E.M.

Table 1. *Magnitude of the systemic perfusion pressure response to the full range of baroreceptor distending pressures applied independently to carotid, coronary or aortic baroreceptors.*

Dog No.	Systemic perfusion pressure response					
	Carotid		Coronary		Aortic	
	kPa	%	kPa	%	kPa	%
1	13.4	63.5	13.1	55.3	4.6	21.2
2	5.1	30.4	3.1	20.7	6.3	28.8
3	14.8	55.2	17.9	56.6	5.2	19.7
4	13.6	56.4	8.9	42	2.8	12.3
5	10.2	60.7	5	23	3.5	18.2
6	15.4	53.8	9.3	32.7	2.5	9.2
7	12.9	59.2	12.6	52.7	6.2	28.4
8	4.5	25.1	3.2	18.3	3.2	15.7
Mean	11.2*	51.4*	9.3*	38.6*	4.3	20.3
± S.E.	1.5	5.3	1.8	5.5	0.5	2.1

* indicates $P < 0.05$ when the response of perfusion pressure in kPa and the percentage response is compared to aortic response. (ANOVA)

(B) Time course of the vascular response

Experimental protocol

For each experiment, the baroreceptor pressure that produced the maximal response during the step increases was used as the stimulus pressure. The test region was exposed to this pressure for durations of 1, 2, 4 or 8 minutes. In some experiments, coronary baroreceptors were also stimulated for periods of only 30 seconds. Pressure perfusing two regions was maintained constant while pressure in the test region was increased in a single step from 8 kPa to the previously defined level and maintained for the desired duration, after which time pressure was reduced in a single step to 8 kPa and the systemic perfusion pressure response was recorded until it had recovered to its previous high level.

Data analysis

The criteria for inclusion of results into the data analysis was that the response of perfusion pressure to the large step change in baroreceptor pressure was greater than 2 kPa and when the baroreceptors were unloaded the perfusion pressure recovered to at least 90 % of its initial level. Once the baroreceptor group was unloaded the systemic pressure was left to recover and the time course was analysed. This recovery of systemic pressure was normalised by expressing it as a percentage of the difference in the systemic pressure before increasing baroreceptor pressure and during the final 10 seconds before it was decreased.

Analysis of variance and Student's unpaired *t* test incorporating both Cochran's and Satterthwaite's methods correcting for unequal variance were used for statistical analysis and group data are expressed as means \pm S.E.M.

Responses

The time for development of maximal systemic responses to large single step increase in baroreceptor pressure from 8 kPa to the predetermined maximal level were for: aortic receptors: 15.4 ± 1.4 s ($n=10$); carotid: 16.3 ± 1.4 s ($n=12$); coronary: 18.5 ± 1.5 s ($n=13$). These times for the minimum perfusion pressure to be achieved were not significantly different from each other. The individual times in each dog are listed in Table 2.

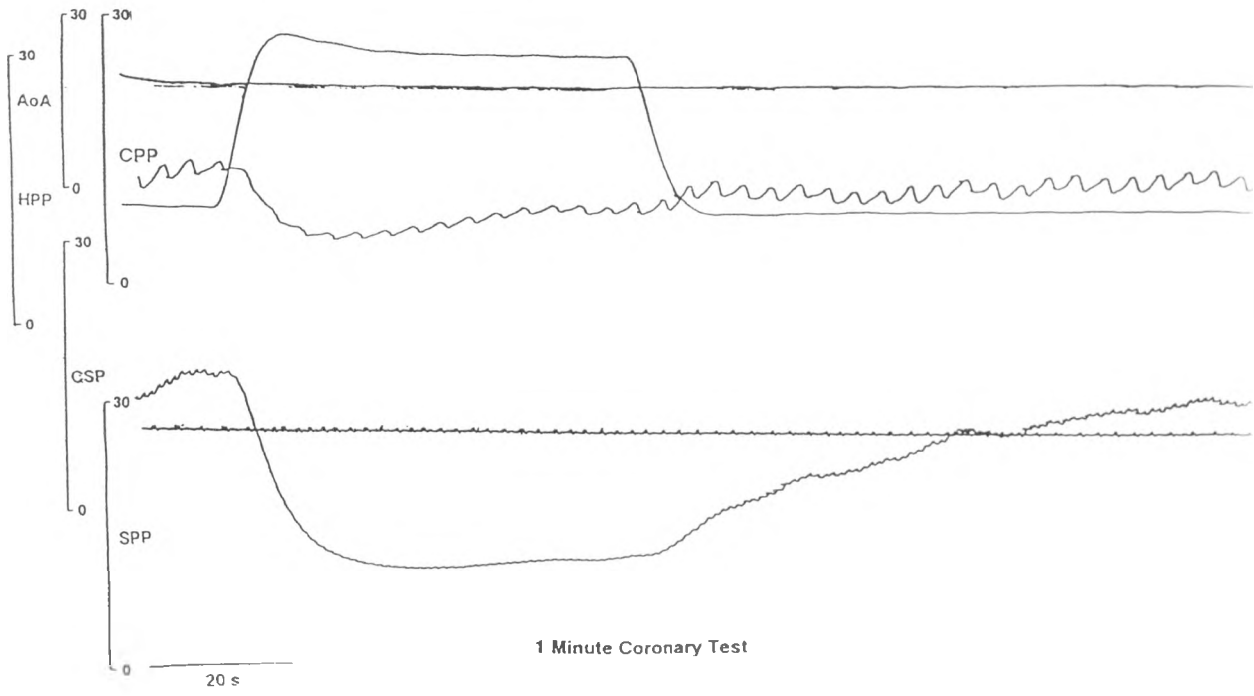
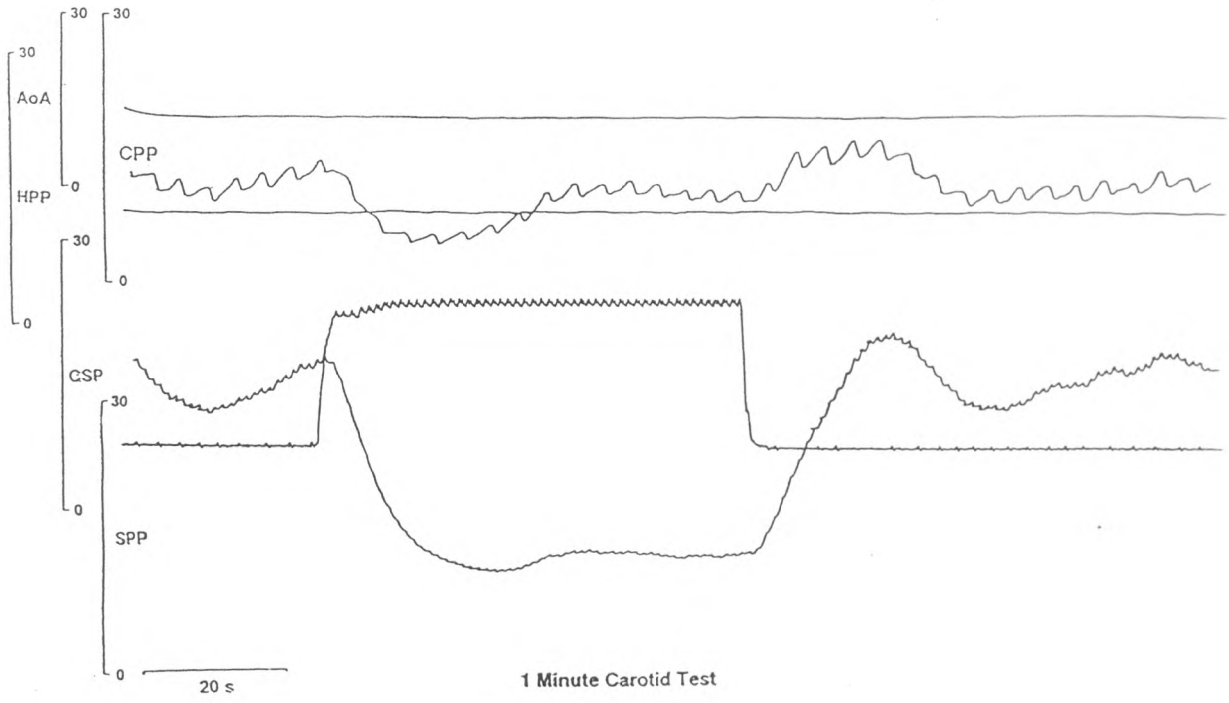
Shown in Figure 5 are results from one animal where carotid pressure (top trace) and coronary pressure (bottom trace) were increased for 1 minute illustrating the systemic pressure response to the pressure changes. Increase in carotid sinus pressure from 8 to 24 kPa caused a decrease in perfusion pressure of 19.4 kPa which was maximal in 22 s. Similarly, an increase in coronary pressure from 8 to 24 kPa decreased perfusion pressure by 18.7 kPa in 33 s. The vasodilatation to both stimuli occurred rapidly.

When the baroreceptor pressures were decreased the times taken for the perfusion pressure to return to its initial level were strikingly different. Following the reduction in carotid pressure from 24 to 8 kPa, systemic pressure recovered rapidly to 90 % of its initial level in 12 s. However, when coronary pressure was lowered to 8 kPa systemic pressure recovered much more slowly and reached 90 % of the initial level in 84 s; i.e. seven times as long as the time for recovery after stimulating carotid receptors.

Table 2. *Time for systemic response to achieve maximum perfusion pressure response to a large step increase in baroreceptor distending pressure.*

Dog No.	Time to maximum systemic perfusion pressure response (s)		
	Carotid	Aortic	Coronary
1			18
2	19		
3	22	12	13
4	17		
5	11		12
6	22	14	33
7		14	
8	16	14	13
9		26	
10	25		23
11	11		
12	13	13	16
13			15
14	16		
15			19
16		20	
17		15	22
18			18
19	12	11	19
20	12	15	
21			19
Mean	18.5	16.3	15.4
S.E.M.	1.5	1.4	1.4

Figure 5. Responses following a 1 minute period of high pressure to carotid baroreceptors (top trace) and coronary baroreceptors (bottom trace). Traces of aortic arch pressure (AoA), coronary perfusion pressure (CPP), head perfusion pressure (HPP), carotid sinus pressure (CSP) and systemic perfusion pressure (SPP). All pressures are in kPa. A large step increase in carotid or coronary pressure resulted in a vasodilatation that was maintained throughout the duration of stimulation. A large step decrease in carotid or coronary pressure resulted in vasoconstriction, but the time course of this was much slower for the coronary test as compared with the carotid test. Pressures distending the two baroreceptor regions not involved in the test were held constant.



In one dog we examined the systemic response to decreasing stimulus pressure to the three baroreceptor regions independently after 1 minute of stimulation. This is shown in Figure 6 where the systemic recovery expressed as a percentage is plotted against time from the decrease in the stimulus pressure. This experiment shows the rapid systemic recovery following a decrease in either aortic arch pressure or carotid sinus pressure with systemic pressure recovering to 50 % of the control level in less than 10 s. In contrast the systemic pressure takes 40 s to reach 50 % and 85 s to achieve 90 % of its initial level following a decrease in coronary pressure. The time taken for perfusion pressure to recover to 90 % of its initial level following independent stimulation of carotid, aortic and coronary baroreceptors for 1 minute are shown in Table 3.

The time course of the vascular response following different periods of stimulation

All three baroreceptor regions were stimulated at the predetermined pressure for periods of 1, 2, 4 and 8 minutes. In addition the coronary baroreceptors were also exposed to the stimulus pressure for 30 seconds.

Figure 7 illustrates the systemic pressure response from one dog following coronary stimulation for 30 seconds and 1 minute. Decreasing the duration of the stimulus had no effect on the rate of the systemic pressure recovery. In this example both systemic pressure responses recovered to the 90 % level in approximately 90 s. In four dogs, systemic pressure recovered to 90 % of its initial level in 89.0 ± 20.4 s following 30 s of stimulation and in 79 ± 14.4 s following 1 minute of stimulation. These values are not significantly different from each other ($P = 0.5$, Student's paired t test) (Table 4)

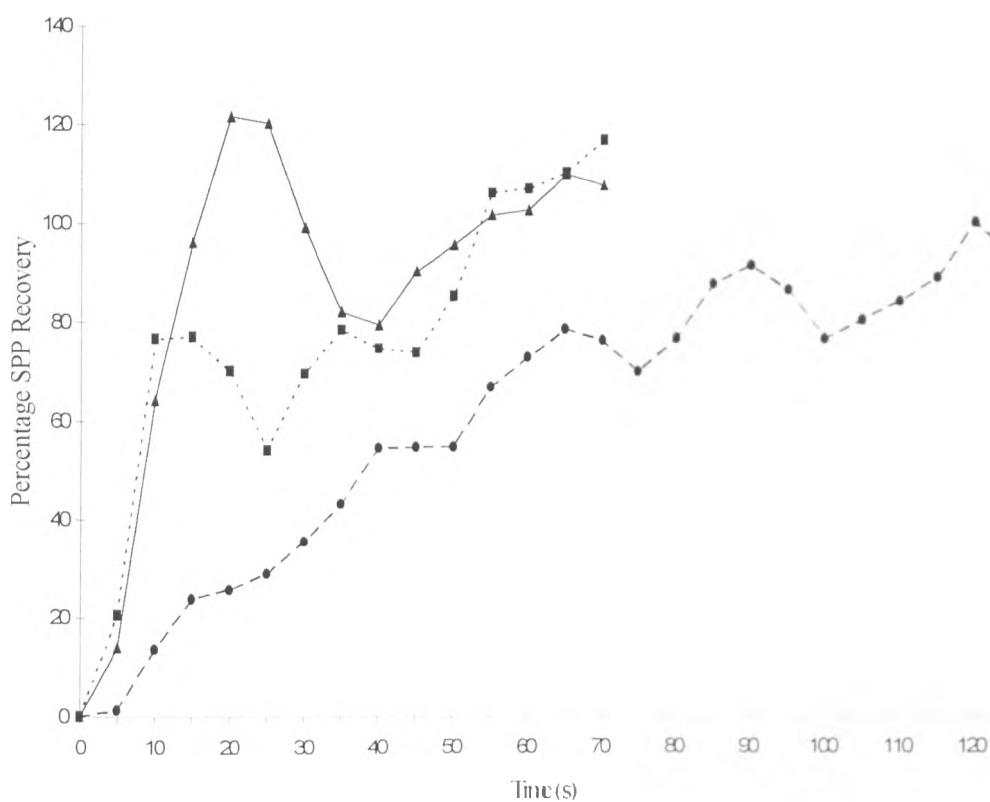


Figure 6. An example from one dog of the recovery of systemic perfusion pressure, after decreases in either aortic arch (■), carotid sinus (▲) or coronary (●) perfusion pressures following one minute at high pressures. Data are expressed as percentages of the maximal response and show the slower response to the decrease in coronary pressure.

Table 3. Time for the systemic perfusion pressure to recover to 90% of its control level following 1 minute of high stimulus pressure applied independently to carotid, coronary or aortic baroreceptors.

Dog No.	Time to 90% perfusion pressure recovery (s)		
	Carotid	Aortic	Coronary
1			107
2	12		
3	27	7	89
4	26		
5	55		57
6	12	46	84
7		7	
8	71	5	73
9		39	
10	26		33
11	9		
12	16	58	18
13			81
14	31		
15			97
16		24	
17		54	77
18			103
19	22	39	56
20	11	36	
21			39
Mean	26.5	31.5	70.3*
S.E.M.	5.4	6.2	7.7

* indicates $P < 0.05$ when coronary recovery is compared to carotid or aortic recovery (Students unpaired t test).

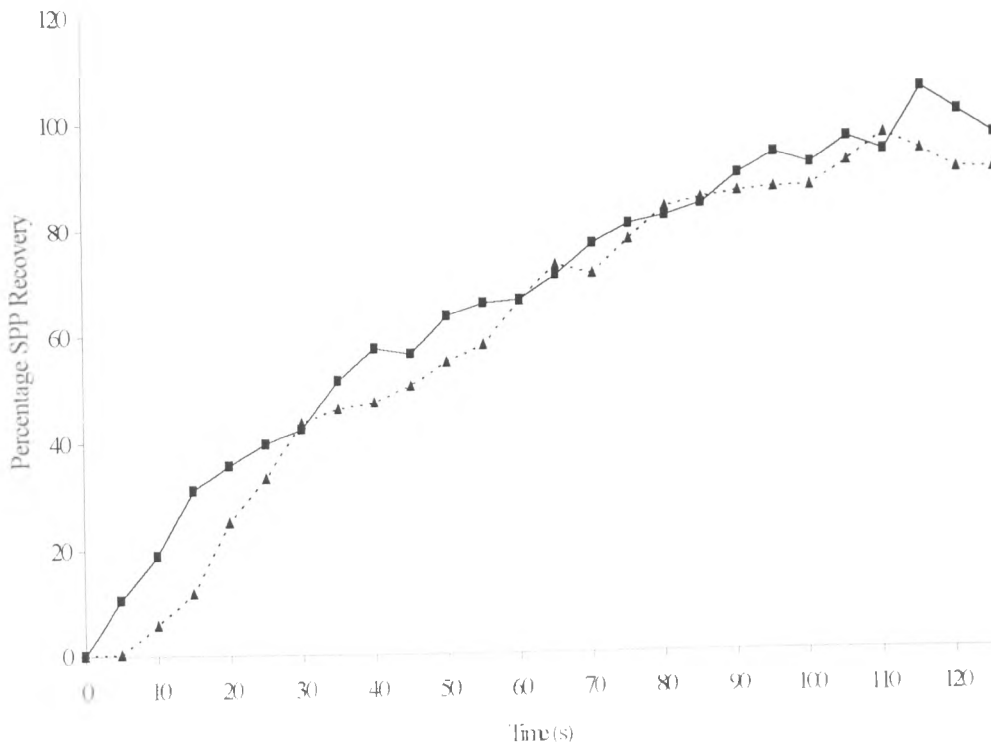


Figure 7. An example from one dog of the recovery of systemic perfusion pressure after decreases in coronary pressures following periods of 30 s (■) and 1 min (▲) at high coronary pressure. Data are expressed as percentages of the maximal responses.

Table 4. *Time for the systemic perfusion pressure to recover to 90% of its control level following 30 s and 1 minute of high stimulus pressure applied to coronary baroreceptors.*

Dog No.	Time to 90% perfusion pressure recovery (s)	
	30 s	1 minute
15	141	97
17	87	77
18	87	103
21	41	39
Mean	89.0	79.0
S.E.M.	20.4	14.4

The individual values of the time taken for systemic pressure to reach 90 % of its control level following stimulation periods of 2, 4 and 8 minutes are given in Tables 5, 6 and 7 respectively. A summary of the time course data comparing the times that systemic pressure took to recover to 90 % of its initial level following decreases in pressures to the three baroreceptor regions after periods of stimulation of up to 8 minutes are given in Table 8. Increasing the duration of stimulus applied to aortic and carotid baroreceptors had no effect on the rate of the resulting recovery of systemic pressure. When pressure applied to the coronary receptors was increased from 1 to 8 minutes although the systemic pressure recovery following coronary unloading was increased, this increase was not significant $P > 0.05$ when assessed with Student's unpaired t test incorporating both Cochran's and Satterthwaite's correction factors.

Table 5. *Time for the systemic perfusion pressure to recover to 90% of its control level following 2 minutes of high stimulus pressure applied independently to carotid, coronary or aortic baroreceptors.*

Dog No.	Time to 90% perfusion pressure recovery (s)		
	Carotid	Aortic	Coronary
3	18	8	89
5	47		
6	12	73	107
7	15	10	39
8	43	3	155
9	15	48	
10	60		
12		51	
13			49
22	13		
23		15	
Mean	27.9	29.7	87.8*
S.E.M.	6.7	10.3	20.9

* indicates $P < 0.05$ when coronary recovery is compared to carotid or aortic recovery (Students unpaired t test).

Table 6. *Time for the systemic perfusion pressure to recover to 90% of its control level following 4 minutes of high stimulus pressure applied independently to carotid, coronary or aortic baroreceptors.*

Dog No.	Time to 90% perfusion pressure recovery (s)		
	Carotid	Aortic	Coronary
3	18	31	47
5	36		
6	7	5	82
7	7		
8	44	13	
9	19		
12		75	
13			56
14	31		
15			90
23		15	82
Mean	23.0	27.8	71.4*
S.E.M.	5.4	12.5	8.4

* indicates $P < 0.05$ when coronary recovery is compared to carotid or aortic recovery (Students unpaired t test).

Table 7. Time for the systemic perfusion pressure to recover to 90% of its control level following 8 minutes of high stimulus pressure applied independently to carotid, coronary or aortic baroreceptors.

Dog No.	Time to 90% perfusion pressure recovery (s)		
	Carotid	Aortic	Coronary
1			241
3	11	19	
5	48		
6	6		32
7	6		
8	41		
10	177		
12		65	
13			136
15			161
16		48	
17		6	
24	19		334
Mean	44.0	34.5	180.8*
S.E.M.	23.0	13.4	50.8

* indicates $P < 0.05$ when coronary recovery is compared to carotid or aortic recovery (Students unpaired t test).

Table 8. Summary of the time for the systemic perfusion pressure to recover to 90% of its control level following a stimulus pressure applied independently to carotid, coronary or aortic baroreceptors.

Time to 90% perfusion pressure recovery (s)			
Duration of stimulus	Carotid	Aortic	Coronary
30 seconds			89.0±20.4 (4)
1 minute	26.5±5.4 (12)	31.5±6.2 (10)	70.3.0±7.7 (13)*
2 minutes	27.9±6.7 (8)	29.7±10.3 (7)	87.8±20.9 (5) *
4 minutes	23.0±5.4 (7)	27.8±12.5 (5)	71.4±8.4 (5) *
8 minutes	44.0±23.0 (7)	34.5±13.4 (4)	180.8±50.8 (5) *

* indicates $P < 0.05$ when coronary recovery is compared to carotid or aortic recovery (Students unpaired t test) Numbers of dogs given in parentheses.

(C) Responses to pulsatile and non-pulsatile coronary pressures

The results from the previous section have shown the much slower systemic pressure recovery following coronary baroreceptor unloading as compared to aortic or carotid baroreceptor unloading. In those tests aortic and carotid receptors were stimulated with non-pulsatile pressures and coronary receptors were exposed to the cardiac pulsations at the same mean pressure. This difference in stimulus type between the three baroreceptor groups could account for the different rates of systemic recovery. To answer this, in 6 dogs it was examined whether stimulating the coronary receptors with non-pulsatile pressures influenced the recovery time of systemic pressure.

Experimental protocol

In these experiments coronary baroreceptors were stimulated with high non-pulsatile pressures for 1 minute. The same criteria and the same method of analysing the time course of the perfusion pressure response were used as previously described in section (B). The time course of the perfusion pressure response from these experiments were compared to those when a pulsatile pressure was used. The modified circuit was used (see Methods and Figure 3) where by the heart was fibrillated and bypassed so no cardiac pulse was generated allowing non-pulsatile pressures to be applied.

Responses

When the stimulus was non-pulsatile increasing pressure from 7.1 ± 0.5 kPa to 25.0 ± 0.7 kPa decreased systemic pressure by 6.8 ± 1.2 kPa ($n=6$). This response was not significantly different from the systemic response of 7.4 ± 1.2 kPa ($n=13$) when the pulsatile stimulus was increased from 8.2 ± 0.3 kPa to 24.4 ± 0.8 kPa. The rate of increase in perfusion pressure following a period at the high level was not influenced by the pulsatile nature of the stimulus. A comparison of the systemic pressure recovery from one dog following a decrease in coronary pressure which had been maintained at a high level for 1 minute using non-pulsatile and pulsatile pressures is illustrated in Figure 8. These similar rates of recovery are exhibited in the group data where after 1 minute at high, non-pulsatile coronary pressure, systemic pressure took 71.1 ± 18.6 s ($n=6$) to reach 90 % of the initial value and this was not significantly different from the recovery times in animals where a pulsatile stimulus was used (70.3 ± 7.7 s, $n=13$) (Table 9).

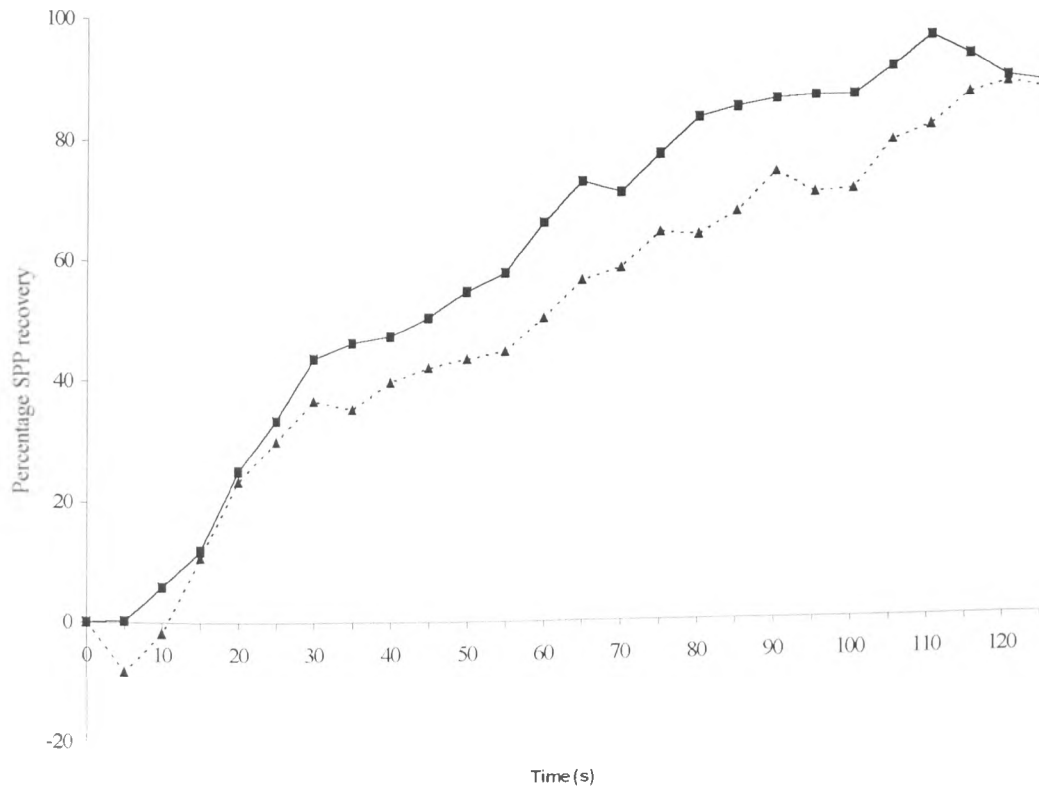


Figure 8. Comparison in one dog of the rate of recovery of systemic perfusion pressure after a decrease in coronary pressure following 1 minute of high pulsatile (■) and non-pulsatile (▲) coronary pressures.

Table 9. *Time for the systemic perfusion pressure to recover to 90% of its control level following 1 minute of either high non-pulsatile or pulsatile stimulus pressure applied to coronary baroreceptors.*

Dog No.	Time to 90% perfusion pressure recovery (s)	
	Non-Pulsatile	Pulsatile
1		107
3		89
5		57
6		84
8		73
10		33
12	13	18
13	52	81
14	112	
15		97
17	116	77
18		103
19		56
20	107	
21	30	39
Mean	71.7	70.3
S.E.M.	18.6	7.7

Discussion

In this series of experiments the surgical and perfusion techniques allowed application of pressure stimuli to the three isolated barosensitive regions: the carotid sinuses, aortic arch and coronary circulation whilst, assessing the resulting vascular resistance responses from the changes in systemic perfusion pressure. The technique of isolating the barosensitive regions enabled comparison of the vascular responses induced by discrete and independent stimulation of the three baroreceptor populations.

It has previously been reported that pressure changes in the pulmonary circulation produces reflex vascular responses (Coleridge & Kidd, 1963; Ledsome & Kan, 1977). These responses were avoided in the current study by incorporating a partial left-heart bypass into the perfusion circuit. This held left ventricular end-diastolic and left atrial pressures relatively constant during large pressure increases applied to the aortic root which increases left ventricular pressure and coronary pressure (Al-Timman & Hainsworth, 1992; Al-Timman *et al.*, 1993).

Pressures applied to coronary baroreceptors and aortic baroreceptors were isolated from each other by snaring the ascending aorta onto the curved stainless steel cannula 0.5 - 1 cm distal to the coronary ostia. The pressure in each region could then be altered independently of the other. During coronary pressure tests 0.5 - 1 cm of the aorta would also be exposed to these pressure changes but it is unlikely that the reflex responses from changes in aortic root pressure would have been initiated by aortic baroreceptors located within the short segment for the following reasons. Coleridge, *et al.*, (1973) have reported that out of 118 vagal afferent fibres projecting from the aortic arch, which were pressure sensitive, only 5 % were located in the

initial 0.5 - 1 cm of the ascending aorta. Furthermore, the dissection of this area and snaring this part of the aorta onto the cannula would most probably have denervated most of those receptors and the snaring would also restrict distension in this part of the aorta.

Pressure applied to the aortic root would alter pressure in the coronary arteries, left ventricle and the short aortic segment. The resulting reflex response to changes in aortic root pressure are due mainly, if not entirely, to receptors in the coronary circulation for reasons explained below.

Electrophysiological and reflex studies have provided evidence for the existence of coronary baroreceptors. Al-Timman *et al.*, (1993) used a preparation that allowed separation of the pressure stimulus between the coronary circulation and the left ventricle. In their study they showed that a combined increase in left ventricular systolic pressure and coronary pressure (aortic root pressure), as used in the present study, decreased systemic and hindlimb perfusion pressures. This denoted a vasodilatation as both regions were perfused at constant flow and vascular resistance responses are assessed from perfusion pressure changes. This is consistent with previously reported results (Challenger *et al.*, 1987; Tutt *et al.*, 1988b; Vukasovic *et al.*, 1989). This study was unique in that pressure in the left ventricle could be altered at constant aortic root pressure and pressure could be changed in the coronary arteries at constant left ventricular pressure. An increase in coronary pressure at a constant left ventricular systolic pressure caused a vascular resistance response which was only a slightly less than the combined response. Changes in left ventricular systolic pressure alone produced only a third of the combined response. As the largest individual response was from a coronary pressure test it was concluded that the

receptors likely to be responsible for this reflex were located in or near the coronary arteries.

The findings of Al-Timman *et al.*, (1993) were further substantiated by an electrophysiological study of the vagal afferents attached to coronary receptors by Drinkhill *et al.*, (1993). The apical ventricular cannula was again used to separate the stimulus between the coronary circulation and the left ventricle. The ten myelinated afferent fibres that were recorded from showed greater sensitivity to changes in mean coronary pressure than to changes in left ventricular systolic pressure. The receptive fields of these fibres were localised by gentle probing and were found to be mainly located to the region of the bifurcation of the circumflex and left anterior descending coronary arteries. This confirms the findings of two earlier studies where a reflex systemic vasodilatation was observed to a brief increase in pressure to a specific region containing the left coronary artery including the bifurcation of the left circumflex and left anterior descending coronary arteries (Okinaka *et al.*, 1963; Kurihara, 1964). When a similar stimulus was applied only to the arteries distal to the bifurcation a systemic vasodilatation was rarely seen.

The earlier observations of a reflex response originating from the coronary arteries is confirmed in the present study. In the bypassed and fibrillated heart pressure applied to the aortic root would not distend the left ventricle as the aortic valve would have remained closed. The effect would be an increase in coronary arterial pressure alone, and the magnitude of the reflex vascular responses to this was not significantly different from tests where both coronary and ventricular pressures changed simultaneously.

The three isolated baroreceptor regions investigated here are all known to contain chemoreceptors as well as baroreceptors (Comroe, 1964; Coleridge, *et al.*,

1970). It is not possible to exclude a contribution from chemoreceptors to the observed vascular response during changes in pressure to each region which in turn alters blood flow to each region. Potential chemoreceptor input was minimized by maintaining a high P_{O_2} level and ensuring that perfusion pressure in each region did not fall below 8 kPa (Lee, Mayou & Torrance, 1964; Biscoe *et al.*, 1970; Sampson & Hainsworth, 1972).

In view of all this evidence, it is reasonable to assume that changing the pressure to the aortic root, as used in the present preparation, is an effective method for applying a discrete stimulus to coronary baroreceptors.

The first part of this study involved the construction of baroreceptor-response curves to determine the baroreceptor pressure that induced the largest systemic response. Carotid and aortic baroreflexes have been characterised to a series of step increases in distending pressure and the response measured as changes in vascular resistance (Angell-James & Daly, 1970; Hainsworth, *et al.*, 1970; Donald & Edis, 1971). These have reported that the response from aortic baroreceptors were smaller in comparison to the carotid response. This is the first study to compare the vascular response from the three areas and reports that the magnitude of the carotid and coronary responses are comparable whereas the aortic response was significantly smaller than either the carotid or coronary responses.

An explanation for the smaller aortic arch induced response may be due to the method employed to isolate and apply pressures to the aortic arch. Isolation of the aortic arch involves dissection around the root of the aorta, origin of the brachiocephalic artery and at the origin of the left subclavian artery. Care was taken during the surgical isolation, however, some nerve damage may have occurred resulting in partial denervation of the arch. A further problem is that the arch is fixed

onto the steel cannula by ties and this is likely to restrict wall deformation and thereby alter the pressure-volume characteristics of the aortic arch. Angell-James, (1971a) examined the effect of reducing the longitudinal tension on the aortic arch in a preparation where baroreceptor afferent activity was recorded before and after severing the descending aorta distal to the origin of the left subclavian artery. Reducing the longitudinal tension increased the threshold pressure and inflexion pressure although the slope of the linear part of the curve remained unchanged. Nevertheless, despite these limitations the results reported here together with earlier data (Angell-James & Daly, 1970; Hainsworth *et al.*, 1970; Donald & Edis, 1971) suggests that aortic baroreceptors are less important in overall baroreceptor function than the other two regions.

The other aspect of our study was to examine the time courses of the reflex vasodilator and vasoconstrictor responses to changes in the level of stimulation of the three baroreflexes. Increasing the distending pressure in each region caused reflex vasodilatation and the time taken for the vasculature maximally to dilate was not significantly different for the three baroreflexes. The rate of vasodilatation reported here is comparable to that presented in an earlier study in anaesthetized dogs where an isolated perfused hindlimb dilated to a maximum in 16 seconds for aortic distension and in 14 seconds for carotid distension (Donald & Edis, 1971). Also, Richter, Keck & Seller (1970) examined the time course of vascular resistance changes in an isolated perfused gracilis muscle with intact innervation in response to electrical stimulation of the carotid sinus nerve, reporting a latency of 10 - 20 seconds for the minimum pressure value to be achieved. The results presented here indicate that the three populations of baroreceptors all respond rapidly to increases in pressure to initiate a

reflex vasodilatation, even though the magnitude of the response differs depending on which group of baroreceptors are stimulated.

Another aspect of baroreflex function is the rate of vasoconstriction after a group of baroreceptors are unloaded. There is no available literature quantifying the rates of vasoconstriction following unloading of any groups of arterial baroreceptors, though the vascular responses from aortic and carotid baroreceptors are known to occur very rapidly.

Decreasing baroreceptor pressure from saturation level to a low level initiates a reflex vasoconstriction, which in this preparation caused systemic perfusion pressure to recover towards its previous level. For all three groups of baroreceptors the rate of recovery of perfusion pressure was slower than the rate of vasodilatation. For carotid responses maximum vasodilatation occurred in 16.3 ± 1.4 s compared to 26.5 ± 5.4 s for 90 % recovery. However, for coronary responses the difference in the times for vasoconstriction and vasodilatation was very much greater. The rate of recovery following the decrease in the stimulus to the three groups of baroreceptors was found to be strikingly different. The rate of recovery following coronary baroreceptor unloading was significantly slower than the response to decreases in the stimulus to aortic or carotid baroreceptors. This finding that the coronary response is delayed compared to aortic and carotid responses is the same for each duration of stimulus applied.

This is the first report showing a delayed vascular response to unloading a specific baroreceptor region. There was an earlier study on reflexes from the left ventricle induced by balloon inflation in the bypassed heart, in which the authors reported that following balloon inflation perfusion pressure to isolated vascular beds recovered to 90 % of the initial level within 1 minute (Zelis, *et al.*, 1977). However, it

is not clear which receptors were being studied and the rate of the response was not quantified and would have been effected by input from aortic and carotid baroreceptors which were exposed to the changes in systemic pressure. Gorman, Cornish & Zucker, (1984) examined the time course of vascular resistance changes in response to intracoronary veratridine injections in conscious dogs. Interestingly, they reported that in sinoaortic denervated dogs the recovery of arterial blood pressure and iliac resistance was prolonged when compared to dogs with intact aortic and carotid baroreceptors. In the sinoaortic denervated state the only major group of functioning arterial baroreceptors would be those in the coronary arteries and it is possible that they are responsible for the delayed vasoconstriction following the reduction in blood pressure observed by Gorman *et al.*, (1984).

In the initial protocol the coronary baroreceptors were exposed to cardiac pulses generated by the heart at a controlled mean pressure, whereas the aortic and carotid baroreceptors were perfused with non-pulsatile pressures. As changes from pulsatile to non-pulsatile pressures applied to carotid baroreceptors have been shown to modify reflex responses (Ead, *et al.*, 1952; Angell-James & Daly, 1970 & 1971) it was possible that the delayed recovery following a period of stimulation of coronary baroreceptors could have been influenced by the nature of the stimulus applied. The protocol was therefore modified to create a full cardiopulmonary bypass and by fibrillating the heart this enabled application of a non-pulsatile pressure stimulus to the coronary baroreceptors. The results from these experiments showed that the slow rate of vasoconstriction following coronary baroreceptor unloading was not dependent on the stimulus being pulsatile.

Carotid and aortic baroreceptors have both been shown to respond rapidly to pressure changes. This is mirrored in recordings of afferent activity from these

baroreceptors, clearly displaying rapid response of afferent activity when pressure is increased and decreased. A possible explanation for the delayed coronary response might be that the baroreceptors remain active signalling a pressure stimulus after the pressure has been lowered to a low level. This question has been examined and the results presented (Drinkhill, *et al.*, 1996). Recordings from coronary vagal afferents display rapid responses to increases and decreases in distending pressure in the coronary arteries. This indicates that prolonged activation of the coronary baroreceptors cannot be responsible for the delayed vasoconstriction.

Other explanations for the prolonged time course could be that central pathways used by the coronary baroreflex impose a delay in the time of recovery of the sympathetic vasomotor activity following coronary baroreceptor stimulation. Another possibility is that the coronary baroreceptors may influence a different population of vasomotor nerves which induce responses with a slower time course. Humoral factors may also be involved in the delayed responses and may account for the further delayed recovery following longer periods of stimulation. However, the current experiments do not allow determination as to which of these reasons is responsible for the slow vasoconstriction following unloading of coronary baroreceptors.

CHAPTER 4.

**RESPONSES OF SYMPATHETIC EFFERENT NEURONAL ACTIVITY TO
CORONARY, CAROTID AND AORTIC BARORECEPTOR STIMULATION.**

Introduction

In the previous chapter there was shown to be a striking difference in the vasoconstrictor response between the coronary baroreceptors and the “classical” aortic arch and carotid sinus baroreceptors. The delay in the vasoconstrictor response from coronary baroreceptor unloading is not due to prolonged activation of the coronary baroreceptors (Drinkhill *et al.*, 1996). As the coronary baroreceptors respond rapidly to pressure changes, the delay in the reflex response resulting in the slow vasoconstrictor component of the coronary baroreflex could be in a central site. A prolonged central inhibition brought about by coronary baroreceptor stimulation may result in extended inhibition of sympathetic vasoconstrictor nervous activity. This type of prolonged inhibition of sympathetic efferent activity has been reported in several studies following sustained increases in arterial pressure brought about by pressor substances (Undesser, Jing-Yun, Lynn & Bishop, 1985; Kenney, Morgan & Mark, 1990) or following electrical stimulation of the aortic nerve (Kunze, 1986). However, there is no evidence as yet which associates this effect to physiological increases in arterial pressure and more specifically to coronary baroreceptors.

The aim of the experiments described in this chapter was to examine the efferent sympathetic discharge responses and vascular responses to loading and unloading of aortic arch, carotid sinus and coronary arterial baroreceptors. Efferent nerve responses were recorded from renal or lumbar nerves. These experiments were designed to resolve the mechanism responsible for the delayed vasoconstriction following coronary baroreceptor unloading.

Methods

Experiments were performed in anaesthetised dogs weighing 15 - 21 Kg. The surgical preparation and the perfusion circuit used in this study are similar to that described in the General Methods section and shown in Figure 2. In some experiments the hind limb on the contralateral side to the lumbar nerves recorded from was vascularly isolated by a previously described method (Challenger *et al.*, 1987). Approximately 4 cm of the femoral vein and artery were exposed and all branches ligated and divided. A nylon snare was placed around each of the three muscle groups at the proximal end of the limb, taking care to exclude the femoral and sciatic nerves and the femoral vessels from the snares. Once the limb was being artificially perfused through a cannula in the femoral artery at constant flow the isolation was completed by tightening the snares with metal cranking devices.

Initially the dog was connected to an abbreviated circuit which allowed all vascular cannulations to be carried out but which excluded all reservoirs and pumps and did not require heparinization of the animal. This technique as previously described (Drinkhill *et al.*, 1993 & 1996) is used during nerve dissection whilst a suitable unit is identified and limits blood loss due to the reduced time that the animal is on full perfusion. The curved stainless steel cannula in the ascending aorta and the cannula in the descending aorta were initially connected and the connections from here to the rest of the perfusion circuit were clamped. Cannulae which were also initially clamped, were inserted into: the left atrium, the central end of the left subclavian artery, the central end of the right common carotid artery, the peripheral end of the left subclavian artery, the central end of the left common carotid artery and the peripheral ends of both common carotid arteries.

Recordings of sympathetic efferent nerve activity were made from either the renal nerves running along the surface of the renal artery or the sympathetic nerve trunk at the level of the 3rd and 4th lumbar sympathetic ganglia. Both of these nerves were surgically exposed through an incision in the left flank of the animal. The nerve from which recordings were to be made was dissected free from the surrounding tissue and covered with warm (37 °C) paraffin oil. The nerve was laid on a black bakelite platform and a binocular microscope (Carl Zeiss Jena CK2, Carl Zeiss Ltd, Welwyn Garden City, Herts, UK; magnification x10 to x40) and fine forceps were used to dissect fine strands from the nerve trunk. These were wrapped around bipolar silver electrodes which were subsequently used to record efferent nerve activity from single or few-fibre preparations. The output from the electrodes was relayed to an amplifier and filtering system (Neurolog system, Digitimer Ltd., Welwyn Garden City, Herts, UK). The action potentials were subsequently displayed on a digital storage oscilloscope (Model OS 1420, Gould Ltd., Hainault, Essex, UK). The signal also passed into a spike processor (Model D130, Digitimer Ltd.) which incorporated an audioamplifier and loudspeaker.

The nerve fibres were tested prior to connection of the animal to the full circuit. Once a satisfactory unit had been found, the animal was heperinized (500 i.u. kg⁻¹, I.V.) and the clamps on the tubing between the abbreviated circuit and the full circuit were removed.

Experimental Protocol

After connecting the animal to the full perfusion circuit a series of pressure tests was performed. Pressures perfusing the coronary and aortic baroreceptors were

held constant at 8 kPa whilst carotid sinus pressure was increased from 8 kPa to a level which produced maximal vasodilatation. It was held at this level for 30 s and then reduced back in a single step to 8 kPa. Efferent sympathetic activity was recorded during this test and until the initial control level had been regained. The pressures perfusing carotid and aortic baroreceptors were then held constant at 8 kPa whilst coronary perfusion pressure was increased in steps from 8 kPa to a level which produced maximal vasodilatation. It was held at this level for 30 s and then reduced. The efferent activity was recorded until the control level was regained. Following this a carotid baroreceptor test was repeated. Experiments were only accepted if the vascular response exceeded 2 kPa and the perfusion pressure and efferent nerve activity recovered to at least 90 % of its initial value following the decrease in baroreceptor pressure.

In some animals the recovery in efferent discharge and systemic perfusion pressure were also examined following loading and unloading of the aortic arch baroreceptors.

The responses of nerve activity and perfusion pressures were normalized by expressing the responses to the increase in baroreceptor pressure as 100 % and calculating the recovery at any time after decreasing the baroreceptor pressure as a percentage of this response

Data are expressed as means \pm S.E.M. and statistical significance was assessed by Student's *t* test for paired data unless otherwise stated.

Results

The experiments reported in this chapter were performed in 12 dogs. After connecting the animal to the full perfusion circuit, pressures were allowed to stabilize and blood gases and pH were analysed and corrected as necessary to attain values of: P_{O_2} 26.2 ± 2.8 kPa; P_{CO_2} 5.3 ± 0.15 kPa and pH 7.4 ± 0.03 . Haematocrit was 19.4 ± 1.0 % ($n=12$).

Responses

Renal Nerves

Recordings were made from six renal sympathetic efferent nerve units in six dogs. Figure 9 shows original traces from one experiment which contrasts the rapid recovery of pressures and renal sympathetic nerve activity following a decrease in carotid pressure with the much slower vascular and neural responses to a decrease in coronary pressure.

In these six dogs, increasing carotid sinus pressure in twelve tests from 8.2 ± 0.1 to 26.0 ± 0.7 kPa resulted in a decrease in systemic perfusion pressure from 17.9 ± 2.0 to 11.1 ± 1.5 kPa and mean renal nerve activity decreased from 12.7 ± 3.7 to 2.2 ± 0.7 impulses s^{-1} . The maximal response of perfusion pressure and nerve activity to the increased carotid pressure were reached in 13.3 ± 1.0 and 1.4 ± 0.2 s respectively. Carotid pressure was held at this high level for 30 s and then decreased to its former level. Following this systemic perfusion pressure recovered to 90 % of

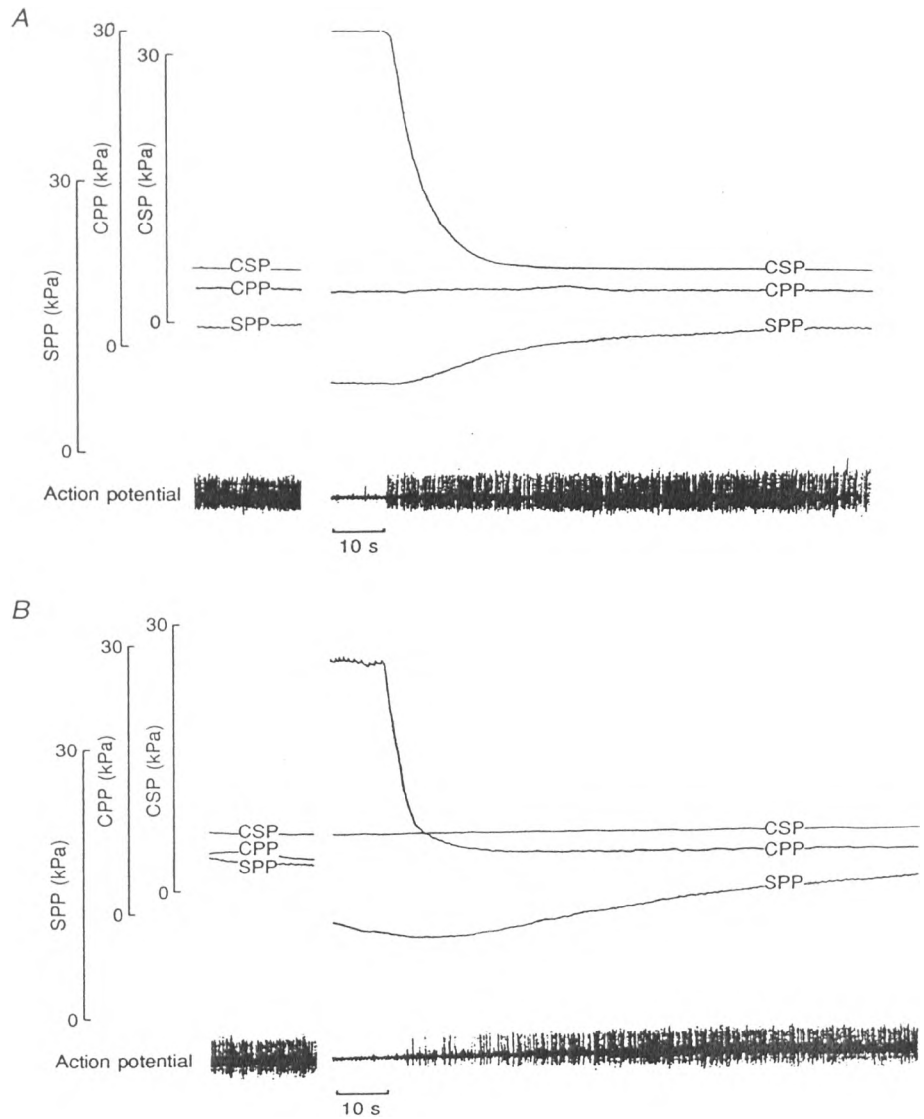


Figure 9. Electrical activity recorded from a strand of the renal nerve showing the responses to decreases in pressure to carotid (*A*) and coronary baroreceptors (*B*). These traces contrast the rapid increases in nerve activity and systemic perfusion pressure following a decrease in carotid pressure (*A*) with the slower responses to the decrease in coronary pressure (*B*). Original records from 1 dog. CSP, carotid sinus pressure; CPP, coronary perfusion pressure; SPP, systemic perfusion pressure.

the control level in 14.8 ± 2.9 s and renal nerve activity returned to 90 % of the initial level in 2.2 ± 0.5 s. Renal nerve activity then further increased to 147.3 ± 20.8 % of the control value within 10 s before decreasing again and stabilising within 35 s.

In these six dogs an increase in coronary perfusion pressure from 8.5 ± 0.2 to 26.1 ± 0.6 kPa resulted in responses of systemic perfusion pressure and renal nerve activity which were not significantly different from the responses to increases in carotid pressure. Systemic perfusion pressure decreased from 18.9 ± 1.9 to 11.4 ± 1.4 kPa and renal nerve activity decreased from 15.0 ± 4.1 to 1.5 ± 0.7 impulses s^{-1} . The systemic perfusion pressure and nerve activity responses to the coronary pressure increases were maximal in 14.5 ± 1.3 and 1.8 ± 0.2 s respectively. These times were not significantly different from those obtained to carotid baroreceptor stimulation. Coronary pressure was held at this high level for 30 s and was then decreased to its initial low level. The time course of the responses of both systemic perfusion pressure and renal nerve activity were completely different from those to decreases in carotid sinus pressure. The return of both pressure and nerve activity to their former levels occurred slowly and there was no overshoot. The times for pressure and neural activity to return to 90 % of the way back to the control values were 110 ± 13 s and 76.2 ± 12 s. Both these times were significantly longer than the recovery times following a decrease in carotid pressure ($P < 0.005$). The times taken for renal nerve activity and systemic pressure to recover to 90 % from individual animals are shown in Table 10.

Figure 10 illustrates data expressed as percentages of the responses to the increases in baroreceptor pressure. Values are shown at 5 s intervals, from tests of a decrease in carotid pressure, followed by a decrease in coronary pressure, and a

subsequent carotid test. This emphasizes the slower responses to the coronary test. The mean data from all 6 dogs are shown in Figure 11 again emphasizing the much slower responses and the absence of overshoot of both efferent activity and systemic perfusion pressure following coronary baroreceptor unloading.

In three of the dogs responses to an increase and decrease in aortic arch pressure were examined. Increasing aortic arch pressure from 7.9 ± 0.1 to 27.6 ± 0.2 kPa decreased efferent renal nerve activity from 37.4 ± 17.6 to 18.0 ± 8.7 impulses s^{-1} . Aortic pressure was maintained at the high level for 30 s after which time it was decreased to its original level and efferent activity returned to within 90 % of its initial value in 2.7 ± 0.7 s.

Table 10. *Time for renal sympathetic efferent nerve activity and systemic perfusion pressure to recover to 90 % of its control level following 30 seconds of high pressure applied independently to carotid and coronary baroreceptors.*

Dog No.	Time to 90 % recovery (s)			
	Carotid		Coronary	
	Activity	SPP	Activity	SPP
1	4	13	77	45
2	1	12	95	125
3	1	9.5	54	113
4	2	25	57	125
5	3	7.5	127	142
6	2	22	47	107
Mean	2.2	14.8	76.2*	110.0*
S.E.M.	0.5	2.9	12.0	13.0

* indicates $P < 0.05$ when times of activity and SPP recovery following a coronary test are compared to carotid activity and SPP recoveries (Students paired t test).

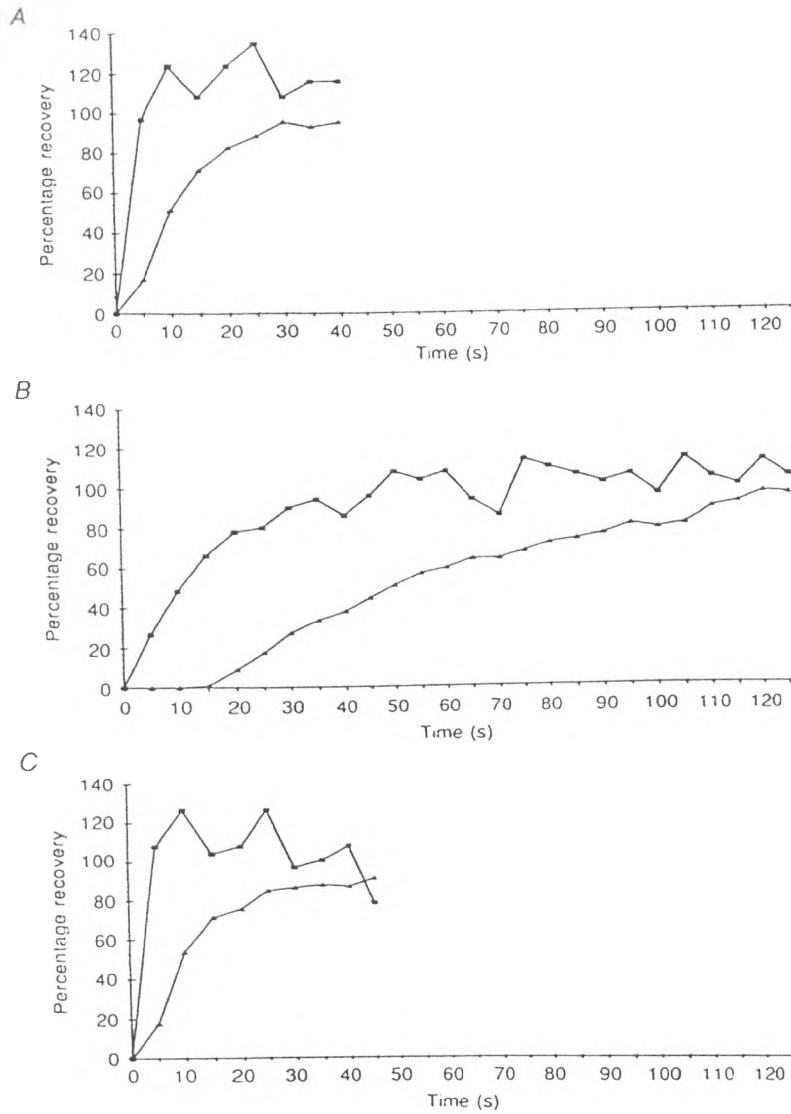


Figure 10. Results from one dog contrasting the time course of the increases in renal efferent nerve activity and arterial perfusion pressure following a decrease in carotid pressure (*A* and *C*) and coronary pressure (*B*). Pressures to each baroreceptor region were maintained at saturation levels for 30 s before decreasing. The coronary test was bracketed by two carotid tests. ■, renal efferent nerve activity; ▲, systemic perfusion pressure.

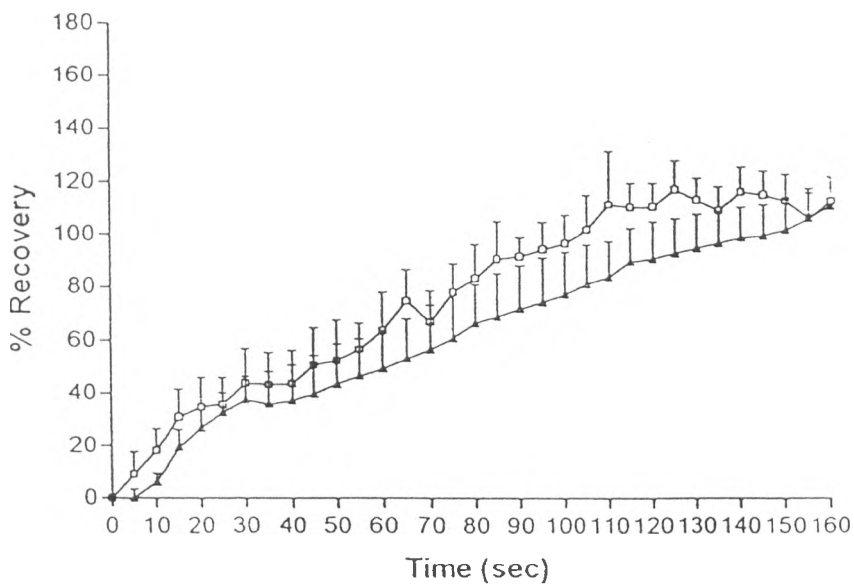
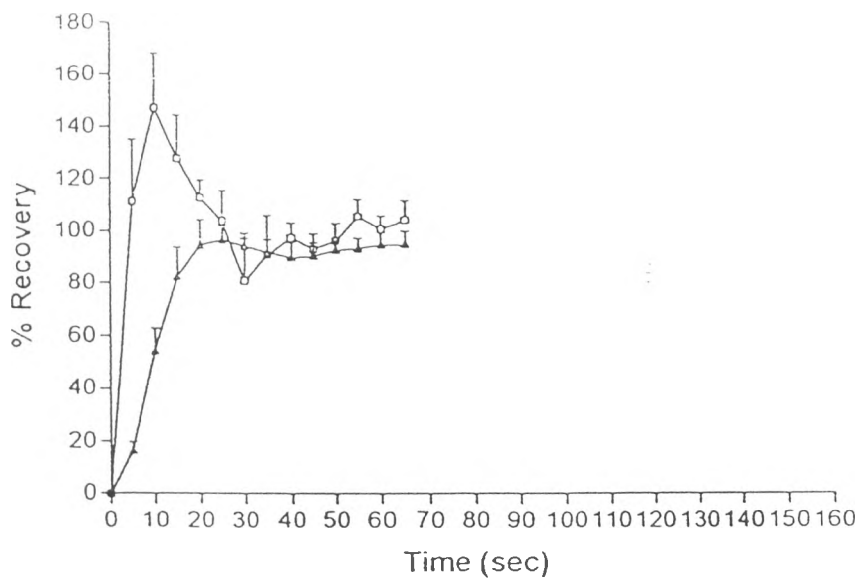


Figure 11. Group data from 6 dogs showing percentage recovery in renal efferent nerve activity (□) and SPP (▲) following a decrease in carotid (top) or coronary (bottom) pressure. Note the rapid increase in efferent nerve activity (with overshoot) following the carotid test is not seen following coronary baroreceptor stimulation. Results show means \pm S.E.M.

Lumbar efferent sympathetic nerves

Recordings were made from six lumbar sympathetic efferent nerves units in six dogs. Figure 12 shows original results from one experiment which contrasts the rapid recovery of pressures and lumbar sympathetic nerve activity following a decrease in carotid pressure with the much slower vascular and neural responses to a decrease in coronary pressure.

In the six dogs increasing carotid sinus pressure from 8.2 ± 0.1 to 27.8 ± 0.7 kPa resulted in a decrease in systemic perfusion pressure of 8.7 ± 1.8 kPa and mean lumbar efferent nerve activity decreased from 11.0 ± 2.8 to 2.8 ± 1.1 impulses s^{-1} . The responses were maximal in 11.6 ± 0.8 and 1.5 ± 0.2 s respectively. Following a decrease in carotid pressure to the initial low level, systemic perfusion pressure and lumbar efferent discharge recovered rapidly to within 90 % of their initial values in 18.0 ± 7.2 and 1.8 ± 0.6 s respectively. Lumbar nerve activity then further increased to 236.8 ± 72.2 % of the control value within 5 s and it remained at a higher level than the control during the recovery period

In the same dogs increasing coronary pressure from 8.4 ± 0.2 to 25.9 ± 0.9 kPa decreased systemic perfusion pressure from 19.3 ± 1.6 to 10.6 ± 0.4 kPa and mean lumbar efferent nerve activity decreased from 15.7 ± 6.9 to 4.1 ± 1.8 impulses s^{-1} ; maximal responses were obtained in 11.5 ± 0.7 and 2.0 ± 0.5 s respectively. Following a decrease in coronary pressure to the initial low level, the times for recovery to 90 % of the initial value were significantly longer than the responses to carotid baroreceptor unloading: 74.8 ± 17.4 s ($P < 0.005$) for systemic perfusion pressure and 30.7 ± 2.1 s ($P < 0.005$) for lumbar activity. No overshoot of either response to coronary

unloading was seen. The individual rates taken for lumbar nerve activity and systemic pressure to recover to 90 % of the initial levels are given in Table 11.

The mean data for recovery of both systemic perfusion pressure and lumbar efferent nerve activity for these six dogs is shown in Figure 13. Again emphasizing the much slower responses and the absence of overshoot of both efferent activity and systemic perfusion pressure following coronary baroreceptor unloading.

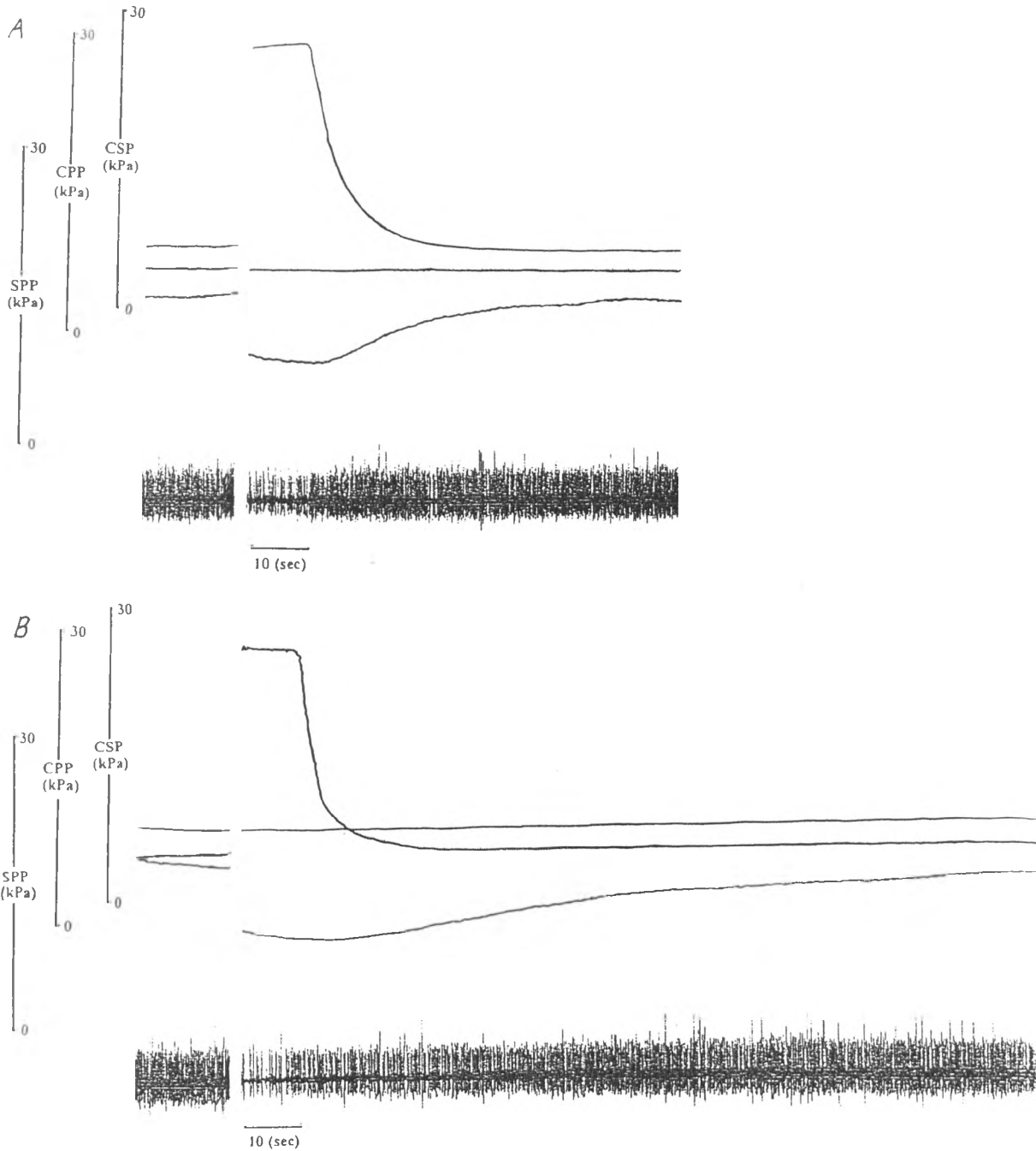


Figure 12. Electrical activity recorded from a strand of the lumbar nerve showing the responses to decreases in pressure to carotid (*A*) and coronary baroreceptors (*B*).

These traces contrast the rapid increases in nerve activity and systemic perfusion pressure following a decrease in carotid pressure (*A*) with the slower responses to the decrease in coronary pressure (*B*). Original records from 1 dog. CSP, carotid sinus pressure; CPP, coronary perfusion pressure; SPP, systemic perfusion pressure.

Table 11. *Time for lumbar sympathetic efferent nerve activity and systemic perfusion pressure to recover to 90% of its control level following 30 seconds of high pressure applied independently to carotid and coronary baroreceptors.*

Dog No.	Time to 90 % recovery (s)			
	Carotid		Coronary	
	Activity	SPP	Activity	SPP
1	1	9	29	50
2	4.5	13.5	33	105
3	1	13	25	25
4	2	54	30	143
5	1	8	27	70
6	1	13	40	56
Mean	1.8	18.4	30.7*	74.8*
S.E.M.	0.6	7.2	2.1	17.4

* indicates $P < 0.05$ when times of activity and SPP recovery following a coronary test are compared to carotid activity and SPP recoveries (Students paired t test).

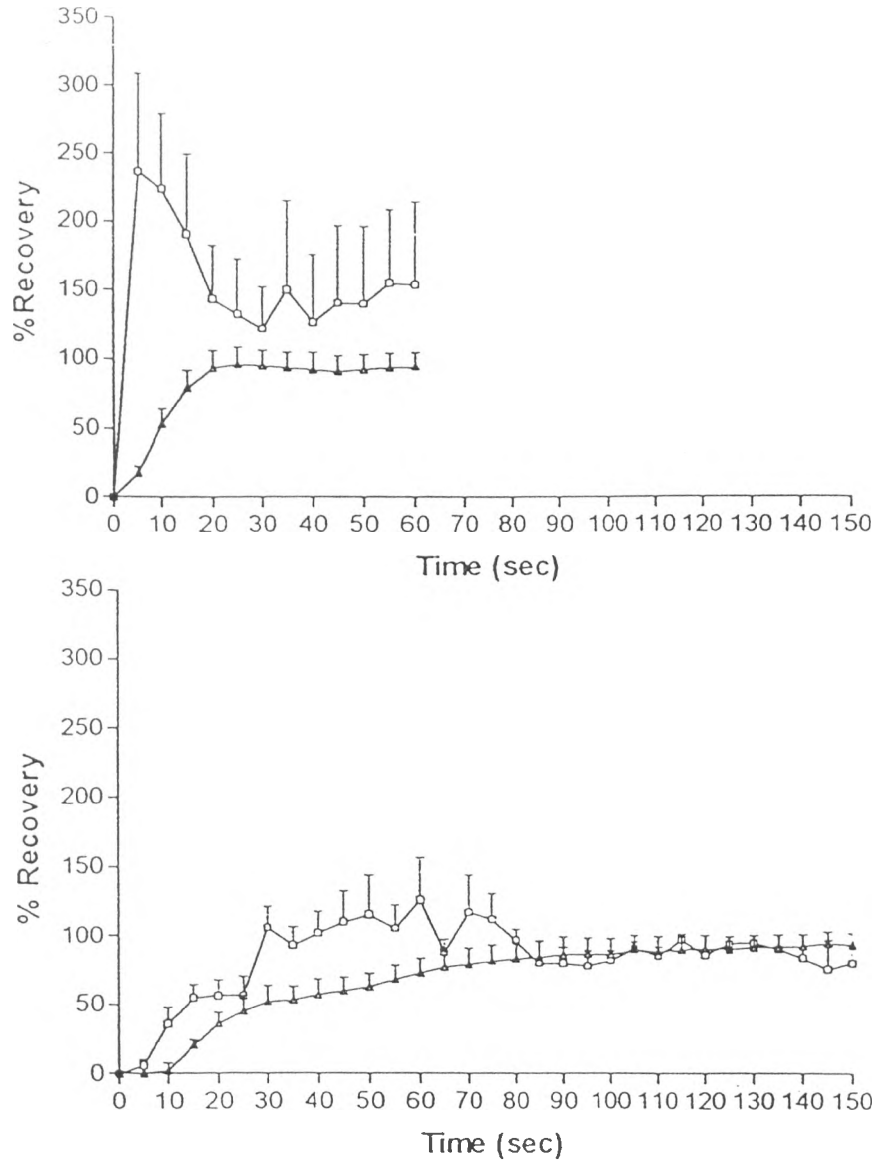


Figure 13. Group data from 6 dogs showing percentage recovery in lumbar efferent nerve activity (□) and SPP (▲) following a decrease in carotid (top) or coronary (bottom) pressure. Note the rapid increase in efferent nerve activity (with overshoot) following the carotid test which is not seen following coronary baroreceptor stimulation. Results show means \pm S.E.M.

Comparison of renal and lumbar responses

A comparison was also made between the recovery time of lumbar and renal efferent discharge following a coronary or carotid baroreceptor test. This showed that there was no significant difference in the times of recovery between renal and lumbar sympathetics to stimulation of carotid baroreceptors (2.2 ± 0.7 s and 1.8 ± 0.6 s respectively). However, recovery of renal sympathetic efferent discharge following coronary baroreceptor stimulation was significantly longer than recovery time of lumbar sympathetic efferent discharge (76.2 ± 12.0 s and 30.7 ± 2.1 s respectively, $P < 0.05$ unpaired t test). This is illustrated in Figure 14.

Hind limb vascular responses

In five dogs vascular responses were assessed in a hind limb in addition to recording systemic responses. The hind limb was vascularly isolated and perfused at constant flow (see General Methods). Responses to unloading carotid and coronary baroreceptors were recorded and the time of recovery of limb perfusion pressure to 90 % of its initial level determined. Following a decrease in coronary pressure limb perfusion pressure recovered to 90 % of the initial level in 90.0 ± 25.2 s which was significantly ($P < 0.05$) slower than the recovery time of 16.6 ± 2.7 s to reach the 90 % level following a reduction in carotid sinus pressure. These times were not significantly different from those observed in the remainder of the systemic circulation (Table 12).

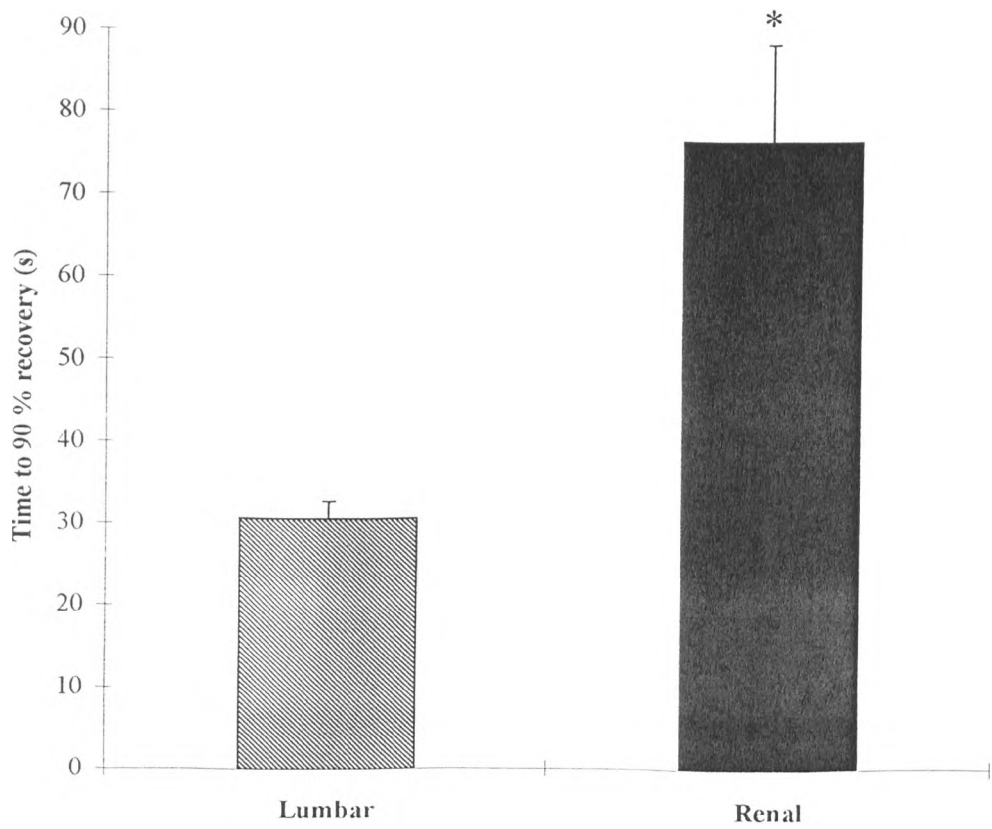


Figure 14. Group results from 6 lumbar tests and 6 renal tests showing the recovery time of renal and lumbar sympathetic efferent nerves following a decrease in coronary pressure. This demonstrates the significant slower recovery of renal nerve activity as compared to lumbar nerve activity (* indicates $P < 0.05$, Student's unpaired t test).

Table 12. *Time for hind limb perfusion pressure (HPP) to recover to 90% of its control level following 30 seconds of high pressure applied independently to carotid and coronary baroreceptors.*

Dog No.	Time to 90 % recovery (s)	
	Carotid	Coronary
	HPP	HPP
1	21	30
2	14	136
3	11	91
4	12	38
5	25	155
Mean	16.6	90.0*
S.E.M.	2.7	25.2

* indicates $P < 0.05$ when time of HPP recovery following a coronary test is compared to carotid HPP recovery (Students paired t test).

Discussion

As discussed in the previous chapter there are two main mechanisms which may account for the delayed vascular responses from coronary baroreceptors. The first possible explanation for this delay is that afferent activity from coronary baroreceptors may persist for some time after coronary pressure had been restored to a low level and the other possible explanation is that coronary baroreceptor stimulation initiates a prolonged central inhibitory mechanism that delays the recovery of sympathetic efferent nerve activity.

The potential mechanism of prolonged activation of coronary baroreceptors has been examined in an electrophysiological study of vagal afferents that respond to changes in coronary pressure and have their receptive fields localised to the coronary arteries (Drinkhill *et al.*, 1996). In that study, activity was recorded from six myelinated vagal afferent fibres whilst coronary pressure was increased from 8 kPa in 4 kPa steps until the response saturated. All six fibres showed an instantaneous increase in discharge following an increase in coronary pressure. Upon decreasing coronary pressure from the high level back to 8 kPa discharge in all six fibres decreased immediately to the initial level. This study has confirmed that coronary baroreceptor afferent activity responds immediately to pressure changes and does not persist after removal of the pressure stimulus. Therefore the delayed vasoconstriction initiated by coronary baroreceptors is not due to prolonged activation of coronary baroreceptors.

The role of a possible prolonged central inhibitory mechanism which causes a subsequent delay in the recovery of sympathetic efferent nerve activity caused by

coronary baroreceptor stimulation has been addressed in this chapter. The efferent pathway of the coronary baroreflex is known to involve sympathetic efferent nerves (Brown, 1966; Challenger *et al.*, 1987). In a series of experiments using perfusion techniques similar to that used in this study, Challenger *et al.*, (1987) demonstrated that by cutting the sciatic and femoral nerves supplying an isolated perfused hind limb the vascular resistance responses to step increases in coronary pressure were abolished. Also, Brown, (1966) used direct cannulation of the left coronary artery to apply a pressure stimulus and he was able to demonstrate reflex decreases in left renal nerve activity to increases in stimulus pressure. However, it is not known whether coronary baroreceptor stimulation affects the same or a different population of sympathetic efferent nerves or whether they induce different discharge patterns in the same nerves. Other studies have reported that receptors concerned with cardiovascular control can have differential effects on sympathetic efferent outflow (Ninomiya, Nisimaru & Irisawa, 1971; Karim, Kidd, Malpus & Penna, 1972).

To examine whether coronary stimulation was inhibiting the recovery of sympathetic efferent activity and affecting different populations of sympathetic nerves electrophysiological recordings were made of renal and lumbar efferent neural discharge during changes in coronary pressure. These responses were compared to changes in carotid sinus pressure and in some animals to changes in aortic arch pressure.

It is clear from recordings of few or single unit of either renal or lumbar nerves that the same unit responded to coronary, carotid and where studied aortic stimulation. Therefore this does not support the hypothesis that the reflex responses from stimulation of coronary receptors are mediated through different sympathetic efferent populations. Even though the same sympathetic efferent fibres responded to

stimulation of all three groups of baroreceptors, the pattern of discharge following unloading of the coronary baroreceptors was quite different from those occurring in response to unloading the other baroreceptors. When carotid sinus or aortic arch pressure was reduced from a high to low (8 kPa) level, efferent discharge increased very rapidly, often in the first few seconds achieving a level far greater than the initial level. This rapid recovery of efferent discharge was associated with a correspondingly rapid rise in systemic pressure sometimes also with an overshoot. Rapid recovery rates of sympathetic efferent activity following carotid baroreceptor stimulation with pressures (Green & Heffron, 1968; Kezdi & Geller, 1968) and electrical stimulation of the carotid sinus nerve (Richter *et al.*, 1970) have been reported, and these are entirely consistent with the present results. In contrast, reducing coronary pressure from a high value to a low (8 kPa) value resulted in a gradual increase in efferent sympathetic discharge and a correspondingly slow increase in systemic pressure. Neither response showed an overshoot.

In this study the recovery of efferent sympathetic nerve activity was related to the recovery of systemic perfusion pressure. However, recordings were made from left renal nerves which innervate the left kidney (DiBona, 1982) and from lumbar nerves which mainly innervate the hind limb (Donald & Ferguson, 1970; Cloninger & Green, 1955). In some dogs a more specific comparison of vascular resistance changes and sympathetic efferent discharge responses were made by relating the response of lumbar nerves to those of perfusion pressure to a vascularly isolated hind limb on the side opposite to the lumbar nerves being studied. The hind limb vascular resistance changes showed a similar time course to the systemic pressure changes.

This demonstration of prolonged inhibition of sympathetic efferent discharge after the cessation of a period of localised stimulation of coronary baroreceptors could

explain the previously reported persistent inhibition of renal sympathetic nerve activity brought about by sustained increases in arterial pressure through the pressor substances, phenylephrine and angiotensin II (Undesser *et al.*, 1985) and phenylephrine (Kenney *et al.*, 1990). Coote & Sato, (1977) have also reported an attenuated inhibition of renal sympathetic nerve activity after increases in arterial pressure through infusion of phenylephrine in rats. However, in this study the duration of sympathetic inhibition was measured from the moment that it was completely abolished to the moment activity reappeared, whereas in the present study the recovery of nerve activity was determined from the time that baroreceptor pressure was reduced.

In the report from Undesser *et al.*, (1985) the delayed recovery of renal activity only became apparent when arterial pressure was raised for periods of 5 minutes or more, which is in contrast to the results presented in this chapter where stimulation of coronary receptors for 30 s resulted in a prolonged inhibition of renal sympathetic nerve activity. Interestingly, Undesser *et al.*, (1985) reported that sectioning the vagus and the aortic nerve, which would have removed the effect of coronary baroreceptors, resulted in a faster recovery of renal nerve activity. Care must be taken in interpreting results from these studies (Coote & Sato, 1977; Undesser *et al.*, 1985; Kenney *et al.*, 1990) as pressor substances have been shown to directly effect baroreceptors and have actions through central pathways and peripheral effects with a number of consequences (Bregel, Anand, Brooks, MacDermott, Peveler, Robinson & Sleight, 1980; Lumbers, Ismay, Lee, McCloskey, Potter & Stevens, 1980; Goldman & Saum, 1984; Munch, Thoren & Brown, 1987; Reid, 1992).

A similar type of prolonged inhibition of renal sympathetic efferent activity has also been demonstrated following electrical stimulation of the left aortic nerve in

rabbits in which the carotid sinus nerves, the right depressor nerve and both vagi were sectioned (Kunze, 1985). The results reported in this chapter differ from those of Kunze, (1986), in that specific physiological stimulation of aortic baroreceptors did not result in prolonged recovery of renal nerve activity. This difference may be ascribed to the difference between the effects of electrical stimulation of a mixed nerve and of applying a more physiological stimulation to a reflexogenic area.

The mechanism responsible for the prolonged inhibition of sympathetic efferent activity following coronary baroreceptor stimulation is not known, however, the delayed response is not apparent in the afferent limb of the reflex arc (Drinkhill *et al.*, 1996) but is present in the sympathetic efferent limb. Therefore a central mechanism must be responsible for the prolonged inhibition of sympathetic efferent discharge.

Glossopharyngeal afferents from the carotid sinuses terminate in the nucleus tractus solitarius (NTS) and vagal afferents from the aortic arch and heart terminate in the NTS and dorsal motor vagal nucleus (DMVN) (Loewy, 1990). Within the NTS there is integration of the afferent signals with different types of interneuronal connections modulated by a variety of neurotransmitters (Dampney, 1994; Spyer, 1994). With specific regard to cardiovascular control, the NTS has projections to vagal preganglionic neurons located mainly in the nucleus ambiguus (Loewy & Spyer, 1990) and sympathetic preganglionic neurones located in the thoracic and upper lumbar segments of the spinal cord (Coote, 1988) which in turn govern efferent parasympathetic and sympathetic outflows.

Long term inhibition of central neurones has been described by Bennet, Goodchild, Kidd & McWilliam, (1988). They recorded from neurones located in the NTS and DMVN in the cat which responded to electrical stimulation of the vagus

nerve. Electrical stimulation of a cardiac or pulmonary vagal branch produced prolonged inhibition of evoked or spontaneous activity of neuronal activity. This inhibition lasted up to 10 s after cessation of the stimulus before a second stimulus could produce a response. No pattern of effects could be attributed to specific vagal branches. The findings of this study do not relate directly to the coronary baroreceptor responses presented in this chapter, but they do indicate that a mechanism exists which is capable of producing a long lasting inhibition of NTS and DMVN neurones following brief activation of vagal cardiac afferents.

How the coronary baroreceptors integrate with the central cardiovascular control system is at present not known. It is clear from the results seen in this chapter and in Chapter 2 that the coronary baroreflex responds to increases in pressure with a time course comparable to that of the aortic arch baroreflex and the carotid sinus baroreflex. This suggests that the central pathways from these three groups of baroreceptors, leading to inhibition of preganglionic sympathetic neurones have similar relay times. However, the inhibition of sympathetic efferent activity persists long after coronary pressure has been reduced, whereas sympathetic efferent activity recovers immediately upon decreasing aortic arch or carotid sinus pressure. The coronary response may be due to the release of an inhibitory neurotransmitter/s which has actions long after its release.

CHAPTER 5.

**VASCULAR RESPONSES TO STIMULATION OF CAROTID, AORTIC
AND CORONARY ARTERY BARORECEPTORS WITH PULSATILE AND
NON-PULSATILE PRESSURES.**

Introduction

There have been numerous studies of the responses of vascular resistance in various regions of the circulation to stimulation of carotid baroreceptors (see Kirchheim, 1976; Sagawa, 1983). These have shown that carotid baroreceptors have buffering capacity for changes in arterial pressure both below and above the normal arterial pressure (Donald & Edis, 1971; Clement & Shepherd, 1972 Coleridge, *et al.*, 1987). It has also been well established that stimulation with pulsatile, rather than non-pulsatile pressures displaces the stimulus-response curve so that lower pressures are required to induce reflex responses, though this effect is dependent on the location of the stimulus pressure on the response-curve, as the pulsatile and non-pulsatile curves diverge at low pressures and merge at high pressures (Ead, *et al.*, 1952; Scher & Young, 1963; Kezdi & Geller, 1968; Koushanpour & McGee, 1969; Angell-James & Daly, 1970 & 1971; Schmidt, *et al.*, 1972; Chapleau & Abboud, 1987).

In contrast to the carotid receptors, less is known of the stimulus-response characteristics of aortic arch baroreceptors. What studies there have been suggest that higher pressures are required in the aortic arch to induce vascular responses (Allison, Sagawa & Kumada, 1969; Hainsworth, *et al.*, 1970; Donald & Edis, 1971; Pelletier, *et al.*, 1972; Pelletier & Shepherd, 1973). However, this may be an artifact resulting from the extensive surgical procedures needed to isolate the aortic arch (see Discussion, Chapter 3). One reason for suspecting this is that studies of afferent discharge from these receptors, in animals not subjected to extensive surgical procedures, have shown that many receptors are active at pressures far below those which have been shown to be necessary to induce reflex changes (Bloor, 1964; Homma & Suzuki, 1966; Aars, 1968; Angell-James, 1971*a, b*; Pelletier *et al.*, 1972;

Arndt, Dörrenhaus & Wiecken, 1975; Brown, *et al.*, 1976; Samodelov, Godehard & Arndt, 1979; Coleridge, Coleridge, Kaufman & Dangel, 1981). A further problem in interpreting the results from previous studies of the aortic reflex is that often no information is provided concerning the pulsatility of the stimulus. However, there have been reports that, unlike the carotid receptors, aortic baroreceptors do not induce greater reflex responses when a pulsatile stimulus is substituted for a non-pulsatile one (Angell-James & Daly, 1970, 1971).

The results in Chapter 3 showed that the vascular responses from coronary baroreceptors are comparable to those from aortic arch and carotid sinus baroreceptors. However, only one aspect of the coronary baroreflex, the rate of vascular resistance changes were examined with non-pulsatile and pulsatile pressures, and was shown to have no effect. It is not yet known whether pulsatility has any effect on other aspects of the reflex responses to changes in coronary arterial pressure.

The experiments in this study had two main objectives and are presented in two parts:

(A) The aim of these experiments was to compare the reflex vascular responses from each baroreceptor region to pulsatile and non-pulsatile stimuli.

(B) The objective of this section was to compare the operating ranges of the three baroreceptor groups. In particular to examine the stimulus-response relationships under conditions which were as physiological and as comparable as possible,

Methods

Experiments were undertaken in anaesthetized dogs weighing 14 - 20 Kg. The surgical preparation and the cardiopulmonary bypass perfusion circuit (Figure 3) used in this study are the same as that described in Chapter 3. The pulsatile pressures were generated and applied by the method previously described in the General Methods chapter.

Results

This series of experiments were performed in 13 dogs. After connection of the perfusion circuit the pressures distending the three baroreceptor regions were held at non-pulsatile values of approximately 8 kPa. The pump perfusing the systemic circulation was adjusted to give a perfusion pressure of about 24 kPa and the preparation was allowed to stabilize for 30 min. During this time blood gases and pH were corrected as necessary to attain values of: P_{O_2} 26.5 ± 3.4 kPa; P_{CO_2} 5.3 ± 0.1 kPa and pH 7.3 ± 0.02 units. Haematocrit was 10.5 ± 0.7 %.

(A) Comparison of the effects of pulsatile and non-pulsatile baroreceptor pressures

This series of experiments were conducted to compare the response of each baroreceptor region to step changes in non-pulsatile and pulsatile distending pressures.

Experimental Protocol

After the initial period to allow stability a series of stimulus-response curves were generated for each baroreceptor region using pulsatile and non-pulsatile pressures. The pressures distending two of the baroreceptor regions were maintained at approximately 8 kPa (non-pulsatile) while the pressure distending the third region was increased in steps of 4 kPa until no further vascular responses were obtained. Pressure was maintained at each step (about 60 s) to obtain a 30 s recording of steady-state responses. In two dogs, coronary pressure was increased in 2 kPa steps from 6 kPa to 12 kPa, then in 4 kPa steps. The pressure was decreased in a single step to the initial value. The pressure steps were initially non-pulsatile. Then pulsatile steps (e.g. Figure 15) were applied at the same mean pressures but with triangular pulses of amplitude 10 kPa and a frequency of 2.5 Hz (150 min^{-1}).

Analysis of the results

Only results from tests in which the overall reflex response to a baroreceptor test exceeded 3 kPa were analysed in detail. Plots were obtained of perfusion pressures against baroreceptor distending pressures for both non-pulsatile and pulsatile stimuli. Two methods were used to analyse the relationships between baroreceptor distending pressures and responses of systemic perfusion pressure. One method involved simply joining the individual points and measuring from the plots. The other approach employed a commercial curve fitting computer program (GraphPad ver. 2.0, GraphPad Software Incorporated, USA) which derived a sigmoid curve to fit mean steady state values for systemic or limb perfusion pressure for each

baroreceptor pressure. The nonlinear regression equation used was Boltzman's sigmoidal, a four parameter sigmoidal function.

Various measurements were made from each curve. Saturation pressure was taken as the baroreceptor pressure corresponding to 95 % of the total perfusion pressure response. The maximum slopes of the curves were determined in two ways: from the slope of the two adjacent points with the steepest gradient, and from the differential of the sigmoid function. The slope represents the ratio of the response to the stimulus, and is commonly used as a measure of the sensitivity of baroreceptor reflexes (Sagawa, 1978, 1983). The inflexion points were the mid-points of the baroreceptor pressures giving maximum slope and the pressures corresponding to the peak differentials of the sigmoid functions. Threshold values are not reported as, in many experiments, particularly those examining the coronary baroreflex, responses were obtained at the lowest pressures tested.

Student's paired and unpaired *t* tests were used for statistical analysis. Values reported are of means \pm S.E.M.

Responses

Satisfactory results were obtained from a total of thirteen dogs; six dogs for coronary responses, five dogs for aortic and six for carotid responses. In some dogs after testing responses to one baroreceptor region it was possible also to test the responses to another. In one animal responses from all 3 regions were studied, in two others responses were obtained from both coronary and aortic baroreceptors.

Coronary responses

Figure 15 shows an example of the reflex responses to step changes in coronary pressure for both pulsatile and non-pulsatile stimuli. Overall, in the six dogs studied, an increase in non-pulsatile coronary pressure from 7.0 ± 0.3 to 25.6 ± 0.9 kPa caused a decrease in systemic perfusion pressure of 6.8 ± 0.4 kPa (30.9 ± 2.0 %). When coronary pressure was pulsatile a similar pressure increase, from 7.3 ± 0.4 to 24.1 ± 0.1 kPa caused a similar response of perfusion pressure of 7.3 ± 1.3 kPa (34.1 ± 4.6 %) (Table 13)

The average response of perfusion pressure to changes in pulsatile and non-pulsatile coronary pressures are compared in Figure 16. None of the perfusion pressures at any of the values of coronary pressure were different between pulsatile and non-pulsatile stimuli. There were also no differences in maximum slopes, inflexion points or saturation points when the curves were analysed by either of the curve fitting methods. The individual values of slope, inflexion pressure and saturation pressure are compared in Table 14, 15 and 16 respectively.

Aortic responses

Five dogs were studied and in these an increase in non-pulsatile aortic pressure from 8.2 ± 0.2 to 29.7 ± 0.8 kPa resulted in a decrease in systemic perfusion pressure of 8.4 ± 1.8 kPa (30.3 ± 6.5 %). The response to a similar increase in pressure when pulsatile, from 8.4 ± 0.2 to 28.7 ± 1.6 kPa caused a similar response of perfusion pressure of 7.2 ± 1.8 kPa (28.9 ± 5.1 %) (Table 13).

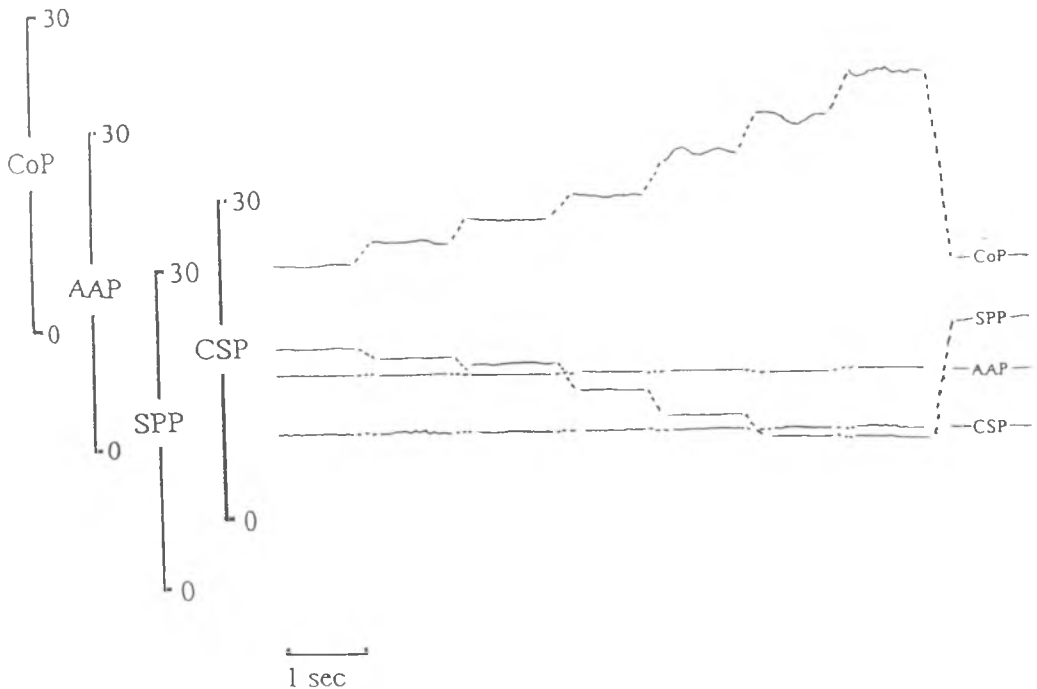
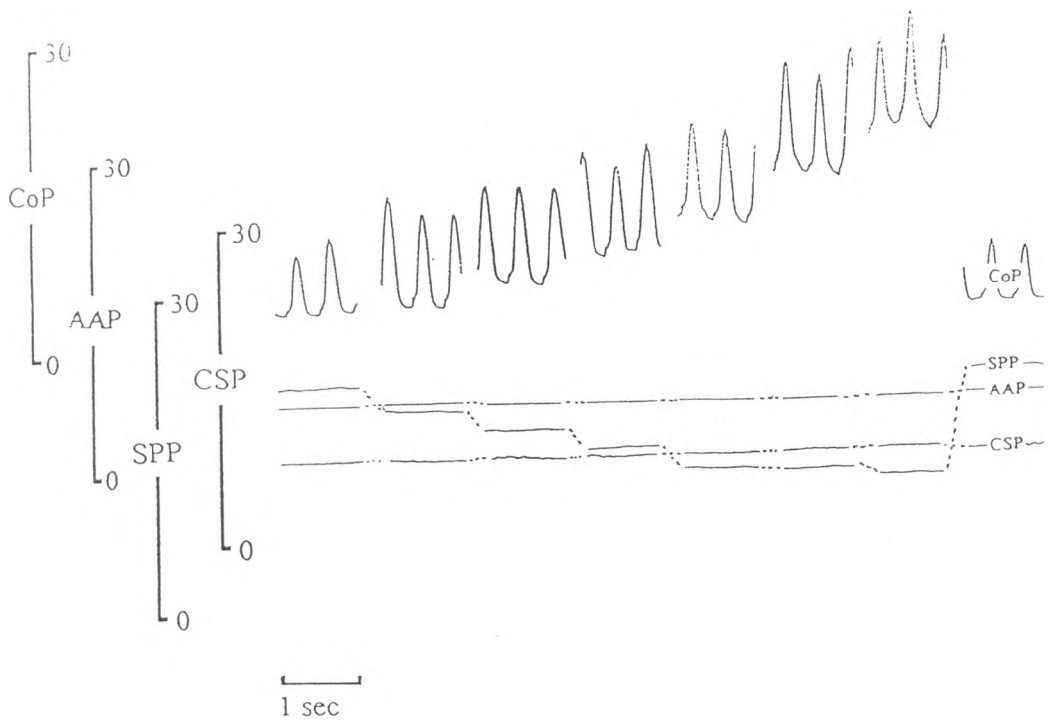
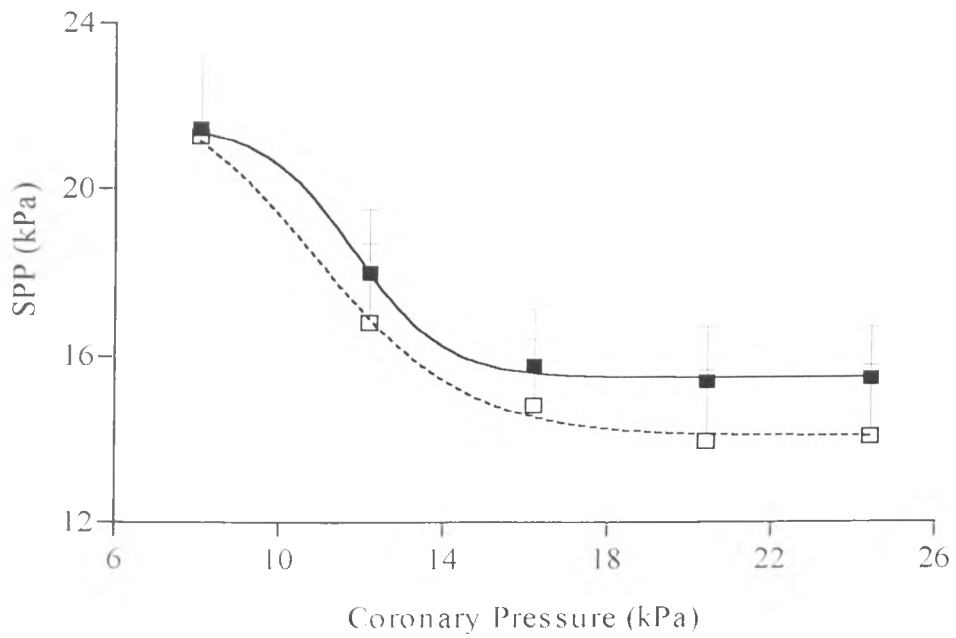


Figure 15. Responses to pulsatile (top traces) and non-pulsatile (bottom traces) pressure increases applied to the coronary baroreceptors in a fibrillated heart. The traces are of aortic arch pressure (AAP), carotid sinus pressure (CSP), coronary pressure (CoP), systemic perfusion pressure (SPP). Note that for both pulsatile and non-pulsatile pressures, overall SPP decreases were similar in both tests. The small fluctuations in CoP in the lower traces are caused by the pump filling the reservoir.

Figure 16. Data from 6 experiments showing the coronary baroreceptor response curves using non-pulsatile (■) and pulsatile (□) pressure stimuli. Responses of systemic perfusion pressure (SPP) are plotted against mean values of coronary pressure.



The average responses to changes in pulsatile and non-pulsatile pressures are compared in Figure 17. There were no differences in the slopes, inflexion points and saturation pressures, but the perfusion pressures at each level of aortic pressure tended to be lower when pulsatile pressures were applied (significant for aortic pressures of 12, 16 and 20 kPa; $P < 0.05$, ANOVA). The individual values of slope, inflexion pressure and saturation pressure are compared in Table 14, 15 and 16 respectively. This indicates a downward displacement of the curve, despite no horizontal displacement.

Carotid responses

In the six dogs studied an increase in non-pulsatile carotid pressure from 7.9 ± 0.3 to 27.1 ± 1.4 kPa decreased perfusion pressure by 10.0 ± 1.5 kPa (40.2 ± 2.8 %). When the carotid pressure was pulsatile, an increase in the mean pressure from 7.8 ± 0.2 to 26.8 ± 1.4 kPa decreased perfusion pressure by 7.6 ± 1.0 kPa (32.0 ± 2.3 %) (Table 13).

The maximum slope of the carotid sinus stimulus-response curve was significantly smaller when the stimulus was pulsatile ($P < 0.05$) and the inflexion point was at a lower carotid pressure ($P < 0.05$). The individual values of slope, inflexion pressure and saturation pressure are compared in Table 14, 15 and 16 respectively. These values are plotted in Figure 18. Overall the curves can be seen to diverge at low and converge at high carotid pressures (Figure 18).

Figure 17. Data from 5 experiments showing aortic arch baroreceptor response curves using non-pulsatile (■) and pulsatile (□) pressure stimuli. Responses of systemic perfusion pressure (SPP) are plotted against mean values of aortic arch pressure. * indicates $P < 0.05$, values of perfusion pressure at pulsatile and non-pulsatile aortic pressures are significantly different (ANOVA).

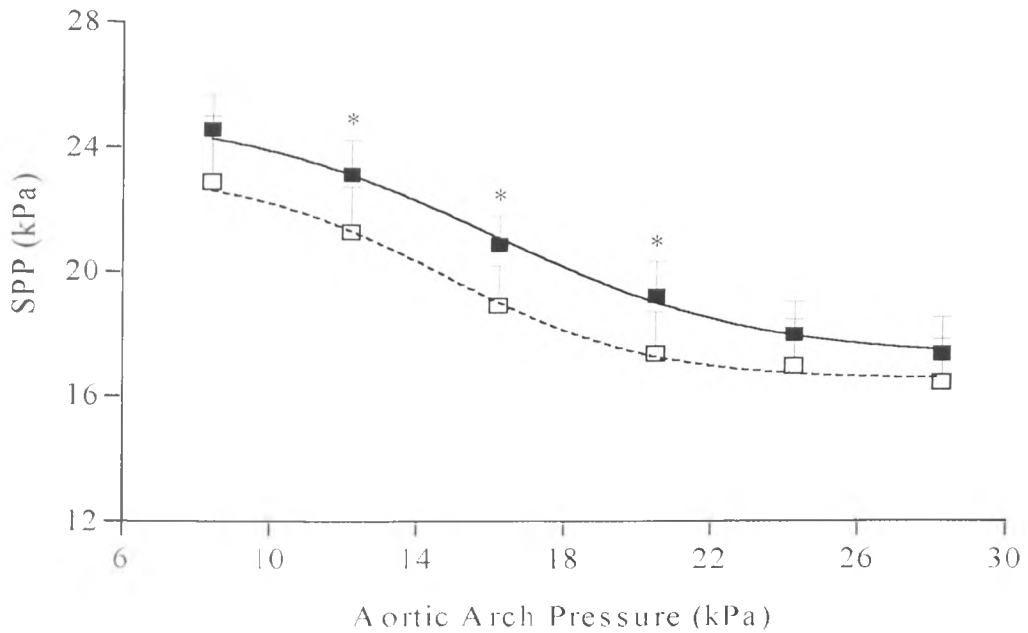


Figure 18. Data from 6 experiments showing carotid sinus baroreceptor response curves using non-pulsatile (■) and pulsatile (□) pressure stimuli. Responses of systemic perfusion pressure (SPP) are plotted against mean values of carotid sinus pressure.

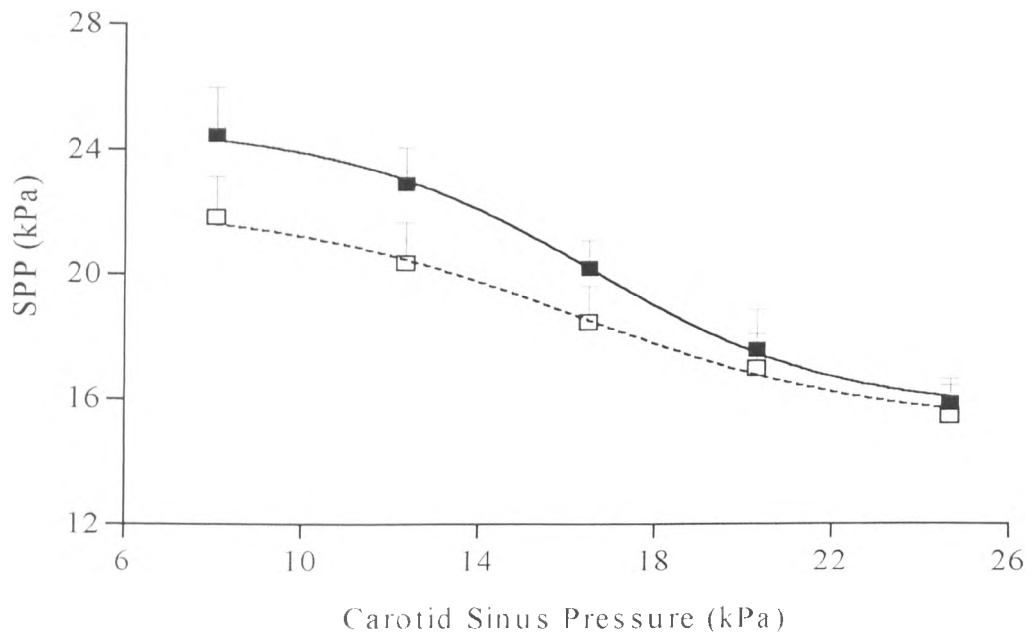


Table 13. *Magnitude of the systemic perfusion pressure response to step increases in non-pulsatile (N-P) and pulsatile pressure applied independently to carotid, aortic and coronary baroreceptors.*

Dog No.	Magnitude of the systemic perfusion pressure response (kPa)					
	Carotid		Aortic		Coronary	
	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile
1	8.1	6.2				
2	14	6.7				
3	5.7	5.6				
4			14.6	13.9		
5					6.2	7.6
6					6.1	13.1
7			9.8	5.6	8.3	6.4
8			8	5.8		
9					7.4	4.1
10	10.6	7.9	5.8	6.8	6.2	5.4
11	7.2	6.9			6.7	7.1
12			4.0	3.7		
13	14.3	12.5				
Mean	10.0	7.6	8.4	7.2	6.8	7.3
S.E.M.	1.5	1.0	1.8	1.8	0.4	1.3

Table 14. Values of maximum slope ($kPa\ kPa^{-1}$) for carotid, aortic and coronary baroreceptors, using non-pulsatile (N-P) and pulsatile stimuli.

Dog No.	Carotid						Aortic						Coronary						
	Sigmoid			Line			Sigmoid			Line			Sigmoid			Line			
	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile	
1	1.27	0.44	0.85	0.74															
2	1.36	0.56	1.38	0.59															
3	0.45	0.44	0.59	0.48															
4					1.76	2.18	1.79	1.82											
5																			
6														1.5	2.09	0.78	1.24		
7					0.53	0.78	0.69	1.0					0.63	1.73	0.65	1.47			
8					0.39	0.32	0.5	0.35					2.16	3.43	1.35	1.29			
9																			
10	1.08	0.48	1.2	0.82	0.43	0.37	0.49	0.4					3.67	2.83	3.3	2.89			
11	0.66	0.52	0.75	0.47									1.1	1.27	1.2	1			
12					0.16	0.44	0.23	0.37					0.89	1.27	0.98	1.6			
13	1.96	1.92	1.67	1.34															
MEAN	1.1	0.7*	1.1	0.7*	0.7	0.8	0.7	0.8					1.7	2.1	1.4	1.6			
S.E.M.	0.2	0.2	0.2	0.1	0.3	0.3	0.3	0.3					0.5	0.4	0.4	0.3			

* indicates $P < 0.05$ compared with corresponding carotid non-pulsatile values (Students paired t test)

Table 15. Values of inflexion pressures (kPa) for carotid, aortic and coronary baroreceptors, using non-pulsatile (N-P) and pulsatile stimuli

Dog No.	Carotid				Aortic				Coronary			
	Sigmoid		Line		Sigmoid		Line		Sigmoid		Line	
	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile
1	19.53	13.35	22.05	9.15								
2	21.34	19.21	22.55	22.18								
3	22.96	18.49	26.45	13.9								
4					16.13	13.05	14.43	13.7				
5									12.29	11.26	14.1	9.75
6									15.52	12.41	13.8	10
7					15.36	11.62	10.68	10.05	10.38	10.97	10.48	9.65
8					17.47	20.49	15.6	18.45				
9									8.6	8.2	7.8	7.4
10	11.99	8.91	10.6	7.35	18.54	19.19	22.53	26.05	10.37	10.77	9.3	10.8
11	20.43	19.91	20.1	18.25					11.06	9.57	14.05	6.75
12					19.19	17.75	12.43	14.4				
13	14.68	13.45	16.23	13.95								
MEAN	18.5	15.5*	19.7	14.1*	17.3	16.4	15.1	16.5	11.41	10.51	11.61	9.11
S.E.M.	1.7	1.8	2.3	2.3	0.7	1.7	2.0	2.7	1.0	0.6	1.1	0.7

* indicates $P < 0.05$ compared with corresponding carotid non-pulsatile values (Students paired t test). † indicates $P < 0.05$ compared with corresponding aortic and carotid values (Students unpaired t test).

Table 16. *Values of saturation pressure (kPa) for carotid, aortic and coronary baroreceptors, using non-pulsatile (N-P) and pulsatile stimuli.*

Dog No.	Saturation pressure (kPa)					
	Carotid		Aortic		Coronary	
	N-P	Pulsatile	N-P	Pulsatile	N-P	Pulsatile
1	23.4	23.4				
2	27.1	24.4				
3	28.0	27.1				
4			23.8	17.8		
5					15.8	18.3
6					25.2	18.7
7			26.5	22.5	14.8	18.5
8			30.5	30.6		
9					15.2	14.6
10	20.0	20.8	25.0	27.3	15.7	14.4
11	29.5	29.0			19.2	17.5
12			28.7	23.8		
13	19.5	18.1				
Mean	24.6	23.8	26.9	24.4	17.6*	17*
S.E.M.	1.7	1.6	1.2	2.2	1.6	0.8

* indicates $P < 0.05$ when values of coronary saturation pressure are compared with corresponding carotid and aortic values (Students unpaired t test).

(B) Comparison of sensitivity ranges for the three baroreceptor areas for both non-pulsatile and pulsatile pressures

Experimental Protocol

Comparisons were made (non-paired) of the inflexion points (Table 15) and 95 % saturation points (Table 16) for each region of the same results reported in the previous section. In order to compare the operating ranges of the three baroreceptor groups it was necessary to reduce possible bias due to variations in the overall magnitudes of the responses. The maximum vasodilatation response from each test was taken to be 100 % and comparisons made of the pressures required to elicit responses from each baroreceptor region.

Responses

The results listed in Table 15 and 16 show that, irrespective of whether the stimuli were pulsatile or non-pulsatile the inflexion and the saturation points for the coronary baroreceptors were lower than the corresponding values for the other two regions. There were no differences in any of the variables between carotid and aortic baroreceptors.

The differences between the coronary and the other baroreceptors are most clearly shown in the normalised plots for both non-pulsatile (Figure 19) and pulsatile stimuli (Figure 20). The coronary receptors clearly operate over a lower pressure range than the other two baroreceptor groups. The normalised curves for the aortic and carotid baroreceptors are almost superimposed.

Figure 19. Comparison of non-pulsatile pressure-response curves from coronary baroreceptors (●), aortic arch baroreceptors (□) and carotid sinus baroreceptors (◆). Total response from each baroreceptor is expressed as 100 %. Note the very similar curves for aortic and carotid receptors but that the coronary curve is to the left of the other curves, and with no clearly defined threshold pressure.

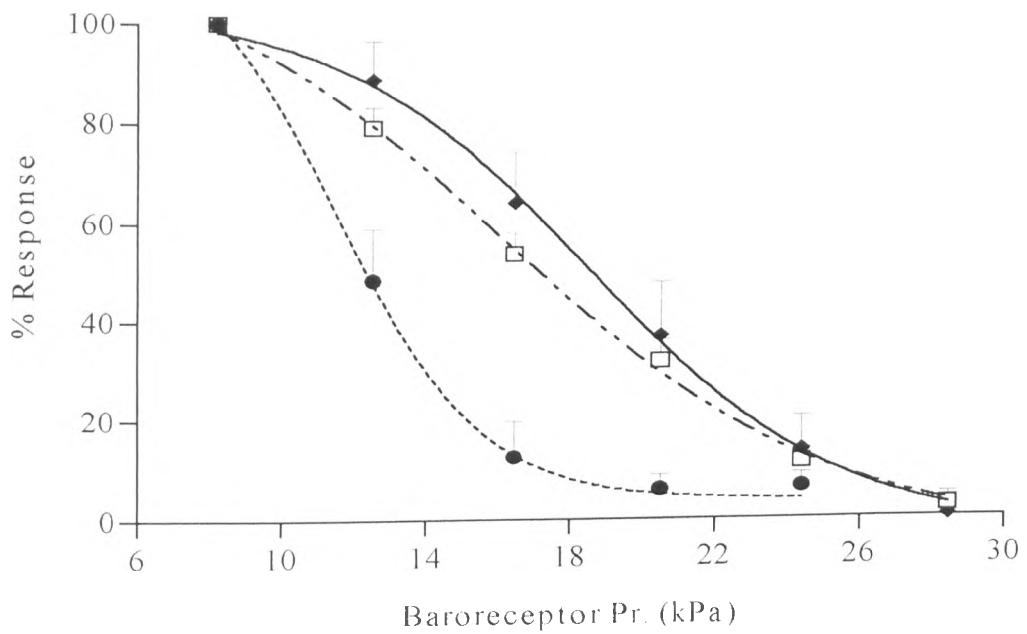
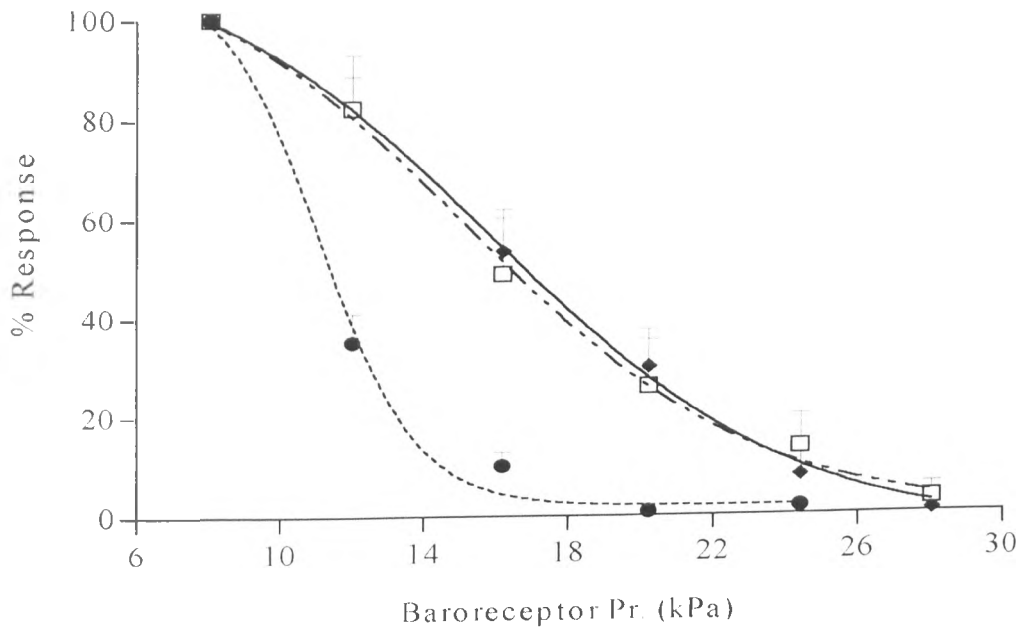


Figure 20. Comparison of pulsatile response curves from coronary baroreceptors (●), aortic arch baroreceptors (□) and carotid sinus baroreceptors (◆). Note the almost perfect overlap of the carotid and aortic curves and the much lower pressures required for the coronary reflexes.



Discussion

As discussed in Chapter 3, the reflex responses from increases in aortic root pressure are attributed primarily to receptors located in the coronary circulation and not to receptors in the left ventricle or the segment of ascending aorta included in this stimulus area. Therefore this study has been able to examine specific barosensitive regions responses to non-pulsatile and pulsatile pressures.

Possibly the most interesting and potentially important observation in the present study is the very low level of pressure required to induce reflex responses from the coronary baroreceptors in comparison to the other two major baroreceptor groups. Usually large reflex responses were observed to the first step change in coronary pressure, from 8 to 12 kPa. In two animals pressure was increased in 2 kPa steps from 6 kPa to 12 kPa after which 4 kPa steps were used and, even with a low starting pressure and smaller increases it was still not possible to determine the threshold pressure for this reflex. Decreasing pressure to even lower levels would be likely to induce confounding responses due to myocardial ischaemia even in the fibrillating heart and activation of chemoreceptors. Furthermore, pressures below 8 kPa must be regarded as being below the physiological range.

The very low range of pressures required to elicit the reflex is actually quite compatible with results from recordings of afferent discharges from the coronary baroreceptors to changes in distending pressure (Drinkhill *et al.*, 1993 & 1996). In both studies it was observed that a similar change in aortic root pressure, decreasing from around 28 to 8 kPa only reduced the average discharge frequency from approximately 22 to 10 impulses⁻¹ indicating that even at the lowest pressure tested, the afferent discharge was far from abolished. Brown, (1965) in an early study of

vagal afferents from coronary baroreceptors also concluded that many afferent fibres remained active even at extremely low coronary pressures produced by coronary occlusion.

It was clearly impossible to determine the threshold coronary pressure for inducing reflex vasomotor responses, therefore the position of the stimulus response curves was assessed by determining the baroreceptor pressures corresponding to the inflexion point and the 95 % saturation point of the curves. However, even though the saturation point could be determined with reasonable confidence the position of the inflexion is dependent on the method used for defining the curves. Two approaches were used. Firstly, the points relating to the *actual results* obtained were simply joined. This approach makes no assumptions about the expected slope of the curve, but it is very dependent on the reliability of each point that was estimated. The second approach was to fit the results to a sigmoid curve using a commercial software package. This assumes that the curve is sigmoid and that the lowest pressure tested is near threshold. Both approaches clearly have disadvantages and even if threshold is not determined they would tend to overestimate rather than underestimate the inflexion value. Nevertheless, it was reassuring to note that both methods provided similar estimates (Tables 14, 15 and 16) and any errors should be common to all experiments.

The similarity between the response curves of the aortic and the carotid reflexes which, when normalised, almost perfectly superimposed is surprising. These similarities were evident during either non-pulsatile or pulsatile perfusion of each area. Comparable effects of pulsatile pressures on reflex responses from aortic and carotid baroreceptors have been reported (Angell-James & Daly, 1970 & 1971). In that study they demonstrated an increase in pulsatile pressure to carotid receptors induced

similar reflex responses as the same increase to aortic receptors, whereas the size of the response was different when the pressure was non-pulsatile. The difference between their non-pulsatile results and those presented here may be explained by their experimental procedure. The aortic baroreceptors were analysed over a limited pressure range and the stimulus area included the bifurcation of the right subclavian and right common carotid arteries which is an area known to contain functional baroreceptors (Nonidez, 1935; Neil, 1956; Okada, 1964). These differences between the experimental protocols complicates comparisons, however, these studies and those reported here both demonstrate comparable responses from carotid and aortic receptors to pulsatile pressures.

The low pressures required to elicit the aortic reflex reported here contrasts with several earlier reports all from anaesthetized dogs using similar perfusion techniques to isolate the aortic arch to those used in this study (Allison *et al.*, 1969; Hainsworth *et al.*, 1970; Donald & Edis, 1971). Indeed it had been suggested that aortic receptors operated in the hypertensive range whereas carotid receptors acted to counter against hypotension (Edis, 1971; Pelletier *et al.*, 1972; Pelletier & Shepherd, 1973). The present results, however, are more compatible with results obtained from recordings of afferent nerve activity from aortic receptors which have shown them to be active at pressures much lower than those reputedly required to cause reflex changes (Bloor, 1964; Homma & Suzuki, 1966; Aars, 1968; Angell-James, 1971*a, b*; Pelletier *et al.*, 1972; Arndt *et al.*, 1975; Samodelov *et al.*, 1979; Coleridge *et al.*, 1981). The discrepancy may be due to methodological problems in isolating the pressure stimulus to the aortic arch (see Chapter 3). In the present study only the more responsive preparations were analyzed (i.e. vascular response > 3 kPa). While this may bias the results, it is likely that only those preparations in which least damage

caused during dissection were selected. The agreement between this study of reflex responses and those of others who have examined afferent nervous activity suggests that the present work may be more representative of what occurs in the intact animal.

The present results suggest a rather different role for the three groups of baroreceptors. They suggest that both carotid and aortic receptors buffer mainly against hypertension and with limited capacity to buffer against hypotension. The coronary receptors would be little affected by increases in pressure above normal, but induce powerful vasoconstrictor responses to counter arterial hypotension. The findings of large reflex responses at very low levels of coronary pressure could explain the observations of Sanders, Mark & Ferguson, (1989). They showed, in humans, that responses of sympathetic efferent activity to decreases in blood pressure were affected much more by 'aortic' than by carotid baroreceptors. 'Aortic' receptors, however, in their experiments, would have included coronary baroreceptors.

The other major aspect of this investigation was to determine whether there were any differences in the responses from each of the baroreceptor areas between non-pulsatile pressure stimuli and pulsatile pressures of the same mean value. It is already known that increasing the amplitude of the carotid pulse at the same mean pressure greatly increases the reflex response of, carotid sinus nerve activity (Ead *et al.*, 1952), renal and splanchnic sympathetic efferent nerve activity (Spickler, Kezdi & Geller, 1967; Kezdi & Geller, 1968) and vascular resistance (Angell-James & Daly, 1970 & 1971). Previous work has also indicated that when a pulsatile stimulus is applied to the carotid receptors the inflexion pressure is decreased and the slope of the stimulus-response curve is less (Ead *et al.*, 1952, Scher & Young, 1963; Angell James & Daly, 1970 & 1971; Schmidt *et al.*, 1972; Chapleau & Abboud, 1987). The results reported here are entirely in agreement with these earlier reports.

Angell James & Daly (1970 & 1971) claimed that the reflex vascular resistance response from aortic baroreceptors distended with pulsatile pressures were not different to those during non-pulsatile perfusion. However in their experiments, it is unclear whether the stimuli to aortic baroreceptors was isolated from coronary receptors, therefore making any interpretation difficult. Interestingly, an *in vitro* study comparing the afferent discharge from aortic receptors stimulated with pulsatile and non-pulsatile pressures reported that the total discharge was greater during pulsatile perfusion due to receptor recruitment (Angell-James, 1971a). The results of the present study agree with those from Angell-James, (1971a) showing that a pulsatile stimulus localised to aortic baroreceptors, did displace the curve downwards, though it did not affect the position of the inflexion of the response curve or its maximum slope. This implies that a pulsatile stimulus is more effective in inducing reflex vasodilatation.

The present report is the first to examine the effects of pulsatility on the responses to stimulation of the coronary baroreceptors. The results showed that changing from non-pulsatile to a pulsatile stimulus did not result in any consistent changes in systemic vascular resistance. In this respect, therefore, coronary baroreceptors were shown to differ from the other two major groups.

There may be three possible explanations for the different behaviour of the coronary receptors. Firstly, coronary receptors have a much lower threshold than the other two groups. This implies that, both at non-pulsatile pressures and throughout the pressure oscillations, the instantaneous value of pressure would remain above threshold for reflex vascular responses. However, recordings of vagal afferent activity from coronary baroreceptors demonstrates that they seem to exhibit a discharge pattern synchronous with the cardiac pulse (Brown, 1965, Drinkhill *et al.*, 1993).

Brown, (1965) concluded that the coronary perfusion pressure pulse did not seem to be an important factor in producing the bursts of activity which coincided with ventricular contraction. He recorded from fibres which appeared to have a threshold pressure below which no activity was seen, though most of the fibres recorded from were active at extremely low coronary pressures. This suggests that some of the coronary receptors recorded from do have a threshold pressure, though overall discharge from several fibres even at the lowest coronary pressure is far from abolished. This contrasts with the other baroreceptor groups for which, when the pressure is low, many receptors would be silent when the pressure is non-pulsatile but would only be excited during the rising phase of a pulsatile stimulus if during part of this phase the pressure was above threshold.

Another possible explanation for the different behaviour of the coronary reflex to pulsatile stimulus may be related to the anatomical location of the receptors. The aortic arch and carotid sinuses are relatively free to distend in response to increases in pressure. The coronary receptors, which are located mainly in the left coronary artery and the proximal parts of its branches (Okinaka *et al.*, 1963; Kurihara, 1964; Brown, 1965; Drinkhill *et al.*, 1993), may have their distension partly restricted by the myocardium. Indeed, in the contracting heart it has been shown that they do respond to a small extent to changes in left ventricular pressure (Drinkhill *et al.*, 1993) although in the present experiments, this effect was prevented by causing the heart to fibrillate.

The third possible explanation is a methodological reason. As it was not possible to define a threshold pressure for the coronary receptors, it is possible that only half of the coronary baroreflex curve was defined. If the coronary receptors were to respond to changes in pulse pressure, like carotid receptors, then at higher

pressures (i.e. the upper segment of the response-curve) both pulsatile and non-pulsatile curves do converge. At lower coronary pressures (< 6 kPa) the two curves may diverge as seen in responses from carotid receptors at pressures at 8 kPa. As discussed earlier in this section lower coronary pressures would be unphysiological. Therefore, this study examined coronary receptor responses over the physiological pressure range and was unable to reveal any differences between non-pulsatile and pulsatile induced responses.

The experiments reported in this chapter have characterised the coronary baroreceptors and compared their function with those of the two other principal baroreceptor groups. The major findings are their very low operating range and their lack of response to changing the pulsatility of the stimulus. This suggests that they may have a different function from the other groups and they are likely to be particularly effective in causing intense vasoconstriction when blood pressure falls to dangerously low levels.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Many previous studies have attributed the reflex responses from left ventricular distension to stimulation of left ventricular mechanoreceptors. However, the interventions used in those studies were poorly localised, resulting in concomitant changes in pressures and volumes in other reflexogenic regions with the heart and lungs and to systemic baroreceptors. Also many of the interventions were unphysiological and steady state responses were not achieved.

Hainsworth and colleagues used perfusion techniques designed to answer many of the above criticisms. Recently they refined their technique to allow distension of the coronary circulation without altering pressure in the left ventricle. They also distended the ventricle without increasing mean coronary pressure (Al-Timman *et al.*, 1993; Drinkhill *et al.*, 1993). These two studies provided conclusive evidence that mechanoreceptors located within the coronary circulation were responsible for initiating the majority if not all of the reflex responses originally ascribed to mechanoreceptors in the left ventricle.

These coronary mechanoreceptors are the only receptors within the cardiopulmonary region to respond across the range of arterial blood pressure. This implies that these receptors have similarities to "classical" systemic baroreceptors located in the aortic arch and carotid sinus. The purpose of the studies in this thesis were to characterise reflex responses from coronary baroreceptors and compare them with responses from aortic arch and carotid sinus baroreceptors. It was hoped that this would provide evidence of the physiological role of coronary baroreceptors.

In this thesis perfusion techniques were used which allowed separate control of pressures perfusing the coronary arteries, aortic arch and carotid sinuses. Pressure to a baroreceptor region was changed independently and the reflex responses were

examined in systemic and isolated hind limb vascular resistance and sympathetic efferent nerve discharge.

Chapter 3 examined the time course of vascular response changes to increases in baroreceptor distending pressure which were maintained for periods up to 8 minutes. At the end of the test period baroreceptor pressure was decreased in a single step and systemic perfusion pressure was allowed to recover to the pre test level.

The time course of the vascular response to increasing pressure to aortic, carotid and coronary baroreceptors was not different. This demonstrated that the three baroreceptor groups all initiate rapid vasodilatation. When the rate of vasoconstriction was examined after reducing baroreceptor pressure it was found that vasoconstriction initiated by coronary baroreceptors was significantly slower than that originating from aortic or carotid baroreceptors. This slow response from coronary receptors was not altered by changing the pressure stimulus from pulsatile to non-pulsatile or by increasing the duration at high coronary pressure from 30 seconds to 8 minutes.

There were a number of possible mechanisms which could account for the slow coronary response. Firstly, coronary receptors may remain active after reducing coronary pressure. The afferent discharge from coronary receptors during changes in coronary pressure was examined by Drinkhill *et al.*, (1996). They concluded that coronary baroreceptor responded immediately to pressure changes and were not therefore responsible for the slow response.

As the afferent limb of the reflex arc was not responsible then it was likely that the delay originated through a central inhibitory mechanism or through coronary receptors activating different sympathetic efferent nerves than carotid or aortic receptors. These putative mechanisms were examined in chapter 4.

Sympathetic efferent discharge was recorded from either lumbar or renal nerves during increases and decreases in pressure to the three groups of baroreceptors independently. Increasing pressure to the carotid or aortic receptors produced immediate inhibition of efferent discharge which recovered almost immediately, sometimes with an overshoot once pressure to carotid or aortic receptors had been decreased. Efferent nerve responses from increasing coronary pressure were similar to aortic and carotid responses. When coronary pressure was reduced both efferent activity and vascular resistance recovered slowly to their previous high levels. All efferent fibres examined in the study responded to stimulation of all three groups of baroreceptors, therefore the slow coronary response was not due to activation of different populations of sympathetic efferent nerves. The mechanism causing the slow response is therefore likely to be located within central pathways activated by coronary receptors. Unfortunately investigation of this mechanism is beyond the scope of the current study, but still requires further analysis.

It was not possible in this study to rule out a contribution from humoral factors released during coronary stimulation, in delaying vascular constriction. Interestingly, coronary receptors appeared to have different effects on lumbar and renal activity, with lumbar activity recovering more rapidly than renal activity. This differential effect on the renal and lumbar sympathetic efferent nerves was not seen during carotid or aortic baroreceptor unloading. This finding suggests that the kidney and possibly the renin-angiotensin system contribute to the slow recovery of vascular resistance following coronary baroreceptor activation.

Another important aspect of baroreceptor function is the response to changes in pulse pressure and the range of arterial pressures over which they operate. These factors were examined in chapter 5. Using the bypassed-fibrillated heart technique it

was possible to apply non-pulsatile pressures to the coronary arteries and compare responses with those during pulsatile perfusion. The response to changing pulse pressure to aortic and carotid baroreceptors was also examined.

The response of carotid baroreceptors to changes in pulse pressure is well documented, with pulsatile carotid pressures shifting the response-curve towards lower pressure and depressing its slope. These results were confirmed in this thesis. Aortic baroreceptors were supposed to respond weakly if at all to changes in pulse pressure. In this study it was found that substituting pulsatile pressure for a non-pulsatile pressures over the mid range of aortic pressures produced greater vasodilatation. Therefore, aortic baroreceptors are sensitive to changes in pulse pressure as indicated by a downward displacement of the stimulus response-curve. The response of coronary baroreceptors to pulsatile and non-pulsatile pressures were found not to be different with both curves overlying each other. Implying that over the pressure range tested coronary baroreceptors are insensitive to changes in arterial pulse pressure. During the coronary pressure tests in the fibrillated hearts the aortic valve would remain closed, thus limiting the pressure change to the coronary circulation. The presence of reflex vascular resistance responses during these changes in aortic root pressure provides further evidence for the origin of the reflex to be the coronary arteries rather than the left ventricle.

The results in this chapter were compared to examine the operating ranges of the three baroreceptor populations. It was demonstrated that coronary baroreceptors induce reflex responses at very low levels of coronary pressure. This level of pressure was insufficient to provoke responses from aortic or carotid receptors. It is clear that coronary baroreceptors operate over a much lower range of arterial pressure than either aortic or carotid receptors, which operate over similar pressure range. These

important observations suggest that coronary baroreceptors have a different regulatory role than either aortic or carotid receptors and that they are likely to function in hypotensive situations whereas the other two receptor groups are likely to buffer against increases in blood pressure.

These findings imply that in normal physiological states, two buffering mechanisms exist. One opposing increases in arterial pressure through the actions of aortic and carotid receptors and the other mechanism opposing decreases in arterial pressure via coronary baroreceptors.

The long term control of mean blood pressure is a contentious issue with the precise role of baroreceptors still to be defined (see Scher, 1977; Shade, Haywood & Bishop, 1991). Coronary baroreceptors however, would act in conjunction with arterial baroreceptors to limit variations in arterial blood pressure. There remains the possibility that arterial baroreceptors only limit short term blood pressure fluctuations and do not set the long term level (Shepherd, 1982). Indeed, in studies in conscious animals in which arterial baroreceptors are denervated blood pressure becomes more variable (Cowley, Liard & Guyton, 1973; Cowley, Quillen & Barber, 1980; Cornish & Gilmore, 1985).

The findings of Eckberg & Sleight, (1992) suggest that since sino-aortic baroreceptors act only to reduce short term fluctuations in pressure, receptors located in the cardiopulmonary region are responsible for setting the long-term mean blood pressure level. The only known receptors in the cardiopulmonary region which respond across the range of arterial pressures are those in the coronary circulation, thus suggesting a role for this group of receptors in the long-term control of blood pressure.

The following list summarises features of coronary arterial baroreceptors that differ with those from either carotid or aortic baroreceptors.

Coronary Baroreceptors

1. No consistent effect on heart rate
2. Loading coronary baroreceptors decreases phrenic nerve activity
3. Unloading coronary baroreceptors initiates a slow vasoconstriction
4. Feature 3 is mirrored in recordings of sympathetic efferent activity
5. Differential effect on lumbar and renal sympathetic efferent nerves
6. Insensitive to changes in coronary pulse pressure over the physiological range
7. Have an extremely low activation threshold

This group of coronary baroreceptors are clearly a different population to those groups of baroreceptors located in and around the aortic arch and carotid sinuses

Further research is necessary to define a role for coronary baroreceptors in cardiovascular control. These investigations might examine whether the operating range of coronary baroreceptors is acutely reset during sustained periods of high

pressure. Acute resetting of both carotid and aortic baroreceptors operating ranges towards the prevailing pressure is well documented (Coleridge, *et al.*, 1981, Kunze, 1981; Undesser, Lynn & Bishop, 1984; Brunner & Kligman, 1992, Munch, 1992; Chen, Chang, Liu & Lin, 1993). This feature of baroreceptors has important implications in their ability to buffer against prolonged changes in blood pressure. Other experiments might investigate the central pathways used by the coronary baroreflex and also the role of the kidney in the vascular responses initiated by coronary receptors

The findings reported in this thesis have further substantiated the existence of baroreceptors within the coronary arteries and have expanded the current knowledge on reflexes originating from these receptors. Certain aspects of their function are unique and are likely to be of importance in the control of blood pressure in the physiological state and may be involved in pathological conditions effecting the cardiovascular system.

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