HYPERTENSIVE MECHANISMS OF BRADYKININ AND ANGIOTENSIN

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by

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PREFACE

Bradykinin is a naturally occurring nonapeptide which is a powerful vasodilator compound, characteristically causing hypotension and a tachycardia on systemic administration. In addition to its direct peripheral vasodepressor action, it also has an equally powerful central action to cause hypertension and a tachycardia which are mediated, in part, by an action on the autonomic nervous system.

The experiments reported in this thesis were conducted to determine more precisely the central site of action of bradykinin and to investigate a number of possible mechanisms which could contribute to the cardiovascular response to centrally-administered bradykinin in the morphine and chloralose anaesthetised greyhound.

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SUMMARY

Since its discovery in the early 1900's, bradykinin has been described as a potent dilator of arterioles in the peripheral circulation as well as in specific vascular beds, e.g. the renal, cerebral and coronary circulations. Subsequent to the widespread vasodilation (which is a direct effect of bradykinin on vascular smooth muscle) that occurs following systemic administration, arterial blood pressure decreases and the resultant stimulus to the baroreceptor reflex mechanisms causes an increase in heart rate and cardiac output. In the morphine and chloralose anaesthetised greyhound, the mechanism of the tachycardia is primarily withdrawal of vagal tone to the heart. The alterations in the cardiovascular parameters that were measured are consistent with previously published work, although the precise mechanism of the increase in heart rate in the greyhound is different to the mechanism in other animals, probably because of the relatively high vagal tone to the heart in the morphine and chloralose anaesthetised greyhound compared with other smaller animals, e.g. rats, rabbits and cats, where an increase in sympathetic activity to the heart seems to be an important mechanism to cause an increase in heart rate.

In addition to its vasodepressor action when administered systemically, bradykinin has also been shown to have a powerful hypertensive action when administered into either the cerebral circulation (via a carotid artery) or directly into the cerebral ventricles. However, the mechanism of this effect of bradykinin is not clear, although there is evidence to suggest involvement of both α and β adrenoceptors. The central site of action of bradykinin has also not been localised, so the experiments described in this thesis were conducted to determine the central site of action and to characterise, in more detail, the mechanism(s) of the

hypertensive response to vertebral artery and carotid artery infusions of bradykinin.

The results of the experiments reported in Chapter 2 indicate that the autonomic nervous system is involved in the hypertensive response and that while selective blockade of α and β adrenoceptors modifies the cardiovascular responses, the vagus nerves are of major importance in determining the responses obtained.

Ablation of the area postrema abolished the central pressor effect of both vertebral and carotid artery bradykinin indicating that the integrity of this area is required for the central pressor effect of bradykinin. It has also been demonstrated that bradykinin has no direct effect on the heart to contribute to the increase in heart rate, so bradykinin is gaining access to central structures to cause the tachycardia. It is also probable that a central cholinergic mechanism and the formation of prostaglandins are intimately involved in the centrally-mediated response to bradykinin and that a direct action in the carotid sinus region or the release of vasopressin do not contribute to the response to centrally-administered bradykinin.

Angiotensin II also has a specific central action mediated via the area postrema to increase arterial blood pressure and while the precise mechanism of action of angiotensin II is still not known, it has recently been suggested that a central opiate mechanism is involved in this response. The results presented in the final section of this thesis indicate that central opiate mechanisms are not involved and also that the greyhound utilises different pathways to other species, even the mongrel dog, in which opiate mechanisms have been shown to be involved in the centrally-mediate response to angiotensin II. While

vertebral artery angiotensin II and bradykinin both act through the area postrema to inhibit cardiac vagal tone, the cardiovascular responses to these peptides show major differences when administered either intravenously or via a carotid artery.

DECLARATION

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any University and that to the best of my knowledge, contains no material previously published by any other person, except where due reference is made in the text.

D. L. WILKINSON

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The experiments described in this thesis are a record of original work conducted in the Department of Physiology at the University of Adelaide during the period 1976-1982.

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During the period of study, the author was employed as a Tutor in the Department of Physiology, University of Adelaide.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Bradykinin is a nonapeptide and belongs to a group of polypeptides collectively called kinins. These compounds share many common physiological properties, including a smooth muscle stimulating effect, as seen in the isolated gut or uterus preparation of various laboratory animals, a powerful depressor action when administered intravenously to an intact animal, and an effect on capillaries to increase membrane permeability. The kinins are also some of the most potent algesic compounds known, characteristically causing intense pain when placed on the base of blisters.

The discovery of the kinins originated in the early 1900's when an unidentified hypotensive substance was found in urine (Abelous and Bardier, 1909). In 1925, Frey also reported that urine contained a substance which, even in small doses, influenced the circulation. This substance was "purified" (Frey, 1926; Frey and Kraut, 1928) and it soon became apparent that there was a similar hypotensive compound in a variety of other tissues and body fluids, including plasma (Frey, 1926) and the pancreas (Frey, Kraut and Schultz, 1930). In fact, it was because the pancreas contained such large amounts of the unknown hypotensive substance that Frey (1930) named it kallikrein, derived from the Greek word for the pancreas, kallikreas.

By 1937, Werle had determined that kallikrein itself was not the active substance, but it released a pharmacologically active compound from a larger plasma precursor (Werle, Gotze and Kepler, 1937). In 1948, Werle and Berek named this active substance kallidin.

Rocha e Silva *et al.* (1949) observed that blood samples from a dog which had previously been injected with snake venom from

Bothrops jararaca, stimulated contractions of the isolated guinea pig ileum. Since the contractions produced were slow, they called the substance bradykinin (from two Greek words, "bradys" meaning slow, and "kinin" meaning to move). Bradykinin was also shown to elicit a powerful depressor response in the anaesthetised rabbit. In addition, evidence was provided that distinguished the new bradykinin from kallikrein, histamine and acetylcholine, and that since trypsin both liberates bradykinin and destroys it if the incubation period is prolonged, it was "probable that bradykinin was the consequence of the rupture of a peptide linkage and that bradykinin itself was a peptide or, at least, contains a peptide linkage, the integrity of which is indespensable to its pharmacological behaviour."

It had thus become established that an enzyme, kallikrein, could act on a larger plasma globulin to release the pharmacologically active peptide, kallidin, and that bradykinin could be released from plasma globulins by the action of trypsin or snake venoms. Furthermore, since the actions of bradykinin and kallidin were similar (both being powerful hypotensive compounds and both causing contraction of the isolated ileum) and their formation, probably from the same substrate (Werle and Berek, 1950), was activated under similar conditions, it was believed they could be the same substance, or at least very closely related. It was not until the structure of these compounds was determined that the small difference was appreciated.

In 1959, Elliott, Lewis and Horton (1960a) isolated the "pure" substance from ox blood treated with trypsin and later (Elliott, Lewis and Horton, 1960b) proposed the following 8 amino acid sequence for bradykinin: Arg-Pro-Pro-Gly-Phe-Ser-Phe-Arg. However, when Boissonnas and co-workers synthesised this compound, it did not possess biological activity similar to that of bradykinin (Boissonnas, Guttman and

Jaquenoud, 1960). The addition of proline at position 7 in the peptide sequence postulated above did, however, produce a peptide that had identical biological activity to bradykinin produced from blood (Boissonnas, Guttmann, Jaquenoud, Konzett and Sturmer, 1960). Elliott, Lewis and Horton (1960c) then determined the amino acid sequence of naturally produced bradykinin and it was found to be identical to the nonapeptide proposed earlier by Boissonnas.

Thus bradykinin is unique in biological science in that the peptide was synthesised, the sequence of which was "arrived at by a quite unusual method of trial and error" (Boissonnas, Guttmann, Jaquenoud, Pless and Sandrin, 1963), before Elliott *et al.* (1960c) had correctly determined the actual amino acid sequence by analytical techniques.

Pierce and Webster (1961) isolated two peptides released by human urinary kallikrein from human plasma, which they called kallidin-10 and kallidin-9. The structures of these two peptides are as follows:

Kallidin-10

H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH

Kallidin-9

H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH

Kallikreins from different tissues and body fluids, e.g. plasma, urine and the pancreas, were originally thought to be the same enzyme. Webster and Pierce (1963) compared kallikreins from human urine, human pancreas, hog pancreas and human plasma and concluded that kallikreins of glandular origin released the decapeptide kallidin-10 (subsequently known as kallidin) from the plasma globulin presursor, kallidinogen, and that this decapeptide is converted to the nonapeptide, kallidin-9

(subsequently known as bradykinin) by plasma aminopeptidase. Kallikrein of plasma origin, however, directly releases kallidin-9 from kallidinogen. They also proposed that trypsin acts on killidinogen to release kallidin-10 and that this is changed to kallidin-9 by further action of trypsin or plasma aminopeptidases. Snake venoms supposedly act in a similar manner to trypsin.

RELEASE OF KININS

As has already been discussed, various factors, e.g. trypsin, snake venom and kallikrein of differing origin, can release bradykinin and kallidin from the precursors, bradykininogen and kallidinogen, collectively called kininogens. Both of these precursors are believed to be identical substrates (Werle and Berek, 1950).

In 1957, Armstrong, Jepson, Keele and Stewart observed that when blood came into contact with glass, it released a substance which caused pain, and contracted the isolated rat uterus and that these actions resembled those of bradykinin.

Hageman factor, which is also activated by contact of blood with glass, is involved in the blood clotting process. Margolis and co-workers have investigated that inter-relationship of blood clotting and peptide release from precursors present in blood (Margolis, 1963; Margolis and Bishop, 1963). They suggest that "activated" Hageman factor acts on a plasma component (kallikreinogen) to release active kallikrein.

When plasma is diluted or allowed to stand, permeability factors are formed (PF/Dil and PF/Age) which increase the permeability of

capillary membranes. According to Miles (1964), the permeability factors probably release kinins by activating endogenous kallikrein.

The mechanism of release of kinins is represented in Figure 1.

INACTIVATION OF KININS

Rocha e Silva *et al.* (1949) experienced considerable difficulty in isolating bradykinin from blood since the same agents that released it (snake venom from *Bothrops jararaca* or trypsin) also destroyed it if incubation was prolonged.

Both kallidin and bradykinin are only active *in vivo* for a short period of time. The most likely enzyme responsible for inactivation of bradykinin is plasma carboxypeptidase B which enzymatically removes the C-terminal arginine (Erdos, Renfrew, Sloane and Wohler, 1963), although a variety of other enzymes, collectively called kininases, can also inactivate bradykinin (Fig. 2). Webster and Pierce (1963) suggested that kallidin could also be inactivated by a serum aminopeptidase which hydrolyses the Lys^1-Arg^2 bond of kallidin, thus forming bradykinin. The bradykinin resulting from this action of aminopeptidase in blood is then rapidly inactivated by carboxypeptidase B in blood, the half-life of bradykinin being only 16-17 seconds in the blood of the cat (Ferreira and Vane, 1967).

Carboxypeptidase B (also known as kininase II), as well as inactivating bradykinin, is also responsible for the enzymatic cleavage of the decapeptide, angiotensin I, to form the pharmacologically active octapeptide, angiotensin II (Yang, Erdos and Levin, 1970; Dorer, Ryan and Stewart, 1974; Dorer, Kahn, Lentz, Levine and Skeggs, 1974;

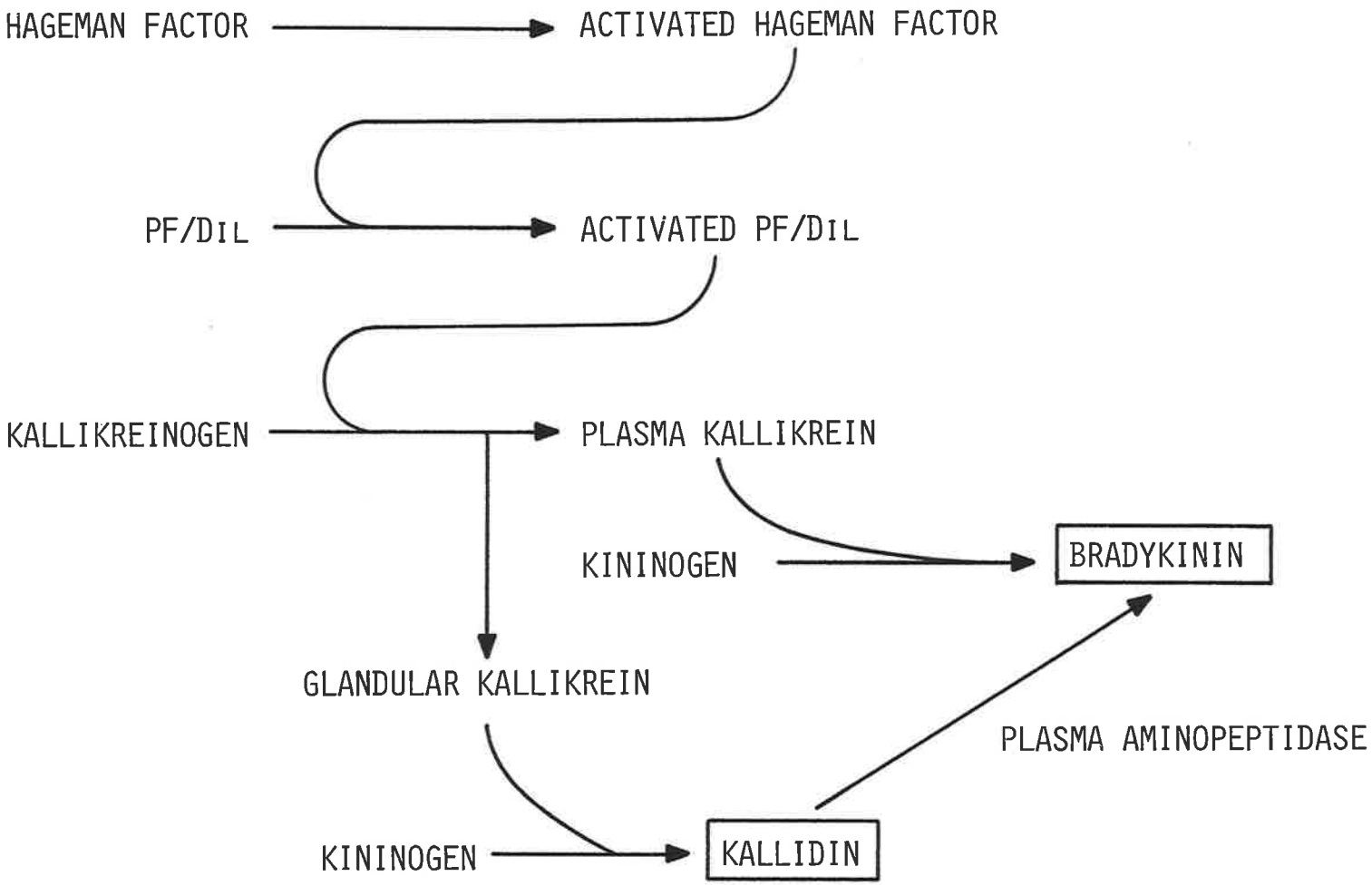

FIGURE 1

FORMATION OF KININS

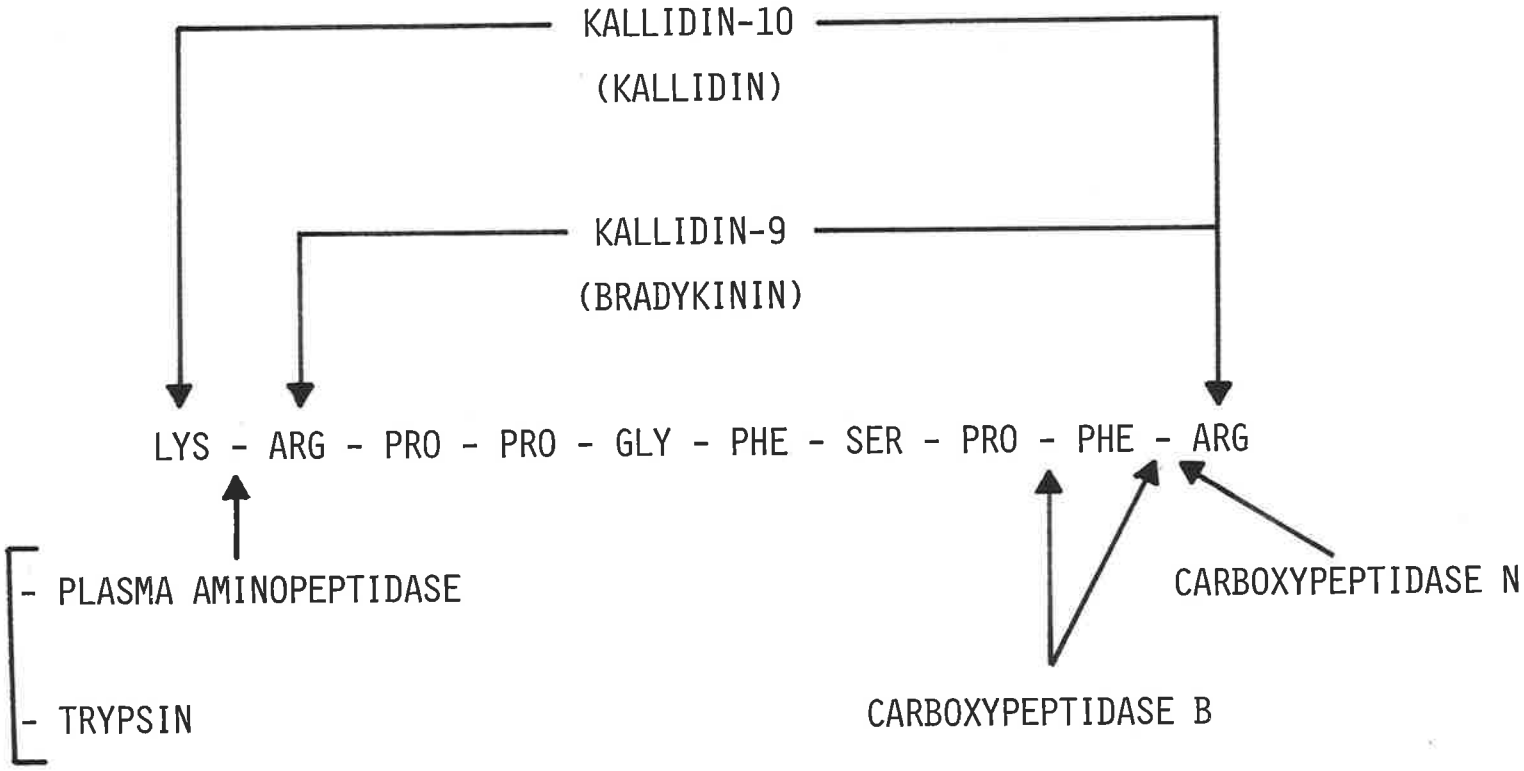

FIGURE 2

DEGRADATION OF KININS

(FROM SCHACHTER, 1964)

Erdos, 1977). Furthermore, most of this hydrolytic activity appears to be within the pulmonary circulation, where most of the circulating bradykinin is inactivated and, similarly, most angiotensin II is formed (Ryan, Roblero and Stewart, 1968; Alabaster and Bakhle, 1972; Erdos, 1977).

A detailed review of the metabolism of kinins has been prepared by Erdos (1966).

EFFECTS OF BRADYKININ

As previously discussed, bradykinin is a powerful hypotensive and smooth muscle-stimulating peptide. In fact, it was these properties that were characteristics of the kininogen-kinin system for many years. Since the elucidation of the structure of bradykinin in 1960 and its subsequent commercial availability, the biological actions have been extensively investigated.

Khairallah and Page (1961 and 1963) compared the effects of bradykinin and angiotensin on smooth muscle of the isolated guinea pig ileum and reported that angiotensin contracted the ileum by both a direct action on the muscle cells and also by stimulating the ganglion cells of Auerbach's plexus, releasing acetylcholine which subsequently contracted the smooth muscle. Bradykinin, however, only caused contraction by a direct action on the muscle cells, this action being unaffected by atropine.

Feldberg and Lewis (1963, 1964 and 1965) demonstrated that the injection of bradykinin into the coeliac artery of anaesthetised cats caused an increase of blood pressure which was abolished following

removal of the suprarenals, from which they concluded that bradykinin released adrenal medullary hormones, probably mainly adrenaline. Since chronic bilateral splanchnicotomy did not alter the effect of bradykinin, they proposed that it was not acting on cholinergic nerves in the adrenal medulla, but directly on the cells to release adrenaline.

In contrast, Comline, Silver and Sinclair (1968) were not able to demonstrate significant release of catecholamines from the bovine adrenal medulla by bradykinin or angiotensin (although bradykinin caused a small increase in adrenaline output) and concluded that these peptides are "probably not normally involved in the release of adrenal medullary hormones." They attributed the discrepancies between their results and those of Feldberg and Lewis to species differences.

GANGLION-STIMULATING EFFECT OF BRADYKININ

As discussed above, bradykinin stimulates the "specialised" sympathetic ganglion, the adrenal medulla. Lewis and Reit (1965) determined that bradykinin also stimulates other sympathetic ganglia, in particular the superior cervical ganglion of the cat. This action of bradykinin is a result of a direct effect on the ganglion cells and does not involve pre-ganglionic nerves or the receptors for acetylcholine. The "bradykinin receptor" was also shown to be different from the "angiotensin receptor" since the ganglia could be made tachyphylactic to bradykinin without having any effect on the ganglion-stimulant action of angiotensin, and vice versa. Trendelenburg (1966) provided further evidence that bradykinin is a non-nicotinic ganglion stimulating compound and that there are minor differences between the ganglion stimulating action of bradykinin and angiotensin.

CARDIOVASCULAR ACTIONS OF BRADYKININ

In 1949, Rocha e Silva, Beraldo and Rosenfeld first reported the hypotensive effect of intravenously injected bradykinin (earlier reports concerning hypotensive substances from various sources were not referring directly to bradykinin, although we now know that bradykinin or kallidin were the unknown substances). In man, bradykinin is one of the most potent vasodilator substances known (Fox, Goldsmith, Kidd and Lewis, 1961). The hypotensive effect and associated reflex tachycardia following intravenous bradykinin have been widely reported in all laboratory animals used (Page and Olmsted, 1961; Olmsted and Page, 1962; Maxwell, Elliott and Kneebone, 1962; Nakano, 1965; Kondo, Okuno, Konishi, Saruta and Kato, 1979). However, there are reports of intravenous bradykinin causing hypotension but a variable heart rate response, i.e. either no change in heart rate, a tachycardia or a bradycardia (Buckley, Bickerton, Halliday and Kato, 1963). Feldberg and Lewis (1964) suggested that following the initial decrease in arterial blood pressure, there was an increase in blood pressure and a tachycardia which was due to adrenaline release from the adrenal medulla. The biphasic nature of the response to bradykinin was also noted by Pearson and Lang (1967) and they attributed the secondary pressor effect to a central action of bradykinin.

In summary, the effects of systemically adminstered bradykinin on the cardiovascular system include hypotension (Konzett and Sturmer, 1960; Capri and Corrado, 1961; Page and Olmsted, 1961; Olmsted and Page, 1962; Maxwell, Elliott and Kneebone, 1962; Buckley, Bickerton, Halliday and Kato, 1963; Feldberg and Lewis, 1964; Nakano, 1965; Harrison, Henry, Paaso and Miller, 1968), which is a direct effect of bradykinin on vascular smooth muscle (Nakano, 1965; Khairallah and Page, 1961; Khairallah and Page, 1963) and an associated reflex tachycardia

(Page and Olmsted, 1961; Olmsted and Page, 1962; Maxwell, Elliott and Kneebone, 1962; Feldberg and Lewis, 1964; Nakano, 1965), increased cardiac output (Page and Olmsted, 1961; Omsted and Page, 1962; Maxwell, Elliott and Kneebone, 1962; Nakano, 1965; Harrison, Henry, Paaso and Miller, 1968) and decreased total peripheral vascular resistance (Page and Olmsted, 1961; Olmsted and Page, 1962; Maxwell, Elliott and Kneebone, 1962; Nakano, 1965; Harrison, Henry, Paaso and Miller, 1968).

The mechanism of the increase in cardiac output is uncertain. Maxwell, Elliott and Kneebone (1962) suggest the tachycardia is primarily responsible, since their calculated stroke volume decreased. In comparison, Harrison, Henry, Paaso and Miller (1968) did not observe a tachycardia and their proposed mechanism of the increase in cardiac output is an increase in stroke volume as a result of an increased venous return, and that the autonomic nervous system plays little part in the cardiovascular responses to systemic bradykinin.

Intravenous bradykinin also causes relaxation of smooth muscle in other vascular beds, including the heart (Maxwell, Elliott and Kneebone, 1962; Nakano, 1965), the kidney (Gill, Melman, Gillespie and Bartter, 1965; McGiff, Itskovitz and Terragno, 1975), the skin and forearm of human subjects (Fox, Goldsmith, Kidd and Lewis, 1961) and the brain (Capri and Corrado, 1961; Rocha e Silva, 1963). This results in increased blood flow through these vascular beds.

In addition to the well-characterised hypotensive effect of intravenous bradykinin, it has also been reported that bradykinin has a powerful hypertensive action and that this effect is mediated via a central action of the peptide (Pearson and Lang, 1967; Lang and Pearson 1968; Pearson, Lambert and Lang, 1969; Lambert and Lang, 1970; Correa and Graeff, 1974; Correa and Graeff, 1975; Correa and Graeff, 1976;

Kondo, Okuno, Konishi, Saruta and Kato, 1979). However, the site and mechanism of action have still not been identified, although several pathways have been postulated. While it is generally accepted that the autonomic nervous system is not involved in the hypotensive response to intravenous bradykinin (Harrison, Henry, Paaso and Miller, 1968), it is believed that the hypertensive response to centrally administered bradykinin does involve the autonomic nervous system. Riccioppo Neto, Corrado and Rocha e Silva (1974) reported that carotid and vertebral artery injections of bradykinin caused hypotension and bradycardia, which is at variance with other reports, although some of these effects may be due to stimulation of perivascular nerve endings and reflex centres rather than a direct action on the central nervous system. Bradykinin injected directly into the cerebral ventricles causes a hypertensive effect which is believed to be due to an α-adrenergic mechanism (Lambert and Lang, 1970; Correa and Graeff, 1974; Correa and Graeff, 1975; Correa and Graeff, 1976). Lambert and Lang (1970) further postulated an α-adrenergic mechanism in conscious rats but primarily a β-adrenergic mechanism in anaesthetised rats. A biphasic response to carotid artery injection of bradykinin was observed by Pearson, Lambert and Lang (1969) who proposed that the initial hypotensive response is caused by inhibition of sympathetic tone to blood vessels and that the subsequent secondary hypertensive response is due to stimulation of cardiac β-receptors. Thus, there is evidence to support both α and β-adrenergic mechanisms in the hypertensive response to centrally administered bradykinin. In addition, it has been suggested that bradykinin may activate parasympathetic centres (Buckley, Bickerton, Halliday and Kato, 1963).

While it has been established that bradykinin has powerful effects on the cardiovascular system, to cause both hypotension and hypertension, (depending on the route of administration), it has not been implicated

in overall control of the cardiovascular system but rather in local circulatory adjustments, e.g. functional and reactive hyperemia (Ungar, 1966). However, bradykinin was not found to be associated with functional hyperemia in skeletal muscle (Hilton and Lewis, 1958; Hilton, 1960; Hilton, 1963). Since the observation by Rocha e Silva (1963) that intravenous injection of large amounts of bradykinin killed guinea pigs with all the manifestations of shock and the reports by Beraldo (1950), Brockelhurst and Lahiri (1963) and Cirstea, Suhaciu and Butculescu (1965) that an increase in kinin levels accompanied anaphylactic shock, it seemed probable that kinin formation might be important in the genesis of some types of shock. The significance of these events can only be evaluated by the use of specific kinin inhibitors, which are currently not available. At this stage, the physiological significance of bradykinin in circulatory control, whether local or total systemic, is still a matter for conjecture, in spite of the fact that bradykinin is one of the most potent pharmacological agents known (Sturmer and Cerletti, 1961; Ungar, 1966) and, potentially, mammalian plasma contains 2-14 μg/ml of bradykinin (Margolis and Bishop, 1963; Diniz and Carvalho, 1963), which means that a healthy man might have 4-11 mg of bradykinin in inactive form in every litre of circulating blood plasma (Erdos, 1966). Nevertheless, it is well established that bradykinin has central actions which result in a powerful hypertensive effect, the mechanism of which is also unclear. The experiments reported in part of this thesis were performed to attempt to determine more precisely the site and mechanism of the hypertensive response to cranial artery infusions of the nonapeptide, bradykinin. The related decapeptide, kallidin, has not been considered in this thesis.

GENERAL METHODS

GENERAL METHODS

(A) *1. Choice of experimental animal:*

The nature of the experiments performed for this thesis necessitated the use of an animal larger than the usual laboratory rat, cat or rabbit for the following reasons:

(a) Since the minimum time for drug infusions was 5 minutes (see Chapter 1), unless the drug solution was concentrated and infused at very slow rates and small volumes, cardiovascular alterations due to volume expansion alone would occur in small animals. Multiple infusions in any single experiment would result in significant expansion of plasma volume with its associated cardiovascular effects and unless the infusion rate was extremely slow, alterations in flow characteristics in the infused vessel would occur.

(b) In order that the distribution of blood to a vascular bed (the brain and hindlimb in the experiments described in this thesis) was not altered or interrupted, the blood vessels were catheterised such that there was no obstruction to blood flow. This was achieved by using catheters with a small external diameter relative to the size of the vessels. In this way, the normal flow and distribution of blood to a vascular bed is preserved and abnormal flow patterns would not be expected to contribute to any of the responses observed. In particular, the normal flow and distribution of blood from the carotid and vertebral arteries would be maintained, which is essential if responses to drug infusions via these routes of administration are to be quantitated and differentiated. The size of the vertebral, carotid and femoral arteries in the rat, mouse or rabbit prohibit direct catheteristation without severe, or even total, obstruction to blood flow, which could conceivably

alter the nature and mechanism of the cardiovascular response to various experimental procedures.

(c) In contrast to humans, small animals such as the rat, cat and rabbit have a higher degree of resting sympathetic tone to the heart and relatively little parasympathetic tone. Therefore, the mechanism(s) by which they change their heart rate during various procedures is likely to be different from those employed by humans. The cardiovascular system of the greyhound, however, appears to respond in a similar manner to humans and mechanisms of action are likely to be more comparable. This is well documented in the case of angiotensin II (Scroop, 1967) and would presumably also apply to bradykinin which is another peptide of similar molecular weight.

(d) In those experiments which involved measurement of cardiac output by the dye-dilution method, withdrawal of sufficient blood to obtain a dye curve would be either impossible or, at least, cause profound changes in the cardiovascular system of small animals. The large blood volume and vessel size of the greyhound permitted the rapid withdrawal of about 50 ml of blood without any noticeable effect on the cardiovascular system.

For the reasons above, the greyhound was chosen as a suitable experimental animal. Greyhounds were used in preference to mongrel dogs because of availability and temperament. In addition, greyhounds (a) are of large, uniform weight (average 30 kg), (b) have minimal subcutaneous fat, which simplifies surgical procedures and minimises the possibility of adipose tissue absorbing various drugs and subsequently slowly releasing these drugs into the circulation, and (c) have a large robust cardiovascular system (since they are bred as a racing animal) allowing them to adapt to surgical trauma and various experimental

procedures. The effect of species differences would also be minimal using only greyhounds as the experimental animal.

(A) 2. *Choice of anaesthetic:*

It is generally believed that drug effects on the cardiovascular system are modified by anaesthesia and that for a given dose of drug the responses obtained vary with the anaesthetic agent. Furthermore, in most circumstances responses obtained under all types of anaesthesia are believed to be depressed when compared with those obtained in conscious animals. However, Wilkinson (1975) showed that anaesthesia induced with chloralose, following morphine premedication, did not depress cardiovascular responsiveness to the test procedures employed (bilateral common carotid artery occlusion and both cranial artery and intravenous infusions of angiotensin II). Unless otherwise stated, the experiments were conducted in greyhounds anaesthetised with 4 g of α-chloralose (Sigma, B.D.H., or Merck) administered intravenously (approximately 120 mg/kg) following premedication with 60 mg intravenous morphine sulphate (David Bull Laboratories, Australia). The chloralose was administered as a 5% solution, prepared by dissolving 4 g sodium tetraborate in 80 ml normal saline (sodium chloride, 0.9% w/v) and then dissolving the chloralose in this solution by heating to 45-50°C. This solution was filtered to remove any undissolved particular matter.

(B) CATHETERISATION OF VESSELS

With the dogs in a state of surgical anaesthesia, a femoral artery, femoral vein, vertebral artery (isolated by blunt dissection through an anterior incision in the root of the neck) and both common carotid

arteries were isolated.

1. Vertebral artery:

All drug infusions into the vertebral artery were given through a 100 cm length of Portex polythene catheter (internal diameter, 0.5 mm, external diameter, 0.8 mm) of which about 1 cm was inserted into the artery through a needle hole of smaller diameter and passed in a centrifugal direction. To prevent accidental withdrawal of the catheter a small square of adhesive tape was attached to it immediately proximal to the site of entry and a ligature passed through the tape and tied loosely around the artery. This held the catheter in place without obstructing blood flow. Leakage from the catheter entry site was avoided by using a puncture needle (Yale, 23G, disposable) with an external diameter smaller than that of the catheter. The dead space in the system was approximately 0.2 ml which represents a delay of about 12 seconds from the beginning of the infusion to entry of drug into the circulation.

2. Carotid artery:

One common carotid artery was catheterised in a similar manner to the vertebral artery. Ligatures were also placed under both common carotid arteries to enable them to be occluded when required.

3. Femoral artery and femoral vein:

A femoral artery and femoral vein were catheterised with 100 cm lengths of Portex polythene catheter (internal diameter, 0.86 mm, external diameter, 1.27 mm) inserted centripetally for a distance of about 15 cm. Again, leakage from the catheter entry site was avoided

by using a puncture needle (Yale, 19G, disposable) of smaller external diameter than that of the catheter. Blood flow was not interrupted in either case by the presence of the catheter. The arterial catheter was used to measure pulsatile arterial blood pressure and the venous catheter for administration of drugs and supplementary anaesthetic. The dead space in the system was 0.58 ml, which represents a delay of about 35 seconds from the beginning of an infusion to entry of drug into the circulation.

(C) RECORDING PROCEDURES

1. Blood pressure:

The femoral arterial catheter, filled with heparinised saline (100 units/ml), was connected to a Statham P23Db pressure transducer, and a continuous record of pulsatile blood pressure was obtained on a Grass Model 7B Polygraph. In addition, mean arterial pressure at a gain 5 times that of the pulsatile pressure was recorded. This secondary trace allowed small changes in blood pressure to be quantitated. Electrical calibration of the pressure-recording system was performed against a mercury manometer.

2. Heart rate:

Heart rate was obtained by using the R wave of a conventional electrocardiogram to trigger a Grass Model 7P4 D tachograph. A secondary trace of heart rate at 4 times the gain was also recorded to allow small changes in heart rate to be quantitated.

(D) INFUSION PROCEDURES

Drugs given by infusion were always administered at the rate of 1 ml/min (usually for 5 minutes) by a motor-driven syringe pump. At the conclusion of the drug infusion period, the catheters were immediately flushed with heparinised normal saline (100 units/ml), again delivered at the rate of 1 ml/min by the motor-driven syringe pump for 2 minutes.

(E) CALCULATION OF RESULTS

1. Blood pressure:

The responses of arterial blood pressure during various procedures, unless otherwise specified, are expressed as the integral of the response (with units of mm Hg x min) which is calculated by planimetry, measuring the area under the response of mean arterial pressure.

2. Heart rate:

Similarly, the responses of heart rate during the various test procedures are expressed as the integral of the response (with units of beats) as calculated by planimetry, measuring the area under the response of heart rate.

CHAPTER 1

NORMAL RESPONSES TO VERTEBRAL ARTERY, CAROTID ARTERY AND INTRAVENOUS INFUSIONS OF BRADYKININ

INTRODUCTION

Rocha e Silva, Corrado and Ramos (1960) demonstrated that bradykinin had a central action when they injected the peptide into the carotid artery of the anaesthetised cat. Similarly, Buckley, Bickerton, Halliday and Kato (1963) demonstrated that bradykinin could cross the blood-brain barrier and affect the cardiovascular system, presumably by a direct central action on parasympathetic centres. Their responses were, however, "variable and unpredictable".

As discussed in the General Introduction, there are many reports of a central action of injections of bradykinin although the mechanism of action is uncertain. Also, since bradykinin has a biphasic effect on blood pressure when administered via the carotid artery (Pearson, Lambert and Lang, 1969), to obtain a "complete" response, it was decided to examine the response to 5 minute infusions of bradykinin into a vertebral artery (which supplies the "cardiovascular areas" in the medulla), a carotid artery and also intravenously to distinguish centrally-mediated effects from the direct peripheral effects of the peptide. An infusion, in contrast to an injection, would allow blood levels of the peptide to stablise and the responses of blood pressure and heart rate to plateau. On the other hand, an injection of bradykinin would almost immediately result in a peak concentration of the peptide which would rapidly decrease with every passage through the lungs, and any secondary response due to continued presence of low concentrations of bradykinin in the circulation would not be observed. Since bradykinin is rapidly (and considerably) destroyed in the pulmonary circulation (Alabaster and Bakhle, 1972; Ryan, Roblero and Stewart, 1968; Erdos, 1977), an intravenous infusion of a small dose of bradykinin would not be expected to have any significant central action. Similarly, arterial administration of small doses of bradykinin into the cerebral circulation

could be expected to have minimal effects in the peripheral circulation following passage through the lungs.

The following experiments were performed, firstly, to determine an appropriate dose of bradykinin that would reasonably distinguish between the central and peripheral effects of the peptide which could then be used in subsequent investigations and, secondly, to characterise the "normal" response to bradykinin infusions via vertebral artery, carotid artery and intravenous routes of administration.

MATERIALS AND METHODS

The experiments were conducted in ex-racing greyhounds, weighing between 26 and 41 kg (mean 29.8 ± 1.5 SEM), anaesthetised with α-chloralose following morphine premedication as described in General Methods. A cuffed endotracheal tube was inserted and artificial respiration was maintained throughout the experiment with a C.F. Palmer positive-pressure respirator. Anaesthesia was supplemented, as required, with small (15-30 mg) intravenous doses of sodium pentobarbitone (Nembutal).

Procedures for catherisation of a vertebral, carotid and femoral artery and a femoral vein, as well as procedures for measurement of blood pressure and heart rate are described in General Methods.

Bradykinin (Sandoz BRS-640), diluted in normal saline (sodium chloride, 0.9% w/v) was infused by a motor driven syringe pump (delivering 1 ml/min) at rates of 1, 2, 5, 10 and 20 μg/min for 5 minutes into each of a vertebral artery, carotid artery and intravenously.

Samples of bradykinin from Sigma, Bachem and the Protein Research Foundation were also infused in a similar manner and at the same rates as Sandoz bradykinin and the results obtained are discussed briefly.

In some dogs, bradykinin was infused via all three routes of administration in the presence of a vertebral artery infusion of the converting enzyme (kininase II) inhibitor, SQ 20,881 (10 μg/min). The infusion of SQ 20,881 was always commenced 5 minutes before any infusion of bradykinin. Administration of this dose of SQ 20,881 (10 μg/min for 5 minutes = 8 nmol/min for 5 minutes) would result in a final blood concentration of 1.6×10^{-9} M, assuming a total blood volume of 2.5 litres (Altman and Dittmer, 1971). This concentration would certainly

not be sufficient to totally inhibit kininase II (Igic, Erdos, Yeh, Sorrells and Nakajima, 1972; Dheung and Cushman, 1973) and at this dose produced only a small potentiation of the bradykinin responses via all routes of administration. Furthermore, this dose of SQ 20,881 could be expected to provide kininase II inhibition for a least the 45 minutes required to perform the bradykinin infusions (Engel, Schaeffer, Gold and Rubin, 1972; Bianchi, Evans, Cobb, Peschka, Schaeffer and Laffan, 1973).

The responses of blood pressure and heart rate are expressed as their integrals and significance, calculated using a Student's t-test for paired observations, was accepted as $p<0.05$.

Drugs used were morphine sulphate (David Bull Laboratories Pty. Ltd., Australia); α-chloralose (Sigma Chemical Company or B.D.H.); Nembutal (Abbott Laboratories); bradykinin (Sandoz BRS-640, Sigma Chemical Company, Bachem Fine Chemicals Inc., Protein Research Foundation); SQ 20,881 (E.R. Squibb & Sons).

RESULTS

1. Blood Pressure:

Bradykinin infusions (Sandoz BRS-640) into either the vertebral artery or carotid artery produced a dose related increase in mean arterial pressure at each of the infusion rates investigated, while intravenous infusions at the same rates produced a dose related decrease in mean arterial pressure. Responses obtained in a single experiment with each route of administration are illustrated in Fig. 1.1. Similar results were obtained in a total of 8 other dogs (although not all doses were repeated in all of the experiments) and the pooled data for each route of aministration is presented in Tables 1.1, 1.2 and 1.3 and combined in Fig. 1.2.

On average, the response to vertebral artery infusion of bradykinin was larger than that obtained with carotid artery infusion, although the difference was only statistically significant ($p<0.05$) at the two highest doses used (10 and 20 µg/min).

Small doses of bradykinin (1 and 2 µg/min) were without significant effect on blood pressure when administered by intravenous infusion, although pressor responses were obtained with both vertebral and carotid artery infusions at the same rates.

2. Heart Rate:

Bradykinin infusions (Sandoz BRS-640) via all three routes of administration caused a tachycardia in a dose related manner. Characteristic responses with all three routes of administration are shown in Fig. 1.1, and pooled data from 9 dogs are presented in

FIGURE 1.1 - The normal responses of pulsatile blood pressure (BP - mm Hg), mean arterial pressure at 5x gain of BP (MAP - mm Hg) and heart rate (HR - beats/min) during a 5 minute infusion of bradykinin (20 μg/min) via a vertebral artery, carotid artery and intravenously.

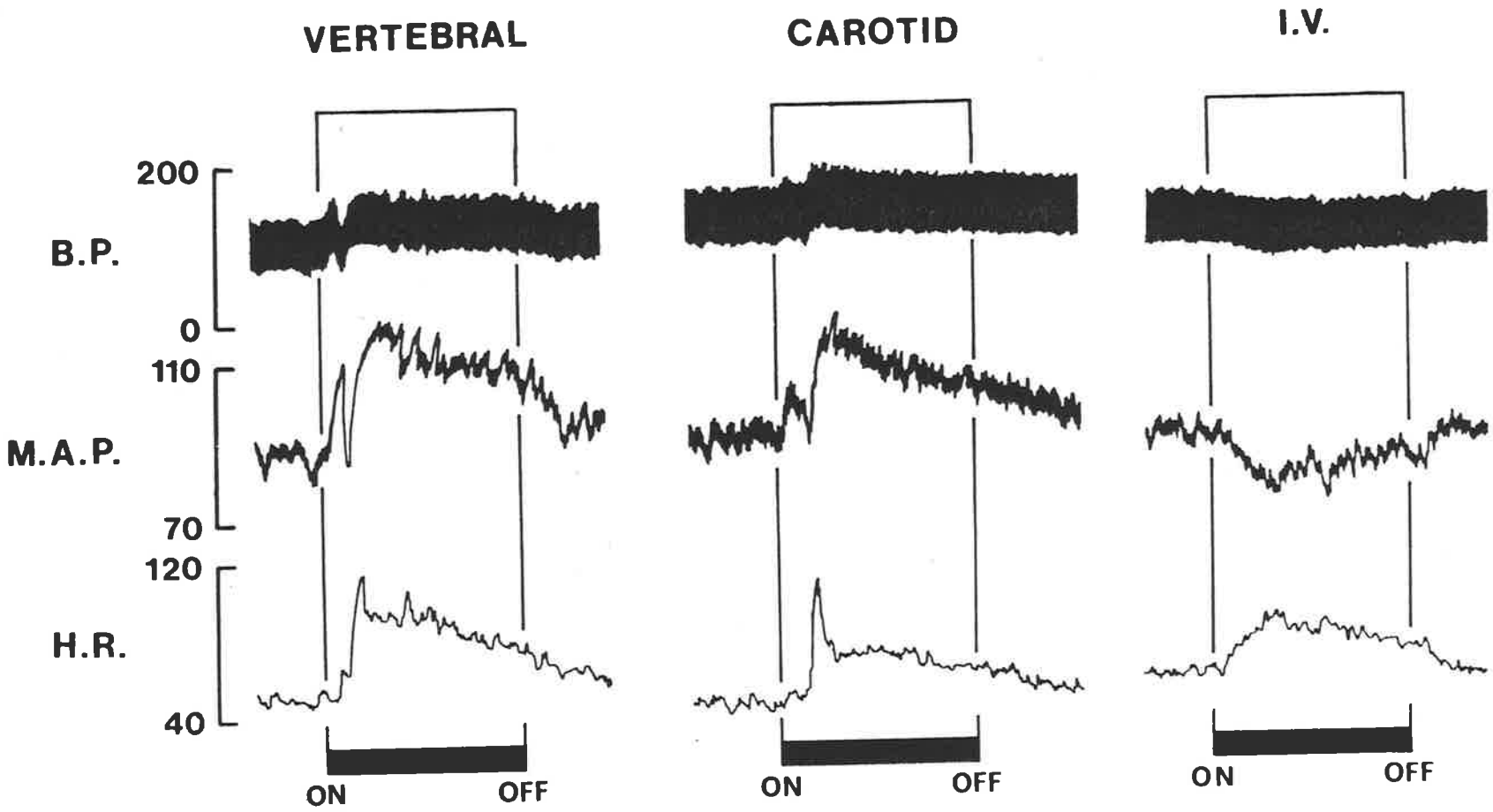
VERTEBRAL
CAROTID
I.V.
200
B.P.
0
110
M.A.P.
70
120
H.R.
40
ON
OFF
ON
OFF
ON
OFF

FIGURE 1.1

FIGURE 1.2 - The relationship between the dose of bradykinin and the change in blood pressure and heart rate during 5 minute infusions at the doses indicated via a vertebral artery (■—■), carotid artery (▲—▲) and intravenously (●—●).

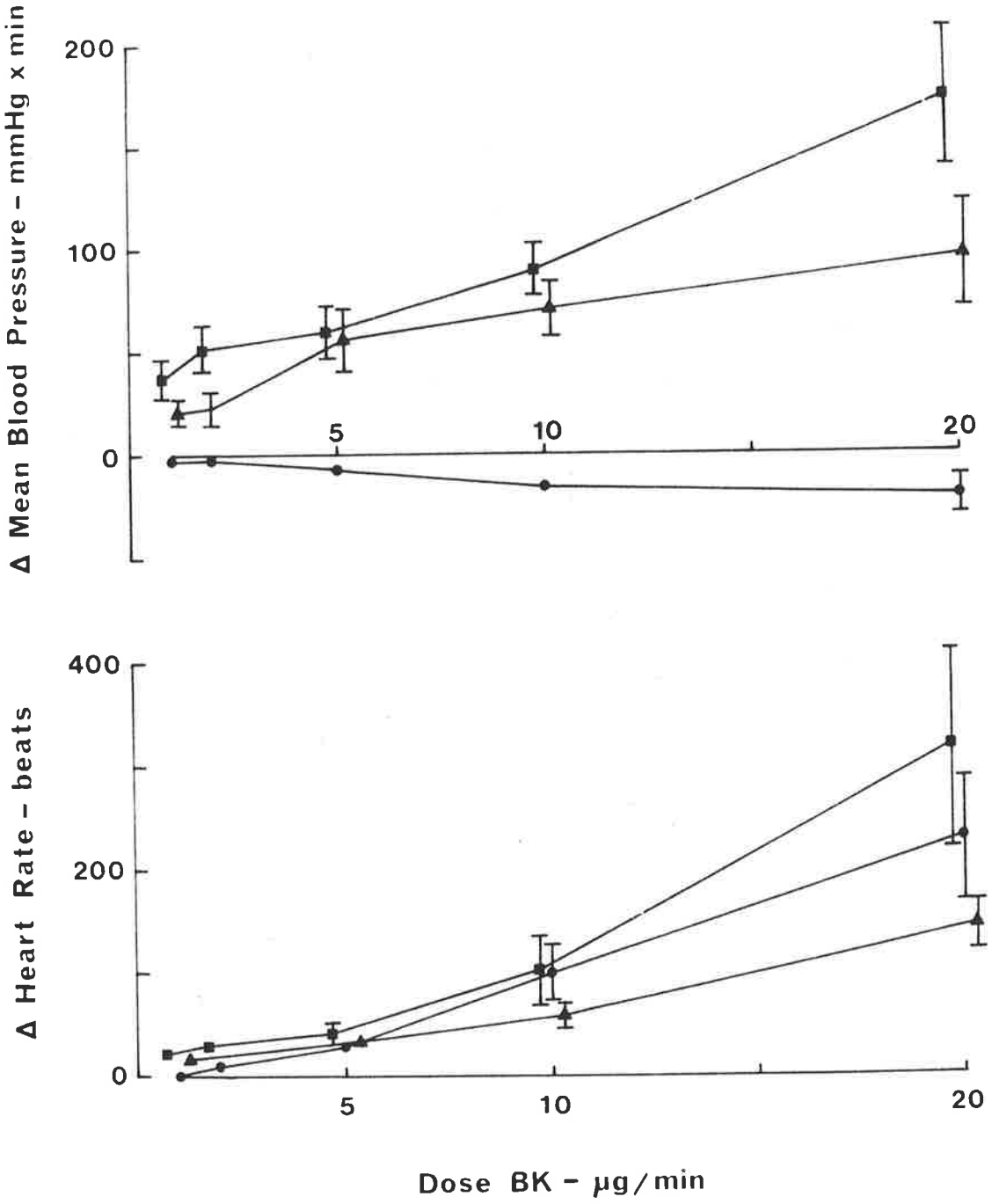
Δ Mean Blood Pressure - mmHg x min
200
100
0
5
10
20
Δ Heart Rate - beats
400
200
0
5
10
20
Dose BK - µg/min

FIGURE 1.2

Tables 1.1, 1.2 and 1.3 and combined in Fig. 1.2. Apart from the vertebral and carotid artery responses during an infusion of 20 μg/min of bradykinin, at each of the infusion rates used there was no significant difference ($p<0.05$) between the tachycardia obtained with any of the routes of administration. However, the response during vertebral artery infusions was, on average, larger than with either carotid artery or intravenous infusions.

3. Blood Pressure Responses During SQ 20,881:

The integrated responses of mean blood pressure during 5 minute infusions of bradykinin (10 μg/min) were significantly ($p<0.05$) potentiated with all three routes of administration in the presence of vertebral artery infusions of SQ 20,881 (10 μg/min). The pooled data from 4 dogs is presented in Table 1.4.

While the absolute changes in mean blood pressure (mm Hg) during infusions of bradykinin via all three routes of administration were not significantly different in the presence of SQ 20,881 compared to control values, they were, on average, larger following SQ 20,881 (Table 1.5). The potentiation of the integrated mean blood pressure responses was, therefore, not entirely due to an increase in the absolute magnitude of the pressor responses, but partly due to an increase in the duration of the response. Both of these factors contribute to the statistically significant potentiation of the integrated mean blood pressure responses (Fig. 1.3).

4. Heart Rate Responses During SQ 20,881:

The integrated responses of heart rate during 5 minute infusions of bradykinin (10 μg/min) were significantly potentiated ($p<0.05$) with

FIGURE 1.3 - The responses of mean blood pressure and heart rate to 5 minute infusions of bradykinin (10 μg/min) via a vertebral artery (VA), carotid artery (CA) and intravenously (IV) before and during a continuous vertebral artery infusion of SQ 20,881 (10 μg/min).

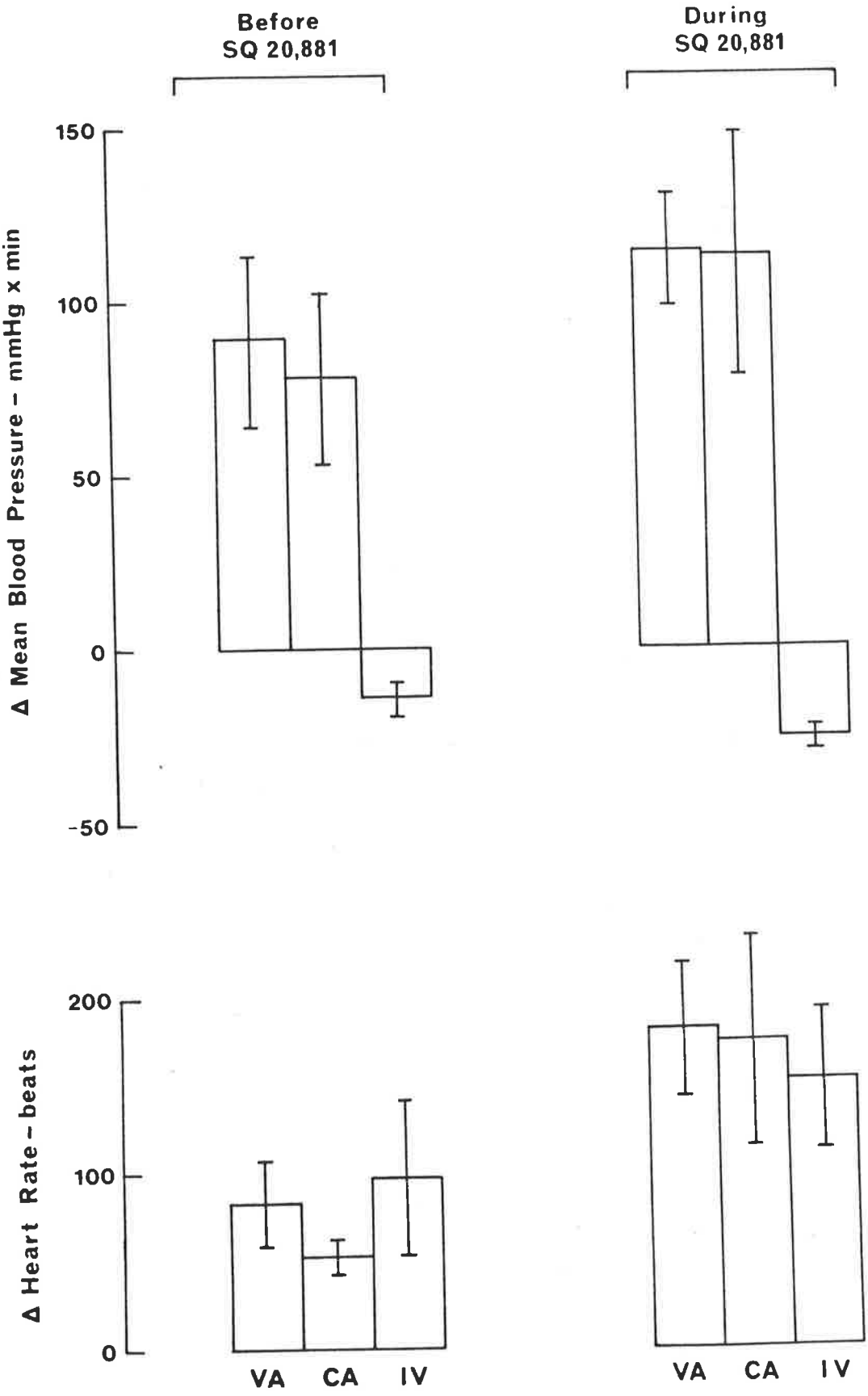
Before
SQ 20,881
During
SQ 20,881
150
100
50
0
-50
Δ Mean Blood Pressure – mmHg x min
200
100
0
Δ Heart Rate – beats
VA
CA
IV
VA
CA
IV

FIGURE 1.3

all three routes of administration in the presence of vertebral artery infusions of SQ 20,881 (10 μg/min). The pooled data from 4 dogs are presented in Table 1.4.

The absolute change in heart rate, while being larger with all 3 routes of administration in the presence of SQ 20,881 compared with control changes, was only statistically significantly larger ($p<0.05$) in the case of carotid artery infusions of bradykinin (Table 1.5). As with the blood pressure responses, the potentiation of the integrated heart rate responses by SQ 20,881 is due, in part, to both a larger absolute heart rate change and a longer duration of the heart rate response.

DISCUSSION

As can be seen from Fig. 1.1, intravenous infusions of bradykinin produced the expected decrease in mean blood pressure, presumably by a direct action of the peptide on blood vessels to cause vasodilation (Rocha e Silva, Beraldo and Rosenfeld, 1949; Fox, Goldsmith, Kidd and Lewis, 1961; Maxwell, Elliott and Kneebone, 1962). The resultant hypotension activates the baroreceptor reflex to cause the associated tachycardia (Page and Olmsted, 1961; Olmsted and Page, 1962). The mechanism of the increased heart rate and involvement of the autonomic nervous system in this response have been examined and the results are presented in Chapter 2 of this thesis.

It is also apparent from Fig. 1.1 that when an amount of bradykinin sufficient to cause a mild hypotensive effect is infused into the cerebral circulation (via a vertebral artery or a carotid artery), a powerful hypertensive effect is observed. Furthermore, the responses obtained with both vertebral artery and carotid artery infusions of bradykinin are biphasic in nature. At the commencement of the infusion, there is a rapid increase in blood pressure and either no change in heart rate or a small tachycardia. The increase in blood pressure is followed by an equally rapid fall in blood pressure toward control values. The total time for the increase in blood pressure and the subsequent decrease in pressure does not normally exceed 30 sec. During the decrease in blood pressure, a pronounced increase in heart rate occurs and this is associated with a further rise in blood pressure, which reaches a maximum value and remains at this value for the duration of the infusion (Fig. 1.1). At the conclusion of the infusion, both blood pressure and heart rate gradually return to control values over a period of about 2 minutes.

The decrease in blood pressure (following the initial rapid increase) in the first 30 sec of the response does not normally fall below the control blood pressure, nor is it always necessarily as dramatic as the vertebral artery response illustrated in Fig. 1.1. However, the response always showed some biphasic characteristics (as described above) and the observed tachycardia did not appear to be associated with the initial part of the response. Although the cranial artery responses are biphasic, they do not have a hypotensive component as observed by Pearson, Lambert and Lang (1969) when they injected bradykinin into the carotid artery of cats. Since the response to continual infusion of the peptide has two components, an initial rapid increase which returns toward control values followed by a sustained increase, previous reports concerning the action of the peptide administered by injection are probably only concerned with the initial part of the response. In addition, the relatively high doses of bradykinin injected in some cases could also have residual peripheral effects to cause hypotension following the initial increase in blood pressure. The infusion rates used in the experiments reported in this thesis would result in minimal peripheral effects after passage through the lungs where bradykinin is largely destroyed, and the length of the infusion is sufficient for blood pressure and heart rate to reach stable values. These plateau values probably represent more closely the effect of circulating endogenous bradykinin. Since it appears probable that the initial response and the subsequent plateau response of blood pressure have different mechanisms (as determined by the lack of a significant heart rate effect during the initial pressor phase), the mechanisms responsible for the plateau phase are probably more indicative of those present during circulation of endogenous bradykinin. For this reason, infusions of bradykinin rather than injections were used in subsequent experiments to determine the mechanisms of the cardiovascular actions of bradykinin, and the infusion rates employed would reasonably distinguish between centrally-mediated

and direct peripheral actions of the peptide.

Feldberg and Lewis (1964) and Pearson and Lang (1967) observed biphasic responses to intravenous injections of bradykinin. Both reported an initial fall in blood pressure followed by hypertension and tachycardia. Feldberg and Lewis suggested release of adrenal medullary adrenaline caused the hypertension and tachycardia, while Pearson and Lang attributed this effect to some central action of bradykinin. In all of the experiments reported in this thesis, intravenous bradykinin produced a purely hypotensive response and an increase in heart rate as typified in Fig. 1.1.

Since the response to cranial artery infusions of bradykinin involves an increase in both blood pressure and heart rate, a centrally-mediated mechanism has been suggested (Pearson and Lang, 1967; Lang and Pearson, 1968; Lambert and Lang, 1970; Correa and Graeff, 1974; Correa and Graeff, 1976; Kondo, Okuno, Konishi, Saruta and Kato, 1979). However the central site of action and the relative involvement of the sympathetic and parasympathetic nervous systems are still not clear, and will be discussed subsequently in this thesis.

BRADYKININ INFUSIONS FOLLOWING SQ 20,881

When bradykinin (10 μg/min) was infused into the vertebral artery, carotid artery and intravenously in the presence of a background vertebral artery infusion of SQ 20,881 (10 μg/min), the integrated responses of blood pressure and heart rate were potentiated in all cases. This potentiation was due to both an increase in the absolute change of blood pressure (although not statistically significant) and heart rate and also to a prolongation of the respective responses.

SQ 20,881 is an inhibitor of kininase II (also known as angiotensin converting enzyme) which is partly responsible for the inactivation of bradykinin in the circulation, mainly in the lungs (Ryan, Roblero and Stewart, 1968; Alabaster and Bakhle, 1972; Erdos, 1977). Inactivation of kininase II, however, will not result in an indefinite response to bradykinin since there are still other active enzymes in the circulation, e.g. plasma aminopeptidase and carboxypeptidase N, but the rate of inactivation of bradykinin is significantly slowed and the responses of blood pressure and heart rate are correspondingly potentiated. As mentioned previously, the dose of SQ 20,881 infused would not be sufficient to completely block all kininase II activity.

The SQ 20,881 was infused into the vertebral artery, but localisation of action to the vertebral artery territory is doubtful since responses with all three routes of administration were significantly potentiated. If the action of SQ 20,881 was limited to the cerebral circulation, responses to intravenous infusions of bradykinin should remain unaffected since these responses reportedly do not involve central actions of the peptide. It thus appears that SQ 20,881 was having its effect in the systemic circulation to generally inhibit (not necessarily totally) the activity of kininase II.

Bradykinin used in the experiments reported so far in this thesis was obtained from Sandoz, Australia (BRS-640). However, they were unable to maintain a supply of sufficient quantity to complete the proposed experiments. Subsequently, bradykinin was obtained from the Sigma Chemical Company, U.S.A. (bradykinin triacetate, Cat. B1377). Infusion of this substance via a vertebral artery, carotid artery and intravenously produced responses that were smaller (about 50%) than those obtained using Sandoz bradykinin. Bradykinin triacetate obtained from Bachem Fine Chemicals Incorporated (California) produced similar

results to those obtained using Sigma bradykinin. A sample of bradykinin was also obtained from the Protein Research Foundation (PRF), Japan, and use for infusion. This substance was as active as Sandoz bradykinin, so it was decided to use PRF bradykinin for the remainder of the experiments. Because of expenditure restraints, the bradykinin was ordered as "bulk" bradykinin triacetate in 25 mg lots. This was then aliquotted and dried (see Appendix 1). Infusions of this substance also produced responses which were smaller in magnitude than those obtained with Sandoz bradykinin and were also smaller than those obtained with pure bradykinin peptide obtained from PRF (even taking into account the difference in molecular weight between "pure" bradykinin and bradykinin triacetate). The reason for the difference in activity between all of the samples used is not clear (see Appendix 1) but, apart from the responses reported in Chapter 1, all subsequent experiments were conducted using bradykinin triacetate from the Protein Research Foundation and every dog served as its own control.

Having characterised the response to vertebral artery, carotid artery and intravenously administered bradykinin, the efferent pathways mediating the responses were investigated and are discussed in detail in Chapter 2.

CHAPTER 2

INVOLVEMENT OF THE AUTONOMIC NERVOUS SYSTEM IN THE CARDIOVASCULAR RESPONSE TO BRADYKININ

INTRODUCTION

Bradykinin has a direct action on vascular smooth muscle to cause vasodilation of peripheral arterioles (Haddy, Emerson, Scott and Daugherty, 1970) and on intravenous administration, this action contributes to the described effects of a decrease in total peripheral vascular resistance, an increase in cardiac output, hypotension and tachycardia (see references in General Introduction). The tachycardia is a reflex effect in response to the decrease in arterial blood pressure and is presumably mediated via an action of the autonomic nervous system, although the relative contributions of the sympathetic and parasympathetic nervous systems have not been quantitated. Since the species of animal used in the experiments reported in the literature have differing degrees of resting sympathetic and parasympathetic tone to the cardiovascular system, the mechanisms responsible for changes in blood pressure and heart rate will be different and species dependent. In these experiments, the mechanisms of the hypotension and tachycardia following intravenous administration of bradykinin have been examined in the morphine and chloralose anaesthetised greyhound, which has a comparable degree of resting sympathetic and vagal tone to man.

Since bradykinin apparently does not readily cross the blood-brain barrier (Bumpus, Smeby, Page and Khairallah, 1964) it is not certain whether bradykinin can act directly on central nervous cells when injected into the blood stream (Correa and Graeff, 1974). To examine the direct effect on bradykinin on central nervous system structures, the peptide was injected directly into the cerebral ventricles (Graeff, Pela and Rocha e Silva, 1969; Pearson, Lambert and Lang, 1969; Lambert and Lang, 1970). Following injection into the cerebral ventricles, bradykinin caused a centrally-mediated pressor effect (Pearson, Lambert and Lang, 1969; Correa and Graeff, 1974, 1975, 1976). Although the

site and mechanism of this central action have not been clearly identified, Lambert and Lang (1970) provide evidence to support both α and β-adrenergic mechanisms.

Centrally-mediated hypertensive responses to carotid artery injections of bradykinin have also been reported (Lang and Pearson, 1968; Pearson and Lang, 1969; Pearson, Lambert and Lang, 1969; Correa and Graeff, 1974, 1975, 1976). In contrast, Ricciopo Neto *et al.* (1974) observed hypotension and bradycardia following intra-carotid injection of bradykinin, but concluded that these effects were "reflex in nature and are probably due to stimulation of paravascular nerve endings distributed along the territory of the occipital artery and possibly located in the meninges." Using cross-circulation techniques, Benetato, Haulica, Muscalu, Bubuianu and Galesanu (1964) reported biphasic responses to carotid artery injection of bradykinin in dogs. It is, however, generally accepted that intra-carotid injection of bradykinin is associated with hypertension and tachycardia, although the site and mechanism of action remain to be determined.

Administration of bradykinin via a vertebral artery has not been widely reported. The vertebral arteries supply the areas postrema with large amounts of blood (Roth and Yamamoto, 1968) and since the areas are "characterised by extreme vascularity" and are deficient in the blood-brain barrier (Klara, Kostrezewa and Brizzee, 1976), accessibility of bradykinin to central structures may be enhanced, or at least different, compared to carotid artery administration. The fenestrated endothelia will allow access to cerebral structures regardless of their molecular size or lipid solubility (Rapoport, 1976). The areas postrema have already been implicated in the centrally-mediated hypertensive response to angiotensin II, another vasoactive peptide of similar molecular weight to bradykinin, whether

infused via a vertebral artery (Joy and Lowe, 1970b) or released from the kidneys *in vivo* (Scroop, Katic, Brown, Cain and Zeegers, 1975). To distinguish possible differences in effect with different routes of administration, bradykinin was infused into both a carotid artery and a vertebral artery in the experiments reported in this thesis.

The following experiments were undertaken in morphine and chloralose anaesthetised greyhounds to examine, in more detail, the cardiovascular responses to cranial artery and intravenous infusions of bradykinin and to identify and quantitate the relative contributions of the various autonomic components.

MATERIALS AND METHODS

The experiments were conducted in ex-racing greyhounds, weighing between 28 and 35 kg (mean 30.7 ± 0.4 s.e.m.), anaesthetised with α-chloralose following morphine premedication as described in General Methods. A cuffed endotracheal tube was inserted and artificial respiration was maintained throughout the experiment with a C. F. Palmer positive-pressure respirator. Anaesthesia was supplemented, as required, with small doses of sodium pentobarbitone (Nembutal).

Procedures for catheterisation of a vertebral, carotid and femoral artery and a femoral vein, as well as procedures for measurment of blood pressure and heart rate are described in General Methods.

MEASUREMENT OF CARDIAC OUTPUT

In addition to blood pressure and heart rate, in six dogs cardiac output was estimated by a dye-dilution technique. In these dogs, a Portex polythene catheter (external diameter, 2.08 mm, internal diameter, 1.57 mm) was advanced toward the heart via a brachial artery and was tied in place. This catheter was used for rapid withdrawal of blood through a densitometer for continuous estimation of dye concentration. An additional large-bore catheter with an internal volume of 2 ml was inserted into a jugular vein and advanced so that the tip was close to the right atrium for injection of dye.

The dye (Cardio-Green, Hynson, Westcott and Dunning Inc.) was rapidly administered via the jugular vein catheter as a 1 mg bolus injection in a volume of 1 ml. Arterial blood, sampled via the brachial artery catheter, was withdrawn at 50 ml/min through a densitomener

(Gilford Instrument, Cuvette densitometer, Model 103 IR), and the densitometer output displayed on a Rikadenki chart recorder. Cardiac output was calculated on an Olivetti Programma 101 desk-top computer according to the method of Hall and Tyler (1971).

CALCULATION OF TOTAL PERIPHERAL RESISTANCE

Total peripheral resistance (TRP) was calculated by dividing mean arterial blood pressure (measured at the time of the cardiac output estimation) by the calculated cardiac output. The units of total peripheral vascular resistance are mm Hg x min x l^{-1}.

PROTOCOL

Bradykinin was administered by infusion at a rate of 20 μg/min for 5 minutes into each of a vertebral artery, carotid artery and intravenously. The experiments reported in this chapter are divided into 3 groups, according to the autonomic blocking procedures outlined below.

GROUP 1 (6 dogs)

(a) Following control infusions of bradykinin via each route of administration investigated, "total" pharmacological blockade of β-adrenergic receptors was achieved by the intravenous administration of propranolol (500 μg/kg). The degree of blockade was assessed by an intravenous injection of 3-5 μg of the β-receptor agonist, isoprenaline, before and after administration of propranolol. Complete abolition of

the control isoprenaline response, which consisted of profound hypotension and tachycardia, by propranolol was accepted as adequate β-receptor blockade. Periodically throughout the experiment, adequacy of blockade was checked by an intravenous injection of 3-5 μg of isoprenaline and additional propranolol (2-5 mg) was injected as required. The responses to bradykinin infusions via all three routes of administration were then examined in the presence of maintained β-receptor blockade.

(b) In the presence of maintained β-receptor blockade, bilateral cooling of the vagus nerves was performed to interrupt transmission of nervous impulses in the vagi. Cooling of the nerves was accomplished by placing U-shaped copper tubes under each nerve and circulating water at -2°C through these tubes. Interruption of nervous transmission by cooling is a reversible procedure and was used in this group of experiments in preference to surgical transection of the nerves. Adequacy of this procedure in blocking vagal transmission was assessed at the conclusion of every experiment by cutting the vagus nerves in the presence of vagal cooling. The absence of any further increase in heart rate or blood pressure indicated complete interruption of vagal transmission by the cooling procedure. When the responses to vertebral artery, carotid artery and intravenous infusions of bradykinin were completed, the vagal cooling was removed.

(c) Bradykinin infusions via each route of administration were then performed in the presence of combined β-receptor blockade (with propranolol) and α-receptor blockade with phentolamine. Adequate α-receptor blockade (as determined by the abolition of the pressor response to a 5 minute intravenous infusion of noradrenaline at a rate of 10 μg/min) was achieved by an intravenous injection of 5-10 mg of phentolamine followed by a continuous infusion of phentolamine

(500 μg/min) for the remainder of the experiment. Adequacy of α-receptor blockade was determined periodically by repeating the infusion of noradrenaline.

(d) Finally, the effect of combined β-receptor blockade, α-receptor blockade and vagal cooling on the responses to bradykinin infusions via each route of administration was determined.

All blocking procedures were checked at the conclusion of the experiment and any dogs not showing complete abolition of the test procedures used were not included in the results.

GROUP 2 (6 dogs)

In another series of experiments, the order of autonomic blockade was altered and the following protocol was used.

(a) Bradykinin infusions were performed in the presence of α-receptor blockade (with phentolamine) in the same manner as in Group 1 experiments.

(b) Bradykinin was infused in the presence of combined α-receptor and β-receptor blockade (with phentolamine and propranolol respectively).

(c) Finally, bradykinin infusions were performed in the presence of combined α-receptor and β-receptor blockade and bilateral vagotomy.

GROUP 3 (6 dogs)

In the final series of experiments, the order of autonomic blockade was again altered and the following protocol was used:

(a) Bradykinin infusions were performed following bilateral vagotomy. (Surgical transection of both vagus nerves was used in preference to bilateral vagal cooling since there was no need for reversal of the vagal block and it was technically simpler than arranging and operating the cooling apparatus).

(b) Bradykinin was infused via each route of administration following bilateral vagotomy and β-receptor blockade with propranolol.

(c) Bradykinin was infused following combined bilateral vagotomy, β-receptor blockade with propranolol and α-receptor blockade with phentolamine.

The responses of blood pressure and heart rate to infusion of bradykinin via each route of administration are expressed as their integrals as measured by planimetry. Cardiac output values are expressed as litres/min and total peripheral resistance as mm Hg x min x l^{-1}.

When comparing responses to bradykinin infusions following the various procedures outlined above, every dog served as its own control, and statistical analysis of the results was performed using a Student's t-test for paired observations, significance being accepted at $p<0.05$.

Drugs used were: morphine sulphate (David Bull Laboratories, Australia); α-chloralose (Sigma Chemical Company or B.D.H.); sodium

pentobarbitone (Nembutal, Abbott Laboratories); bradykinin triacetate (Protein Research Foundation, Japan); propranolol hydrochloride (Inderal, I.C.I.); isoprenaline hydrochloride (Isuprel, Winthrop Laboratories, Australia); phentolamine mesylate (Regitine, Ciba-Geigy, Australia); noradrenaline (Levophed, Winthrop Laboratories, Australia); indocyanine green (Cardio-Green, Hynson, Westcott and Dunning Inc.).

RESULTS

(A) GROUP 1

1. Blood pressure:

Infusion of bradykinin (20 µg/min for 5 minutes) into either a vertebral artery or a carotid artery caused an increase of blood pressure in both cases (Tables 2.1, 2.2 and Fig. 2.1). The pressor response during vertebral artery infusion (102.8 ± 15.5 mm Hg x min) was significantly larger than that obtained during carotid artery infusion (61.4 ± 16.7 mm Hg x min). Intravenous infusion of bradykinin at the same rate produced the expected decrease in blood pressure in all dogs (Table 2.3 and Fig. 2.1).

Following β-adrenergic receptor blockade with propranolol, the increase in blood pressure during vertebral artery infusions of bradykinin was reduced to approximately 50% of the control response and this difference was statistically significant ($p<0.05$). While the increase in blood pressure during carotid artery infusion of bradykinin following propranolol was, on average, slightly smaller than the control response, the difference was not statistically significant. However, the pressor response to carotid artery infusion was still significantly smaller than the response to vertebral artery infusion of bradykinin. The decrease in blood pressure during intravenous infusion of bradykinin was unchanged following β-receptor blockade with propranolol.

Bilateral vagal cooling, in the presence of β-receptor blockade with propranolol, resulted in a further significant decrease in the magnitude of the pressor response to vertebral artery infusion of bradykinin (from 48.0 ± 7.9 to 6.6 ± 5.4 mm Hg x min) such that with

FIGURE 2.1 - The responses of blood pressure during vertebral artery (VA), carotid artery (CA) and intravenous (IV) infusions of bradykinin (20 μg/min for 5 minutes) in (A) the intact dog (control responses) and then (B) following β-adrenoceptor blockade with propranolol (500 μg/kg intravenously), (C) following propranolol and bilateral vagal cooling, (D) following propranolol and α-adrenoceptor blockade with phentolamine (5 mg, then 500 μg/min continuously by intravenous infusion) and, finally, (E) following propranolol, phentolamine and bilateral vagal cooling.

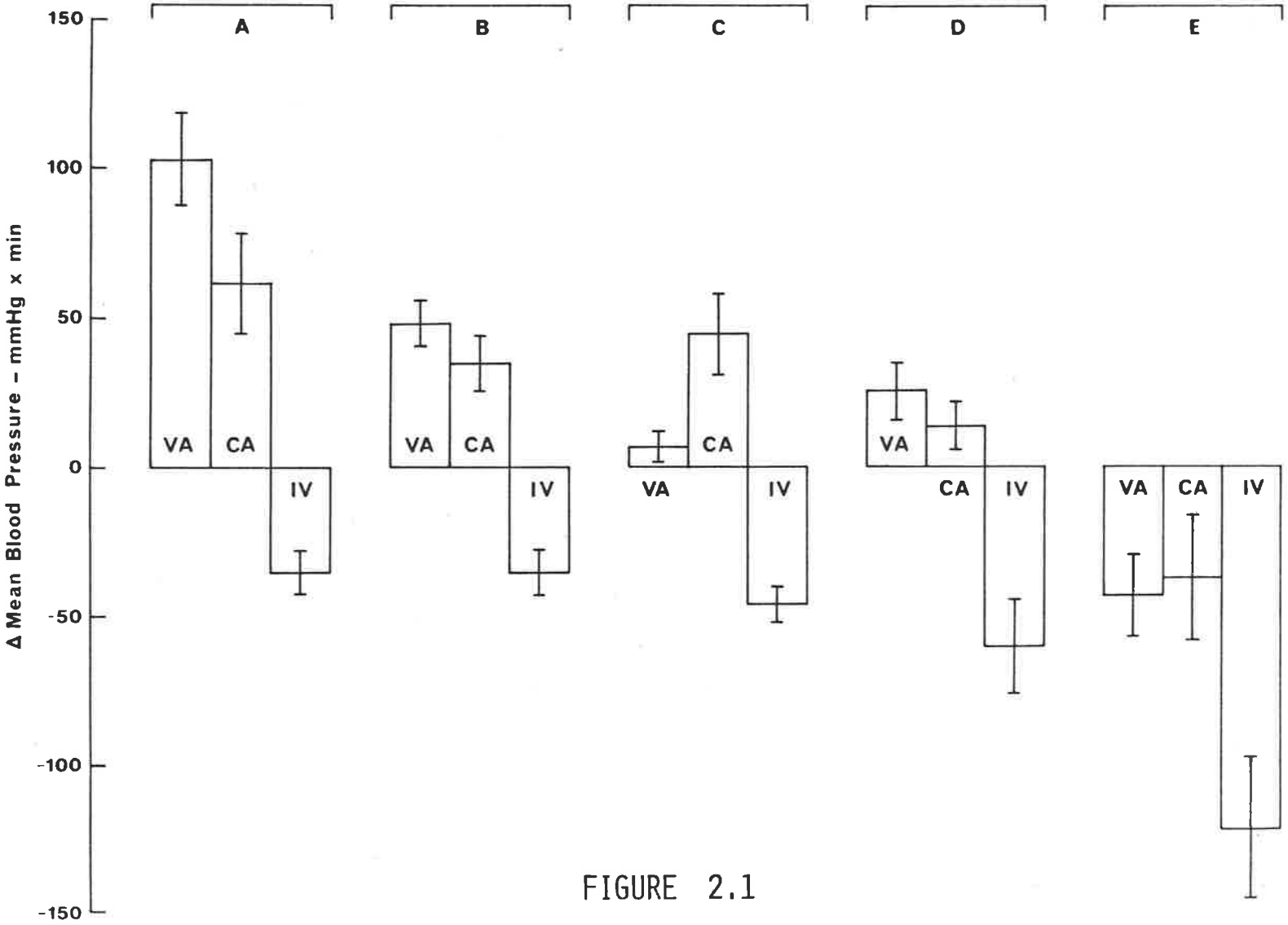
A
B
C
D
E
150
100
50
0
-50
-100
-150
Δ Mean Blood Pressure - mmHg x min
VA
CA
IV

FIGURE 2.1

this combined treatment, the pressor response was almost totally abolished and was not significantly different from zero. The blood pressure responses during carotid artery and intravenous infusions of bradykinin were not further altered by the addition of bilateral vagal cooling to the protocol and were still not significantly different from control responses.

The combined treatment of β-receptor blockade (with propranolol) and α-receptor blockade (with phentolamine) resulted in a significantly smaller pressor response to vertebral artery infusions of bradykinin compared with control responses (Table 2.1 and Fig. 2.1). Furthermore, the pressor response to vertebral artery infusions of bradykinin following this combined treatment was significantly smaller than that obtained with β-receptor blockade alone. Although the addition of phentolamine to the protocol did not result in a significantly smaller pressor response to carotid artery infusions of bradykinin compared to the response obtained with propranolol alone, the response following the combined treatments was significantly smaller than the control responses (Table 2.2). The depressor response to intravenous infusions of bradykinin in the presence of combined β-receptor and α-receptor blockade were, on average, marginally larger than both the control responses and the responses following β-receptor blockade alone, although the difference in both cases was not statistically significant.

Finally, the combined treatments of propranolol, phentolamine and bilateral vagal cooling resulted in depressor responses to bradykinin when infused via all three routes of administration. The depressor responses to vertebral artery and carotid artery infusions of bradykinin were not significantly different (Tables 2.1 and 2.2) although the depressor response to intravenous infusion of bradykinin was significantly larger than both the vertebral artery and carotid

artery responses. Whereas the depressor response to intravenous infusion of bradykinin was not greatly affected by any previous treatments, it was significantly larger than the control response following the final combined treatments (Table 2.3).

2. Heart rate:

Infusion of bradykinin (20 μg/min for 5 minutes) into each of a vertebral artery, carotid artery and intravenously caused a pronounced tachycardia in all 3 cases (Tables 2.1, 2.2 and 2.3 and Fig. 2.2). The response to vertebral artery infusion of bradykinin was statistically significantly larger than the carotid artery response, but was not significantly different from the heart rate response during intravenous infusion (see Fig. 2.2).

Following β-receptor blockade with propranolol, a tachycardia was still observed during bradykinin infusion via all three routes of administration, although the responses were significantly smaller than the control responses in all three cases. There was no significant difference between the responses obtained with all three routes of administration following β-receptor blockade (Tables 2.1, 2.2 and 2.3 and Fig. 2.2).

The combined treatment of β-receptor blockade with propranolol and bilateral vagal cooling resulted in almost complete abolition of the heart rate responses during bradykinin infusion via all three routes of administration. The responses obtained were not significantly different from zero (Tables 2.1, 2.2 and 2.3 and Fig. 2.2).

Combined β-receptor blockade (with propranolol) and α-receptor blockade (with phentolamine) resulted in a significantly smaller

FIGURE 2.2 - The responses of heart rate during vertebral artery (VA), carotid artery (CA) and intravenous (IV) infusions of bradykinin (20 μg/min for 5 minutes) in (A) the intact dog (control responses) and then (B) following β-adrenoceptor blockade with propranolol (500 μg/kg intravenously), (C) following propranolol and bilateral vagal cooling, (D) following propranolol and α-adrenoceptor blockade with phentolamine (5 mg, then 500 μg/min continuously by intravenous infusion) and finally, (E) following propranolol, phentolamine and bilateral vagal cooling.

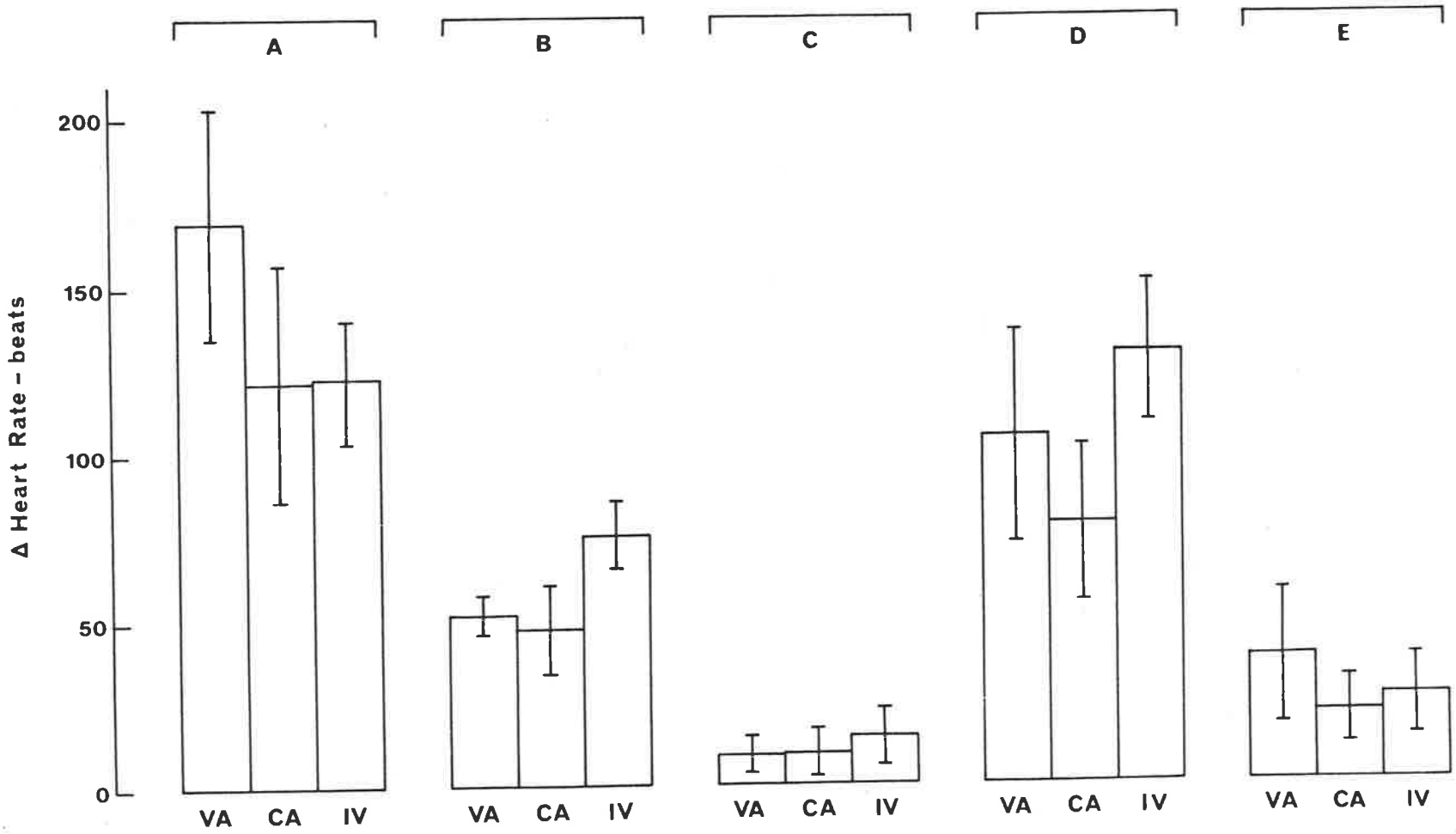

FIGURE 2.2

tachycardia during bradykinin infusion via a vertebral artery and carotid artery compared with control responses. The heart rate response during intravenous infusion was not significantly different from control responses. Furthermore, the responses to vertebral artery and carotid artery infusions of bradykinin following combined β-receptor and α-receptor blockade were not significantly different from the tachycardia obtained following β-receptor blockade alone. However, the tachycardia observed during intravenous infusion of bradykinin following combined β-receptor and α-receptor blockade was significantly greater than that obtained following β-receptor blockade alone.

Finally, the combined treatment of β-receptor and α-receptor blockade and bilateral vagal cooling resulted in significantly smaller heart rate responses than those obtained with combined β-receptor and α-receptor blockade. Although the responses were, on average, larger than those obtained following β-receptor blockade and bilateral vagal cooling, the difference was not statistically significant. The tachycardia responses following the final combination of blocking procedures were, surprisingly, significantly larger than zero and possible explanations for these results will be discussed.

(B) GROUP 2

1. Blood pressure:

Infusion of bradykinin (20 μg/min for 5 minutes) into either a vertebral artery or a carotid artery caused an increase of blood pressure in both cases (Table 2.4, 2.5 and Fig. 2.3). While the responses to vertebral artery infusions were, on average, larger

FIGURE 2.3 - The responses of blood pressure during vertebral artery (VA), carotid artery (CA) and intravenous (IV) infusions of bradykinin (20 μg/min for 5 minutes) in (A) the intact dog (control responses), (B) following α-adrencoeptor blockade with phentolamine (5 mg, then 500 μg/min continuously by intravenous infusion), (C) following phentolamine and β-adrencoceptor blockade with propranolol (500 μg/kg intravenously) and (D) following phentolamine, propranolol and bilateral vagotomy.

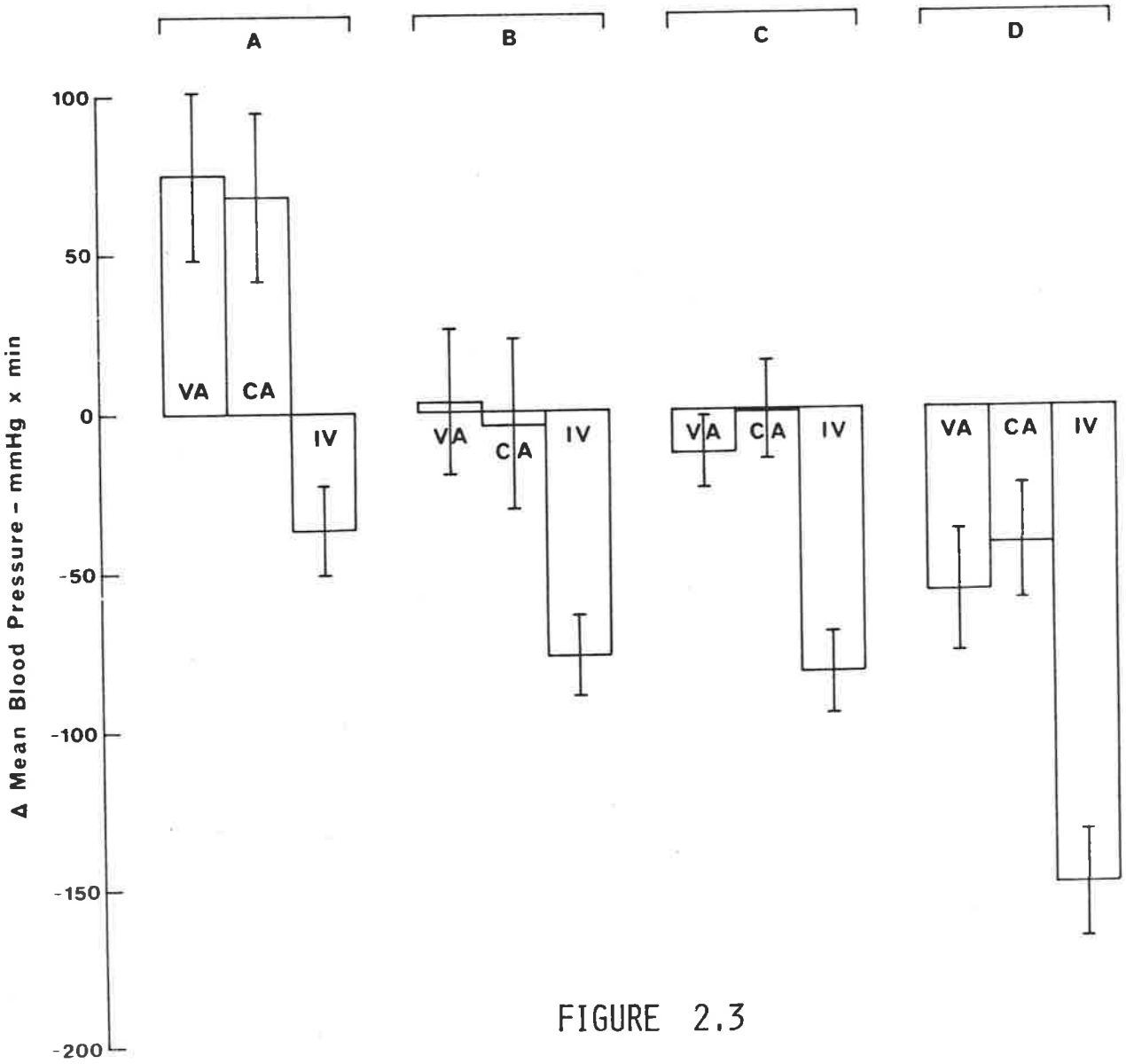
A
B
C
D
100
50
0
-50
-100
-150
-200
Δ Mean Blood Pressure - mmHg x min
VA
CA
IV

FIGURE 2.3

than the responses to carotid artery infusions, the difference was not statistically significant. Intravenous infusion of bradykinin at the same rate produced a decrease in blood pressure (Table 2.6).

The addition of phentolamine did not cause statistically significant alterations in either resting blood pressure (before, 92.7 ± 5.0; after, 87.7 ± 5.2 mm Hg) or heart rate (before, 62.3 ± 7.7; after, 129.5 ± 32.4 beats/min), but following α-receptor blockade with phentolamine, the pressor responses obtained during vertebral artery and carotid artery infusions of bradykinin were significantly reduced compared to control responses and were not significantly different from zero. The depressor response during intravenous infusion of bradykinin was significantly greater following α-receptor blockade.

Additional blockade of β-receptors with propranolol, in the presence of α-receptor blockade with phentolamine, did not cause any further significant change in the blood pressure responses (compared to the responses following α-receptor blockade alone) with any of the routes of administration of bradykinin. These responses should be identical with those obtained in Group 1 experiments following the same pre-treatments. However, in Group 1 experiments a small pressor response was obtained during vertebral artery bradykinin, while in Group 2 experiments a small depressor response was obtained and these responses were statistically significantly different (unpaired t-test, $p<0.05$). The responses during carotid artery and intravenous infusions of bradykinin were not significantly different in Group 1 and Group 2 experiments following the combined treatment of propranolol and phentolamine.

Bilateral vagotomy, in the presence of α and β-receptor blockade, resulted in depressor responses to infusions of bradykinin via all

three routes of administration. Addition of bilateral vagotomy to the protocol resulted in a significantly larger depressor response to intravenous infusions of bradykinin in comparison to the responses obtained with α and β-receptor blockade. The depressor response to intravenous infusion of bradykinin was significantly larger than the depressor responses obtained with either vertebral or carotid artery infusions of bradykinin, which were not significantly different. The responses to bradykinin infusions via all three routes of administration following the combined treatments of propranolol and phentolamine were not significantly different from the responses obtained in Group 1 experiments following the same combined treatments.

2. Heart rate:

Bradykinin infusions via all three routes of administration caused a pronounced tachycardia in all cases and there was no significant difference between the responses with any route of administration, although, on average, the responses obtained during vertebral artery infusion of bradykinin were larger than those obtained with either carotid artery or intravenous infusions of bradykinin (Tables 2.4, 2.5, 2.6 and Fig. 2.4).

Following α-receptor blockade with phentolamine, the tachycardia obtained during bradykinin infusion via each route of administration was reduced compared with control responses, although this reduction was only statistically significant with vertebral artery and intravenous administration of bradykinin.

Subsequent administration of propranolol, in the presence of phentolamine, caused no further significant effect on the heart rate responses to infusion of bradykinin via all three routes of administration.

FIGURE 2.4 - The responses of heart rate during vertebral artery (VA), carotid artery (CA) and intravenous (IV) infusions of bradykinin (20 μg/min for 5 minutes) in (A) the intact dog (control responses), (B) following α-adrenocceptor blockade with phentolamine (5 mg, then 500 μg/min continuously by intravenous infusion), (C) following phentolamine and β-adrenoceptor blockade with propranolol (500 μg/kg intravenously) and (D) following phentolamine, propranolol and bilateral vagotomy.

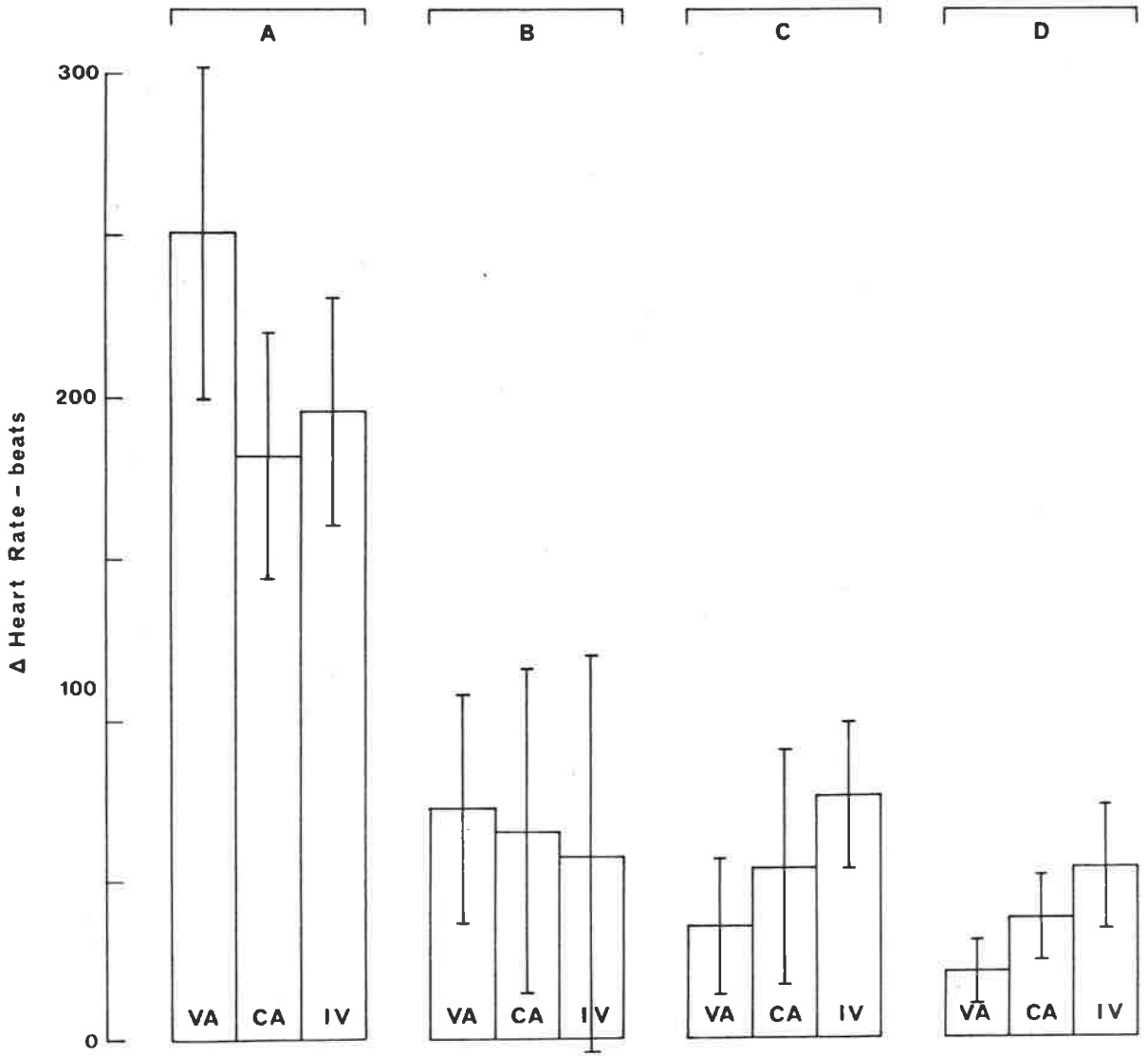
A
B
C
D
300
200
100
0
Δ Heart Rate - beats
VA
CA
IV
VA
CA
IV
VA
CA
IV
VA
CA
IV

FIGURE 2.4

Bilateral vagotomy, in the presence of propranolol and phentolamine, caused no further significant effect on the heart rate responses compared with propranolol and phentolamine treatment alone, although the responses were, on average, slightly reduced. Following the combined treatment of vagotomy, propranolol and phentolamine, the heart rate responses were all significantly higher than zero and possible reasons for these results will be discussed.

3. Cardiac output:

Infusion of bradykinin (20 μg/min for 5 minutes) via either a vertebral artery, carotid artery or intravenously caused a significant increase in cardiac output in all cases and there was no significant difference between the changes in cardiac output with each route of administration (Tables 2.7, 2.8 and 2.9 and Fig. 2.5).

Following α-receptor blockade with phentolamine, the increase in cardiac output during infusion of bradykinin was reduced with each route of administration, although the difference was only statistically significant with the vertebral route of administration.

Administration of propranolol, in the presence of phentolamine, did not result in any further significant changes in the response of cardiac output to bradykinin infusions with any route of administration (compared with the responses obtained following phentolamine alone), although the response to carotid artery infusion of bradykinin was now significantly different from the control response (Table 2.8).

Following the combined treatments of phentolamine, propranolol and bilateral vagotomy, the increase of cardiac output was, on average, lower than control values, but the difference was only statistically

FIGURE 2.5 - The changes in mean blood pressure (mm Hg), cardiac output (ΔC.O. - litres/min) and total peripheral vascular resistance (ΔT.P.R. - mm Hg x min x l^{-1}) during vertebral artery (VA), carotid artery (CA) and intravenous (IV) infusions of bradykinin (20 μg/min for 5 minutes) in (A) the intact dog (control responses) and then (B) following α-adrenoceptor blockade with phentolamine (5 mg, then 500 μg/min continuously by intravenous infusion), (C) following phentolamine and β-adrenoceptor blockade with propranolol (500 μg/kg intravenously) and (D) following phentolamine, propranolol and bilateral vagotomy.

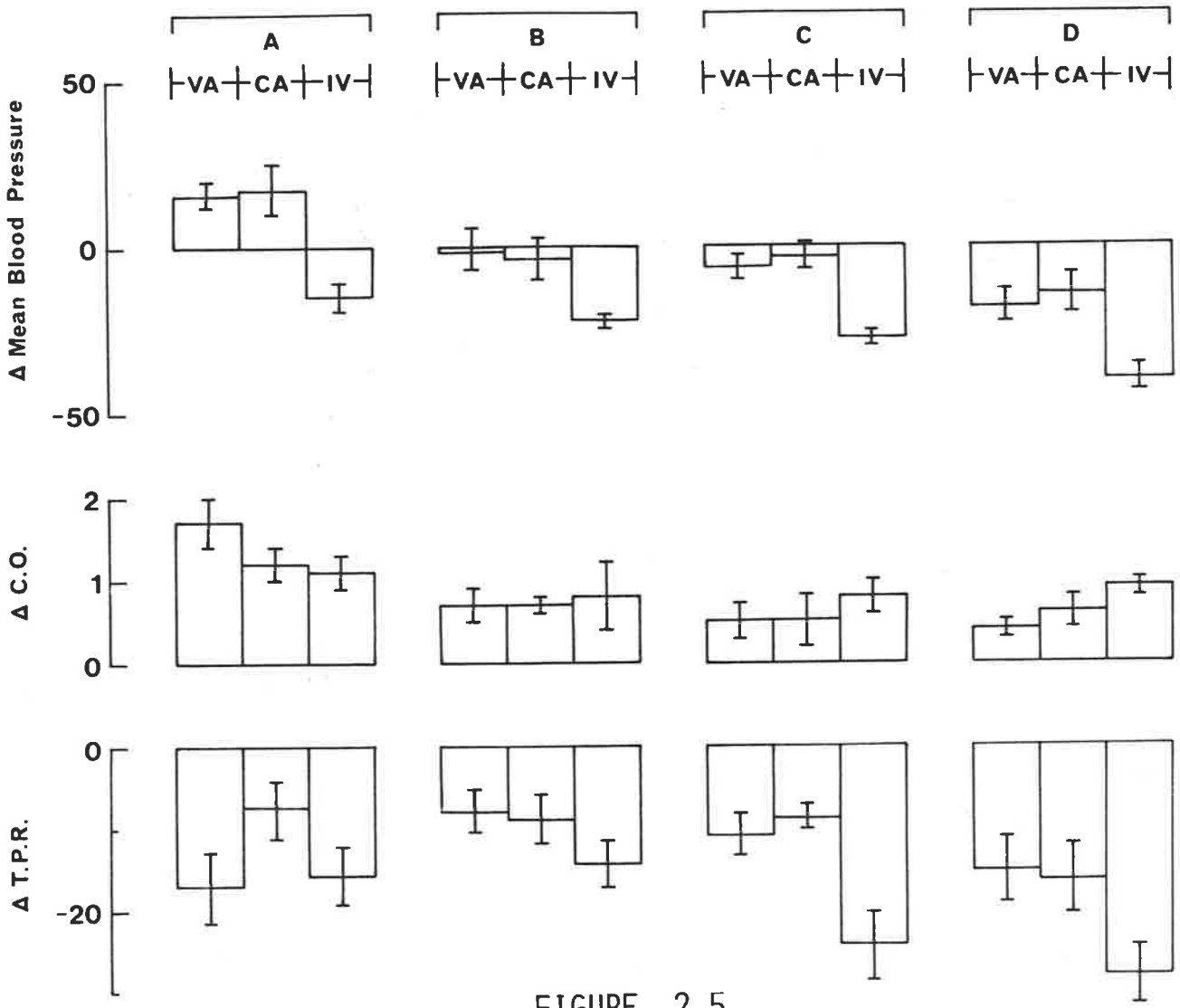

FIGURE 2.5

significant with the vertebral artery route of administration.

4. Total peripheral vascular resistance:

Infusion of bradykinin (20 μg/min for 5 minutes) into either a vertebral artery, carotid artery and intravenously caused a decrease in total peripheral vascular resistance in all cases and there was no significant difference between the changes in total peripheral resistance with each route of administration (Tables 2.7, 2.8 and 2.9 and Fig. 2.5).

Following administration of phentolamine, the decrease of total peripheral vascular resistance during bradykinin infusions via all three routes of administration was not significantly different from control values (Tables 2.7, 2.8 and 2.9 and Fig. 2.5).

Propranolol, in addition to phentolamine, resulted in a significantly larger decrease in total peripheral resistance during intravenous infusions of bradykinin while the responses during vertebral and carotid artery infusions of bradykinin were still not significantly altered from control responses.

The addition of bilateral vagotomy to the above protocol did not result in any further significant effects on total peripheral resistance, the only significant alteration from control values occurring on intravenous infusion of bradykinin.

(C) GROUP 3

1. Blood pressure:

Infusion of bradykinin (20 µg/min for 5 minutes) into either a vertebral artery or a carotid artery caused an increase in blood pressure (Tables 2.13 and 2.14 and Fig. 2.6) and the response to vertebral artery infusion of bradykinin was significantly larger than the response to carotid artery infusion. Intravenous infusion of bradykinin caused a decrease in blood pressure in all cases (Table 2.15 and Fig. 2.6). None of these responses were significantly different from those obtained in Group 1 or Group 2 experiments.

Following bilateral vagotomy, depressor responses to bradykinin infusions were obtained with each route of administration and, furthermore, the depressor response to intravenous infusion was significantly greater (4-5 times) than the control response.

When propranolol was administered, in the presence of bilateral vagotomy, the responses to bradykinin infusions via either a carotid artery or intravenously were not further changed from the responses obtained with vagotomy alone, but the depressor response to vertebral artery infusions of bradykinin was significantly smaller.

The combined treatment of bilateral vagotomy, propranolol and phentolamine produced responses that were not significantly different from those obtained following bilateral vagotomy alone. The responses to vertebral artery and carotid artery infusions of bradykinin were not significantly different from each other, but the depressor response to intravenous infusion of bradykinin was significantly larger than that obtained during either vertebral artery or carotid artery infusion of

FIGURE 2.6 - The responses of blood pressure during vertebral artery (VA), carotid artery (CA) and intravenous (IV) infusions of bradykinin (20 μg/min for 5 minutes) in (A) the intact dog (control responses) and then (B) following bilateral vagotomy, (C) following bilateral vagotomy and β-adrenoceptor blockade with propranolol (500 μg/kg intravenously) and (D) following bilateral vagotomy, propranolol and α-adrenoceptor blockade with phentolamine (5 mg, then 500 μg/min continuously by intravenous infusion).

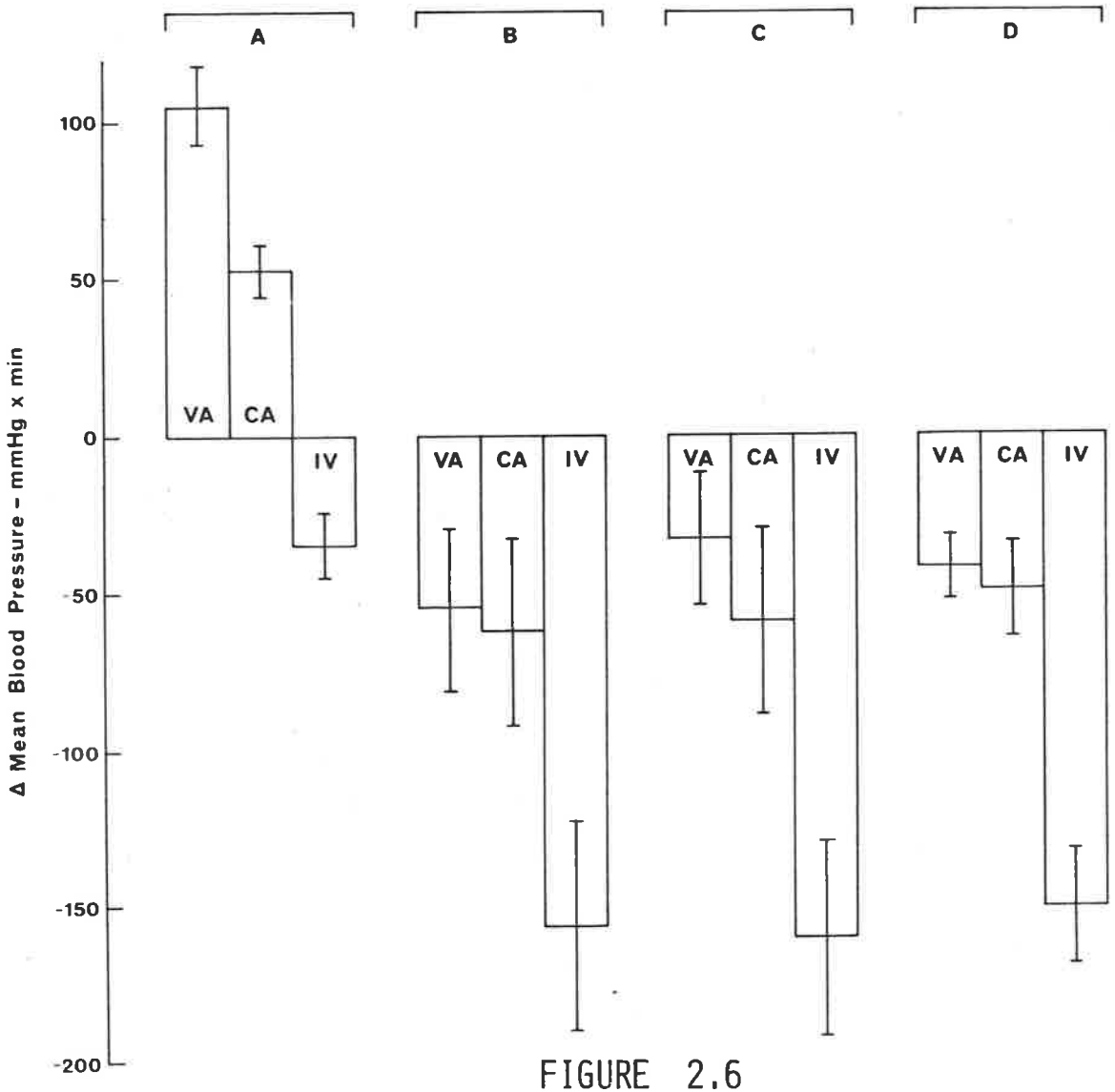
A
B
C
D
100
50
0
-50
-100
-150
-200
Δ Mean Blood Pressure - mmHg x min
VA
CA
IV

FIGURE 2.6

bradykinin. Again, the responses following these final treatments were not significantly different from those obtained in either Group 1 or Group 2 experiments following the same combined treatments.

2. Heart rate:

Bradykinin infusions (20 μg/min for 5 minutes) via a vertebral artery, carotid artery and intravenously caused a marked tachycardia in all cases. The responses to vertebral artery and intravenous infusions were not significantly different, but both were significantly larger than the response to carotid artery infusion of bradykinin (Tables 2,13, 2.14 and 2.15 and Fig. 2.7).

Bilateral vagotomy did not cause any significant alteration in the responses (compared to control responses) to bradykinin infusions via any of the routes of administration and there was now no significant difference between the responses obtained with all routes of administration.

Administration of propranolol, following bilateral vagotomy, completely abolished the tachycardia previously obtained with all routes of administration.

Surprisingly, following the combined treatment of bilateral vagotomy, propranolol and phentolamine, a tachycardia was again obtained during bradykinin infusions via all three routes of administration. Although the responses were considerably (and significantly) reduced from control values, they were all significantly larger than the responses obtained following vagotomy and propranolol (Fig. 2.7).

FIGURE 2.7 - The responses of heart rate during vertebral artery (VA), carotid artery (CA) and intravenous (IV) infusions of bradykinin (20 μg/min for 5 minutes) in (A) the intact dog (control responses) and then (B) following bilateral vagotomy, (C) following bilateral vagotomy and β-adrenoceptor blockade with propranolol (500 μg/kg intravenously) and (D) following bilateral vagotomy, propranolol and α-adrenoceptor blockade with phentolamine (5 mg, then 500 μg/min continuously by intravenous infusion).

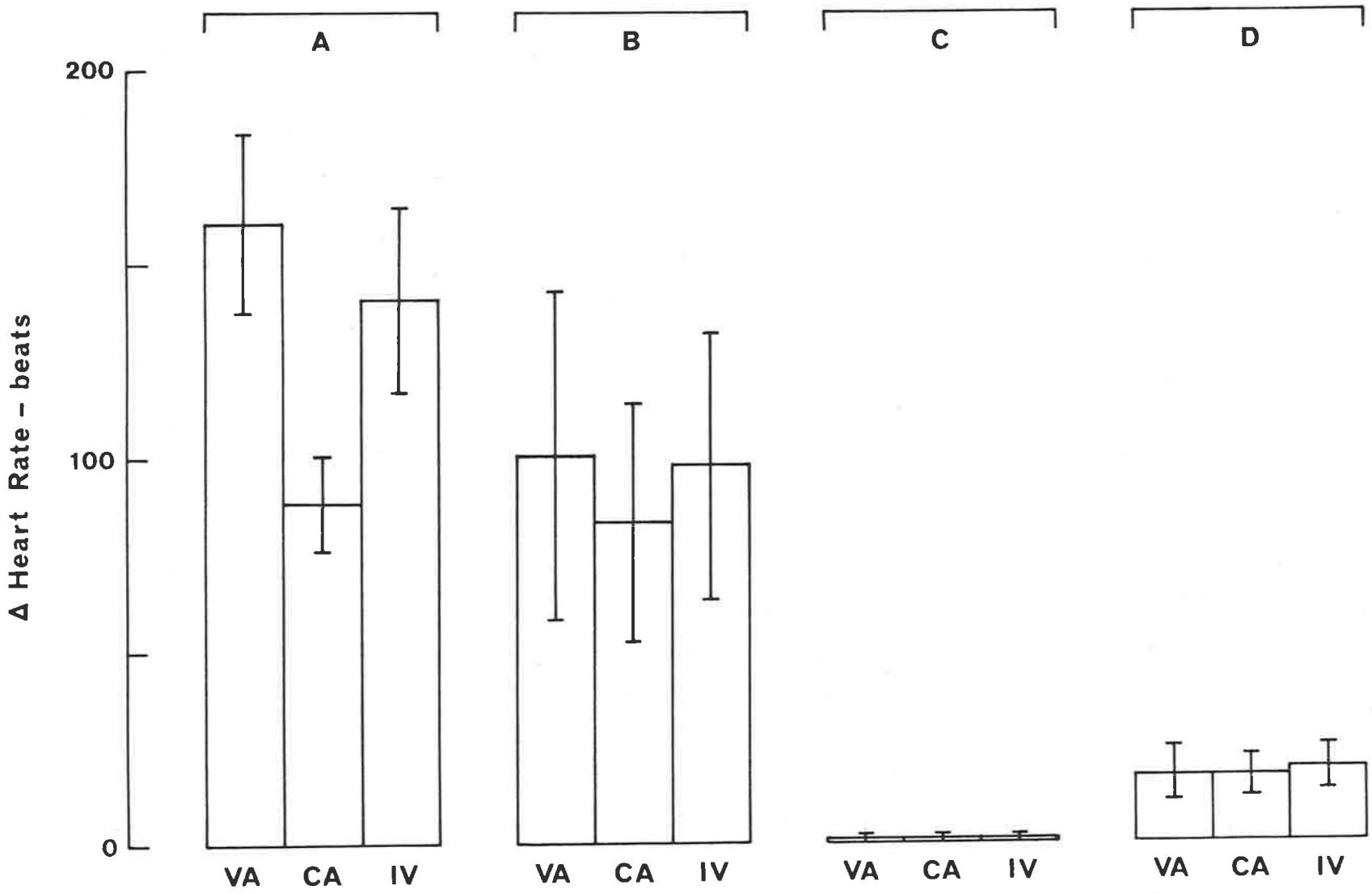
A
B
C
D
200
100
0
Δ Heart Rate – beats
VA
CA
IV
VA
CA
IV
VA
CA
IV
VA
CA
IV

FIGURE 2.7

DISCUSSION

1. Vertebral artery responses:

Infusion of bradykinin via a vertebral artery caused a tachycardia and hypertension in all dogs in each group of experiments. In addition, the results from Group 2 experiments showed an increase in cardiac output and a decrease in calculated total peripheral vascular resistance during vertebral artery infusion of bradykinin. Since total peripheral vascular resistance decreased in all cases, the hypertensive response is presumably a direct result of the increase in cardiac output (Table 2.7).

In Group 1 experiments, propranolol reduced the magnitude of the tachycardia to 31% of the control value (Table 2.1 and Fig. 2.2) and this was associated with a similar decrease to 46% of the control value in the pressor response (Table 2.1 and Fig. 2.1), suggesting stimulation of cardiac sympathetic nerves to increase heart rate and cardiac output has a significant contribution to the pressor response to vertebral artery infusions of bradykinin. The increase in cardiac output is due to the tachycardia, since calculated stroke volume is decreased (compared to control values) during vertebral artery infusion of bradykinin (Table 2.10). Complete abolition of both the tachycardia and the pressor response was achieved by simultaneous blockade of cardiac sympathetic nervous effects (with propranolol) and inhibition of cardiac parasympathetic effects by vagal cooling, further suggesting that the pressor response to vertebral artery bradykinin is mediated by an increase in heart rate and cardiac output. Furthermore, from these results there appears to be a dual nervous mechanism responsible for the tachycardia, involving both stimulation of the cardiac sympathetic nerves and inhibition of vagal tone to the heart.

Blockade of α-adrenoceptors with phentolamine, in addition to β-adrenoceptor blockade with propranolol, caused a further small, but significant (compared to β-receptor blockade alone) decrease in the magnitude of the pressor response to vertebral artery bradykinin, suggesting stimulation of α-adrenoceptors contributes, in part, to the increase in blood pressure. Further evidence that α-receptor stimulation contributes to the increase in blood pressure is provided in Group 2 experiments (Table 2.4) where phentolamine alone significantly reduced the pressor effect of vertebral artery bradykinin. In these experiments, phentolamine also reduced the responses of heart rate and cardiac output, indicating a possible effect of phentolamine to reduce sympathetic stimulation to the heart. Yamaguchi and Kopin (1980) also observed that the pressor response to sympathetic nerve stimulation was reduced by phentolamine due to non-selective blockade of both α_1 and α_2 adrenoceptors.

A peripheral effect of phentolamine on vascular α_1-receptors to prevent an increase in total peripheral vascular resistance, and therefore arterial blood pressure, as suggested by Pearson *et al.* (1969) and Lambert and Lang (1970), is unlikely since total peripheral vascular resistance was always decreased during vertebral artery infusions of bradykinin (Table 2.7) and therefore could not contribute to any pressor effect.

When stimulated, pre-synaptic α_2-receptors cause a decrease in the amount of noradrenaline released from pre-synaptic noradrenergic nerve terminals by a negative feedback mechanism (Farnebo and Hamberger, 1971; Kirpekar and Puig, 1971; Langer, Adler, Enero and Stefano, 1971; Starke, 1971). Blockade of α_2-receptors by phentolamine would, therefore, be expected to increase post-synaptic sympathetic nerve activity by increased stimulation of α_1-receptors. However, phentolamine

is also an α_1-receptor antagonist, so any effect of phentolamine on ganglionic α-receptors is unlikely to explain the observed effect of an increase in heart rate. Furthermore, the amount of bradykinin circulating in the systemic circulation during vertebral artery infusion would be minimal and not sufficient to cause sufficient ganglionic stimulation to produce such large increases in arterial blood pressure and heart rate.

Bradykinin also depletes brain structures of noradrenaline (Capek, Masek, Sramka, Krsiak and Svek, 1969), but again this effect would remove the central α-receptor inhibition of the cardiovascular system and should therefore result in potentiation of the central effects of bradykinin and not, as observed, a decrease in the response.

Correa and Graeff (1974) proposed a central α-receptor stimulant action of bradykinin to cause an increase of both blood pressure and heart rate to intraventricular bradykinin. However, they reported that intraventricular noradrenaline also caused an increase of blood pressure and heart rate, which is at variance with other workers (Takahashi and Bunag, 1981; De Jong, Zandberg and Bohus, 1975; Bhargava, Mishra and Tangri, 1972) who suggest that the central action of noradrenaline on α-receptors, located in either the medulla oblongata (Hausler, 1974), the nucleus tractus solitarius or the locus coeruleus (Young and Kuhar, 1980), is to cause depression of the cardiovascular system. This latter effect is now generally accepted. Consequently, to cause the observed effects of an increase in blood pressure and heart rate (i.e. a cardiovascular stimulant effect), bradykinin would need to exert an inhibitory effect on central α-receptors which would result in sympathetic stimulation, and blockade of this central effect (with phentolamine) would reduce any changes in blood pressure and heart rate. The mechanism of any inhibitory effect of bradykinin on

central nervous system α-receptors is unknown and, at present, only speculative. As mentioned by Correa and Graeff (1974), species differences and the choice of anaesthetic agent may also cause differences in the results obtained by different workers.

In Group 2 experiments, the addition of propranolol, following previous administration of phentolamine (Table 2.4, Figs. 2.3 and 2.4), did not further significantly alter either the heart rate or blood pressure responses to vertebral artery bradykinin, suggesting stimulation of cardiac β-receptors does not contribute to the pressor response obtained after α-receptor blockade. The remaining tachycardia, which would contribute to the increase in cardiac output following this combined treatment, is presumably mediated by decreased vagal tone to the heart (assuming "total" β-receptor blockade with propranolol). The results from Group 1 experiments (following propranolol and phentolamine) also provide further evidence for a significant effect of withdrawal of vagal tone to the heart to cause the observed tachycardia since the heart rate response was only marginally attenuated from control values following this combined treatment. In fact, the tachycardia obtained during vertebral artery infusion of bradykinin following propranolol and phentolamine was, on average, larger than that obtained following propranolol alone but the difference was not significantly different. This effect may possibly reflect a central action of phentolamine to remove the central α-receptor inhibition on the cardiovascular system, although this effect does not appear to be of significance in the response to bradykinin, as discussed above.

Using the isolated-head preparation in rats, Takahashi and Bunag (1981) observed that phentolamine pre-treatment (via a carotid artery)

eliminated the initial depressor response to carotid artery injections of bradykinin and also potentiated the secondary pressor phase of the response. While they propose that the secondary pressor effect is mediated primarily by prostaglandin synthesis in the brain, they suggest that the larger effect following phentolamine pre-treatment can be explained by removal of the centrally-mediated cardiovascular inhibition caused by bradykinin-stimulated noradrenaline release in the brain. A similar mechanism could presumably operate during vertebral artery administration of bradykinin. However, in the experiments reported in this thesis, the pressor response to vertebral artery infusions of bradykinin was reduced, and not potentiated, following phentolamine administration. Furthermore, a depressor effect was never observed in any of these experiments. The dose of bradykinin used by Takahashi and Bunag was about 50 μg/kg (assuming an average rat weight of 200 g) which is 70-80 times larger than the dose of bradykinin used in the experiments reported in this thesis (650-700 ng/kg/min, assuming an average dog weight of 30 kg). Furthermore, since the dose of bradykinin was administered by slow infusion in the experiments reported in this thesis (and not given by injection as in the experiments reported by Takahashi and Bunag), the instantaneous blood concentrations of bradykinin would differ considerably. The initial vasodepression resulting from a 10 μg injection of bradykinin (Takahashi and Bunag, 1981) could conceivably arise from a large amount of bradykinin stimulating centrally-mediated withdrawal of sympathetic vasomotor tone. If high local concentrations of bradykinin (which would be achieved following the injection of a large dose) are required to cause this effect, they may not be observed during slower administration of smaller amounts of the peptide over a longer period of time. Hence the method (and route) of administration may explain why only pressor effects were observed in the experiments reported in this thesis.

In summary, it appears as though bradykinin is having a central effect on both sympathetic and vagal structures to cause an increase in cardiac output and arterial blood pressure. While phentolamine may have a central α-receptor blocking effect, it is probable that the major effect is at peripheral α_1 and α_2 receptors to reduce post-synaptic sympathetic nervous activity to the heart (Yamaguchi and Kopin, 1980). This effect of phentolamine would explain the reduction in the magnitude of the pressor response and the tachycardia to vertebral artery bradykinin. Since blockade of peripheral vascular α-receptors with phentolamine would cause a reduction in resting sympathetic tone to blood vessels, the resulting peripheral vasodilation and vascular pooling of blood could also contribute to the smaller increase of cardiac output during vertebral artery infusions of bradykinin.

From the results discussed above, there is evidence that activation of both α and β-adrenoceptors contributes to the pressor response to vertebral artery bradykinin. Whether activation of these receptors is a result of catecholamine release (mainly adrenaline) from adrenal medullary cells cannot be determined from these experiments. Since bradykinin is a potent releaser of adrenal meduallary hormones in cats (Feldberg and Lewis, 1963, 1964, 1965) and also stimulates sympathetic nerves (Severs and Daniels-Severs, 1973; Ganten, Unger, Rockhold, Schaz and Speck, 1979; Takahashi and Bunag, 1981), both of these effects may be involved, to varying degrees, in the activation of systemic α and β-adrenoceptors. However, since intravenous infusion of bradykinin always resulted in hypotension, it appears unlikely that adrenaline release from the adrenal medulla contributes significantly to any hypertensive effect of bradykinin, and that the pressor effect is mediated via a central action of bradykinin to stimulate the sympathetic nervous system and inhibit vagal nuclei.

Following either bilateral vagal cooling or vagotomy, (Group 1 and 2 experiments, respectively), in the presence of α and β-receptor blockade vertebral artery infusions of bradykinin caused hypotension and a small (statistically insignificant) tachycardia. This remaining tachycardia, following α and β-receptor blockade and interruption of vagal transmission, is probably a result of incomplete β-adrenergic receptor blockade. While the doses of propranolol and phentolamine were sufficient to completely abolish the test responses to isoprenaline and noradrenaline respectively, this does not necessarily indicate 100% pharmacological blockade of α and β-receptors, even though blockade appeared to be considerable. Any "unblocked" adrenergic receptors could still contribute to the small tachycardia observed. Surgical transection of both vagus nerves and also the spinal cord at the level of C2 would totally abolish all sympathetic and parasympathetic activity and would verify this point. Similarly, the small (but statistically significant) tachycardia obtained during vertebral artery bradykinin following bilateral vagotomy, propranolol and phentolamine in Group 3 experiments could be the result of incomplete β-receptor blockade.

While the responses from Group 1 and 2 experiments indicate involvement of the autonomic nervous system in the hypertensive response to vertebral artery bradykinin, the relative contribution of α and β-receptor stimulation and inhibition of vagal transmission to the response is not absolutely clear. In Group 1 experiments, propranolol reduced the pressor effect of vertebral artery bradykinin by about 70% and subsequent phentolamine caused a further small, but significant effect. However, in Group 2 experiments, phentolamine alone significantly reduced the mean pressor response to only 4% of the mean control response and subsequent administration of propranolol caused a further small, but non-significant, reduction in the pressor response in 5 out of 6 dogs, such that the response was now slightly depressor. It appears,

therefore, that the contribution of α and β-receptor effects to the response could depend on the nature of any previous treatments. However, the combined treatment of propranolol and phentolamine did not result in large depressor responses of similar magnitude to the control intravenous responses. If centrally-mediated effects on the autonomic nervous system were responsible for the pressor effect, then pharmacological blockade of the peripheral autonomic nervous system receptors should abolish this effect and the circulating bradykinin should then produce systemic vasodilation and a hypotensive response in a similar manner to an intravenous infusion, after allowing for any pulmonary inactivation of the peptide following cranial artery administration before it reaches the systemic circulation. This effect was not observed in either Group 1 or Group 2 experiments. In Group 1 experiments, a small pressor response persisted following α and β-receptor blockade, while in Group 2 experiments, a small depressor response was obtained following the same treatments. These different results in the two groups of experiments presumably indicate that pharmacological blockade of α and β-receptors is incomplete and the degree of blockade is slightly different in the two groups.

Vagal cooling or vagotomy, whether alone or following propranolol or phentolamine, resulted in depressor responses to vertebral artery infusions of bradykinin of similar magnitude to control intravenous responses. This effect would seem to indicate an important role for the vagus nerves in the expression of a pressor response to vertebral artery infusions of bradykinin. The results from Group 1 and 2 experiments, as discussed above, suggest that withdrawal of vagal tone to the heart and the consequent increase in cardiac output are of significance in producing the observed increase in blood pressure. However, since some sympathetic nerves are also located in a common sheath with the vagus nerves (Miller, Christensen and Evans, 1964),

bilateral vagal cooling or vagotomy would also result in the removal of some sympathetic influences on the heart as well as complete abolition of any parasympathetic effects on the heart. So the procedures used in these experiments to interrupt vagal (parasympathetic) transmission are not "pure", and the contribution of the vagal sympathetic nerves to the total cardiovascular response to bradykinin infusions cannot be determined from these experiments. A comparison of the effects of atropine on the responses would unmask any vago-sympathetic influences, assuming atropine administration can totally inhibit vagal parasympathetic nervous transmission and that atropine will not exert any central effect of its own which would modify the cardiovascular response to bradykinin.

The results from Group 3 experiments provide supportive evidence for the role of the vagus nerves in the blood pressure response to vertebral artery infusion of bradykinin. Following vagotomy, an increase of heart rate and cardiac output mediated by withdrawal of vagal tone is not possible, and since the response of blood pressure is now depressor, a major contributor to the pressor effect of vertebral artery bradykinin appears to be an increase of cardiac output as a result of withdrawal of vagal tone to the heart.

In addition, vagotomy significantly potentiated the depressor response to intravenous infusion of bradykinin (Table 2.15). The depressor response to vertebral artery infusion following vagotomy was only 35% of the intravenous response, representing about 65% destruction of bradykinin as it traverses the pulmonary circulation.

The tachycardia remaining following bilateral vagotomy was completely abolished by subsequent propranolol administration, indicating strong sympathetic stimulation during the infusion of bradykinin to

maintain the heart rate response at approximately control levels. In comparison to Group 1 experiments, where β-receptor stimulation was responsible for about 50% of the control heart rate response, in Group 3 experiments the sympathetic effect following vagotomy would seem to be potentiated since the tachycardia obtained was not significantly reduced from control values. Since the blood pressure decreased during vertebral artery bradykinin (following vagotomy), an additional stimulus to the sympathetic nervous system would arise from stimulation of the carotid sinus baroreceptors, which would contribute to the substantial tachycardia obtained. The aortic arch baroreceptors are non-functional as a result of interruption of the afferent pathway of the reflex by vagotomy.

Vertebral artery infusions of bradykinin always resulted in an increase of heart rate and arterial blood pressure. Since the pressor response was associated with a tachycardia and not a reflex bradycardia, this effect would indicate either (a) that bradykinin has markedly attenuated the normal baroreceptor reflexes or (b) that its central stimulating action is so powerful that it over-rides any compensatory effects of the baroreceptor reflex or (c) that it has a powerful direct cardiac stimulant action. The effect of bradykinin on the isolated heart has been examined and the results are discussed in Chapter 3.

It has been suggested by several workers that another peptide, angiotensin II, may have a central action to modulate the actions of the arterial baroreceptor reflex (Sweet and Brody, 1970; Fukiyama, 1973; Goldstein, Heitz, Shaffer and Brody, 1974; Marker, Miles and Scroop, 1980). Similarly, there is evidence that bradykinin injected into the lateral cerebral ventricles of conscious rats impairs normal baroreceptor function (Unger, Rockhold, Yukimura, Rettig, Rascher and Ganten, 1981). The possible contribution of a baroreceptor-modulating

effect of bradykinin has been investigated and will be discussed in Chapter 3.

As reported by other workers, and discussed above, bradykinin has a powerful central action to cause a tachycardia and an increase in arterial blood pressure. Whether this effect of bradykinin is a direct action of the peptide on central nervous system structures or whether it is mediated via the release of some other endogenous compound cannot be determined from the experiments described in this chapter.

It has been suggested that intracerebroventricular bradykinin (Kondo, Okuno, Konishi, Saruta and Kato, 1979) and also carotid artery injections of bradykinin (Takahashi and Bunag, 1981) both cause pressor effects which are mediated by prostaglandin biosynthesis in the central nervous system. The effect of vertebral artery administration of indomethacin, an inhibitor of endogenous prostaglandin synthesis, on the responses of blood pressure and heart rate to vertebral artery infusions of bradykinin has been investigated and will be discussed in Chapter 3.

In addition, bradykinin administration via either a vertebral artery (Harris and Rocha e Silva Jnr., 1965) or a carotid artery (Rocha e Silva Jnr. and Malnic, 1962; Rocha e Silva Jnr. and Malnic, 1964; Harris and Rocha e Silva Jnr., 1965) has been demonstrated to release anti-diuretic hormone (ADH). Since ADH has a vasoconstrictor effect, in addition to its anti-diuretic action on the kidney, if sufficient quantities were released during vertebral artery infusions of bradykinin, this hormone could possibly contribute to the pressor response obtained. This possibility was investigated but, as discussed later in Chapter 3, it appears unlikely that centrally-mediated release of ADH during vertebral artery infusions of bradykinin contributes to

the hypertensive effect obtained.

In summary, the pressor response obtained during vertebral artery infusion of bradykinin is mediated mainly by an increase in cardiac output as a consequence of the observed tachycardia which, in turn, is mediated primarily by withdrawal of cardiac vagal tone, although stimulation of sympathetic nerves to the heart may have a minor role, indicating that the central effect of bradykinin is not confined to the vagal nuclei. Sympathetically-induced vasoconstriction is not important since total peripheral vascular resistance was always decreased during vertebral artery infusion of bradykinin and did not differ from control values following any of the treatments used. These results indicate that vertebral artery infusions of bradykinin have specific cardiovascular effects which appear to be mediated by the central nervous system.

The possibility of involvement of other effects of bradykinin e.g. releasing prostaglandins or a direct cardiac stimulant action to cause the hypertensive response, will be discussed subsequently.

2. Carotid artery responses:

Infusion of bradykinin via a carotid artery caused an increase of both blood pressure and heart rate in all dogs in each group of experiments. In addition, the results from Group 2 experiments showed an increase in cardiac output and a decrease in calculated total peripheral vascular resistance during control carotid artery infusions of bradykinin. Since total peripheral vascular resistance decreased in all cases, the hypertensive response is presumably a direct result of the increase in cardiac output (Table 2.8) which, in turn, is due entirely to the observed tachycardia since calculated stroke volume

decreased during control carotid artery infusions of bradykinin (Table 2.11).

In Group 1 experiments, the administration of propranolol did not cause a statistically significant change in the magnitude of the pressor response to carotid artery infusion of bradykinin, but the magnitude of the tachycardia was reduced by about 50%. These results suggest that stimulation of cardiac β-receptors does not contribute significantly to the pressor response observed. Furthermore, subsequent interruption of vagus nerve transmission by bilateral vagal cooling, in the presence of propranolol, also had no effect on the hypertensive effect of carotid artery infusions of bradykinin, even though the tachycardia was essentially abolished following this combined treatment. While alterations in total peripheral vascular resistance were not assessed in Group 1 experiments, the results from Group 2 experiments suggest that total peripheral resistance would probably be decreased. Presumably, then, the hypertensive response following propranolol and bilateral vagal cooling must be due to an increase in cardiac output mediated by an increase in stroke volume (since calculated total peripheral vascular resistance decreased and heart rate did not change). While stroke volume was decreased during control infusions of bradykinin, following various autonomic blocking procedures in Group 2 experiments it was shown to increase during bradykinin infusions (Table 2.11), and while it was not measured in Group 1 experiments, it is postulated that stroke volume must have increased to cause the hypertensive response. This increase in stroke volume cannot be a result of cardiac sympathetic nerve stimulation, since β-receptor blockade has been maintained with propranolol, and possibly reflects an increase in venous return to the heart as a result of widespread peripheral vasodilation (as evidenced by the reduction in calculated total peripheral vascular resistance). Even in the absence of any changes in heart rate, withdrawal of the inhibitory effects of the vagus nerves on the heart

could also conceivably result in an increased force of contraction of the myocardium and an increase in stroke volume.

Subsequent administration of phentolamine, in the presence of propranolol, caused a slight reduction in the magnitude of the average pressor response and a slight increase in the magnitude of the tachycardia (compared to the responses following propranolol alone) during carotid artery infusions of bradykinin (Table 2.2), but in both cases the difference was not statistically significant. The pressor response was, however, now significantly reduced from control values (without a significant decrease in the magnitude of the tachycardia), indicating possible involvement of α-receptor stimulation in the hypertensive response to carotid artery bradykinin. Further evidence for an α-receptor effect was obtained in Group 2 experiments, where phentolamine alone significantly reduced the pressor response. The tachycardia following phentolamine was, on average, larger than that following propranolol alone and this effect would indicate considerable withdrawal of vagal tone to the heart is probably responsible for the increase in heart rate and cardiac output following blockade of both α and β-adrenergic receptors. As discussed previously with regard to the responses obtained during vertebral artery infusions of bradykinin, these experiments are unable to distinguish between peripheral or centrally-mediated α-receptor effects. Blockade of peripheral α-receptors with phentolamine would prevent centrally-mediated bradykinin-induced vasoconstriction and might, therefore, prevent pressor responses if they were the result of peripheral vasoconstriction. However, in all cases calculated total peripheral vascular resistance was decreased below control values during carotid artery infusions of bradykinin, suggesting peripheral vasoconstriction, mediated by some central action of bradykinin, is not important in the expression of the presssor responses obtained. Similarly, blockade of central α-receptors would

be expected to remove inhibition of the cardiovascular system and therefore potentiate the stimulant actions of bradykinin. This was, however, not observed, and the previous discussion pertaining to the α-receptor effects during vertebral artery infusions of bradykinin is also applicable to the effects observed during carotid artery infusions.

Further support for involvement of the autonomic nervous system in the hypertensive response to carotid artery bradykinin was obtained following the combined treatments of propranolol, phentolamine and bilateral vagal cooling in Group 1 experiments. Following these autonomic blocking procedures, the response to carotid artery infusion of bradykinin was now depressor. Since the response following propranolol and phentolamine alone was pressor, the vagus nerves appear to have an important role in the expression of a pressor response to carotid artery infusions of bradykinin since the response of blood pressure was depressor only after inhibition of vagus nerve transmission. Similar results were obtained in Group 2 experiments. The difference in magnitude of the response between carotid artery bradykinin and intravenous bradykinin represents about 70% extraction of bradykinin in the pulmonary circulation.

The importance of the vagus nerves in the expression of a pressor response to carotid artery infusion of bradykinin is further exemplified by the results from Group 3 experiments, where bilateral vagotomy alone resulted in depressor responses to carotid artery infusions of bradykinin. Subseqeunt pharmacological blockade of α and β-adrenergic receptors produced no further alteration in the blood pressure response, although the addition of propranolol resulted in complete abolition of the heart rate response. However, since the depressor responses to carotid artery infusions of bradykinin in Group 3 experiments following bilateral vagotomy were essentially identical to those obtained in both Group 1

and Group 2 experiments following the final combined treatments of propranolol, phentolamine and bilateral vagal cooling (or bilateral vagotomy), these results suggest that the vagus nerves are the preferential pathway in the hypertensive response to carotid artery bradykinin, but the results of both Group 1 and Group 2 experiments also indicate a possible contribution of sympathetic nervous system effects, although the relative importance of α and β-adrenergic effects is not absolutely clear and appears to be dependent, in part, on the nature of any previous treatments. An unexplained effect on the response to carotid artery infusions of bradykinin was observed following propranolol and bilateral vagal cooling in Group 1 experiments compared with the response following bilateral vagotomy and propranolol in Group 3 experiments. In Group 1 experiments, a pressor response persisted (which was not significantly different from the control response) while in Group 3 experiments, a large depressor response was obtained. In both cases, there was no significant heart rate response. This effect is difficult to explain, and even though vagal cooling is probably not as effective as vagal section, and there may still be some β-receptors not blocked by the dose of propranolol administered, the test procedures used indicated satisfactory inhibition of β-receptor and vagal effects, so it is highly improbable that insufficient blockade of autonomic effects is able to explain the different results obtained. In any case, the pressor response in Group 1 experiments was not altered from control values by vagal cooling and proranolol, while the heart rate effects were essentially eliminated, indicating the blocking procedures employed were probably satisfactory.

Carotid artery infusions of bradykinin always resulted in an increase in heart rate and blood pressure. As discussed previously with regard to vertebral artery infusions of bradykinin, since the

pressor response to carotid artery infusions of bradykinin is also associated with a tachycardia and not a reflex bradycardia, this would indicate either (a) that bradykinin has markedly attenuated the normal baroreceptor reflex, or (b) that its central stimulating effect is so powerful that it over-rides any compensatory effects of the baroreceptor reflex, or (c) that is has a powerful direct cardiac stimulant action. The possible contribution of a baroreceptor modulating effect of carotid artery infusions of bradykinin has been investigated and will be discussed in Chapter 3.

A possible effect of carotid artery infusion of bradykinin to release prostaglandins or anti-diuretic hormone has also been investigated and the results will be discussed in Chapter 3.

In summary, from the results discussed in this chapter, it appears that the pressor response to carotid artery infusions of bradykinin is mediated primarily by an increase in cardiac output which is dependent mainly on an increase in heart rate due to withdrawal of vagal tone to the heart. (This mechanism is similar to the mechanism of the pressor response to vertebral artery infusions of bradykinin). However, stimulation of α and β-adrenergic receptors may have a minor role in the expression of a pressor response to carotid artery infusion of bradykinin as evident from the results in Group 1 and Group 2 experiments, but it is only following the interruption of vagal transmission that the centrally-mediated pressor effects are removed and the depressor response remaining resembles that to intravenously administered bradykinin.

3. Intravenous responses:

Control intravenous infusions of bradykinin produced a decrease in arterial blood pressure and an associated tachycardia in all dogs in each group of experiments. The results from Group 2 experiments also showed an increase in cardiac output and a decrease in calculated total peripheral vascular resistance during intravenous infusions of bradykinin (Table 2.9 and Fig. 2.5), which is a result of the direct action of bradykinin to dilate peripheral arterioles (Nakano, 1965; Haddy, Emerson, Scott and Daugherty, 1970). Since arterial blood pressure decreased and cardiac output increased, the observed hypotensive effect is presumably a direct result of the decreased total peripheral vascular resistance. Furthermore, the increase in cardiac output is a direct result of the tachycardia since calculated stroke volume was decreased from control values during intravenous infusions of bradykinin (Table 2.12). These effects are generally in accordance with results published by other workers (Konzett and Sturmer, 1960; Capri and Corrado, 1961; Page and Olmsted, 1961; Maxwell, Elliott and Kneebone, 1962; Olmsted and Page, 1962; Buckley, Bickerton, Halliday and Kato, 1963; Feldberg and Lewis, 1964; Nakano, 1965; Harrison, Henry, Paaso and Miller, 1968).

The magnitude of the depressor response to intravenous infusions of bradykinin was unchanged following propranolol, propranolol and vagal cooling and a combination of propranolol and phentolamine pre-treatments. It was not until all sympathetic and parasympathetic effects were eliminated in Group 1 experiments with the final combined treatments of propranolol, phentolamine and bilateral vagal cooling that a significant potentiation in the magnitude of the depressor response was obtained, but the tachycardia was altered following each of the pre-treatments used. Since the depressor effects were not

altered while at least one component of the autonomic nervous system remained functional, these results suggest that the baroreceptor reflex is probably acting to prevent large deceases in arterial blood pressure during intravenous infusions of bradykinin and when all autonomic nervous system effects are blocked, the reflex effects are removed and the depressor response is potentiated. To investigate this possibility, the carotid sinus baroreceptors were denervated and the response to infusion of bradykinin was examined. These results will be discussed subsequently in Chapter 3.

Furthermore, in all groups of experiments, pre-treatment with propranolol, either alone or in combination with the other autonomic blocking procedures, produced no significant alteration in the magnitude of the depressor response obtained (prior to propranolol administration), indicating β-adrenergic stimulation does not contribute significantly to the depressor effect, although it contributes, in part, to the associated tachycardia.

In contrast to the results in Group 1 experiments, α-adrenergic receptor blockade with phentolamine produced a significantly larger depressor response to intravenous infusions of bradykinin in Group 2 experiments (Table 2.6 and Fig. 2.3). Since this was associated with a decrease in the magnitude of the tachycardia and no significant alteration in the response of either cardiac output or total peripheral vascular resistance (Table 2.9 and Fig. 2.5), these results again suggest that the baroreceptor reflex is normally active to prevent precipitous decreases in arterial blood pressure and that an increase in heart rate is a major mechanism whereby the fall in blood pressure is limited. However, this mechanism is not supported by the results from Group 1 experiments, where the combined treatments of propranolol and bilateral vagal cooling totally abolished the heart rate response

yet there was no alteration in the magnitude of the depressor response.

The mechanism of the decrease in the magnitude of the tachycardia during intravenous bradykinin following phentolamine is not clear, but it appears as though phentolamine is somehow blocking the reflex increase in heart rate, and since an increase in heart rate is primarily responsible (as determined in Group 2 experiments) for limiting the fall in blood pressure, a larger depressor response results. Whether the effect of phentolamine is a central effect or a peripheral effect to prevent stimulation of cardiac sympathetic nerves cannot be determined from these experiments. Following the administration of propranolol and phentolamine in Group 2 experiments, an intravenous infusion of bradykinin resulted in a significantly larger decrease in total peripheral resistance (Table 2.9) compared with both the control value and the change following phentolamine administration alone, yet neither the change in cardiac output nor arterial blood pressure were significantly altered by the addition of propranolol. Since the decrease in total peripheral resistance is much larger following propranolol and the increase in cardiac output is the same, the hypotensive response to intravenous bradykinin might have been expected to be significantly potentiated. However, while the response was potentiated by 26% (Fig. 2.5), the difference was not statistically significant.

While the depressor responses obtained during intravenous infusions of bradykinin in Group 1 and Group 2 experiments indicate involvement of the autonomic nervous system in the response, the relative contribution of cardiac and vascular sympathetic effects and cardiac vagal effects is not clear and probably depends, in part, on the nature of any previous treatments. However, in Group 3 experiments, bilateral vagotomy alone resulted in a significant potentiation of the depressor effect, and this response was not further altered by

subsquent administration of phentolamine or propranolol, indicating a primary role of the vagus nerves in preventing a large decrease in blood pressure. But complete abolition of the heart rate response (following vagotomy and propranolol) did not further alter the depressor response, suggesting the tachycardia, associated with the decrease in blood pressure, does not limit the fall in blood pressure, and that the magnitude of the depressor response could be determined by some other effect of the vagus nerves.

Bradykinin has also been demonstrated to stimulate sensory nerve endings in the heart (Neto, Brasil and Antonio, 1974; Needleman, 1976; Staszewska-Barczak, Ferreira and Vane, 1976; Staszewska-Barczak and Dusting, 1977), and some of the sensory nerves involved are located in the vagus, while others travel from the heart and great vessels along sympathetic pathways to the spinal cord (Brown, 1979). However, it is unlikely that the effects obtained during intravenous bradykinin are the result of stimulation of vagal chemosensitive afferents, since the coronary chemoreflex (mediated via vagal afferents) results in bradycardia and hypotension (Neto, Brasil and Antonio, 1974) and these effects were never observed during intravenous administration of bradykinin. Similarly, stimulation of chemosensitive sympathetic afferent fibres probably does not have an important contribution to the responses obtained, since stimulation of sympathetic afferents reflexly causes an increase in sympathetic efferent outflow, producing cardiac stimulation (Schwartz, Foreman, Stone and Brown, 1976; Staszewska-Barczak and Busting, 1977). This reflex could conceivably contribute to the observed tachycardia, but the direct vasodilator effect of bradykinin on vascular smooth muscle predominates over any reflex increase in sympathetic vasoconstrictor tone and causes a decrease in total peripheral vascular resistance and hypotension. However, intracoronary injection of bradykinin (Neto, Brasil and

Antonio, 1974) does not cause excitatory effects when the vagus nerves are intact. Hence vagal influences on the heart predominate over sympathetic excitatory effects when both vagal and sympathetic afferents are stimulated by bradykinin. Furthermore, the method of administration or application of bradykinin also determines the nature of the response obtained. Epicardial application of bradykinin (Uchida and Murao, 1974; Staszewska-Barczak, Ferreira and Vane, 1976) causes a tachycardia and an increase in arterial blood pressure (which is potentiated following vagotomy), whereas an intracoronary injection of bradykinin causes a decrease in blood pressure. The amount of bradykinin entering the coronary circulation during an intravenous infusion of bradykinin would be small (following passage through the pulmonary circulation) and would, therefore, have a minor effect, if any, on the responses reported in this Chapter.

In summary, the hypotensive effect of intravenous bradykinin is due to its direct action on vascular smooth muscle to cause widespread vasodilation and the associated tachycardia and increased cardiac output are baroreceptor reflex effects to minimise the decrease in blood pressure. The results from Group 3 experiments indicate that the vagus nerves are of major importance in determining the normal response to bradykinin, but the results from Group 1 and 2 experiments suggest stimulation of α and β-adrenergic receptors has a minor role in the responses obtained.

SUMMARY

1. The responses of blood pressure, heart rate, cardiac output and total peripheral vascular resistance to 5 minute infusions of bradykinin (20 μg/min) via a vertebral artery, carotid artery and intravenously were examined in the morphine-chloralose anaesthetised greyhound.

2. Bradykinin infused via a vertebral artery and a carotid artery caused an increase in arterial blood pressure, heart rate and cardiac output and a decrease in calculated total peripheral vascular resistance. Intravenous infusions of bradykinin resulted in a decrease in arterial blood pressure and total peripheral vascular resistance and an associated tachycardia and increase in cardiac output.

3. The pressor response obtained during vertebral artery infusion of bradykinin is mediated by an increase in cardiac output as a consequence of the associated tachycardia which, in turn, is mediated primarily by withdrawal of cardiac vagal tone. Sympathetically-induced vasoconstriction is not involved. The results indicate that vertebral artery infusions of bradykinin have specific cardiovascular effects which appear to be mediated by the central nervous system.

4. The pressor response to carotid artery infusions of bradykinin is also mediated primarily by an increase in cardiac output which is dependent mainly on an increase in heart rate due to withdrawal of vagal tone to the heart. Sympathetic stimulation of α and β-receptors has only a minor role in the expression of the pressor response.

5. The hypotensive effect of intravenous bradykinin is due to its direct action on vascular smooth muscle to cause widespread

vasodilation, and the associated tachycardia and increased cardiac output are reflex effects to minimise the decrease in blood pressure. The vagus nerves are of major importance in determining the normal response to intravenous bradykinin.

CHAPTER 3

INVOLVEMENT OF OTHER FACTORS IN THE CARDIOVASCULAR RESPONSE TO BRADYKININ

SECTION 1

Direct Actions of Bradykinin on the Isolated Perfused Rabbit Heart

INTRODUCTION

As discussed in Chapter 2 of this thesis, infusion of bradykinin via all three routes of administration investigated, viz. vertebral artery, carotid artery and intravenously, resulted in an increase in both heart rate and cardiac output. With regard to vertebral artery and carotid artery infusions of bradykinin, evidence has been presented suggesting that the pressor response obtained during bradkyinin infusions via these routes of administration is dependent on the increase in cardiac output. Since calculated stroke volume decreased during control bradykinin infusions in these experiments, the observed increase in cardiac output could be the result of several factors. An increase in venous return of blood to the right atrium (Nakano, 1965) and, in the absence of any vasoconstriction in the pulmonary vascular bed (Maxwell, Elliott and Kneebone, 1962), an increase in the volume of blood entering the left ventricle could result in an increase in cardiac output (due to the Frank-Starling relationship for cardiac contraction) even in the absence of any increase in heart rate. This mechanism acting alone, therefore, implies an increase in stroke volume, which was not observed, so the increase in cardiac output must result from stimulation of the heart by some other mechanism. Since bradykinin always resulted in a decrease in total peripheral resistance (see Chapter 2), according to Guyton *et al.* (1973), this effect alone would result in an increase in venous return and cardiac output (assuming right atrial pressure does not increase greatly). Again, if this mechanism was operative, the increase in venous return would cause the observed increase in cardiac output by increasing heart rate since stroke volume was decreased. The increase in heart rate in this case could presumably be a reflex effect in response to stimulation of both right and left atrial stretch receptors by the increased volume of blood returning to the heart, but the relative contribution of this effect cannot be determined from these experiments.

The results in Chapter 2 suggest substantial involvement of the vagus nerves in the heart rate response (which is a centrally-mediated action of bradykinin and not a reflex effect since arterial blood pressure increased concomitantly), although a direct effect of bradykinin on the heart cannot be excluded by these experiments. In the presence of "total" pharmacological and surgical blockade of the nervous system (using the procedures discussed in Chapter 2), the tachycardia was essentially abolished yet the cardiac output was still increased. This result is suggestive of a possible direct positive inotropic effect of bradykinin on cardiac muscle, although an increase in the myocardial contractile force by the Frank-Starling mechanism could also contribute to the increase in cardiac output in the intact dog following these procedures.

During intravenous infusion of bradykinin, the decrease in arterial blood pressure was accompanied by an increase in heart rate which is largely due to activation of the baroreceptor reflex and its consequent effects on the autonomic nervous system. However, a direct effect of bradykinin on the heart could also contribute to the observed cardiac effects.

Accordingly, the experiments presented in this section were performed to examine the effect of bradykinin on the isolated perfused rabbit heart.

MATERIALS AND METHODS

New Zealand white rabbits weighing 2.0-2.5 kg were injected intravenously with 1000 units of heparin 5 minutes before being sacrificed by cervical dislocation. Their hearts were immediately removed and perfused retrograde through an aortic cannula with oxygenated (100% O_2) Locke solution (37°C) of the following composition: sodium chloride 45 g, potassium chloride 2.1 g, glucose 5 g, sodium bicarbonate 2.5 g and calcium chloride 0.6 g, all being dissolved in 5 litres of distilled water. The perfusion rate was measured by collecting the effluent into a graduated measuring cyclinder for 30 second periods, and was adjusted so that the perfusion rate in the normally-beating heart was 20 ml/min. The perfusion pressure, measured 2 cm proximal to the aortic valve using a Statham P23Dc transducer was constant at 45 mm Hg (and determined by the height of the Locke reservoir above the aortic valve), and changes in perfusion rate were measured during the various procedures described. Thus dilation of coronary blood vessels should result in an increase in the perfusion rate and coronary vasoconstriction a decrease in the measured perfusion rate.

Changes in myocardial tension (expressed in grams weight) were measured by attaching a Statham FTO3 isometric force transducer to the apex of the heart and the response was monitored on a Grass Model 7B Polygraph. The diastolic tension was maintained throughout the experiment at 2.0-2.5 g. The electrical signal from the FTO3 transducer was also used to trigger a Grass Model 7P4D tachograph to monitor instantaneous changes in heart rate.

All drugs to be administered were dissolved in physiological saline (sodium chloride 0.9% w/v) and were injected into the perfusion line

5 cm proximal to the aortic valve in a volume of 0.1 ml. Injection of 0.1 ml saline was without effect on any of the monitored variables.

Drugs used were: adrenaline acid tartrate (David Bull Laboratories, Australia), bradykinin triacetate (Protein Research Foundation, Japan).

RESULTS

Injection of adrenaline (1 μg) into the perfusion catheter caused an increase in the mean force of contraction of the myocardium (5.4 ± 0.5 g, mean ± SEM) and also a marked increase (105.0 ± 14.3 beats/min) in the heart rate (Table 3.1). These effects are consistent with the β-receptor stimulant properties of adrenaline, so adrenaline was included in the protocol to demonstrate that the isolated heart preparation was capable of changing its rate and/or force of contraction. Following injection of adrenaline, no alteration in the perfusion rate was observed.

Injection of bradykinin into the perfusion cannula, in doses ranging from 1 ng to 20 μg, did not change the tension developed during contraction, the heart rate or the perfusion rate at any of the doses used (Table 3.1).

DISCUSSION

The results from the experiments on the isolated rabbit heart clearly indicate that bradykinin does not have any direct action on the heart to alter either the force of contraction or the heart rate. An injection of 20 μg of bradykinin into the coronary arteries (via the perfusion cannula), which was also the amount infused (20 μg/min) in the experiments discussed previously in Chapter 2, was without any effect on the heart. Since the amount of bradykinin entering the coronary circulation following a 20 μg/min infusion into a systemic artery or vein *in vivo* would be considerably less than 20 μg (allowing for inactivation during passage through the pulmonary circulation), a direct effect of bradykinin to cause, or at least contribute to, the cardiac effects discussed in Chapter 2 is of no physiological consequence.

These results are at variance with the conclusion of Rosas *et al.* (1965) who suggest that "it seems reasonable to assume that in the intact animal (intravenous) bradykinin also has a direct cardiac action." However, their conclusion is based on observations of changes in cardiac output, stroke volume and heart rate (in the rat) before and after ganglion blockade and not on any measurement of cardiac contractility e.g. peak dP/dt, or even an isolated rat heart preparation. Nakano (1965) also reported an increase in myocardial contractile force during intravenous administration of bradykinin in anaesthetised open-chest dogs, measured by directly attaching a strain-guage to the left ventricle. Harrison *et al.* (1968) reported "little direct myocardial effect was noted", and Maxwell *et al.* (1962) found no significant change in left ventricular work in dogs. These conflicting results could conceivably be due to (a) species differences, (b) anaesthetic influences, or (c) the method of measuring contractile force e.g. open-chest anaesthetised dogs, as used by Nakano, may respond differently

to dogs not subject to pneumothorax.

Therefore, the tachycardia observed during infusion of bradykinin via a vertebral artery or a carotid artery is presumably a direct effect of bradykinin on central structures to cause stimulation of cardiac sympathetic nerves and/or inhibition of vagal centres. As discussed in Chapter 2, withdrawal of cardiac vagal tone is the predominant effect, although sympathetic stimulation may contribute to a lesser extent.

The heart rate effects during intravenous infusion of bradykinin are generally accepted as being reflex responses of the autonomic nervous system arising from the baroreceptor response to decreased arterial blood pressure. Again, the results discussed in Chapter 2 suggest withdrawal of cardiac vagal tone predominates over sympathetic stimulation, but a direct effect of bradykinin to stimulate the heart could not be excluded by the experiments discussed in Chapter 2. However, the results presented here indicate that there would be no contribution of direct effects of bradykinin on the heart and that the tachycardia is purely a reflex effect resulting from the decrease in arterial blood pressure. A small, direct centrally-mediated cardiac stimulant effect may also be present, but this effect is probably minimal since stimulation of central structures by bradykinin causes hypertension (and not hypotension as observed) in association with the tachycardia.

Following injection of either adrenaline or bradykinin into the perfusion cannula, the perfusion rate did not change and remained constant at 20 ml/min. However, the method of measuring the coronary perfusion rate may not have been satisfactory to determine small changes in coronary perfusion even if they occurred. It is well documented that bradykinin does result in a decrease in coronary

vascular resistance and an increase in coronary flow (Maxwell, Elliot and Kneebone, 1962; Nakano, 1965; Needleman, Marshall and Sobel, 1975; Needleman, 1976; Antonio and Rocha e Silva, 1962), and that this effect may be both a direct action of bradykinin on the coronary vasculature or also stimulating the release of other vasodilator substances by bradykinin. There is considerable evidence that bradykinin converts endogenous arachidonate in the heart into the vasodilator substance, prostacyclin (PGI_2) (Needleman, Bronson, Wyche, Sivakoff and Nicolaou, 1978; Schror, Moncada, Ubatuba and Vane, 1978), although it was previously believed to be prostaglandin E_2 (Needleman, 1976; Needleman, Marshall and Sobel, 1975) and that it is the PGI_2 released by bradykinin that mediates the coronary vasodilation. So, although bradykinin is capable of inducing dilation of the coronary vasculature, either directly or through the formation of PGI_2, it did not have any effect on either the myocardial contractile force or on the heart rate, and the cardiovascular effects observed in the experiments reported in Chapter 2 are mediated by the autonomic nervous system or possibly, as will be discussed subsequently, the formation or release of other substances , e.g. anti-diuretic hormone or prostaglandins.

SECTION 2

Effect of Carotid Sinus Denervation on the Cardiovascular Responses to Bradykinin

INTRODUCTION

Benetato *et al.* (1964), using cross-circulation techniques in anaesthetised dogs, suggest that denervation of the carotid sinus baroreceptors changes the cardiovascular response to intracarotid injection of bradykinin from a biphasic response to one that is purely pressor. Riccioppo Neto *et al.* (1974), however, reported that injection of bradykinin into the carotid artery of anaesthetised dogs caused bradycardia and hypotension and that these responses were not altered following section of the sinusal nerve nor by intracranial sectioning of the roots of the vagus nerves. They further suggest that these effects are reflex due to stimulation of paravascular nerve endings and that the nature of the sensory nerve terminals could be similar to the "chemoreceptors for pain" described by Lim *et al.* (1962). Even though the control responses to carotid artery injections of bradykinin were different in nature in the above two reports, and both were different to the responses reported in Chapter 2 of this thesis, it is possible that the carotid sinus baroreceptor reflex (or even a chemoreceptor effect mediated by the carotid bodies) might contribute to, or modify, the response to carotid artery administration of bradykinin.

Furthermore, Marker *et al.* (1980) have demonstrated that another peptide of similar molecular weight, angiotensin II, attenuates the depressor action of carotid-sinus nerve stimulation, although the mechanism or "the role of this action is unproven".

It is conceivable, therefore, that bradykinin may reduce the effectiveness of the carotid sinus baroreceptor reflex in response to the pressor effect obtained during both vertebral artery and carotid artery infusion of bradykinin, and potentiate the centrally-mediated hypertensive and heart rate effects of the peptide.

The influence of the carotid sinus baroreceptors on the responses to vertebral and carotid artery infusions of bradykinin were examined in a short series of experiments in the anaesthetised greyhound and the results are presented and discussed in this section. The tachycardia obtained during intravenous administration of bradykinin is reportedly a baroreflex-mediated response to the decrease in arterial pressure. Similarly, the magnitude of the depressor response is determined by (a) the direct peripheral vasodilator effect of bradykinin, and (b) the degree of baroreceptor compensation to this response. Accordingly, the depressor response to intravenous bradykinin should be potentiated in the absence of the baroreceptor reflex mechanisms. To verify this prediction, the carotid sinus baroreceptors were denervated and the cardiovascular response to intravenous infusion of bradykinin was examined, and the results are also discussed in this section.

MATERIALS AND METHODS

The experiments were conducted in ex-racing greyhounds, weighing between 30 and 33 kg (31.8 ± 0.75 kg, mean ± SEM), anaesthetised with α-chloralose following morphine pre-medication as described in General Methods. A cuffed endotracheal tube was inserted and artificial respiration was maintained throughout the experiment with a C.F. Palmer positive-pressure respirator. Anaesthesia was supplemented, as required, with small doses of sodium pentobarbitone (Nembutal).

Procedures for catheterisation of a vertebral, carotid and femoral artery and a femoral vein, as well as procedures for measurement of blood pressure and heart rate, are described in General Methods. In addition, loose ligatures were placed around both common carotid arteries to enable these vessels to be occluded during the experiment.

Both carotid sinuses were isolated by blunt dissection at the origin of the internal carotid artery, and the carotid sinus nerves identified by the characteristic depressor response evoked by electrical stimulation (5-10 volts, 20-40 Hz, 1 msec duration from a Grass S48 stimulator). Loose ligatures were placed under both nerves to enable access for surgical section later in the experiment.

Bradykinin (20 μg/min for 5 minutes) was infused into each of a vertebral artery, carotid artery and intravenously to obtain control responses of blood pressure and heart rate. In addition, the responses to a 5 minute period of occlusion of the left carotid artery and, separately, the right carotid artery were obtained.

Once all control responses were obtained, the carotid sinus nerve on the opposite side to the carotid artery infusion of bradykinin was

sectioned. Removal of this carotid baroreceptor function was verified by the lack of effect of occlusion of the ipsilateral carotid artery. The infusions of bradykinin via all 3 routes of administration were then repeated. Subsequently, the other carotid sinus nerve was sectioned, and the absence of any change in blood pressure and heart rate during a 5 minute period of occlusion of the ipsilateral carotid artery indicated complete denervation of the carotid sinus baroreceptor. Finally, the infusions of bradykinin were repeated in the absence of both carotid sinus baroreceptors.

Drugs used were: morphine sulphate (David Bull Laboratories, Australia), α-chloralose (Sigma), sodium pentobarbitone (Nembutal, Abbott Laboratories), bradykinin triacetate (Protein Research Foundation, Japan).

RESULTS

(A) VERTEBRAL ARTERY RESPONSES

Infusion of bradykinin via a vertebral artery caused an increase in mean arterial blood pressure and heart rate in all dogs (Table 3.2 and Fig. 3.1). Following surgical transection of the contralateral carotid sinus nerve (i.e. the nerve on the contralateral side of the carotid artery infusion), there was a significant rise in the resting mean arterial blood pressure (77.5 ± 5.3 to 89.3 ± 6.2 mm Hg) with no significant change in the resting heart rate (42.0 ± 8.2 to 43.0 ± 10.1 beats/min), but the responses of blood pressure and heart rate during vertebral artery infusion of bradykinin were not significantly different from the control responses (Table 3.2 and Fig. 3.1), although they were, on average, reduced in magnitude. Section of the remaining carotid sinus nerve caused a further significant elevation of the resting blood pressure (from 89.3 ± 6.2 to 113.3 ± 5.9 mm Hg) and also a significant rise in the resting heart rate (from 43.0 ± 10.1 to 64.0 ± 9.0 beats/min), but there was still no statistically significant effect on the responses of both blood pressure and heart rate during vertebral artery infusion of bradykinin, although the hypertensive effect was, on average, smaller than the control responses while the heart rate response was larger (Table 3.2 and Fig. 3.1).

(B) CAROTID ARTERY RESPONSES

Infusion of bradykinin via a carotid artery caused an increase in mean arterial pressure and heart rate in all dogs (Table 3.3 and Fig. 3.1). Following surgical transection of the contralateral carotid sinus nerve, there was a significant rise in the resting mean arterial

FIGURE 3.1 - The responses of mean blood pressure and heart rate to 5 minute infusions of bradykinin (20 µg/min) via a vertebral artery (VA), carotid artery (CA) and intravenously (IV) in the intact dog and then following transection of firstly, the left carotid sinus nerve and then transection of the remaining carotid sinus nerve. All carotid artery infusions were via the right carotid artery.

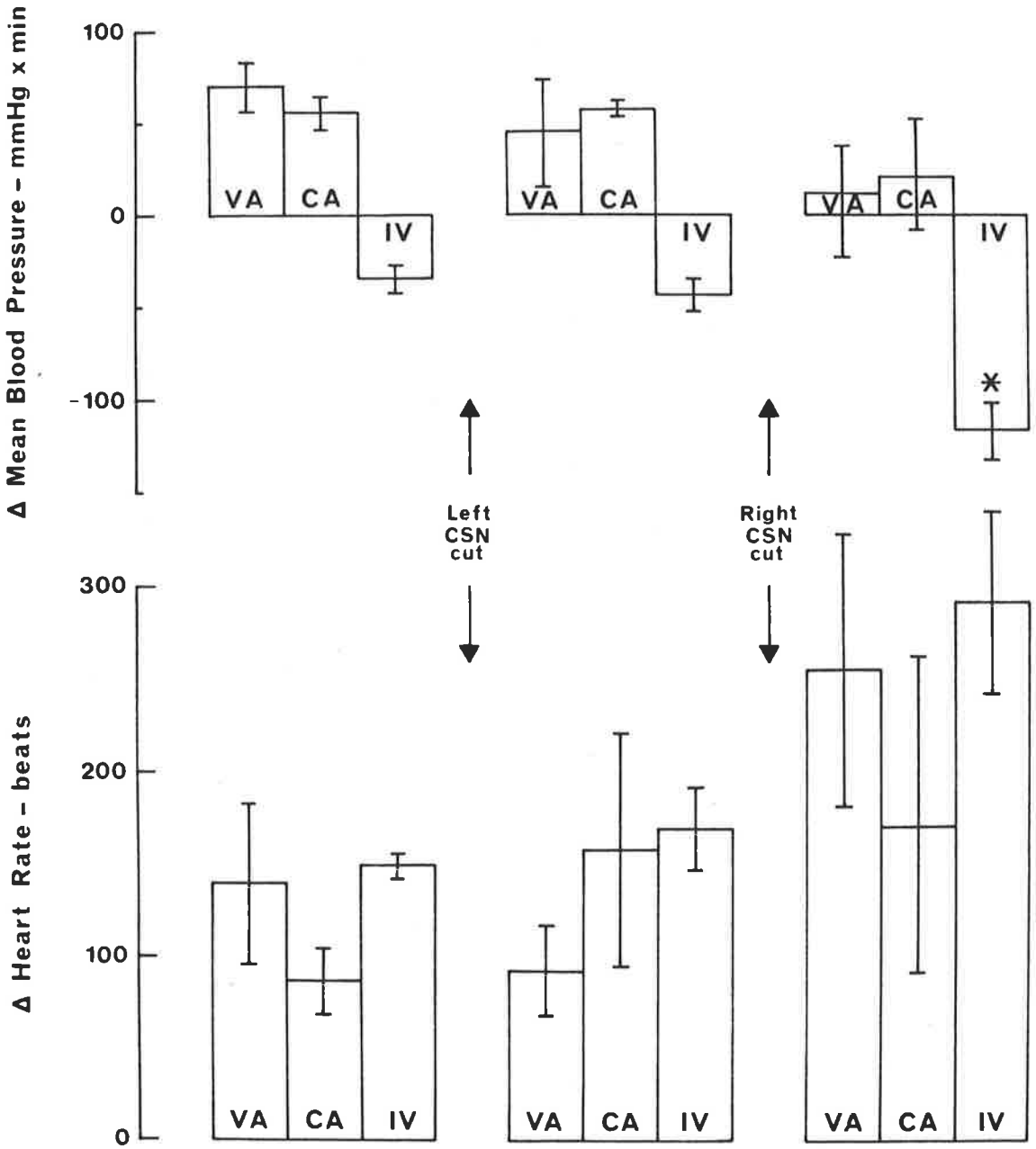
Δ Mean Blood Pressure - mmHg x min
100
0
-100
VA
CA
IV
*
Left CSN cut
Right CSN cut
Δ Heart Rate - beats
300
200
100
0
VA
CA
IV

FIGURE 3.1

blood pressure (from 77.0 ± 6.5 to 92.3 ± 5.5 mm Hg) with no significant alteration in the resting heart rate (from 48.5 ± 13.9 to 41.8 ± 11.5 beats/min), but the responses of blood pressure and heart rate during carotid artery infusion of bradykinin were not significantly different from the control responses (Table 3.3 and Fig. 3.1). Section of the remaining carotid sinus nerve produced a further significant elevation in the resting level of mean arterial blood pressure (from 92.3 ± 5.5 to 113.0 ± 5.4 mm Hg) and a significant rise in the resting heart rate (from 41.8 ± 11.5 to 69.0 ± 12.9 beats/min), but there was still no statistically significant effect on the responses of both blood pressure and heart rate during carotid artery infusion of bradykinin, although the hypertensive effect was, on average, smaller than the control response while the heart rate response was larger (Table 3.3 and Fig. 3.1).

(C) INTRAVENOUS RESPONSES

Intravenous infusion of bradykinin via a femoral vein caused a decrease in mean arterial blood pressure and an increase in heart rate in all dogs (Table 3.4 and Fig. 3.1). Following section of the contralateral carotid sinus nerve, there was a significant elevation in the resting mean arterial blood pressure (from 78.3 ± 6.4 to 92.5 ± 5.7 mm Hg) with no significant change in the resting heart rate (from 46.8 ± 12.5 to 47.8 ± 12.9 beats/min), but the responses of blood pressure and heart rate during intravenous bradykinin were not significantly different from the control responses (Table 3.4 and Fig. 3.1). Section of the remaining carotid sinus nerve caused a further significant elevation in the resting mean arterial blood pressure (from 92.5 ± 5.7 to 111.3 ± 6.3 mm Hg) and a significant rise in the resting heart rate (from 47.8 ± 12.9 to 81.5 ± 14.9 beats/min),

but there was still no statistically significant effect on the heart rate responses during intravenous bradykinin, although the responses were, on average, larger than the control responses. However, the depressor response during an intravenous infusion of bradykinin following denervation of both carotid sinuses was significantly larger than the control response (Table 3.4 and Fig. 3.1).

In summary, section of one or both carotid sinus nerves produced no statistically significant effects on either the blood pressure or heart rate responses during vertebral artery or carotid artery infusions of bradykinin, although the trend was towards smaller pressor responses and larger heart rate responses, especially following bilateral nerve section. Following bilateral carotid sinus nerve section, the depressor response to intravenous infusion of bradykinin was statistically significantly larger and this was associated with a larger heart rate response, although this latter difference was not statistically significant.

DISCUSSION

The results from these experiments indicate that the carotid sinus baroreceptors and carotid body chemoreceptors are probably not involved to a significant extent in the expression of the pressor response and tachycardia during vertebral artery and carotid artery infusions of bradykinin and, furthermore, they do not appreciably modify the response to bradykinin infusion via these two routes of administration. Since carotid sinus nerve section affected the responses to both vertebral artery and carotid artery infusions in a similar manner, this suggests that bradykinin is not having its effect in the carotid sinus region (including the carotid body chemoreceptors). If bradykinin was affecting sensory nerve endings located in either the carotid sinus or the carotid body, section of the carotid sinus nerves should have different effects on the blood pressure and heart rate responses during carotid artery infusion of bradykinin compared to vertebral artery infusion since the vertebral arteries do not perfuse the carotid sinus region. It is further suggested that bradykinin stimulation of chemosensitive nerve endings in the carotid bodies is not involved in the pressor response to vertebral artery or carotid artery bradykinin since stimulation of these sensory nerve endings causes bradycardia, a decrease in myocardial contractility, both of which are mediated via vagal efferents, and an increase in total peripheral resistance (Berne and Levy, 1981) which are the opposite effects to those observed during cranial artery infusions of bradykinin.

Marker *et al.* (1980) have provided evidence that the octapeptide, angiotensin II, "has a specific ability to attenuate the depressor response to carotid sinus nerve stimulation such that the response to a given stimulus is reduced in the presence of angiotensin II in the vertebral circulation." From the experiments reported in this section, it is unlikely that bradykinin has a similar action to attenuate the

baroreceptor reflex since the pressor effect of cranial artery bradykinin tended to be reduced following sinus nerve section. If bradykinin was attenuating the baroreceptor reflex, the pressor effects should be larger following denervation of the baroreceptors. Thus involvement of the carotid sinus baroreceptors, or even the carotid body chemoreceptors (since the afferent sensory nerves for the carotid body chemoreflex are also located in the carotid sinus nerve), is not an important mechanism in determining the cardiovascular response to vertebral artery or carotid artery bradykinin. The absence of potentiation of the responses following bilateral carotid sinus nerve section could conceivably be due to the influence of the baroreceptors located in the aortic arch. If these receptors were not active, the direct centrally-mediated hypertensive effect of bradykinin would not be opposed by the normal baroreceptor-mediated compensatory mechanisms and the pressor response and heart rate response would be potentiated. This effect was not observed, so either (a) bradykinin has no "direct" effect on the carotid sinus region and/or (b) "other" baroreceptors can compensate in the absence of the carotid sinus baroreflex.

The effect of bilateral carotid sinus nerve section on the responses to intravenous bradykinin is as expected and indicates that the baroreceptor reflex is normally active to prevent large decreases in arterial pressure. Furthermore, as long as one carotid sinus baroreceptor remains functional (as well as the aortic arch baroreceptors), the responses of blood pressure and heart rate are not altered from control responses, and it is not until both carotid sinus baroreceptors are denervated that the baroreceptor compensation is removed and a larger decrease in blood pressure results. Associated with this larger hypotensive response is an increase in the magnitude of the heart rate response, which presumably indicates increased activity of the remaining baroreceptor reflex originating in the aortic arch in an attempt to

minimise the hypotension.

In summary, these results indicate that there is no direct effect of bradykinin administered via a vertebral artery or a carotid artery on sensory nerve endings located either in the carotid sinus or carotid body and that bradykinin, unlike angiotensin II, does not appear to attenuate the normal baroreceptor reflex since bilateral carotid sinus nerve section did not alter the blood pressure and heart rate responses to bradykinin in a significant manner. However, the magnitude of the depressor response during intravenous bradykinin is determined, in part, by a compensatory action mediated via the carotid sinus baroreceptors since the response of blood pressure (and also heart rate) is potentiated following bilateral carotid sinus nerve section.

SECTION 3

Cardiovascular Effects of Anti-Diuretic Hormone: A Mediator of the Response to Bradykinin?

INTRODUCTION

In addition to its powerful anti-diuretic properties mediated via an action on the renal tubules, vasopressin (anti-diuretic hormone, ADH) and also the related peptide, octapressin, have a direct action on blood vessels to cause vasoconstriction (Feruglio, Greco, Cesano, Indovina, Sardi and Chiandussi, 1964; Longo, Morris, Smith, Beck and Assali, 1964; Maxwell, 1965; Delaney, Goodale, Cheng and Wangensteen, 1966; Nakano, 1967; Corliss, McKenna, Sialer, O'Brien and Rowe, 1968; Commarato and Lum, 1969; Ericcson, 1972a, 1972b; Nakano, 1973) and an increase in arterial blood pressure in a variety of animals, including dogs (Longo, Morris, Smith, Beck and Assali, 1964; Maxwell, 1965; Emmerson, 1966; Nakano, 1967; Corliss, McKenna, Sialer, O'Brien and Rowe, 1968; Ericcson, 1971, 1972a, 1972b, 1972c, 1972d). Heart rate is also decreased (Longo, Morris, Smith, Beck and Assali, 1964; Maxwell, 1965; Emmerson, 1966; Nakano, 1967) as is stroke volume and cardiac output (Feruglio, Greco, Cesano, Indovina, Sardi and Chiandussi, 1964; Longo, Morris, Smith, Beck and Assali, 1964; Maxwell, 1965; Delaney, Goodale, Cheng and Wangensteen, 1966; Nakano, 1967; Corliss, McKenna, Sialer, O'Brien and Rowe, 1968; Commarato and Lum, 1969; Ericcson, 1971, 1972a, 1972d) during intravenous administration of vasopressin or octapressin. Furthermore, the vasoconstriction produced by vasopressin is a direct action on the smooth muscle of blood vessels and does not involve nervous or humoral mechanisms (Nakano, 1973).

Bradykinin has also been demonstrated to stimulate the release of anti-diuretic hormone (Rocha e Silva Jnr. and Malnic, 1962, 1964; Harris and Rocha e Silva Jnr., 1965) and this effect is mediated by a central action of bradykinin and is not a secondary effect due to the hypotension resulting from the intravenous administration of bradykinin, since release of ADH was obtained following injection of small amounts

of bradykinin via a carotid artery (Rocha e Silva Jnr. and Malnic, 1962, 1964; Harris and Rocha e Silva Jnr., 1965) or a vertebral artery which were without effect on intravenous administration (Harris and Rocha e Silva Jnr., 1965).

Since vasopressin has an action on the cardiovascular system to cause an increase in arterial blood pressure, and it is released by bradykinin, it is possible that vasopressin release could contribute to the hypertensive effects obtained during vertebral artery and carotid artery administration of bradykinin. Unfortunately, the release of vasopressin by bradykinin could not be monitored directly in these experiments (in the absence of an assay procedure), but the cardiovascular effects of vasopressin infusion (at various doses) via a vertebral artery, carotid artery and intravenously were examined in the anaesthetised greyhound and, in combination with results published previously by other workers, inferences have been made with regard to a possible contribution of the cardiovascular effects of vasopressin to the responses obtained during bradykinin infusions.

MATERIALS AND METHODS

The experiments were conducted in ex-racing greyhounds, weighing between 29 and 32 kg (mean 30.3 ± 0.8 SEM) anaesthetised with α-chloralose following morphine pre-medication as described in General Methods. A cuffed endotracheal tube was inserted and artificial respiration was maintained throughout the experiment with a C.F. Palmer positive-pressure respirator. Anaesthesia was supplemented, as required, with small doses of sodium pentobarbitone (Nembutal).

Procedures for catheterisation of a vertebral, carotid and femoral artery and a femoral vein, as well as procedures for measurement of blood pressure and heart rate, are described in General Methods.

Synthetic arginine-vasopressin was infused into each of a vertebral artery, carotid artery and intravenously at doses of 5, 20, 50 and 100 mU/min for 5 minutes, and the responses of mean arterial blood pressure and heart rate are expressed as their integrals, as described in General Methods.

RESULTS

Infusion of synthetic arginine-vasopressin at the rate of 5 mU/min for 5 minutes (average 0.17 mU/kg/min) via a vertebral artery, carotid artery and intravenously did not result in any alteration in either blood pressure or heart rate. Increasing the infusion rate to 20, 50 and 100 mU/min for 5 minutes (average 0.67, 1.67 and 3.33 mU/kg/min respectively) via the same three routes of administration usually resulted in an increase in blood pressure and a decrease in heart rate (Table 3.5). The responses of blood pressure were, however, somewhat variable and no meaningful dose-response relationship was observed with any of the routes of administration. On the other hand, the responses of heart rate did exhibit a dose-response effect, the bradycardia being larger with increasing doses of vasopressin via all routes of administration (Table 3.5). This pattern of responses suggests that the bradycardia is not simply a reflex effect in response to an increase in arterial pressure. In some dogs, a large bradycardia was obtained with no alteration in blood pressure so no baroreflex effects could contribute to the bradycardia.

It should be mentioned that the total number of dogs used to obtain the results for this section was small (n=3), and not all of the doses of vasopressin were infused in each dog to avoid any problems with tachyphylaxis. However, the aim of this short series of experiments was not to investigate the cardiovascular effects of vasopressin in any detail (since these have been reported in the literature), but to gain some idea of the blood levels of vasopressin that have to be achieved to obtain a pressor effect and attempt to correlate this data to the blood levels already measured by other workers under a variety of conditions (e.g. infusion of bradykinin) to determine whether vasopressin release could possibly contribute to the cardiovascular actions of bradykinin. In the three dogs investigated, it can be seen that relatively large

amounts of vasopressin must be infused to obtain a pressor effect via any of the routes of administration and, as will be discussed subsequently, the blood levels of vasopressin achieved during these infusions are far in excess of those measured by other workers during bradykinin infusions. Thus the three dogs used satisfied the aims of the experiments, but because of the variability in the results, no comparative statistics were attempted and, in fact, were not required to allow conclusions to be drawn from the results.

DISCUSSION

Assuming an average blood volume for a dog of 86.2 ml/kg (Altman and Dittmer, 1971), the total blood volume of a 30 kg greyhound would be approximately 2.5 litres. Since the half-life of vasopressin is about 5 minutes (Lauson, 1967), infusion of 5 mU/min (0.17 mU/kg/min) of vasopressin for 5 minutes would result in the addition of about 25 mU to the circulation of the dog, or an increase in the concentration of circulating vasopressin of 10 μU/ml blood. The concentration of vasopressin in the normal conscious dog is about 0.9 μU/ml (Bonjour and Malvin, 1970), so even an infusion of 5 mU/min results in a significant elevation of plasma vasopressin levels. However, at plasma levels of about 10 μU/ml, no cardiovascular effects were observed following vasopressin administration via any of the three routes investigated.

Similarly, infusion of 20 mU/min (0.67 mU/kg/min), 50 mU/min (1.67 mU/kg/min) and 100 mU/min (3.3 mU/kg/min) of vasopressin for 5 minutes would result in plasma levels of approximately 40, 100 and 200 μU/ml respectively. At these higher infusion rates, the responses of blood pressure and heart rate showed considerable variation between dogs, although there was a tendency toward hypertension and bradycardia. Because of the variation, no clear dose-response relationship was evident for either blood pressure or heart rate and no comparative statistics were performed.

Although an increase in arterial blood pressure could be obtained with the higher infusion rates of vasopressin via each of the three routes of administration, it still remains to be demonstrated that bradykinin could stimulate vasopressin release in sufficient quantities to cause, or at least contribute to, the hypertension during bradykinin infusion. Harris and Rocha e Silva (1965) have shown that an intravenous

injection of bradykinin (32 μg/kg) results in an average increase in vasopressin levels of 55.7 ± 25.8 μU/ml blood. However, injection of this amount of bradykinin (32 μg/kg) was associated with a decrease in arterial blood pressure of 90-96 mm Hg, which would provide an additional powerful stimulus for vasopressin release in addition to any direct action of bradykinin to release vasopressin. The dose of intravenous bradykinin required by Harris and Rocha e Silva to consistently release vasopressin (32 μg/kg) was 48 times higher than the amount of bradykinin infused in the experiments reported in this Section (660 ng/kg/min). Therefore, if 32 μg/kg increased the amount of circulating vasopressin by 55 μU/ml, an infusion of 660 ng/kg/min would result in negligible release of vasopressin. Furthermore, Cowley, Monos and Guyton (1974) found that elevation of plasma vasopressin levels by 4-80 μU/ml elevated arterial blood pressure by less than 5 mm Hg. It appears, therefore, that vasopressin release does not contribute in any significant manner to the cardiovascular responses to intravenous bradykinin. If vasopressin did contribute at all, it would presumably only limit the fall in blood pressure since intravenous bradykinin is depressor and vasopressin has a pressor effect mediated via direct vasoconstriction.

In contrast to the effect of intravenous injection of bradykinin, Harris and Rocha e Silva (1965) demonstrated that injection of only 2 μg/kg of bradykinin into a carotid artery of anaesthetised cats consistently released vasopressin which resulted in an average increase in plasma levels of 87.4 ± 30.2 μU/ml (n=5) which were not significantly different to those obtained following intravenous injection of 32 μg/kg of bradykinin. However, these investigators also observed large (12-44 mm Hg) depressor responses to carotid artery bradykinin which would also result in vasopressin release. The infusion rate of bradykinin in the experiments reported in this Section was only 660 ng/kg/min, and

even if this dose released about the same amount of vasopressin as the higher (2 μg/kg) dose used by Harris and Rocha e Silva, the results of Cowley, Monos and Guyton again suggest that this amount of vasopressin release would cause a minimal (less than 5 mm Hg) rise in arterial blood pressure. It is, therefore, highly improbable that carotid artery infusions of bradykinin at the low dose used in the experiments reported in this thesis would result in the release of sufficient amounts of vasopressin to contribute in any significant way to the large hypertensive effect of carotid artery bradykinin. The amount of vasopressin released in the experiments of Harris and Rocha e Silva is probably due to the combined effects of the direct central action of bradykinin to stimulate vasopressin release and also to the hypotension which they reported. In the experiments reported in this Section, carotid artery infusion of bradykinin always resulted in an increase in arterial blood pressure and this would, if anything, inhibit vasopressin release, again suggesting that the release of vasopressin would not be of sufficient magnitude to cause any significant change in blood pressure during carotid artery infusion of bradykinin.

Harris and Rocha e Silva (1965) also reported that vertebral artery injection of 2 μg/kg and 8 μg/kg of bradykinin resulted in elevation of plasma vasopressin levels by 10.0 ± 9.3 (n=4) and 96.0 ± 42.4 μU/ml (n=4) respectively, which, as discussed above, is not sufficient to cause any significant change in blood pressure. They also, however, obtained a decrease in blood pressure following vertebral artery injection of bradykinin which could exaggerate the vasopressin release following vertebral artery bradykinin. As discussed above with regard to the effects of carotid artery bradykinin, from the results presented in this Section and in combination with the quantitative results concerning vasopressin release published by other workers, it seems highly improbable that vasopressin release occurs in sufficient amounts to contribute to the

hypertensive effects of vertebral artery bradykinin.

It must be stressed that the above discussion is somewhat "theoretical" and to verify that vasopressin release is not significant in contributing to the cardiovascular responses to vertebral artery, carotid artery and intravenous infusions of bradykinin, it would be necessary to either, (a) measure the blood levels of vasopressin during the infusions of bradykinin and then subsequently infuse vasopressin in amounts sufficient to achieve the same blood level and examine the cardiovascular effects of this calculated dose of vasopressin, or (b) infuse bradykinin in the presence of a specific vasopressin inhibitor to determine whether vasopressin release has an important contribution to the bradykinin responses obtained, or (c) infuse bradykinin into hypophysectomized dogs.

The above discussion indicates, at least with regard to the responses of blood pressure, that vasopressin release is probably not an important factor in determining the cardiovascular response to bradykinin infusions. Further investigation of the effects of vasopressin and bradykinin on other cardiovascular parameters also suggests that the vasopressin effects are minimal. For example, during vertebral artery, carotid artery and intravenous infusions of bradykinin, a tachycardia was observed in all cases, yet vasopressin infused via these same routes of administration caused a bradycardia, indicating that the heart rate effects of vasopressin do not contribute to the heart rate effects of bradykinin, but they could modify the magnitude of the response. If this is the case, than a specific vasopressin antagonist would be required to unmask any modifying effects of vasopressin on the bradykinin responses, but this has not been performed and no definite quantitative conclusions can be made from the results presented in this Section.

The mechanism of the bradycardia during vasopressin infusion is not

clear. Varma *et al.* (1969) have suggested that the bradycardia following intravenous vasopressin is partly due to a peripheral depressant action on the heart and partly to vagal stimulation mediated via the baroreceptor reflex in response to an increase in arterial blood pressure. However, with low doses of vasopressin infused in these experiments, a bradycardia was observed in the absence of any change in blood pressure suggesting reflex vagal stimulation is not involved. A direct action on the heart or a cardio-inhibitory action mediated via an effect on central structures are other possibilities but cannot be confirmed or dismissed by these experiments. Varma *et al.* (1969) provide evidence that only a small contribution to the bradycardia obtained following intravenous vasopressin is from a direct action of vasopressin on central cardio-inhibitory structures, but the majority of vagal stimulation is reflex. This mechanism, however, does not explain the bradycardia obtained in the absence of any change in blood pressure as reported in this Section. The bradycardia following intraventricular vasopressin is reportedly due to "a stimulating action on the cardio-inhibitory neurons in the region of the vagal nuclei." (Varma *et al.*, 1969). Further experiments are still necessary to determine the mechanism of the vasopressin-induced bradycardia.

The responses of cardiac output and total peripheral vascular resistance are also different during infusion of bradykinin compared with vasopressin. Bradykinin infusion via each of a vertebral artery, carotid artery and intravenously resulted in an increase in cardiac output and a decrease in total peripheral vascular resistance (see Chapter 2) but vasopressin infusion via the same three routes caused the opposite effects in these parameters. viz. a decrease in stroke volume and cardiac ouput and an increase in total peripheral vascular resistance. These effects further suggest minimal involvement of vasopressin in the responses to bradykinin administration.

In summary, it has been demonstrated that vasopressin has an action on the cardiovascular system but that these effects would oppose, rather than contribute to, the cardiovascular effects of bradykinin. Thus vasopressin release may modify the responses to bradykinin, but it is probable, although no direct measurements were made, that the amount of vasopressin released by bradykinin is small and that the "direct" effects of bradykinin predominate to cause the observed responses. As discussed, the use of a vasopressin antagonist, or even hypophysectomized dogs, would be required to establish the precise role of vasopressin in the responses to bradykinin and this has not been determined.

SECTION 4

Central Actions of Bradykinin: Involvement of the areas postrema

INTRODUCTION

Angiotensin II is a vasoactive peptide of similar molecular weight to bradykinin which, in contrast to the peripheral vasodilator action of bradykinin, is a powerful vasoconstrictor compound (Braun-Menendez *et al.*, 1940; Page and Helmer, 1940; Bumpus, Schwartz and Page, 1951; Page and Bumpus, 1961; Peart, 1965; Ungar, 1966; Lowe and Scroop, 1970; Reit, 1972; Peach, 1977) acting directly on the vascular smooth muscle of resistance vessels. In addition to its direct action on blood vessels to cause vasoconstriction and a consequent increase in mean arterial blood pressure, angiotensin II also has a significant central effect which contributes to the pressor response. In the morphine and chloralose anaesthetised greyhound, infusion of angiotensin II into a vertebral artery caused an increase of blood pressure, heart rate and cardiac output with no change in total peripheral resistance (Lowe and Scroop, 1969). Since the low doses of angiotensin administered via a vertebral artery (8-32 ng/min) to cause these effects were without effect when given intravenously, these authors concluded that angiotensin "can produce effects on the circulation by a specific action in the area of distribution of the vertebral artery." The low doses of angiotensin were also without effect when administered via a carotid artery. Furthermore, withdrawal of vagal tone to the heart was the major effect of vertebral artery angiotensin to cause the observed cardiovascular responses (Scroop and Lowe, 1969) and any contribution of the sympathetic nervous system could only be demonstrated when vagal pathways were eliminated.

It has subsequently been demonstrated that ablation of the area postrema abolishes, or at least highly significantly reduces, the pressor response to vertebral artery angiotensin (Joy and Lowe, 1970a, 1970b; Scroop *et al.*, 1971) but this procedure is without effect on the response to intravenous angiotensin, other vasoactive compounds (e.g. prostaglandin

$F_{2\alpha}$, and acetylcholine) or carotid artery occlusion. The response to endogenously generated angiotensin following renal artery constriction was also significantly reduced following ablation of the area postrema (Scroop *et al.*, 1975). Thus an intact area postrema is essential for the centrally-mediated cardiovascular effects of angiotensin which are important in the overall cardiovascular response to angiotensin.

It has been shown from the results in Chapter 2 of this thesis that bradykinin also has a powerful central action to cause hypertension and a tachycardia. The direct action of bradykinin on vascular smooth muscle to cause dilation and a decrease in blood pressure is evident during intravenous administration, but administration via either a vertebral artery or a carotid artery has a central action to cause an increase in blood pressure. Since the central action of angiotensin is mediated via an action on the area postrema, the experiments reported in this Section were conducted to determine whether bradykinin also exerts its central action via the area postrema, which is situated in the caudal portion of the medulla near the obex and is deficient in the blood-brain barrier (Klara *et al.*, 1976) and should allow access of bradykinin to cerebral structures (Rapoport, 1976).

MATERIALS AND METHODS

The experiments were conducted in ex-racing greyhounds, weighing between 28 and 31 kg (mean 30.3 ± 0.5 SEM), anaesthetised with α-chloralose following morphine pre-medication as described in General Methods. A cuffed endotracheal tube was inserted and artificial respiration was maintained throughout the experiment with a C.F. Palmer positive-pressure respirator. Anaesthesia was supplemented, as required, with small doses of sodium pentobarbitone (Nembutal).

Procedures for catheterisation of a vertebral, carotid and femoral artery and a femoral vein, as well as procedures for measurement of blood pressure and heart rate, are described in General Methods.

Control responses to 5 minute infusions of bradykinin via a vertebral artery, carotid artery and intravenously were obtained. In addition, the responses of blood pressure and heart rate to a 5 minute infusion of angiotensin II (32 ng/min) via a vertebral artery were obtained. These latter responses were used in these experiments as the control for ablation of the area postrema, which was approached dorsally through the fourth ventricle and ablated under direct vision by thermocoagulation. The absence of any significant response of both blood pressure and heart rate during vertebral artery infusion of angiotensin following ablation was accepted as evidence that the areas postrema were satisfactorily coagulated. Since no histological procedures were undertaken, the above "indirect" evidence of satisfactory ablation was used, and if any dogs exhibited large increases in either resting blood pressure or resting heart rate, they were excluded from the results since damage to central structures other than the area postrema had probably occurred. The area postrema is adjacent to other

central structures which are involved in the control of the cardiovascular system e.g. the nucleus tractus solitarius and the dorsal motor nucleus of the vagus, and these areas, if damaged, result in hypertension (Barnes and Ferrario, 1981). Following ablation of the areas postrema, the infusions of bradykinin via each route of administration were repeated. The responses of blood pressure and heart rate are expressed as their integrals, as described in General Methods.

Drugs used were: α-chloralose (Sigma), morphine sulphate (David Bull Laboratories, Australia), angiotensin II (Hypertensin, Ciba) and bradykinin triacetate (Protein Research Foundation, Japan).

RESULTS

Infusion of angiotensin via a vertebral artery caused an increase in mean arterial blood pressure and heart rate in all dogs (Table 3.6 and Fig. 3.2). Following ablation of the area postrema, the responses of both blood pressure and heart rate during angiotensin infusions were significantly reduced from control values and were now not significantly different from zero. The resting level of blood pressure was, however, slightly elevated following ablation of the area postrema (before, 107.4 ± 5.5 mm Hg; after, 127.8 ± 6.2 mm Hg), as was the resting heart rate (before, 36.2 ± 3.3 beats/min; after, 56.4 ± 5.6 beats/min) and both of these changes were statistically significant.

Infusion of bradykinin via a vertebral artery and a carotid artery also caused an increase in mean arterial blood pressure and heart rate while intravenous bradykinin caused a decrease in mean arterial blood pressure and an associated increase in heart rate (Table 3.6 and Fig. 3.2). Following ablation of the area postrema, the blood pressure responses during vertebral artery and carotid artery infusions of bradykinin were significantly reduced from control values such that they were not significantly different from zero in either case. The blood pressure responses during intravenous bradykinin were not significantly altered following area postrema ablation.

The heart rate responses during vertebral artery and carotid artery infusions of bradykinin were, on average, larger following area postrema ablation but the difference was not statistically significant in either case. The heart rate responses during intravenous bradykinin were also larger following area postrema ablation and this difference was statistically significant (Table 3.6 and Fig. 3.2).

FIGURE 3.2 - The responses of mean blood pressure and heart rate to 5 minute infusions of bradykinin (20 μg/min) via a vertebral artery (VA), carotid artery (CA) and intravenously (IV) and a 5 minute vertebral artery infusion of angiotensin II (AII - 32 ng/min) before and after area postrema ablation (APA). (* significant difference compared to control responses, $p<0.05$).

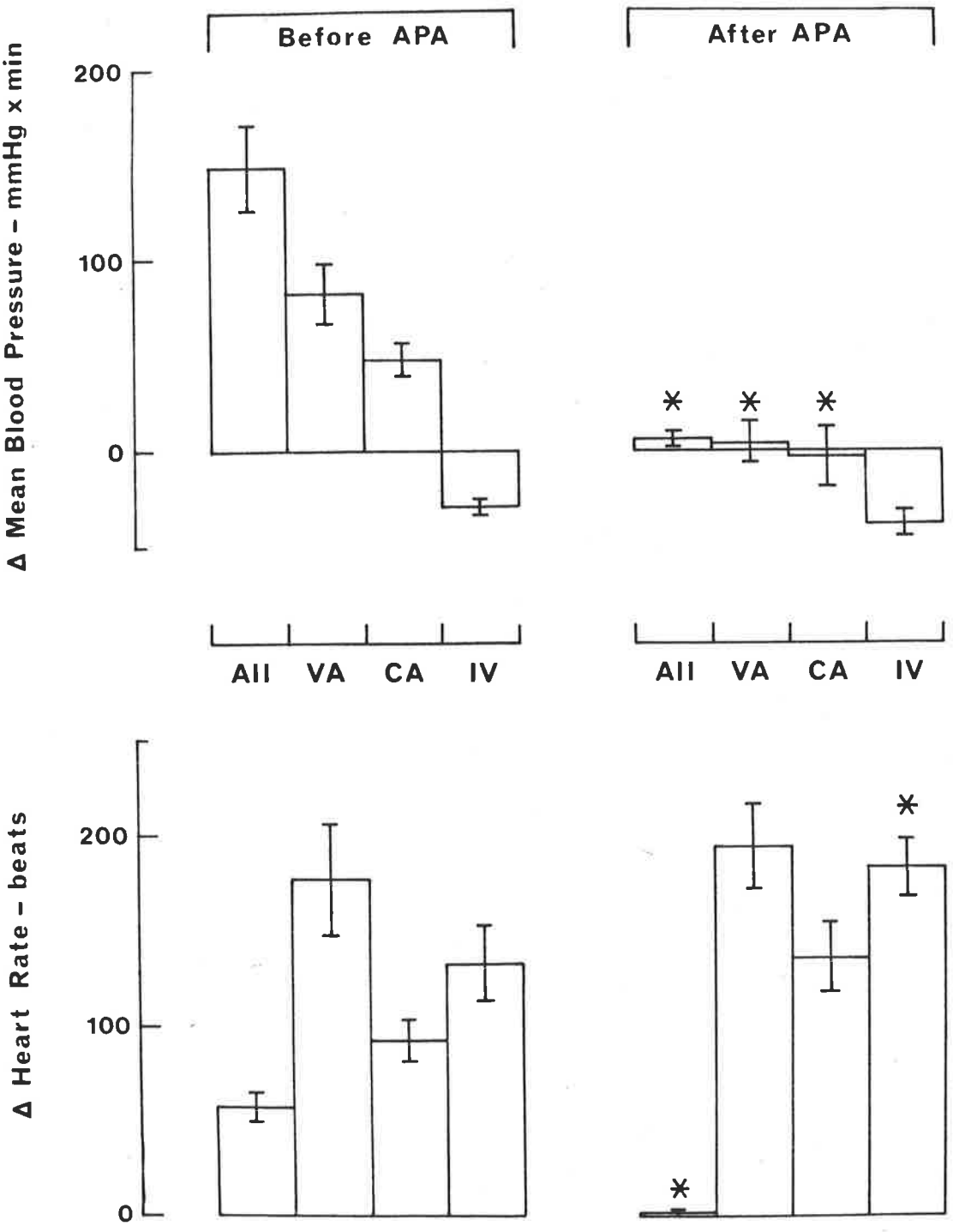
Before APA
After APA
Δ Mean Blood Pressure – mmHg x min
200
100
0
AII
VA
CA
IV
Δ Heart Rate – beats
200
100
0

FIGURE 3.2

DISCUSSION

The infusions of angiotensin II via a vertebral artery were only used in these experiments to determine whether thermocoagulation of the areas postrema was adequate. Since it has been shown previously that this procedure can abolish the blood pressure and heart rate responses to vertebral artery angiotensin II (Joy and Lowe, 1970a, 1970b; Scroop *et al.*, 1971), the areas postrema were progressively thermocoagulated until the angiotensin responses were abolished, or at least highly significantly reduced from control values. It was then assumed that the areas postrema could not contribute to any responses during subsequent bradykinin infusions.

Following ablation of the areas postrema, both resting blood pressure and resting heart rate showed small, but statistically significant, increases. When coagulating the areas postrema, it was found that the amount of coagulation required to abolish the angiotensin responses yet cause no change in resting blood pressure and heart rate was critical. Consequently, in these experiments, progressive coagulation was performed until such time as the angiotensin responses were essentially eliminated and only small, if any, changes in resting blood pressure and heart rate occurred. It seemed preferable to eliminate the angiotensin effects and at the same time accept small increases in resting blood pressure and heart rate rather than maintain these resting levels and still have some residual angiotensin effects. In any case, the alterations in the resting values were small, so if any damage to adjacent neural structures (e.g. vagal nuclei) did occur, it was minimal and alone would not be expected to significantly modify the bradykinin responses. Furthermore, any baroreceptor pathways in the nucleus tractus solitarius or surrounding neural tissue do not appear to have been damaged since the reflex tachycardia during intravenous bradykinin was preserved and,

in fact, slightly potentiated. While no other test procedures were used in these experiments, it has previously been demonstrated that ablation of the area postrema has no effect on the responses to vertebral artery infusions of $PGF_{2\alpha}$, acetylcholine and carotid occlusion (Joy and Lowe, 1970b) and specifically abolished the cardiovascular response to vertebral artery angiotensin II.

Area postrema ablation did not significantly affect the depressor response to intravenous bradykinin. Since the depressor effect of bradykinin is the result of the direct vasodilator action of bradykinin on peripheral vascular smooth muscle (see Chapter 1 and Chapter 2 of this thesis), the lack of effect of area postrema ablation further suggests that any central effects of intravenous bradykinin do not have an important role in the expression of the response. The slight potentiation of the depressor response following area postrema ablation may reflect a small contribution of central effects to the normal response, but it is normally small because the amount of bradykinin reaching central structures during intravenous administration would be small after passing through the pulmonary circulation where bradykinin is largely and rapidly destroyed. Since the central effects are pressor, the small contribution of these effects would limit the fall in blood pressure during intravenous infusion of bradykinin and when these effects are removed by area postrema ablation, the depressor response is potentiated. The larger depressor response would also provide a stronger stimulus to the baroreceptor reflex to potentiate the tachycardia, as was observed in these experiments.

The pressor responses to vertebral artery and carotid artery bradykinin were abolished by area postrema ablation, indicating that the integrity of the area postrema is essential for the centrally-mediated pressor effects of bradykinin. In some dogs the responses were

significantly reduced, but remained slightly pressor, whereas in other dogs a small depressor response was obtained. In the latter case, it is probable that bradykinin has entered the peripheral circulation to cause vasodilation, while in the other dogs the small pressor response probably represents incomplete destruction of the area postrema. So, at least with regard to the effects on blood pressure, it seems that bradykinin and angiotensin may have a "common pathway" to both cause hypertension via a central mechanism. Furthermore, the preferential efferent pathway in the responses to both bradykinin and angiotensin seems to be withdrawal of cardiac vagal tone.

However, examination of the heart rate responses to both of these peptides reveals considerable differences in their mechanisms of action. Area postrema ablation abolishes both the blood pressure and heart rate responses to vertebral artery angiotensin, but the heart rate responses to both vertebral artery and carotid artery bradykinin were preserved even though the blood pressure response was abolished. A further difference between the actions of bradykinin and angiotensin is evident when comparing the responses to vertebral artery and carotid artery administration of the peptides. Bradykinin causes hypertension and a tachycardia with both routes of administration, while administration of 32 ng/min of angiotensin II via a carotid artery caused essentially no effect, yet this same dose was strongly pressor when administered via a vertebral artery (Lowe and Scroop, 1969). These authors therefore suggested that the central site of action of angiotensin is within the area of distribution of the vertebral artery, and this site was subsequently localised to the area postrema. Since the area postrema is supplied largely by the vertebral artery with minimal supply from the carotid arteries, it is surprising that both vertebral artery and carotid artery infusions of bradykinin cause similar responses that are both abolished by area postrema ablation. This is in direct contrast to the

effects of angiotensin where carotid artery infusions have negligible effects and the response resembles that to intravenous infusion. That is, the angiotensin responses strongly suggest that the blood supply to the areas postrema is of vertebral artery origin and there is minimal "spillover" from the carotid circulation to the vertebral. The bradykinin responses, however, indicate that with this peptide there could be considerable "spillover" from the carotid circulation to allow bradykinin to reach the area postrema. If this were the case, it would explain why carotid artery bradykinin produced responses which are of similar nature but smaller in magnitude, since not all of the bradykinin infused into the carotid artery would reach the area postrema. Because bradykinin is a powerful dilator of blood vessels, including cerebral vessels (Rocha e Silva, 1963; Lumley, Humphrey, Kennedy and Coleman, 1982), it is possible that cerebral vasodilation could increase the accessibility of carotid artery bradykinin to areas that are normally supplied by the vertebral artery. On the other hand, the vasoconstrictor peptide, angiotensin II, would tend to reduce any communication between the carotid and vertebral circulations and prevent carotid artery angiotensin from reaching the area postrema, even though total vertebral artery blood flow is not altered (Lowe and Scroop, 1969).

Another major difference between the effects of angiotensin and bradykinin is that the heart rate responses to vertebral artery and carotid artery infusions of bradykinin are preserved following area postrema ablation whereas those to vertebral artery angiotensin are abolished. The mechanism of this effect of bradykinin, in the absence of any increase in blood pressure, is not clear. The results from Chapter 2 suggested that it was predominantly the increase in heart rate and the consequent increase in cardiac output that was responsible for the increase in arterial blood pressure, yet following area postrema ablation the heart rate response is preserved (and, in fact, slightly

potentiated) and the blood pressure response is essentially eliminated. Since the centrally-mediated effects on blood pressure were prevented by ablation of the area postrema, it is unlikely that any heart rate mechanisms requiring an intact area postrema would remain. If the central actions of bradykinin are mediated solely through the area postrema, then the observed tachycardia following area postrema ablation is presumably not a central effect, or if it is, it utilises sites other than the area postrema. As shown in Chapter 3 (Section 1) of this thesis there is no direct effect of bradykinin on the heart that could account for the increase in heart rate. It is also unlikely that the tachycardia is the result of stimulation of sympathetic ganglia. Although bradykinin has been shown to stimulate sympathetic ganglia (Lewis and Reit, 1965; Trendelenburg, 1966), if there was significant sympathetic stimulation, arterial pressure should increase as a result of the increase in heart rate and also an increase in total peripheral resistance resulting from sympathetic stimulation of blood vessels. Bradykinin-stimulated release of catecholamines from the adrenal medulla has also been demonstrated in cats (Feldberg and Lewis, 1963, 1964, 1965) although the effect was minimal in calves (Comline, Silver and Sinclair, 1968). However, the amount of bradykinin reaching the adrenal medulla during vertebral artery or carotid artery infusion of bradykinin would be small (after passage through the pulmonary circulation) and therefore would not cause release of catecholamines in sufficient quantities to account for the large tachycardia. Epicardial bradykinin has been demonstrated to cause a reflex increase in blood pressure and heart rate (Staszewska-Barczak, Ferreira and Vane, 1976). However, direct application of 5 μg of bradykinin in their experiments only resulted in small increases in heart rate, so stimulation of epicardial nociceptors alone cannot account for the tachycardia observed in the experiments reported in this thesis. This nociceptive reflex is also associated with an increase in blood pressure, and this effect further suggests that bradykinin is

not stimulating cardiac pain receptors to cause the increase in heart rate following area postrema ablation since blood pressure did not change.

Bradykinin, as well as dilating the major resistance vessels, also has a constrictor action on the superior and inferior venae cavae (Regoli and Barabe, 1980). The combination of these two effects would result in an increase in venous return, right and left atrial pressures and pulmonary arterial pressure, as measured by Nakano (1965). The increased stretch of the right atrium could result in an increase in heart rate via the Bainbridge reflex or a direct effect of stretching the sino-atrial node cells. Stretch receptors located in the left atrium and pulmonary artery could also conceivably mediate the effects on heart rate. The magnitude of the possible contribution of these mechanisms to the increase in heart rate during bradykinin infusions is unknown.

Since cardiac output was not measured (and total peripheral vascular resistance was not calculated) in these experiment, the reason that blood pressure did not change in spite of the large tachycardia cannot be precisely determined, and the mechanism of the tachycardia following area postrema ablation also cannot be determined from these experiments.

In summary, these experiments provide further evidence that the blood pressure response during intravenous bradykinin is a direct peripheral effect of the peptide, and is not dependent on any centrally-mediated effects, while the blood pressure responses during vertebral artery and carotid artery infusions of bradykinin are the result of a central action of the peptide and are dependent on the integrity of the area postrema. The heart rate effects during cranial artery infusions of bradykinin are not altered by area postrema ablation and

the mechanism of these responses is not clear.

SECTION 5

Involvement of Cholinergic Mechanisms in the Responses to Bradykinin

INTRODUCTION

It has been demonstrated in previous experiments reported in this thesis that bradykinin administered via either a vertebral artery or a carotid artery has an action on central nervous system structures to cause an increase in heart rate and blood pressure. While the precise location of these central structures remains unknown, the experiments reported in Section 4 of this chapter indicate that the integrity of the area postrema is essential for bradykinin to exert its central effects. The area postrema is deficient in the blood-brain barrier (Klara *et al.*, 1976) so bradykinin could have access to central structures through this region (Rapoport, 1976). Since there are structures in close proximity to the area postrema e.g. the nucleus ambiguus and the dorsal vagal nucleus, which have very high concentrations of acetylcholine, choline acetyltransferase and acetylcholinesterase (Kobayashi, Brownstein, Saavedra and Palkovits, 1975; Koelle, 1954; Palkovits and Jacobowitz, 1974), it is conceivable that bradykinin could obtained access to central nervous system structures through the area postrema and subsequently stimulate a central cholinergic pathway to cause, or contribute to, the cardiovascular responses to vertebral artery and carotid artery bradykinin.

It has been shown previously that the intraventricular administration of cholinergic agents causes a pressor response in rats and it has further been suggested that an imbalance between the central cholinergic pressor and the noradrenergic depressor mechanisms may be involved in the neurogenic initiation of essential or spontaneous hypertension (Yamori, 1976). Activation of these central cholinergic pressor pathways by bradykinin would presumably act primarily to reduce cardiac vagal tone in the morphine and chloralose anaesthetised greyhound to cause a tachycardia and hypertension, as discussed previously in this thesis.

To investigate the possibility that the cardiovascular response to bradykinin infusions is mediated via acetylcholine release in the brain, the effect of an inhibitor of acetylcholinesterase (physostigmine) on the cardiovascular response to bradykinin infusion was examined, and the results are discussed in this Section.

MATERIALS AND METHODS

The experiments were conducted in ex-racing greyhounds, weighing between 29 and 32 kg (mean 30.0 ± 0.7 SEM), anaesthetised with α-chloralose following morphine pre-medication as described in General Methods. A cuffed endotracheal tube was inserted and artificial respiration was maintained throughout the experiment with a C.F. Palmer positive-pressure respirator. Anaesthesia was supplemented, as required, with small doses of sodium pentobarbitone (Nembutal).

Procedures for catheterisation of a vertebral, carotid and femoral artery and a femoral vein, as well as procedures for measurement of blood pressure and heart rate are described in General Methods.

Control responses to 5 minute infusions of bradykinin via a vertebral artery, carotid artery and intravenously were obtained. In addition, the responses of blood pressure and heart rate to a 5 minute vertebral artery infusion of acetylcholine (250 μg/min) were obtained. Following the administration of physostigmine (200 μg/min for 5 minutes) via a vertebral artery, the vertebral artery infusion of acetylcholine was repeated and potentiation of the responses indicated adequate inhibition of acetylcholinesterase by the dose of physostigmine. Finally, the infusions of bradykinin via each of the routes of administration were repeated in the presence of physostigmine. At the conclusion of the bradykinin infusions, acetylcholine was again infused via a vertebral artery to confirm that cholinesterase inhibition was maintained.

The responses of blood pressure and heart rate are expressed as their integrals, as described in General Methods. Statistical comparisons were performed using the Student's t-test for paired observations, with significance being accepted at $p<0.05$.

Drugs used were: morphine sulphate (David Bull Laboratories, Australia), α-chloralose (Sigma), sodium pentobarbitone (Nembutal, Abbott Laboratories, Australia), bradykinin tri-acetate (Protein Research Foundation, Japan), acetylcholine chloride (Laboratories Lematta and Boinott, Paris) physostigmine sulphate (eserine sulphate, Sigma).

RESULTS

Infusion of bradykinin via a vertebral artery and a carotid artery caused an increase in blood pressure (71.5 ± 9.3 and 37.5 ± 5.6 mm Hg x min, respectively) and heart rate (124.8 ± 60.9 and 112.5 ± 35.4 beats, respectively), while intravenous infusion of bradykinin caused a decrease in blood pressure (-30.5 ± 5.1 mm Hg x min) and an increase in heart rate (154.0 ± 43.3 beats - see Table 3.7 and Fig. 3.3).

Control infusions of acetylcholine via a vertebral artery also caused an increase in heart rate (21.5 ± 10.2 beats/min) and blood pressure (16.5 ± 1.8 mm Hg) and following the administration of physostigmine via a vertebral artery, the heart rate response was significantly potentiated (139.0 ± 20.9 beats/min) while the blood pressure response was now depressor (-33.8 ± 6.9 mm Hg). However, in 2 dogs, there was an initial pressor response which lasted for about 1 minute, and this was followed by a decrease in arterial pressure below control levels. The mechanisms of these effects is dicussed subsequently. (Note that the responses to acetylcholine are not expressed as integrals of the responses, but as absolute changes in blood pressure (mm Hg) and heart rate (beats/min). The reason is that the changes in heart rate during vertebral artery acetylcholine were so large following eserine that both the resting heart rate and the maximum heart rate achieved could not be accommodated within the same sensitivity range on the recorder. Thus an integrated value could not be obtained, but the absolute resting and peak heart rates could be measured during the infusion.)

Following cholinesterase inhibition with eserine, the blood pressure responses to both vertebral artery and carotid artery infusion of bradykinin were statistically significantly reduced from

FIGURE 3.3 - The responses of mean blood pressure and heart rate to 5 minute infusions of bradykinin (20 μg/min) via a vertebral artery (VA), carotid artery (CA) and intravenously (IV) before and after a vertebral artery infusion of eserine (200 μg/min for 5 minutes). (* significant difference compared to control responses, $p<0.05$).

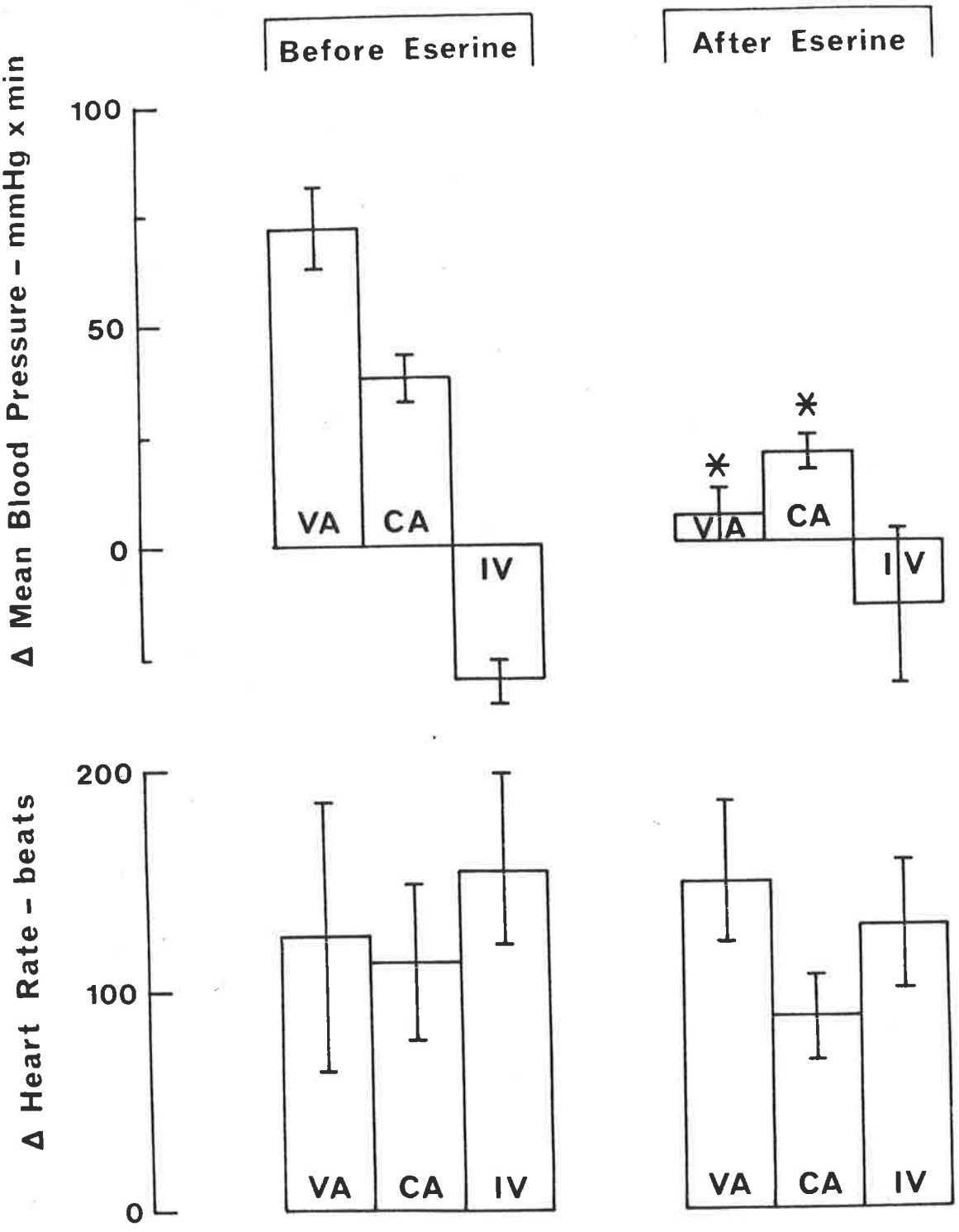
Before Eserine
After Eserine
Δ Mean Blood Pressure – mmHg x min
100
50
0
VA
CA
IV
*
*
Δ Heart Rate – beats
200
100
0
VA
CA
IV

FIGURE 3.3

control values, while the responses to intravenous bradykinin were not significantly altered by the administration of eserine (Table 3.7 and Fig. 3.3). However, in one dog the blood pressure response to intravenous bradykinin was slightly pressor and while this response was at variance with the other results, it was a consistent response in that particular dog. The heart rate responses to bradykinin infusion were not significantly altered from control values with any of the three routes of administration (Table 3.7 and Fig. 3.3).

DISCUSSION

Infusion of acetylcholine via a vertebral artery caused an increase in blood pressure and heart rate which is a central effect of acetylcholine to activate pressor mechanisms (Krstic, 1978). While the responses to vertebral artery bradykinin following physostigmine, which consisted of a predominant depressor response and a tachycardia, did not consist simply of a potentiation of the control responses, they still indicate significant potentiation of the effects of acetylcholine. It has been reported that intracerebroventricular acetylcholine has a biphasic effect, consisting of an initial pressor effect and a subsequent prolonged depressor effect. The secondary depressor response has been attributed to mobilization of catecholamines in the brain via an effect of acetylcholine on central muscarinic receptors (Krstic, 1978) and these catecholamines have a powerful hypotensive action (De Jong, Zandberg and Bohus, 1975). Thus inhibition of acetylcholinesterase in these experiments is probably causing noradrenaline release in the brain which causes the decrease in arterial blood pressure. The large potentiation of the heart rate response during vertebral artery acetylcholine following physostigmine presumably reflects a prolonged effect of acetylcholine on cardio-excitatory structures and this has a dominant effect over any central noradrenergic cardio-inhibition. Since the increase in heart rate during vertebral artery acetylcholine was potentiated following physostigmine, although it was not measured in these experiments, cardiac output would also probably be increased. This increase in cardiac output and decrease in blood circulation time could conceivably result in some acetylcholine entering the peripheral circulation to directly dilate peripheral blood vessels and decrease total peripheral resistance which could also contribute to the observed decrease in blood pressure. Both the blood pressure and heart rate responses during vertebral artery infusion of acetylcholine following

physostigmine are therefore consistent with a prolonged action of acetylcholine due to acetylcholinesterase inhibition, and while not performed in every dog, an intravenous infusion of the same dose of acetylcholine (250 μg/min for 5 minutes) caused very large depressor responses following physostigmine which is also consistent with considerable inhibition of acetylcholinesterase.

The pressor response obtained during vertebral artery infusion of bradykinin was essentially eliminated following physostigmine administration, but the heart rate response was not significantly altered from control values. It appears, therefore, that bradykinin could be activating a cholinergic mechanism in the brain and that the acetylcholine which is released could be subsequently stimulating a noradrenergic vasodepressor pathway and that stimulation of this latter pathway prevents any change in blood pressure. During the control responses to bradykinin, any acetylcholine released in the brain would activate a cholinergic pressor mechanism (similar to the mechanism of the pressor response to vertebral artery acetylcholine) to inhibit cardiac vagal tone and cause an increase in heart rate, cardiac output and blood pressure. However, when the action of acetylcholinesterase is inhibited, acetylcholine could conceivably then activate the noradrenergic vasodepressor mechanism to eliminate the normal blood pressure response. The mechanism by which the central vasodepressor mechanism is able to selectively eliminate the blood pressure response (and not affect the heart rate response) presumably involves vasodilation of peripheral blood vessels since the heart rate response is not altered, but an effect to reduce cardiac output by a reduction in stroke volume cannot be eliminated since this variable was not monitored. Even though it appears probable that vertebral artery bradykinin stimulates a central cholinergic mechanism, this effect is exaggerated by physostigmine and may involve subsequent noradrenaline release. In the absence of acetylcholinesterase inhibition, activation

of the noradrenergic depressor pathways is probably minimal, but a central action of the bradykinin-induced release of acetylcholine to contribute to the increase in heart rate could still occur. However, these experiments are unable to determine the magnitude of the contribution of central mechanisms to the tachycardia.

The responses of blood pressure and heart rate during carotid artery infusions of bradykinin following physostigmine showed the same pattern of changes as with vertebral artery infusions i.e. a significant attenuation of the blood pressure response with no change in the magnitude of the heart rate response, suggesting similar mechanisms may be involved in the responses to carotid artery bradykinin. The results from Section 4 of this thesis also suggest that the mechanisms are similar since ablation of the area postrema affected the responses to both vertebral artery and carotid artery bradykinin in a similar way. Since bradykinin administered by these routes seems to act via the area postrema, a similar effect on the responses following physostigmine is not surprising. Thus carotid artery bradykinin also could gain access to central structures through the area postrema and then activate a cholinergic mechanism in the same way as vertebral artery bradykinin.

Although physostigmine affected the responses to vertebral artery and carotid artery bradykinin in a similar way, the reduction in the magnitude of the blood pressure response during carotid artery bradykinin was not as great as that seen with vertebral artery bradykinin. This different effect presumably reflects a difference in the amount of bradykinin reaching the area postrema as discussed previously in Section 4 of this chapter. Since the postulated mechanism of the reduction in the pressor response during vertebral artery bradykinin was bradykinin-stimulated acetylcholine release which subsequently released noradrenaline

and activated central depressor mechanisms, if less bradykinin reached the area postrema during carotid artery infusion, then less acetylcholine would be released in the brain and the subsequent activation of noradrenergic depressor mechanisms (which would oppose the pressor effects of bradykinin) would not be as intense. If this was the case, then the balance of pressor and depressor effects would be in favor of the pressor mechanism, whereas with vertebral artery bradykinin, a more intense stimulation of the depressor mechanism is sufficient to completely abolish any pressor response.

The blood pressure and heart rate responses during intravenous bradykinin were not significantly altered from control values following the administration of physostigmine. This result suggests that the responses to intravenous bradykinin do not involve a cholinergic mechanism and, as discussed previously in this thesis, has a direct action on vascular smooth muscle to cause vasodilation. It also indicates that intravenous bradykinin does not cause the release of significant amounts of acetylcholine in the brain, probably because the amount of bradykinin reaching the brain (specifically, the area postrema) is small. If significant amounts of systemically administered bradykinin did reach the brain, the responses would be modified considerably.

It is perhaps surprising that the heart rate response to intravenous bradykinin was not significantly modified since the tachycardia is a baroreceptor reflex effect in response to the hypertension. As acetylcholine is a transmitter in the baroreflex arc (Palkovits, 1981), physostigmine might have been expected to potentiate any central effect of acetylcholine and, as a result of this potentiation of the baroreceptor effect, potentiate the heart rate response during intravenous bradykinin. However, this was not observed, possibly indicating either (a) the amount of physostigmine in areas involved in the baroreceptor reflex

was insufficient to produce a significant potentiation of the baroreceptor reflex effects or (b) activation of central noradrenergic cardio-inhibitory mechanisms may also have occurred to balance any centrally-mediated cholinergic cardiac stimulant effects.

In summary, the results from these experiments suggest that during vertebral artery and carotid artery administration of bradykinin, the peptide gains access to central cardiovascular areas through the area postrema (or has an action at the area postrema) to subsequently stimulate a cholinergic pathway (unknown at present), of which the predominant effect is inhibition of cardiac vagal tone to increase heart rate, cardiac output and arterial blood pressure. While the evidence presented to support an action of a central cholinergic pathway is largely indirect from potentiation of the effects of acetylcholine, the responses obtained are consistent with the proposed involvement of a central cholinergic pathway to produce the observed cardiovascular responses, but the precise action of such a mechanism cannot be determined from these experiments. During intravenous infusions of bradykinin following physostigmine, the cardiovascular responses were not altered from control values suggesting minimal, if any, involvement of cholinergic mechanisms.

SECTION 6

The Effect of Inhibition of Prostaglandin Synthesis on the Cardiovascular Responses to Bradykinin

INTRODUCTION

Prostaglandins are derived from polyunsaturated fatty acids, in particular arachidonic acid, which is incorporated as a structural component of phospholipids in cell membranes of all tissues in the body (Dusting, Moncada and Vane, 1979). Chemical or mechanical stimulation of cell membranes by a variety of stimuli cause release of the various prostaglandins and their powerful effects on the cardiovascular system make them possible mediators of the actions of bradykinin.

When injected intravenously, arachidonic acid reduces arterial pressure (Rose, Johnson, Ramwell and Kot, 1974) and these authors suggested that the mechanism of action is through conversion of arachidonic acid to an intermediate in the biosynthesis of prostaglandin E_2 (PGE_2). It has subsequently been demonstrated that the predominant vascular metabolite of arachidonic acid is prostacyclin (PGI_2) which mediates the hypotension following intravenous arachidonic acid administration (Moncada and Vane, 1977; Mullane, Dusting, Salmon, Moncada and Vane, 1979). Prostaglandins are also formed from exogenous arachidonic acid in the isolated heart (Needleman, 1976) and it was suggested that PGE_2 was the main metabolite which caused dilation of the coronary vessels (Needleman, Marshall and Sobel, 1975). However, since the discovery of prostacyclin, it now appears as though PGI_2 is the primary active metabolite of arachidonic acid (Schror, Moncada, Ubatuba and Vane, 1978; Needleman, Bronson, Wyche, Sivakoff and Nicolaou, 1978) which mediates dilation of the coronary vessels (Dusting, Moncada and Vane, 1977). Other prostaglandins and metabolites of arachidonic acid also have effects on the heart and blood vessels of all regional circulations (Dusting, Moncada and Vane, 1979).

Homogenates or slices of brain have been shown to synthesise various prostaglandins, including PGE_2, $PGF_{2\alpha}$ and PGD_2 (MacDermot, Blair and Cresp, 1981). Furthermore, PGE_2 modulates noradrenaline release from rat cerebral cortex (Hillier and Templeton, 1980) and also inhibits vasoconstriction elicited by sympathetic nerve stimulation (Hedqvist, 1979). Prostaglandins are released in the vicinity of autonomic neuroeffector junctions and influence the release of transmitter from nerve terminals, suggesting that prostaglandins are important as modulators of autonomic neuroeffector transmission (Hedqvist, 1977). Prostacyclin is also inhibitory on noradrenergic transmission in the autonomic nervous system (Hedqvist, 1979).

Bradykinin has been shown to stimulate the release of prostaglandins (Blumberg, Denny, Marshall and Needleman, 1977; McGiff, Terragno and Malik, 1972) and these prostaglandins have been implicated as mediators of the pressor effect of centrally-administered bradykinin (Kondo, Okuno, Konishi, Saruta and Kato, 1979; Takahashi and Bunag, 1981) and that PGE_2 may also contribute to the depressor response to intravenous bradykinin (Murthy, Waldron and Goldberg, 1978), although Kondo *et al.* (1979) found that the contribution of vasodepressor prostaglandins to the hypotensive response to intravenous bradykinin was minimal. Pearson and Lang (1969) reported that acetylsalicylic acid had no effect on the pressor responses to intracarotid bradykinin. Although these authors used acetylsalicylic acid for its analgesic properties to determine whether the cardiovascular responses were reflex in response to pain caused by bradykinin, acetylsalicylic acid is also a potent inhibitor of prostaglandin synthesis (Vane, 1971). Their results, therefore, suggest no involvement of prostaglandins in the pressor response to bradykinin.

Since (a) bradykinin has been shown to release various prostaglandins

from different tissues, including the brain, (b) bradykinin has a specific central action to cause hypertension (as discussed in Section 4 of this chapter) and (c) prostaglandins can modulate transmitter release from nerve terminals, both in the periphery and in the brain, it is likely that prostaglandin synthesis could contribute to the cardiovascular effects of bradykinin. To determine whether prostaglandins do contribute to the cardiovascular responses obtained during infusion of bradykinin via a vertebral artery, carotid artery and intravenously, the infusions were performed in the presence of an inhibitor of prostaglandin synthesis and the results are presented in this section.

MATERIALS AND METHODS

The experiments were conducted in ex-racing greyhounds, weighing between 25 and 34 kg (mean 28.9 ± 1.2 SEM, n=10), anaesthetised with α-chloralose following morphine pre-medication as described in General Methods. A cuffed endotracheal tube was inserted and artificial respiration was maintained throughout the experiment with a C.F. Palmer positive-pressure respirator. Anaesthesia was supplemented, as required, with small doses of sodium pentobarbitone (Nembutal).

Procedures for catheterisation of a vertebral, carotid and femoral artery and a femoral vein, as well as procedures for measurement of blood pressure and heart rate, are described in General Methods.

Control responses to 5 minute infusions of bradykinin (20 μg/min) via a vertebral artery, caroitd artery and intravenously, and a 5 minute infusion of angiotensin II (32 ng/min) via a vertebral artery were obtained. An infusion of indomethacin (3 mg/min for 30 min) was then administered via a vertebral artery to inhibit prostaglandin synthesis. This dose of indomethacin (3 mg/kg) has been shown to produce considerable (if not total) competitive and non-reversible inhibition of prostaglandin syntesis in both dogs and man (Flower, 1974). Furthermore, this dose of indomethacin is sufficient to inhibit endogenous prostaglandin synthesis in both the central nervous system and the periphery (Kondo, Okuno, Konishi, Saruta and Kato, 1979). Following blockade of endogenous prostaglandin synthesis, the infusions of angiotensin and bradykinin were repeated.

Indomethacin (150 mg) was dissolved in the following solution to give a final concentration of 3 mg/ml: ethanol (9 ml), normal saline (35 ml), 0.715% sodium carbonate (6 ml).

The responses of blood pressure and heart rate are expressed as their integrals, as described in General Methods, and statistical significance ($p<0.05$) was determined using a Student's t-test for paired observations.

Drugs used were: morphine sulphate (David Bull Laboratories, Australia), α-chloralose (Sigma), bradykinin tri-acetate (Protein Research Foundation, Japan), angiotensin II (Hypertensin, Ciba), indomethacin (Sigma), sodium pentobarbitone (Nembutal, Abbott Laboratories, Australia).

RESULTS

Infusion of angiotensin II via a vertebral artery caused an increase in arterial pressure (92.8 ± 14.4 mm Hg x min) and heart rate (13.6 ± 6.6 beats). Following a vertebral artery infusion of indomethacin, these responses were not significantly altered from control values (Table 3.8 and Fig. 3.4).

Vertebral artery and carotid artery infusions of bradykinin also resulted in an increase in arterial blood pressure (79.1 ± 7.6 and 63.5 ± 9.5 mm Hg x min, respectively) and heart rate (79.2 ± 14.1 and 61.7 ± 8.5 beats, respectively), while intravenous bradykinin caused a decrease in blood pressure (-24.3 ± 4.2 mm Hg x min) and a tachycardia (107.4 ± 27.9 beats). Following a vertebral artery infusion of indomethacin, the blood pressure responses to both vertebral artery and carotid artery infusions of bradykinin were significantly reduced, but the heart rate responses were not altered from control values. Indomethacin had no effect on the hypotensive effect of intravenous bradykinin, although the tachycardia was significantly potentiated following indomethacin administration (Table 3.8 and Fig. 3.4).

FIGURE 3.4 - The responses of mean blood pressure and heart rate to 5 minute infusions of bradykinin (20 μg/min) via a vertebral artery (VA), carotid artery (CA) and intravenously (IV) and a 5 minute vertebral artery infusion of angiotensin II (AII - 32 ng/min) before and after a vertebral artery infusion of indomethacin (3 mg/min for 30 minutes). (* significant difference compared to control responses, $p<0.05$).

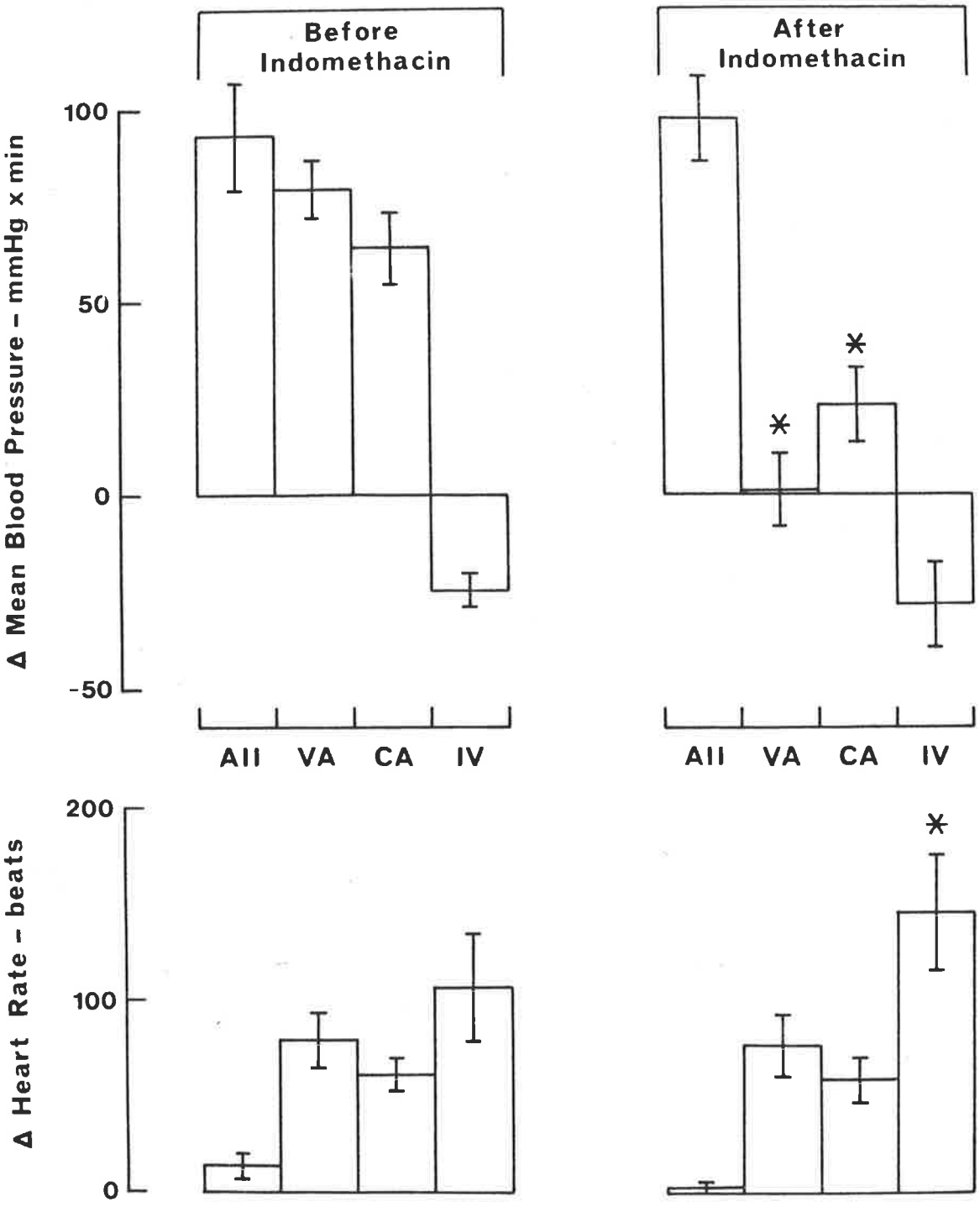
Before Indomethacin
After Indomethacin
Δ Mean Blood Pressure - mmHg x min
100
50
0
-50
All
VA
CA
IV
Δ Heart Rate - beats
200
100
0
*

FIGURE 3.4

DISCUSSION

The results presented in this section indicate that prostaglandin synthesis is an important mechanism in the hypertensive responses obtained during vertebral artery and carotid artery infusions of bradykinin since the response to vertebral artery bradykinin was abolished, and the response to carotid artery bradykinin was reduced by about 65% following indomethacin. These results support the conclusions of Kondo *et al.* (1979) who have presented evidence that the central pressor effect of intracerebroventricular bradykinin was greatly attenuated by indomethacin, indicating prostaglandin involvement in the response. Since inhibition of prostaglandin synthesis was likely to be non-specific and involve both peripheral and central inhibition, the results from these experiments cannot distinguish between central nervous system and peripheral effects of prostaglandin synthesis, although the results of Kondo *et al.* (1979) indicate that the pressor effect of bradykinin is mediated mainly by prostaglandin synthesis in the central nervous system. However, the depressor effect of intravenous bradykinin was unchanged following administration of indomethacin providing supportive evidence for the conclusions in previous sections of this thesis that the hypotensive effect of intravenous bradykinin results from a direct action of bradykinin on vascular smooth muscle to cause vasodilation. Thus examination of the blood pressure effects alone suggests prostaglandins are largely responsible for the hypertensive effects of centrally-administered bradykinin, but consideration of the heart rate responses, in conjunction with previous results presented in this thesis, suggests that the mechanism of the effects of bradykinin is more complex than simply involving the effects of prostaglandins on the cardiovascular system. Inhibition of endogneous prostaglandin synthesis by indomethacin did not alter the magnitude of the tachycardia during either vertebral

artery or carotid artery infusions of bradykinin, suggesting that prostaglandins are not involved in this response and that withdrawal of cardiac vagal tone is probably responsible, as discussed in Chapter 2. It thus appears as though prostaglandins contribute to the increase in blood pressure, but not to the increase in heart rate.

It has been established that prostaglandin E_1 (PGE_1) and prostaglandin E_2 (PGE_2) reduce noradrenaline release from sympathetic nerve terminals (Hedqvist and Wennmalm, 1971; Wennmalm, 1971). Furthermore, this inhibition of transmitter release is a pre-junctional mechanism since the PGE's have no effect on the responses to exogenously applied noradrenlaine. Similarly, PGE_2 has been tentatively proposed to inhibit noradrenaline release from rat cerebral cortex slices *in vitro*, although extrapolation of this effect to an *in vivo* situation in other species should be treated with caution. Inhibition of prostaglandin synthesis with indomethacin has also been shown to cause an increase in the evoked noradrenaline release from rat cerebral cortex slices (Hillier and Templeton, 1980). These modulating effects of prostaglandins on transmitter release from sympathetic nerves or noradrenergic neurons in the central nervous system, in conjunction with the prostaglandin-releasing activity of bradykinin, strongly suggest that bradykinin-stimulated prostaglandin release in the brain could result in removal of central noradrenergic inhibition of the cardiovascular system to cause hypertension and tachycardia. However, it is also possible that prostaglandins released by bradykinin could have direct excitatory effects on the cardiovascular system. The experiments reported in this section cannot distinguish between these possible actions.

There is an apparent anomaly in the mechanism of the responses

of blood pressure and heart rate. The increase in blood pressure during cranial artery administration of bradykinin was either abolished (vertebral artery) or greatly reduced (carotid artery) by indomethacin, implicating prostaglandin synthesis as an important mechanism, whether via a central or a peripheral action. The mechanism of the pressor effects of cranial artery bradykinin involved primarily an increase in cardiac output (as a result of withdrawal of cardiac vagal tone) since total peripheral vascular resistance was always decreased (see Chapter 2 of this thesis) and it is suggested that prostaglandins may be mediators of these effects. However, inhibition of prostaglandin synthesis specifically affects the blood pressure response and does not alter the magnitude of the tachycardia, so it appears as though bradykinin could still cause vagal inhibition (by a non-prostaglandin mechanism) to cause the tachycardia, yet some other effect of prostaglandin inhibition prevents any increase in blood pressure. The mechanism of this effect and its site of action are unknown, although it could be mediated through the area postrema, as discussed subsequently. The possible interactions between bradykinin, prostaglandin synthesis and the modulating effect of prostaglandins on noradrenaline release from central neurons and the sympathetic nervous system requires further investigation before any mechanism for the action of bradykinin can be confidently proposed. As discussed in Section 5 of this chapter, a central cholinergic mechanism may also be involved.

It should be pointed out that other workers considering the mechanism of central pressor effects have not measured (or, at least not published) heart rate responses in addition to blood pressure responses, and their conclusions concerning the mechanism of the pressor effect do not include an analysis of the heart rate contribution. The effects on blood pressure obtained in the experiments reported in

this Section agree with previous workers i.e. the pressor effect is reduced following indomethacin suggesting prostaglandins contribute to the increase in blood pressure (Kondo, Okuno, Konishi, Saruta and Kato, 1979; Takahashi and Bunag, 1981), but the mechanism becomes more complicated when, for example, inhibition of prostaglandin synthesis abolishes the blood pressure effect but has no effect on the heart rate response, and it was believed (see Chapter 2) that the increase in blood pressure was a result of the increased heart rate and cardiac output.

In summary, these results suggest that the pressor effects of vertebral artery and carotid artery bradykinin are mediated by prostaglandins but the heart rate responses do not involve prostaglandin synthesis. The nature of the prostaglandin(s) involved in the pressor response is not known, but the central actions of PGE_2 suggest that this prostaglandin could be involved. Furthermore, the responses reported in this Section following indomethacin administration are similar (especially those during vertebral artery bradykinin) to those reported in Section 4 of this chapter following ablation of the area postrema, suggesting that the site of the central action of bradykinin is the area postrema and that subsequent prostaglandin synthesis mediates the cardiovascular responses observed, especially the resulting hypertension. The responses to intravenous bradykinin were not greatly affected by either indomethacin or area postrema ablation, and any small changes that did occur can be explained by the direct effect of bradykinin on vascular smooth muscle to cause vasodilation and the subsequent response of baroreceptor homeostatic mechanisms to alterations in arterial blood pressure. Prostaglandin synthesis is not an important mechanism in the cardiovascular response to intravenous bradykinin.

CONCLUSIONS

The results of the experiments presented in this Chapter indicate the following:

1. Bradykinin has no direct positive chronotropic or inotropic effects on the isolated rabbit heart.

2. The cardiovascular responses to vertebral artery and carotid artery bradykinin do not involve stimulation of "receptors" in the carotid sinus region (including the carotid bodies) and it is probable that bradykinin does not attenuate the baroreceptor reflex. The responses to intravenous bradykinin were modified by carotid sinus nerve section, which is consistent with the fact that the hypotension is a direct action of bradykinin to cause vasodilation and that the tachycardia is a baroreceptor reflex effect to minimise the hypotensive response.

3. Release of vasopressin by bradykinin does not contribute to the cardiovascular responses obtained during vertebral artery, carotid artery and intravenous infusions of bradykinin, especially at the dose of bradykinin used in these experiments.

4. The pressor effects of vertebral artery and carotid artery infusions of bradykinin were abolished by thermal ablation of the area postrema, indicating that the integrity of the area postrema is essential for the centrally-mediated pressor effects of cranial artery bradykinin. However, the depressor response to intravenous bradykinin does not require the area postrema pathways.

5. A central cholinergic mechanism may be involved in the responses to vertebral artery and carotid artery infusions of bradykinin. It is suggested that bradykinin is acting, or gaining access to other central structures, through the area postrema to possibly activate a central cholinergic pathway. The depressor response to intravenous bradykinin does not involve cholinergic mechanisms.

5. Inhibition of endogenous prostaglandin synthesis by indomethacin resulted in complete abolition of the pressor response to vertebral artery bradykinin and a large reduction in the response to carotid artery bradykinin. It is suggested that the central site of action of cranial artery bradykinin is the area postrema and subsequent stimulation of prostaglandin synthesis in the brain mediates the pressor responses, largely by inhibition of cardiac vagal tone to increase cardiac output. The depressor response to intravenous bradykinin does not involve prostaglandins as mediators of the effect.

CHAPTER 4

THE ROLE OF ENDOGENOUS OPIATES IN THE PRESSOR RESPONSE TO ANGIOTENSIN II

INTRODUCTION

It is well documented that angiotensin II has a central action to cause an increase in arterial blood pressure (Scroop and Lowe, 1969; Lowe and Scroop, 1969; Ferrario, Dickinson and McCubbin, 1970). Scroop and Lowe (1969) showed in the morphine and chloralose anaesthetised greyhound that the "major cardiovascular effects of vertebral artery infusions of angiotensin were due to withdrawal of vagal tone to the heart" and that "only when the vagal pathways were blocked could a definite contribution from the sympathetic nervous system be demonstrated". Furthermore, an intact area postrema is required for the hypertensive response to vertebral artery angiotensin II (Joy and Lowe, 1970a, 1970b; Scroop, Katic, Joy and Lowe, 1971; Ferrario, Gildenberg and McCubbin, 1972; Gildenberg, Ferrario and McCubbin, 1973). However, Ferrario *et al.* (1972) suggest that vertebral artery angiotensin II in mongrel dogs augments sympathetic vasomotor activity through an action at the area postrema. It thus appears that the precise mechanism of action of angiotensin is different in morphine-chloralose anaesthetised greyhounds compared with mongrel dogs, although the pressor response to vertebral artery angiotensin is mediated via an action at the area postrema in both species. Szilagyi and Ferrario (1980, 1981) also suggest that the central actions of angiotensin II are not prominant unless morphine is used as part of the anaesthetic.

Recently, Pert *et al.* (1975) have shown that opiate receptors are present in the area postrema and Armstrong *et al.* (1979) have identified enkephalin-containing neurones in the same area. Feldberg and Wei (1981) placed filter paper on the dorsal surface of the brainstem at the obex and application of 40 μg (or less) of morphine to the filter paper caused bradycardia and hypotension, which is a similar response to subcutaneous morphine although much higher doses are required via the

latter route of administration. However, injection of morphine into the third ventricle caused a large tachycardia and a small increase in blood pressure, suggesting morphine has an action in the third ventricle (probably on the hypothalamus) to stimulate cardiac sympathetic nerves. Hence Szilagyi and Ferrario (1980, 1981) have suggested that a central action of the endogenous opiate system may be modulating the effect of angiotensin to cause the pressor effect since they could not obtain this effect if morphine was not part of the anaesthetic regime. In a previous series of experiments in this laboratory (Wilkinson, 1975), we found no significant difference in the pressor response to vertebral artery infusions of angiotensin II in the morphine and chloralose anaesthetised greyhound compared with the chloralose anaesthetised greyhound, which is at variance with the results of Szilagyi and Ferrario (1980, 1981) although in our experiments the increase in heart rate was larger in the morphine pre-treated dogs than in those anaesthetised with chloralose alone. Our results, therefore, do not support those of Ferrario *et al.* in mongrel dogs and do not implicate morphine or the endogenous opiate system in the pressor response to vertebral artery angiotensin II. The following experiments were performed using the opiate receptor antagonist, naloxone, to investigate the possible involvement of an opiate mechanism in the pressor response to vertebral artery and intravenous angiotensin II.

MATERIALS AND METHODS

The experiments were divided into three groups according to the method of anaesthesia and the route of administration of naloxone.

GROUP 1

These experiments were conducted in ex-racing greyhounds (n=10) anaesthetised with α-chloralose following morphine pre-medication as described in General Methods. A cuffed endotracheal tube was inserted and artificial respiration was maintained throughout the experiment with a C.F. Palmer positive-pressure respirator. Anaesthesia was supplemented as required, with small doses of sodium pentobarbitone (Nembutal).

Procedures for catheterisation of a vertebral artery, a femoral artery and a femoral vein, as well as procedures for measurement of blood pressure and heart rate, are described in General Methods. In addition, loose ligatures were placed around both common carotid arteries to enable these vessels to be occluded during the experiment.

Angiotensin II was administered by continuous infusion at the rate of 1 ml/min for 5 minutes via a vertebral artery at doses of 4, 8, 16 and 32 ng/min (0.133 - 1.067 ng/kg/min) and intravenously at doses of 32 and 250 ng/min (1.067 and 8.333 ng/kg/min, respectively). The responses of blood pressure and heart rate to a 5 minute period of bilateral common carotid artery occlusion were also determined. All of the above procedures were then repeated following a vertebral artery infusion of the opiate receptor antagonist, naloxone, at the rate of 1 μg/min for 5 minutes.

GROUP 2

These experiments (n=5) were again performed in ex-racing greyhounds anaesthetised with α-chloralose following morphine pre-medication and the experimental protocol involving vertebral artery and intravenous infusions of angiotensin II and bilateral common carotid artery occlusion was identical to that employed in Group 1 experiments. However, in these experiments, naloxone was administered intravenously at the same dose as in Group 1 experiments.

GROUP 3

This group of experiments (n=5) were performed in ex-racing greyhounds anaesthetised with α-chloralose without any previous morphine pre-medication. The protocol for vertebral artery and intravenous infusions of angiotensin II and bilateral common carotid artery occlusion was again identical to Group 1 experiments and naloxone was administered via a vertebral artery at the rate of 1 μg/min for 5 minutes.

In each group of experiments, the responses of blood pressure and heart rate are expressed as their integrals, as described in General Methods and a Student's t-test for paired observations was used for statistical analysis of the results, with significance being accepted at $p<0.05$.

Drugs used were: morphine sulphate (David Bull Laboratories, Australia), α-chloralose (Sigma), sodium pentobarbitone (Nembutal, Abbott Laboratories), angiotensin II (Hypertensin, Ciba), naloxone hydrochloride (Narcan, Endo Laboratories, Australia).

RESULTS

GROUP 1

Control infusions of angiotensin II via a vertebral artery caused an increase in arterial blood pressure at each of the doses used in these experiments (Table 4.1 and Fig. 4.1). These pressor responses were accompanied by an increase in heart rate (Table 4.2 and Fig. 4.2), although with the two lowest doses used (4 and 8 ng/min) the change in heart rate was small and not statistically significantly different from zero.

Intravenous infusion of angiotensin II (32 ng/min) resulted in a small pressor response (Table 4.1 and Fig. 4.1) which was only 20% of the magnitude of the pressor response obtained during vertebral artery infusion of the same dose. There was no statistically significant change in heart rate during intravenous infusion of this dose of angiotensin II. Intravenous infusion of a higher dose of angiotensin II (250 ng/min) caused a larger pressor response (Table 4.1 and Fig. 4.1) and while the heart rate was, on average, slightly reduced from control values during this infusion, the difference was not significantly different from zero (Table 4.2 and Fig. 4.2).

A 5 minute period of bilateral common carotid artery occlusion was associated with a large increase in arterial blood pressure (Table 4.1 and Fig. 4.1) and a small increase in heart rate (Table 4.2 and Fig. 4.2).

Following the vertebral artery infusion of naloxone, the resting blood pressure showed a small, non-significant increase from 83.1 ± 4.9 mm Hg to 94.1 ± 7.3 mm Hg and the resting heart rate also showed a small,

FIGURE 4.1 - The responses of mean blood pressure during 5 minute infusions of angiotensin II via a vertebral artery and intravenously at the doses indicated (ng/min) and a 5 minute period of bilateral common carotid artery occlusion (BCO) before and after a vertebral artery infusion of naloxone (1 μg/min for 5 minutes) in morphine and chloralose anaesthetised greyhounds. (* significant difference, $p<0.05$).

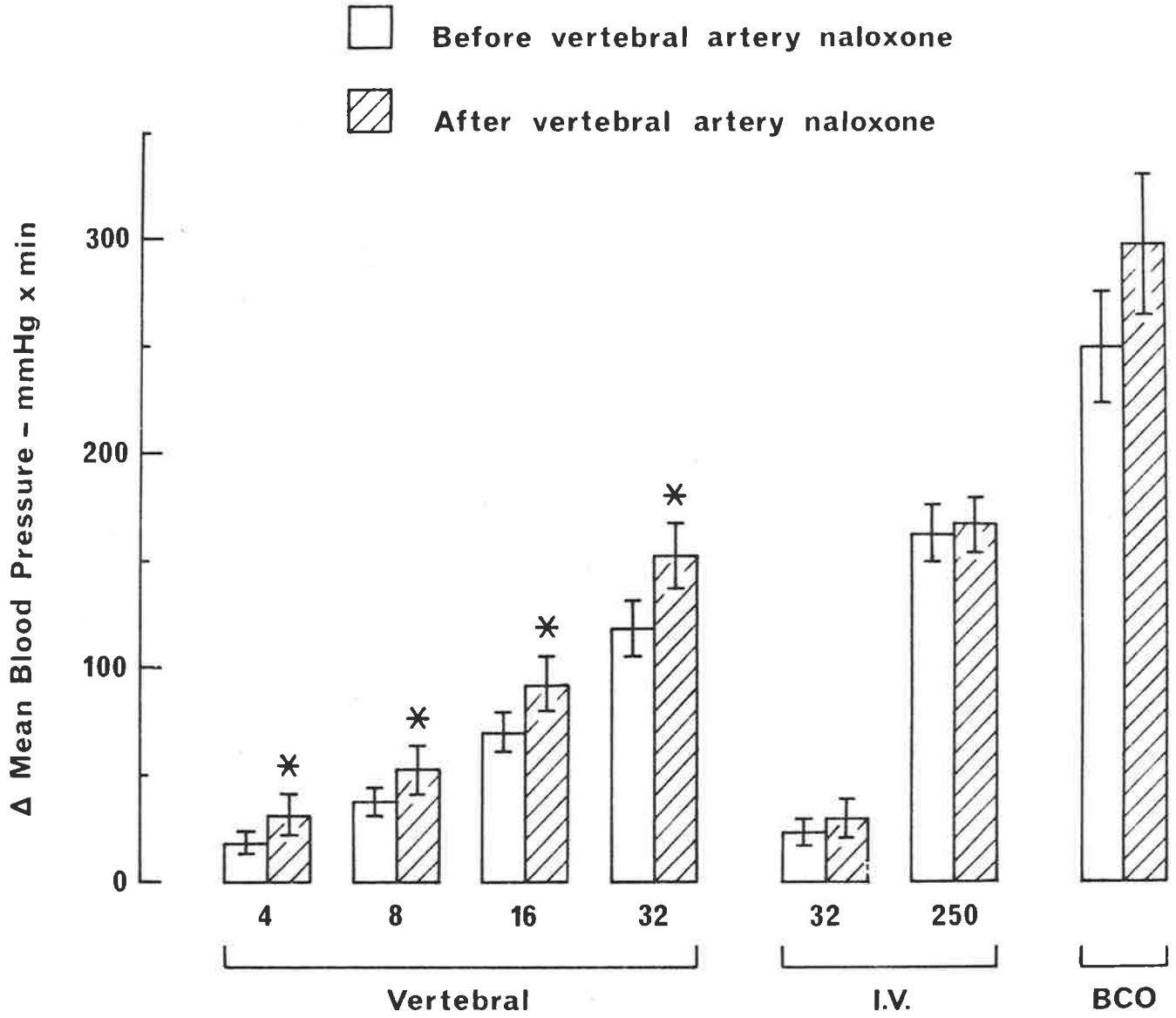
Before vertebral artery naloxone
After vertebral artery naloxone
Δ Mean Blood Pressure - mmHg x min
300
200
100
0
*
*
*
*
4
8
16
32
32
250
Vertebral
I.V.
BCO

FIGURE 4.1

FIGURE 4.2 - The responses of heart rate during 5 minute infusions of angiotensin II via a vertebral artery and intravenously at the doses indicated (ng/min) and a 5 minute period of bilateral common carotid artery occlusion (BCO) before and after a vertebral artery infusion of naloxone (1 μg/min for 5 minutes) in morphine and chloralose anaesthetised greyhounds. (* significant difference, $p<0.05$).

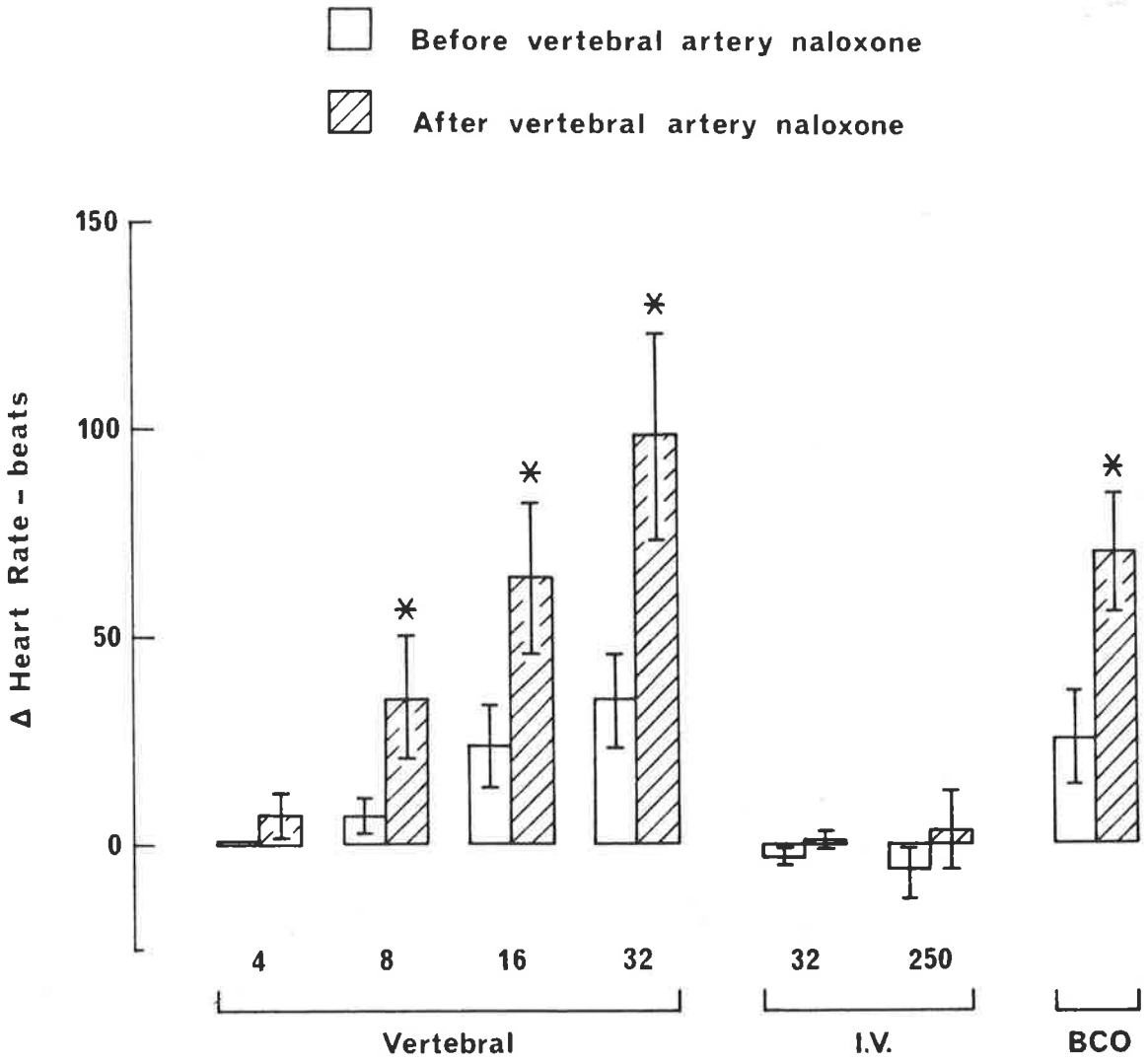
Before vertebral artery naloxone
After vertebral artery naloxone
Δ Heart Rate - beats
150
100
50
0
*
*
*
*
*
4
8
16
32
32
250
Vertebral
I.V.
BCO

FIGURE 4.2

non-significant increase from 36.2 ± 1.7 beats/min to 44.9 ± 7.3 beats/min. The pressor responses to vertebral artery infusions of angiotensin II at all of the doses investigated were significantly potentiated from control values (Table 4.1 and Fig. 4.1) and these effects were associated with an increase in the magnitude of the tachycardia with all doses investigated, although the difference was only significant with the three highest doses used (Table 4.2 and Fig. 4.2). There was no significant alteration in the magnitude of either the blood pressure or the heart rate responses during intravenous infusion of angiotensin following naloxone at either of the doses investigated, nor was the pressor response during bilateral common carotid artery occlusion significantly different from control values, although the associated tachycardia was significantly larger following naloxone administration (Table 4.2 and Fig. 4.2).

GROUP 2

Control infusions of angiotensin II via a vertebral artery caused an increase in arterial blood pressure at each of the doses used in these experiments (Table 4.3 and Fig. 4.3) and these responses were not significantly different from the corresponding responses in Group 1 experiments. An increase in heart rate also occurred during vertebral artery infusions of all doses of angiotensin II, although only with the highest dose (32 ng/min) was the change in heart rate significantly different from zero (Table 4.4 and Fig. 4.4). Again, none of the heart rate responses were significantly different from the corresponding responses obtained in Group 1 experiments.

Intravenous infusion of a small dose of angiotensin II (32 ng/min) resulted in a small pressor response which was only 14% of the magnitude

FIGURE 4.3 - The responses of mean blood pressure during 5 minute infusions of angiotensin II via a vertebral artery and intravenously at the doses indicated (ng/min) and a 5 minute period of bilateral common carotid artery occlusion (BCO) before and after an intravenous infusion of naloxone (1 μg/min for 5 minutes) in morphine and chloralose anaesthetised greyhounds. (* significant difference, $p<0.05$).

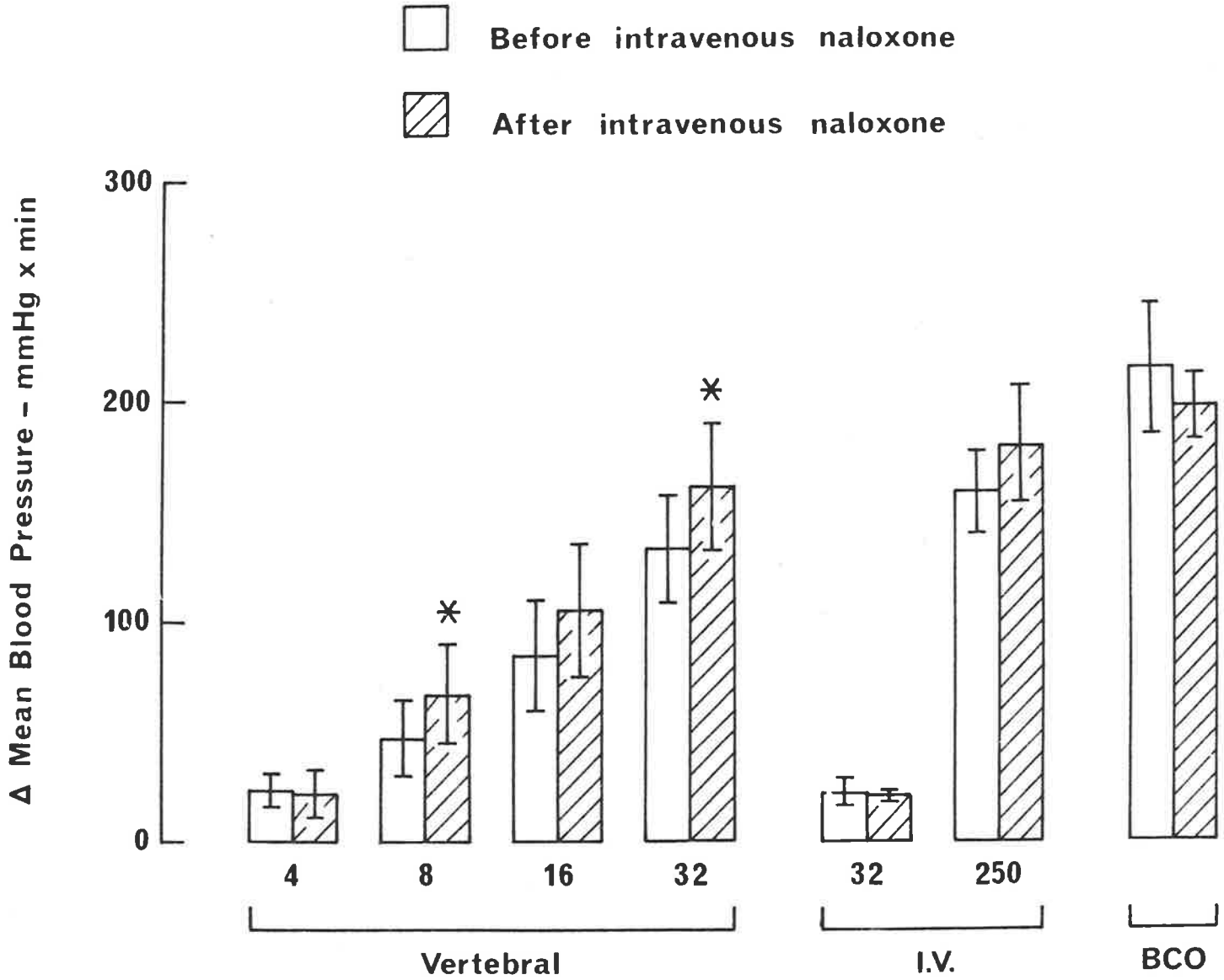
Before intravenous naloxone
After intravenous naloxone
Δ Mean Blood Pressure – mmHg x min
300
200
100
0
*
*
4
8
16
32
32
250
Vertebral
I.V.
BCO

FIGURE 4.3

of the pressor response obtained during vertebral artery infusion of the same dose (Table 4.3 and Fig. 4.3). The larger dose of angiotensin II (250 ng/min) caused a larger pressor response when infused intravenously. On average, the heart rate decreased below control values during intravenous infusion of both doses of angiotensin, although in both cases the small change was not significantly different from zero (Table 4.4 and Fig. 4.4).

During the 5 minute period of bilateral common carotid artery occlusion, there was a large increase in arterial blood pressure (Table 4.3 and Fig. 4.3) and a smaller non-significant increase in heart rate (Table 4.4 and Fig. 4.4). These responses of blood pressure and heart rate were not significantly different from those obtained in Group 1 experiments.

Following the intravenous administration of naloxone, the resting blood pressure showed a small, non-significant increase from 83.1 ± 4.9 mm Hg to 94.1 ± 7.3 mm Hg and the resting heart rate also showed a small, non-significant increase from 36.2 ± 1.7 beats/min to 44.9 ± 7.3 beats/min, but both the blood pressure and heart rate responses during vertebral artery infusions of 8, 16 and 32 ng/min of angiotensin II were, on average, potentiated, although the only statistically significant difference occurred in the blood pressure responses to infusion of 8 and 32 ng/min of angiotensin. The blood pressure and heart rate responses to 4 ng/min of angiotensin infused via a vertebral artery, as well as the responses to both doses of intravenous angiotensin and bilateral common carotid artery occlusion were essentially unaltered following intravenous administration of naloxone (see Tables 4.3, 4.4 and Figs. 4.3, 4.4).

FIGURE 4.4 - The responses of heart rate during 5 minute infusions of angiotensin II via a vertebral artery and intravenously at the doses indicated (ng/min) and a 5 minute period of bilateral common carotid artery occlusion (BCO) before and after an intravenous infusion of naloxone (1 μg/min for 5 minutes) in morphine and chloralose anaesthetised greyhounds. (* significant difference, $p<0.05$).

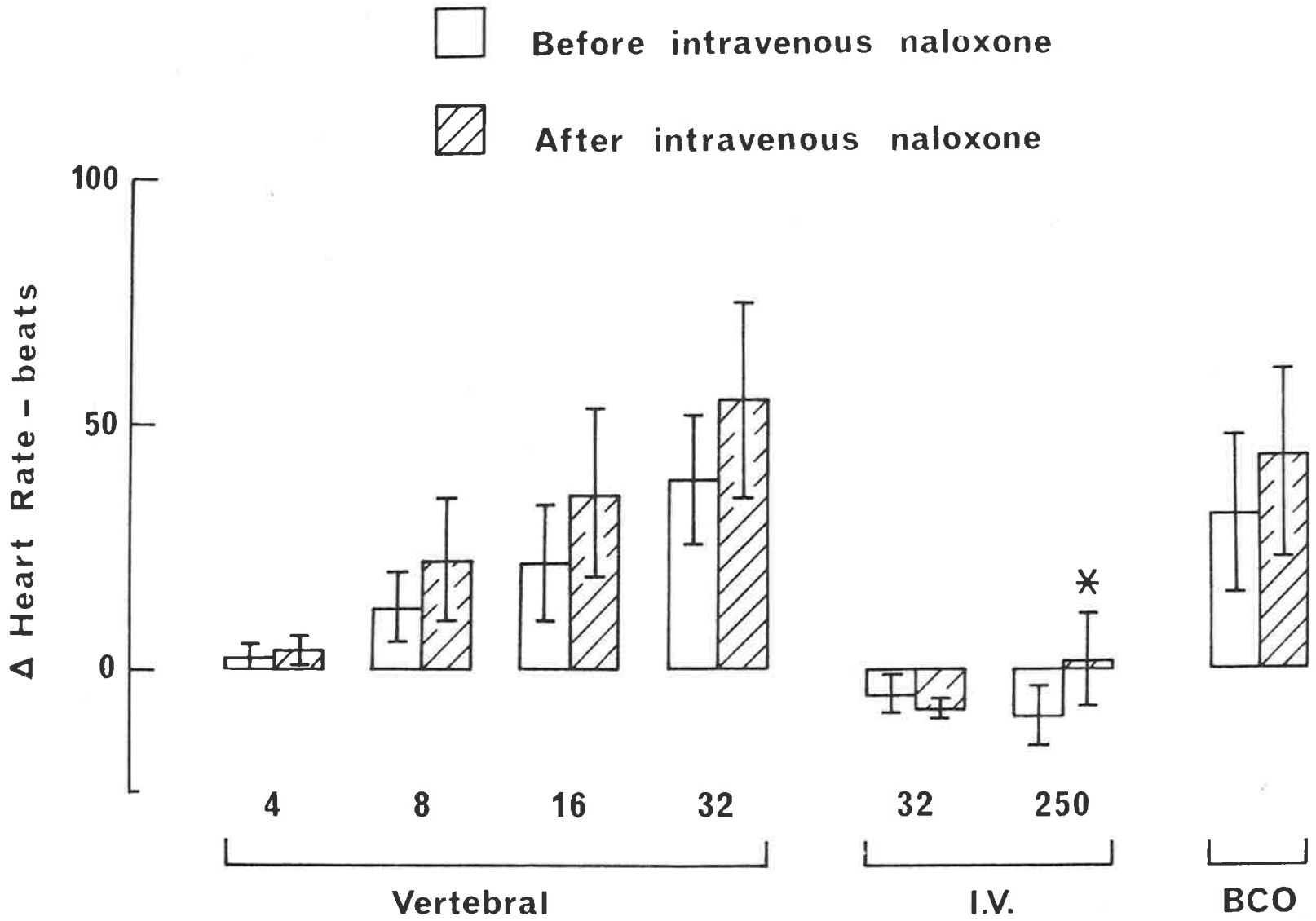
Before intravenous naloxone
After intravenous naloxone
Δ Heart Rate – beats
100
50
0
4
8
16
32
32
250
*
Vertebral
I.V.
BCO

FIGURE 4.4

GROUP 3

Control infusions of angiotensin II via a vertebral artery caused an increase in blood pressure and heart rate while intravenous angiotensin caused an increase in blood pressure and a small decrease in heart rate (Table 4.5, 4.6 and Figs. 4.5, 4.6). While the control responses of blood pressure were, on average, slightly larger in Group 1 experiments than in Group 3 experiments, this difference was not statistically significant (unpaired t-test, $p<0.05$). However, the control heart rate responses were significantly larger in Group 3 experiments during vertebral artery infusions of all doses of angiotensin II than in Group 1 experiments, but there was no significant difference between the heart rate responses during intravenous angiotensin II or bilateral carotid occlusion in the two groups of experiments.

In these dogs, which did not receive morphine as part of the anaesthetic regime, the administration of naloxone via a vertebral artery had no significant effect on the responses of blood pressure and heart rate during any of the procedures investigated (Tables 4.5, 4.6 and Figs. 4.5, 4.6). The resting levels of blood pressure were also essentially unchanged from control values (from 131.8 ± 3.3 to 131.4 ± 5.0 mm Hg), as was the resting heart rate (from 83.8 ± 13.2 to 87.0 ± 16.6 beats/min). However, in these dogs the resting blood pressure and heart rate were both significantly higher than those in morphine and chloralose dogs.

FIGURE 4.5 - The responses of mean blood pressure during 5 minute infusions of angiotensin II via a vertebral artery and intravenously at the doses indicated (ng/min) and a 5 minute period of bilateral common carotid artery occlusion (BCO) before and after a vertebral artery infusion of naloxone (1 μg/min for 5 minutes) in chloralose anaesthetised greyhounds.

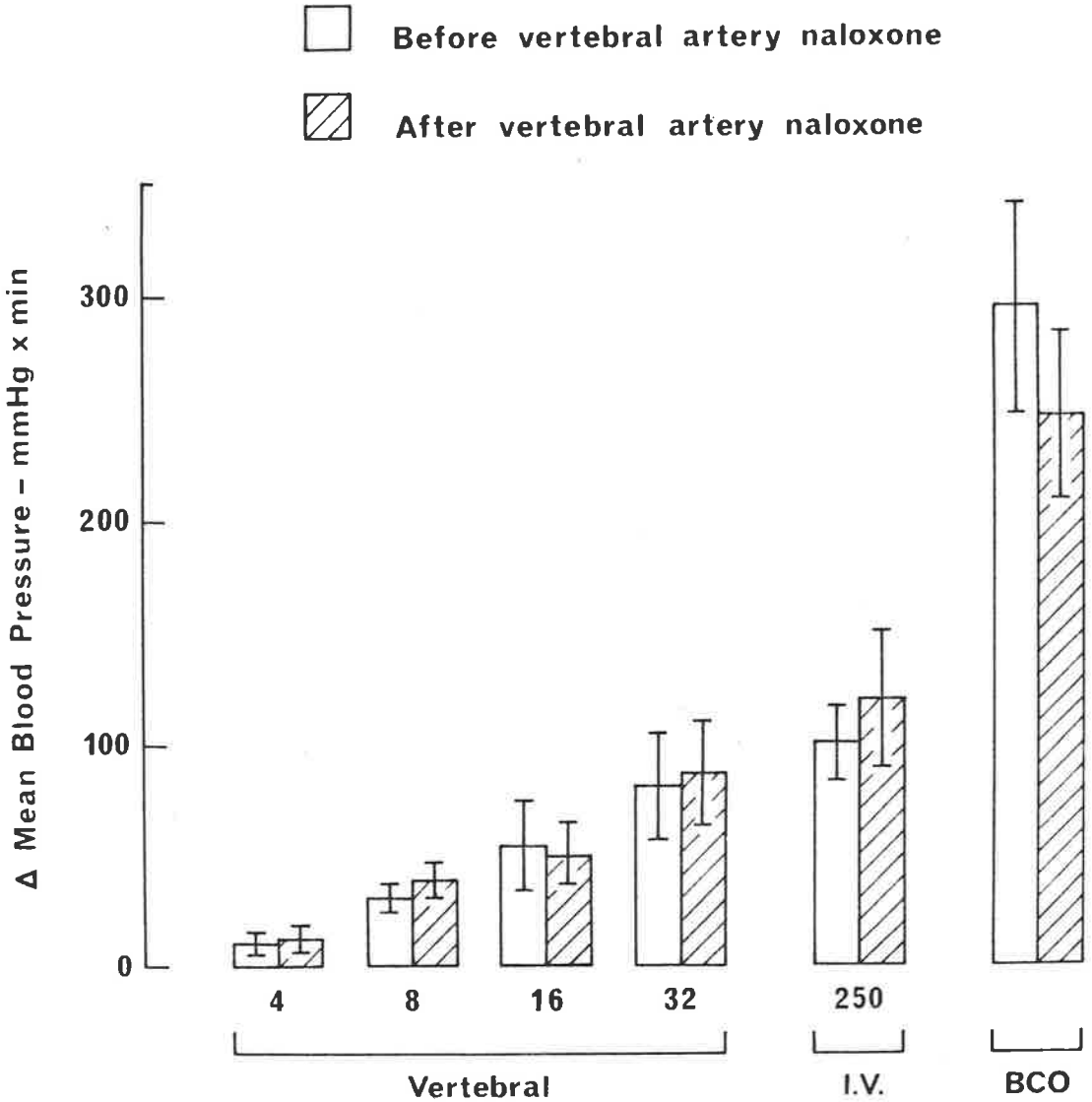
Before vertebral artery naloxone
After vertebral artery naloxone
Δ Mean Blood Pressure – mmHg x min
300
200
100
0
4
8
16
32
250
Vertebral
I.V.
BCO

FIGURE 4.5

FIGURE 4.6 - The responses of heart rate during 5 minute infusions of angiotensin II via a vertebral artery and intravenously at the doses indicated (ng/min) and a 5 minute period of bilateral common carotid artery occlusion (BCO) before and after a vertebral artery infusion of naloxone (1 μg/min for 5 minutes) in chloralose anaesthetised greyhounds.

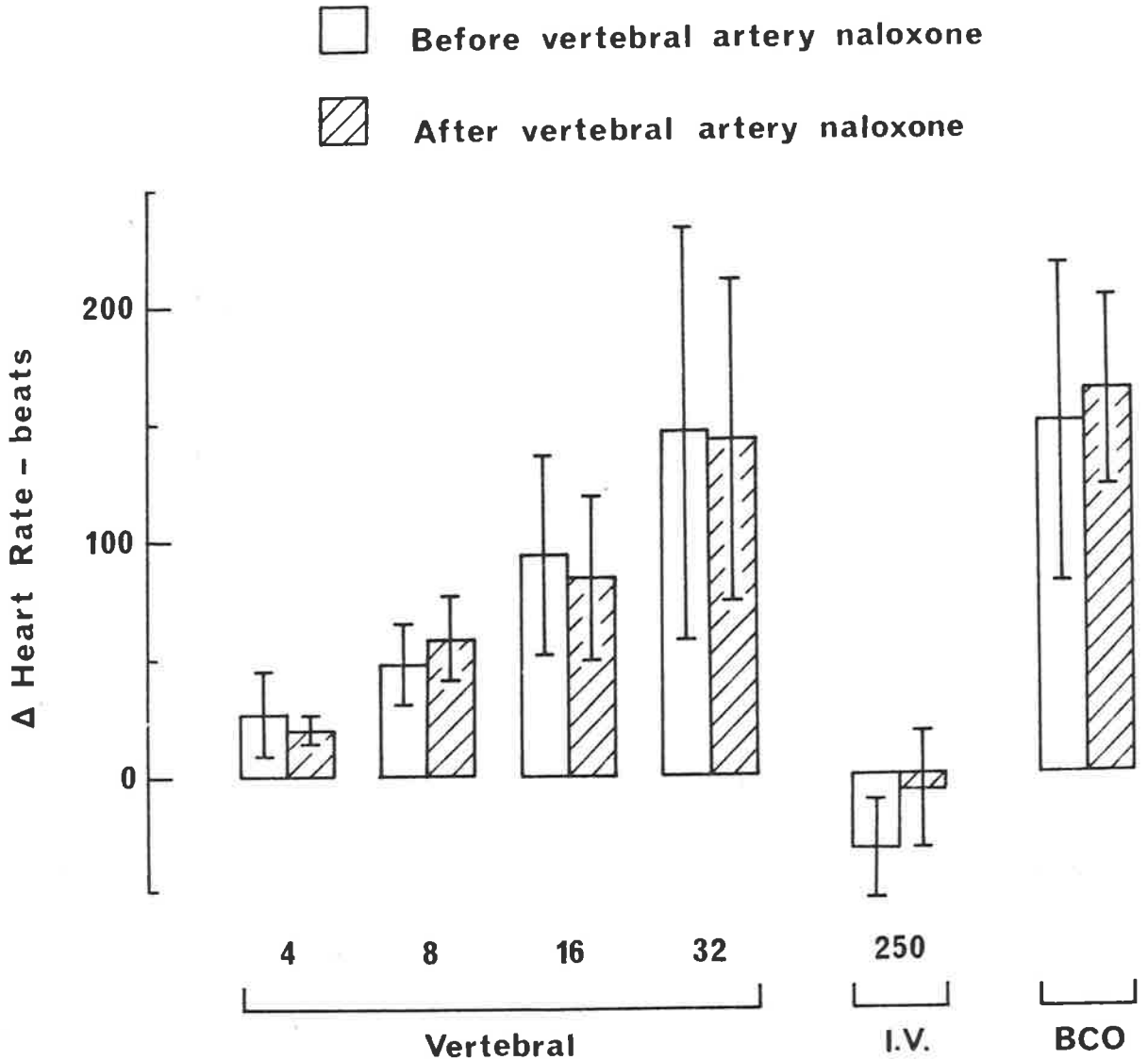
Before vertebral artery naloxone
After vertebral artery naloxone
Δ Heart Rate - beats
200
100
0
4
8
16
32
250
Vertebral
I.V.
BCO

FIGURE 4.6

DISCUSSION

Vertebral artery infusions of angiotensin II caused an increase in blood pressure and heart rate at all doses investigated in each of the different groups of experiments. Furthermore, there was no statistically significant difference in the magnitude of the blood pressure responses obtained in those dogs anaesthetised with morphine and chloralose compared to those anaesthetised with chloralose alone. These results confirm those obtained previously in this laboratory (Wilkinson, 1975), but are at variance with those of Szilagyi and Ferrario (1980, 1981), who observed a potentiation of the pressor response to vertebral artery angiotensin II when morphine was administered to mongrel dogs anaesthetised with chloralose alone.

In the experiments reported in this Chapter, the pressor responses to vertebral artery infusions of angiotensin II obtained in morphine and chloralose anaesthetised greyhounds were significantly potentiated following the vertebral artery infusion of a low dose of naloxone. A similar effect was obtained following an intravenous infusion of the same low dose of naloxone and the responses following vertebral artery naloxone were no different to those following intravenous naloxone. Since the pressor responses to intravenous angiotensin II were not significantly altered following either vertebral artery or intravenous naloxone administration, the effect of naloxone to potentiate the responses to vertebral artery angiotensin II is not due to an alteration in the peripheral vascular responsiveness to angiotensin II and is, therefore, probably a central action of naloxone. However, since the pressor responses to bilateral common carotid artery occlusion were not significantly altered following naloxone administration, the responsiveness of the baroreceptor reflex cardiovascular control mechanisms is not altered, yet naloxone still potentiates the blood pressure responses

to vertebral artery angiotensin. These results suggest that the endogenous opiate system could have an inhibitory effect on the central action of vertebral artery angiotensin II in the greyhound, which is opposite to the findings of Szilagyi and Ferrario (1980, 1981) in mongrel dogs, who reported that intravenous naloxone reduced the pressor responses to vertebral artery angiotensin II in both morphine-chloralose and chloralose anaesthetised dogs and concluded that both morphine and the endogenous opiate system potentiate the central action of angiotensin II. Further evidence for an inhibitory action of morphine on the central actions of angiotensin II is provided by the heart rate responses to vertebral artery angiotensin II in group 1 experiments in morphine and chloralose anaesthetised dogs and in Group 3 experiments in chloralose anaesthetised dogs (Tables 4.2, 4.6 and Figs. 4.2, 4.6). Following blockade of the effects of morphine in Group 1 experiments with naloxone, the heart rate responses to vertebral artery angiotensin II were not significantly different from those obtained in Group 3 experiments where morphine was not incorporated into the anaesthetic regime, suggesting morphine has an inhibitory effect on the response of heart rate.

The results reported in this Chapter obtained in greyhounds anaesthetised with chloralose alone are also at variance with those of Szilagyi and Ferrario. Naloxone had no effect on the pressor responses to vertebral artery angiotensin in these experiments but Szilagyi and Ferrario obtained a significant reduction in the magnitude of the pressor responses in mongrel dogs. These results in the chloralose anaesthetised greyhound suggest that the endogenous opiate system is not of major importance in the pressor response to vertebral artery angiotensin II (at least in the chloralose anaesthetised greyhound and that the effect of naloxone in the morphine and chloralose anaesthetised greyhound may simply be the result of blocking the central cardiovascular effects of exogenous morphine. So the

results presented in this Chapter suggest that that endogenous opiate system is not involved in the response to vertebral artery angiotensin II in the chloralose anaesthetised greyhound and that if morphine is used as part of the anaesthetic regime the pressor responses to angiotensin II will be, if anything, reduced in magnitude. Both of these results directly contradict those of Szilagyi and Ferrario.

The most likely explanation for the different results from our laboratory and those of Szilagyi and Ferrario is the different experimental animal employed in the experiments. It has already been shown that the increase in blood pressure during vertebral artery angiotensin in the mongrel dog is the result of an increase in sympathetic vasomotor activity through an action of angiotensin at the area postrema, whereas in the greyhound it is primarily withdrawal of cardiac vagal tone and a consequent increase in cardiac output. This difference in mechanism of action of angiotensin II, as well as the apparent different involvement of opiate mechanisms in mongrel dogs compared to the greyhound (as discussed in this Chapter), suggest that the mechanism of the pressor effect of vertebral artery angiotensin is different in the two species of dog, although an action at the area postrema is common to both. The precise mechanism of action of angiotensin II in the greyhound is still unknown, but probably does not involve the endogenous opiate system.

CONCLUDING REMARKS

CONCLUDING REMARKS

Bradykinin has been recognised as a powerful dilator compound since its discovery early in the 20th century. Following the determination of its peptide sequence in 1960 and its subsequent commercial availability in pure form, it was also reported that bradykinin elicited a powerful hypertensive response when administered via a carotid artery or directly into the cerebral ventricles. Even though a considerable amount of research has been conducted to define the physiological pathways for the cardiovascular responses to both systemically and centrally-administered bradykinin, the mechanism(s) involved in the centrally-mediated response have still not been identified, although involvement of the autonomic nervous system has been implicated in many species. However, there is conflicting evidence concerning the relative involvement of the various receptors in the autonomic nervous system.

From the experiments reported in Chapter 2 of this thesis using the morphine and chloralose anaesthetised greyhound as the experimental animal, the hypertensive response to both vertebral artery and carotid artery bradykinin is mediated by an increase in cardiac output which, in turn, is mediated by an increase in heart rate due primarily to a decrease in vagal efferent activity to the heart. However, some contribution of the sympathetic nervous system to the observed cardiovascular effects is possible since pharmacological blockade of α and β-receptors did modify the responses but not to the extent where all central effects were eliminated. Furthermore, the order of α and β-receptor blockade indicated differing contributions from these receptors. This difference strongly suggests that the removal of one of the possible pathways may result in the animal subsequently using a different pathway to cause the same effect. For example, if an animal

used an increase in heart rate as the mechanism for a particular response and this increase in heart rate was caused equally by an increase in sympathetic activity to the heart and a concurrent decrease in parasympathetic activity, following pharmacological inhibition of sympathetic activity, the animal could utilise only the remaining parasympathetic pathway to cause the same change in heart rate. Given this possibility, one would conclude from the above experiment that the sympathetic nervous system was not involved in the particular response because the animal subsequently utilised an alternative pathway to cause the same final effect. This "switching pathways" phenomenon could conceivably explain the conflicting conclusions from Chapter 2 experiments following the administration of propranolol and phentolamine in the different groups of experiments.

Nevertheless, it has been established that the autonomic nervous system is involved in the hypertensive responses to vertebral artery and carotid artery bradykinin, and that the vagus nerves are of major importance. The central site of action is the area postrema since the integrity of this area is essential for the hypertensive response and a central cholinergic mechanism (unknown at present) and the formation of prostaglandins in the brain are also probably involved in this response.

While both bradykinin and angiotensin, another peptide of similar molecular weight, have been shown to have specific centrally-mediated actions to cause hypertension, they clearly do not have the same mechanism of action, although some aspects of their actions appear to be the same. Both vertebral artery angiotensin II and bradykinin act at the area postrema to cause an increase in arterial blood pressure, the primary mechanism being an increase in heart rate and cardiac output as a result of withdrawal of cardiac vagal tone. However,

major differences in the effects of these two peptides on the cardiovascular system are apparent following carotid artery administration. Angiotensin II is only weakly active and has no central action when administered via a carotid artery, whereas bradykinin is still strongly pressor via this route of administration and appears to utilise a similar mechanism of action to bradykinin administered via a carotid artery. Since the vertebral arteries and carotid arteries supply different areas of the brain, the apparent "common mechanism" of action of vertebral artery and carotid artery bradykinin was surprising, and possible explanations are presented in this thesis.

One further aspect of the central action of angiotensin II is also discussed in Chapter 4. The precise action of angiotensin II is not known, but the endogenous opiate system has been suggested to potentiate the actions of angiotensin II. Evidence is presented in this thesis to suggest that morphine, and probably the endogenous opiates, attenuate the actions of angiotensin II.

Even though bradykinin is a powerful hypotensive and hypertensive substance, depending on the route of administration, and that humans potentially have massive amounts of bradykinin in the circulation (in inactive form), the involvement of bradykinin in control of the circulation or in the possible pathogenesis of hypertension is unproven. If hyperbradykininism was to be subsequently identified as a cause of hypertension, some possible mechanisms of this effect have been examined and discussed in this thesis. The availability of a specific bradykinin antagonist would assist in determining a physiological role for bradykinin.

A P P E N D I C E S

APPENDIX 1

APPENDIX 1

Samples of bradykinin obtained from different sources produced blood pressure and heart rate responses of different magnitudes when infused via a vertebral artery, carotid artery and intravenously. Sandoz BRS-640 and Protein Research Foundation (PRF) compounds were pure bradykinin peptide (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) while Sigma, Bachem and "bulk" PRF samples were bradykinin triacetate (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg.2AcOH.$4H_2O$). Even allowing for the different molecular weights (pure bradykinin 1060 and bradykinin triacetate 1252) the effects of the samples on the cardiovascular system differed considerably in magnitude.

Sandoz bradykinin was obtained in solution in sealed glass ampoules (90 μg/ml) and was diluted in normal saline (sodium chloride, 0.9% w/v) immediately before infusion into the dog. Sigma and "pure" PRF bradykinin were obtained in sealed containers containing 1 mg of powder while Bachem and "bulk" PRF bradykinin were obtained in sealed containers containing 25 mg of powder. In the case of Bachem and "bulk" PRF bradykinin, the powder was dissolved in distilled water and aliquotted into tubes such that each tube contained 500 μg of bradykinin peptide (i.e. allowance was made for the contribution of 2AcOH.$4H_2O$ to the weight of the compound). These tubes were then evaporated to dryness, either by freeze-drying or Vortex-evaporating under reduced pressure, capped tightly and stored at -20°C. It is unlikely that the above procedure would result in loss of biological activity since samples of another peptide (angiotensin II) prepared in the same manner could be stored for long periods without loss of activity compared to a "fresh" sample.

To further examine possible differences between the various samples, 10 μg of each was spotted onto thin layer cellulose paper and developed

in the following solvent: n-Butanol, 15 ml; Pyridine, 10 ml; acetic acid, 3 ml; distilled water, 12 ml. Two plates were developed as described above. One was stained using the Sakaguchi:α-naphthol technique (Dawson, Elliott, Elliott and Jones, 1959a) and one sprayed with 0.2% ninhydrin (Dawson *et al.*, 1959b). The Sakaguchi method stains for arginine, while ninhydrin detects amino acids. With both techniques, four spots (corresponding with the four samples spotted) were obtained and all four spots moved the same distance. According to these tests, the samples appeared to be identical and pure (using Sandoz BRS-640 as the standard for "purity") and no difference in biological activity would be expected.

The reason for differences in activity are still not known. It was therefore decided to use bradykinin from the same supplier for all remaining experiments in Chapters 2 and 3, and we chose bradykinin triacetate from the Protein Research Foundation, Japan.

TABLES

TABLE 1.1

The relationship between the dose of bradykinin and the change in mean blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during 5 minute vertebral artery infusions at the rate indicated.

DOG	INFUSION RATE (μg/min)									
	1		2		5		10		20	
	BP	HR	BP	HR	BP	HR	BP	HR	BP	HR
1	61	26	35	48	37	79	91	264	-	-
2	31	0	41	0	69	53	88	111	-	-
3	12	17	24	19	35	19	52	31	-	-
4	25	42	57	40	55	48	59	73	-	-
5	27	13	60	39	57	46	101	131	-	-
6	68	32	95	33	123	55	144	97	-	-
11	-	-	-	-	40	0	85	17	145	286
15	-	-	-	-	-	-	-	-	227	470
16	-	-	-	-	-	-	-	-	146	196
MEAN	37.3	21.7	52.0	29.8	59.4	42.9	88.6	103.4	172.7	317.3
SEM	9.9	6.6	11.2	7.8	12.5	10.5	12.3	33.5	33.3	98.8

TABLE 1.2

The relationship between the dose of bradykinin and the change in mean blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during 5 minute carotid artery infusions at the rate indicated.

DOG	INFUSION RATE (μg/min)									
	1		2		5		10		20	
	BP	HR	BP	HR	BP	HR	BP	HR	BP	HR
1	10	0	20	17	45	28	60	116	-	-
2	9	12	10	12	22	33	62	67	-	-
3	24	30	21	22	42	43	33	32	-	-
4	23	27	42	23	38	27	70	46	-	-
5	41	19	46	30	53	30	72	66	-	-
6	24	20	0	18	134	65	136	68	-	-
11	-	-	-	-	61	29	56	18	137	168
15	-	-	-	-	-	-	-	-	70	156
16	-	-	-	-	-	-	-	-	80	106
MEAN	21.8	18.0	23.2	20.3	56.4	36.4	69.9	59.0	95.7	143.3
SEM	5.2	4.9	8.0	2.8	14.8	5.6	13.0	12.9	25.6	23.3

TABLE 1.3

The relationship between the dose of bradykinin and the change in mean blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during 5 minute intravenous infusions at the rate indicated.

DOG	INFUSION RATE (μg/min)									
	1		2		5		10		20	
	BP	HR	BP	HR	BP	HR	BP	HR	BP	HR
1	-20	0	0	0	-13	28	-17	137	-	-
2	0	0	-10	46	-16	64	-26	130	-	-
3	0	0	0	0	0	16	-4	55	-	-
4	0	0	0	14	-9	34	-13	212	-	-
5	0	0	0	0	5	37	-18	74	-	-
6	0	0	0	5	-7	22	-24	48	-	-
11	-	-	-	-	-10	24	-12	42	-34	178
15	-	-	-	-	-	-	-	-	-11	324
16	-	-	-	-	-	-	-	-	-16	186
MEAN	-3.3	0	-1.7	10.8	-7.1	32.1	-16.3	99.7	-20.3	229.3
SEM	3.7	0	1.8	8.1	3.0	6.4	3.1	25.6	8.6	58.0

TABLE 1.4

Responses of mean blood pressure (BP - mm Hg x min) and heart rate (HR - beats) to 5 minute infusions of bradykinin (10 μg/min) via a vertebral artery (VA), carotid artery (CA) and intravenously (IV) before, and in the presence of, vertebral artery infusions of SQ 20,881 (10 μg/min)

DOG	BP (mm Hg x min)						HR (beats)					
	BEFORE SQ			DURING SQ			BEFORE SQ			DURING SQ		
	VA	CA	IV	VA	CA	IV	VA	CA	IV	VA	CA	IV
3	52	33	-4	111	73	-25	31	32	55	114	110	160
4	59	70	-13	78	62	-20	73	46	212	260	78	244
5	101	72	-18	122	122	-26	131	66	74	212	312	84
6	144	136	-24	146	195	-33	97	68	48	138	196	120
MEAN	89.0	77.8	-14.8	114.3	113.0	-26.0	83.0	53.0	97.3	181.0	174.0	152.0
SEM	24.6	24.7	4.9	16.3	35.0	3.1	24.3	9.9	44.6	38.8	60.1	39.7

TABLE 1.5

The changes of blood pressure (BP - mm Hg) and heart rate (beats/min) to 5 minute infusions of bradykinin (10 μg/min) via a vertebral artery (VA), carotid artery (CA) and intravenously (IV) before, and in the presence of, a vertebral artery infusion of SQ 20,881 (10 μg/min).

DOG	ΔBP (mm Hg)						ΔHR (beats/min)					
	BEFORE SQ			DURING SQ			BEFORE SQ			DURING SQ		
	VA	CA	IV	VA	CA	IV	VA	CA	IV	VA	CA	IV
3	11	6	-6	26	12	-26	10	10	24	45	41	81
4	13	18	-23	16	14	-25	16	14	58	62	40	72
5	19	14	-10	20	27	-7	37	18	23	35	71	21
6	35	33	-29	44	52	-27	34	18	42	62	76	62
MEAN	19.5	17.8	-17.0	26.5	26.3	-21.3	24.3	15.0	36.8	51.0	57.0	59.0
SEM	6.3	6.5	6.3	7.1	10.6	5.5	7.7	2.2	9.6	1.7	11.1	15.3

TABLE 2.1

The effects of propranolol, propranolol and vagal cooling, propranolol and phentolamine, propranolol and phentolamine and vagal cooling on the responses of mean arterial blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during vertebral artery infusions of bradykinin.

DOG	NORMAL		PROPRANOLOL		PROPRANOLOL AND VAGAL COOLING		PROPRANOLOL AND PHENTOLAMINE		PROPRANOLOL AND PHENTOLAMINE AND VAGAL COOLING	
	BP	HR	BP	HR	BP	HR	BP	HR	BP	HR
16	146	196	72	51	22	27	16	198	-86	41
17	112	182	49	33	14	12	25	64	-55	47
18	110	263	43	54	0	0	36	119	-23	0
19	78	110	28	65	0	0	0	31	-37	0
20	68	95	48	56	-3	7	49	110	-18	98
MEAN	102.8	169.2	48.0	51.8	6.6	9.2	25.2	104.4	-43.8	37.2
SEM	15.5	34.2	7.9	5.9	5.4	5.6	9.4	31.6	13.8	20.3

TABLE 2.2

The effects of propranolol, propranolol and vagal cooling, propranolol and phentolamine, propranolol and phentolamine and vagal cooling on the responses of mean arterial blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during carotid artery infusions of bradykinin.

DOG	NORMAL		PROPRANOLOL		PROPRANOLOL AND VAGAL COOLING		PROPRANOLOL AND PHENTOLAMINE		PROPRANOLOL AND PHENTOLAMINE AND VAGAL COOLING	
	BP	HR	BP	HR	BP	HR	BP	HR	BP	HR
16	80	106	68	57	58	33	7	130	-104	41
17	51	84	23	15	0	0	12	31	-17	20
18	105	247	23	31	49	0	40	117	-33	0
19	55	89	27	85	73	0	0	28	11	0
20	16	81	32	49	42	14	9	84	-45	42
MEAN	61.4	121.4	34.6	47.4	44.4	9.4	13.6	78.0	-37.6	20.6
SEM	16.7	35.4	9.5	13.3	13.7	7.3	7.7	23.7	21.3	10.4

TABLE 2.3

The effects of propranolol, propranolol and vagal cooling, propranolol and phentolamine, propranolol and phentolamine and vagal cooling on the responses of mean arterial blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during intravenous infusions of bradykinin.

DOG	NORMAL		PROPRANOLOL		PROPRANOLOL AND VAGAL COOLING		PROPRANOLOL AND PHENTOLAMINE		PROPRANOLOL AND PHENTOLAMINE AND VAGAL COOLING	
	BP	HR	BP	HR	BP	HR	BP	HR	BP	HR
16	-16	186	-15	97	-51	17	-112	113	-205	40
17	-45	96	-58	93	-53	42	-37	131	-108	35
18	-25	121	-38	52	-64	0	-33	188	-80	0
19	-50	95	-29	58	-38	0	-55	71	-107	0
20	-40	113	-38	77	-45	12	-67	145	-114	51
MEAN	-35.2	122.2	-35.6	75.4	-46.2	14.2	-60.8	129.6	-122.8	25.2
SEM	7.1	18.7	7.8	10.1	6.0	8.6	15.9	21.4	23.9	11.9

TABLE 2.4

The effects of phentolamine, phentolamine and propranolol, phentolamine and propranolol and bilateral vagotomy on the responses of mean arterial blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during vertebral artery infusions of bradykinin.

DOG	NORMAL		PHENTOLAMINE		PHENTOLAMINE AND PROPRANOLOL		PHENTOLAMINE AND PROPRANOLOL AND VAGOTOMY	
	BP	HR	BP	HR	BP	HR	BP	HR
24	58	369	-45	0	-27	0	-60	0
26	6	118	-2	150	-36	0	-38	8
27	183	265	82	139	33	111	-140	10
28	50	98	0	145	-15	77	-28	19
29	82	338	39	0	-6	0	-20	23
30	69	315	-56	0	-29	25	-62	64
MEAN	74.7	250.5	3.0	72.3	-13.3	35.5	-58.0	20.7
SEM	26.4	51.7	23.1	35.5	11.2	21.3	19.5	10.2

TABLE 2.5

The effects of phentolamine, phentolamine and propranolol, phentolamine and propranolol and bilateral vagotomy on the responses of mean arterial blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during carotid artery infusions of bradykinin.

DOG	NORMAL		PHENTOLAMINE		PHENTOLAMINE AND PROPRANOLOL		PHENTOLAMINE AND PROPRANOLOL AND VAGOTOMY	
	BP	HR	BP	HR	BP	HR	BP	HR
24	-37	112	-31	0	-28	0	-31	0
26	64	278	-20	0	-28	0	-73	21
27	146	125	81	115	51	202	-106	56
28	84	89	44	276	33	100	9	69
29	75	211	-10	0	-4	0	-34	12
30	76	276	-90	0	-27	20	-22	68
MEAN	68.0	181.8	-4.3	65.2	-0.5	53.7	-42.8	37.7
SEM	26.5	37.8	26.8	50.6	15.5	36.8	18.2	13.6

TABLE 2.6

The effects of phentolamine, phentolamine and propranolol, phentolamine and propranolol and bilateral vagotomy on the responses of mean arterial blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during intravenous infusions of bradykinin.

DOG	NORMAL		PHENTOLAMINE		PHENTOLAMINE AND PROPRANOLOL		PHENTOLAMINE AND PROPRANOLOL AND VAGOTOMY	
	BP	HR	BP	HR	BP	HR	BP	HR
24	-99	272	-104	0	-94	52	-128	12
26	-39	238	-52	0	-120	66	-189	14
27	-21	217	-49	344	-48	153	-209	83
28	-28	55	-53	0	-67	104	-114	80
29	-13	152	-109	0	-64	0	-123	20
30	-20	238	-96	0	-108	82	-146	113
MEAN	-36.7	195.3	-77.2	57.3	-83.5	76.2	-151.5	53.7
SEM	14.2	35.5	12.8	62.8	12.6	23.0	17.3	19.5

TABLE 2.7

The effects of (A) phentolamine, (B) phentolamine and propranolol, and (C) phentolamine, propranolol and bilateral vagotomy on the responses of cardiac ouput (C.O. - litres/min) and total peripheral vascular resistance (T.P.R. - mm Hg x min x l^{-1}) before and during a 5 minute vertebral artery infusion of bradykinin (20 μg/min).

	C.O.								T.P.R.							
	NORMAL		A		B		C		NORMAL		A		B		C	
	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING
	1.69	2.96	1.98	2.36	1.42	1.64	1.48	2.04	58.6	37.8	35.4	24.6	54.2	42.7	76.4	47.5
	2.17	3.41	4.48	5.33	2.43	3.43	3.32	3.67	38.3	24.9	19.2	13.9	30.9	19.5	32.8	25.6
	1.45	4.20	2.17	3.18	2.08	3.03	2.93	2.96	64.8	29.0	35.5	29.9	41.3	30.4	49.8	35.8
	2.91	3.77	3.94	4.25	2.74	3.03	2.66	3.04	30.6	26.3	26.4	24.5	39.8	34.3	43.6	35.9
	3.10	5.42	2.94	4.00	1.87	1.96	2.39	2.58	33.9	23.8	33.0	27.3	58.8	55.1	48.5	41.1
	1.98	3.60	1.98	3.60	1.72	2.29	1.84	2.62	50.5	32.5	40.9	22.0	49.4	27.9	58.7	32.8
MEAN	2.22	3.89	2.92	3.79	2.04	2.56	2.44	2.82	46.1	29.1	31.7	23.7	45.7	35.0	51.6	36.5
SEM	0.27	0.35	0.44	0.41	0.20	0.29	0.28	0.22	5.7	2.2	3.2	2.2	4.2	5.1	6.0	3.0

TABLE 2.8

The effects of (A) phentolamine, (B) phentolamine and propranolol, and (C) phentolamine, propranolol and bilateral vagotomy on the responses of cardiac output (C.O. - litres/min) and total peripheral vascular resistance (T.P.R. - mm Hg x min x l^{-1}) before and during a 5 minute carotid artery infusion of bradykinin (20 μg/min).

	C.O.								T.P.R.							
	NORMAL		A		B		C		NORMAL		A		B		C	
	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING
	1.69	2.60	1.78	2.35	1.38	1.47	-	-	51.5	29.2	41.6	27.7	60.1	51.0	-	-
	2.52	4.88	5.09	5.45	2.90	4.56	3.52	3.46	32.9	20.1	14.3	11.4	28.6	16.0	28.9	23.9
	2.03	2.88	2.25	2.96	1.84	2.54	2.46	3.31	44.8	42.4	34.2	31.4	48.4	39.0	56.9	30.5
	3.00	3.93	3.73	4.63	2.54	2.78	2.32	2.86	29.7	28.8	27.9	24.6	40.9	39.6	50.0	42.3
	3.16	3.85	2.61	3.41	1.73	2.04	1.99	2.98	34.2	34.8	37.9	27.9	64.7	53.4	57.8	33.9
	2.16	3.37	2.16	3.37	2.01	2.03	1.91	2.59	47.2	38.6	42.5	21.8	45.8	37.9	59.2	40.5
MEAN	2.43	3.59	2.94	3.70	2.07	2.57	2.44	3.04	40.1	32.3	33.1	24.1	48.1	39.5	50.6	34.2
SEM	0.23	0.34	0.51	0.47	0.23	0.44	0.29	0.16	3.6	3.3	4.3	2.9	5.3	5.4	5.6	3.4

TABLE 2.9

The effects of (A) phentolamine, (B) phentolamine and propranolol and (C) phentolamine, propranolol and bilateral vagotomy on the responses of cardiac output (C.O. - litres/min) and total peripheral vascular resistance (T.P.R. - mm Hg x min x l^{-1}) before and during a 5 minute intravenous infusion of bradykinin (20 μg/min).

	C.O.								T.P.R.							
	NORMAL		A		B		C		NORMAL		A		B		C	
	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING
	1.82	2.31	1.66	2.34	1.24	1.82	-	-	46.2	26.0	44.0	20.1	70.2	34.6	-	-
	4.34	5.75	4.72	4.31	2.95	3.33	2.75	3.66	19.6	10.6	13.3	10.7	29.5	17.7	38.9	16.9
	1.66	3.40	2.21	3.52	1.77	2.96	2.60	3.02	48.2	17.1	31.7	13.9	49.7	18.2	53.8	28.5
	2.90	4.16	3.86	5.98	2.40	4.12	2.44	3.65	31.7	20.4	27.2	15.2	42.1	19.2	45.5	23.0
	2.92	3.84	2.68	3.29	2.08	2.16	1.54	2.36	37.7	27.6	38.4	23.7	49.5	36.1	72.7	30.5
	2.43	3.30	2.43	3.30	1.72	2.54	2.21	3.30	43.2	30.3	28.3	16.9	55.2	24.8	48.4	20.3
MEAN	2.85	3.79	2.93	3.79	2.03	2.82	2.31	3.20	37.8	22.0	30.5	16.8	49.4	25.1	51.9	23.8
SEM	0.44	0.47	0.47	0.51	0.24	0.34	0.21	0.24	4.4	3.0	4.3	1.9	5.5	3.4	5.7	2.5

TABLE 2.10

The effects of phentolamine, phentolamine and propranolol, phentolamine and propranolol and bilateral vagotomy on the responses of stroke volume (ml) before and during vertebral artery infusions of bradykinin.

DOG	NORMAL		PHENTOLAMINE		PHENTOLAMINE AND PROPRANOLOL		PHENTOLAMINE AND PROPRANOLOL AND VAGOTOMY	
	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING
24	37	20	15	18	21	24	24	32
26	78	38	75	51	26	37	36	39
27	56	47	54	42	47	48	31	29
28	50	40	41	33	22	22	19	21
29	54	36	13	17	18	19	23	23
30	31	25	11	16	17	22	20	24
MEAN	51.0	34.3	34.8	29.5	25.2	28.7	25.5	28.0
SEM	7.4	4.5	11.8	6.6	5.0	5.1	3.0	3.0

TABLE 2.11

The effects of phentolamine, phentolamine and propranolol, phentolamine and propranolol and bilateral vagotomy on the responses of stroke volume (ml) before and during carotid artery infusions of bradykinin.

DOG	NORMAL		PHENTOLAMINE		PHENTOLAMINE AND PROPRANOLOL		PHENTOLAMINE AND PROPRANOLOL AND VAGOTOMY	
	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING
24	22	24	12	16	23	25	-	-
26	66	44	30	32	31	49	39	36
27	72	41	54	40	48	41	26	31
28	50	45	43	29	22	20	16	18
29	47	37	11	15	17	19	18	27
30	33	24	10	15	21	20	21	24
MEAN	48.3	35.8	26.7	24.5	27.0	29.0	24.0	27.2
SEM	8.5	4.3	8.4	4.8	5.0	5.7	4.6	3.4

TABLE 2.12

The effects of phentolamine, phentolamine and propranolol, phentolamine and propranolol and bilateral vagotomy on the responses of stroke volume (ml) before and during intravenous infusions of bradykinin.

DOG	NORMAL		PHENTOLAMINE		PHENTOLAMINE AND PROPRANOLOL		PHENTOLAMINE AND PROPRANOLOL AND VAGOTOMY	
	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING
24	23	18	12	16	28	30	-	-
26	72	50	28	26	35	36	31	40
27	59	37	53	28	57	40	28	27
28	48	56	56	23	22	30	17	22
29	42	41	12	14	20	21	14	21
30	31	25	14	16	19	23	24	26
MEAN	45.8	37.8	29.2	20.5	30.2	30.0	22.8	27.2
SEM	8.1	6.5	9.2	2.6	6.5	3.3	3.6	3.8

TABLE 2.13

The effects of bilateral vagotomy, bilateral vagotomy and propranolol, bilateral vagotomy and propranolol and phentolamine on the responses of mean arterial blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during vertebral artery infusions of bradykinin.

DOG	NORMAL		VAGOTOMY		VAGOTOMY AND PROPRANOLOL		VAGOTOMY AND PROPRANOLOL AND PHENTOLAMINE	
	BP	HR	BP	HR	BP	HR	BP	HR
48	95	147	0	30	10	0	-6	0
49	160	200	0	183	30	0	-38	9
50	122	201	-49	152	-43	0	-54	27
51	67	87	-156	0	-112	6	-73	40
52	98	223	-107	0	-67	0	-61	0
53	92	105	-19	240	-17	0	-23	28
MEAN	105.7	160.5	-55.2	100.8	-33.2	1.0	-42.5	17.3
SEM	13.0	22.9	26.0	42.5	21.3	1.0	10.2	6.8

TABLE 2.14

The effects of bilateral vagotomy, bilateral vagotomy and propranolol, bilateral vagotomy and propranolol and phentolamine on the responses of mean arterial blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during carotid artery infusions of bradykinin.

DOG	NORMAL		VAGOTOMY		VAGOTOMY AND PROPRANOLOL		VAGOTOMY AND PROPRANOLOL AND PHENTOLAMINE	
	BP	HR	BP	HR	BP	HR	BP	HR
48	58	67	50	59	32	0	15	19
49	86	104	-47	144	10	0	-39	4
50	33	65	-66	122	-119	0	-74	23
51	46	91	-176	0	-154	0	-85	35
52	36	139	-88	0	-80	0	-73	0
53	55	64	-49	176	-46	5	-41	22
MEAN	52.3	88.3	-62.7	83.5	-59.5	0.8	-49.5	17.2
SEM	7.9	12.1	29.8	30.7	29.6	0.8	15.0	5.3

TABLE 2.15

The effects of bilateral vagotomy, bilateral vagotomy and propranolol, bilateral vagotomy and propranolol and phentolamine on the responses of mean arterial blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during intravenous infusions of bradykinin.

DOG	NORMAL		VAGOTOMY		VAGOTOMY AND PROPRANOLOL		VAGOTOMY AND PROPRANOLOL AND PHENTOLAMINE	
	BP	HR	BP	HR	BP	HR	BP	HR
48	-14	49	-34	45	-22	0	-63	25
49	-33	225	-149	191	-240	0	-180	4
50	-14	121	-128	148	-187	0	-181	22
51	-28	143	-291	26	-216	0	-171	38
52	-81	175	-180	0	-167	0	-143	0
53	-39	133	-161	179	-138	6	-172	26
MEAN	-34.8	141.0	-157.2	98.2	-161.7	1.0	-151.7	19.2
SEM	10.1	23.9	33.9	34.3	31.5	1.0	18.6	5.9

TABLE 3.1

The changes in mean tension developed during contraction (ΔT - grams) and heart rate (ΔHR - beats/min) following administration of adrenaline or bradykinin to the isolated rabbit heart.

DRUG / EXPT. NO.	ΔT 1	2	3	4	5	MEAN ± SEM
Adrenaline 1 µg	7	5.4	3.8	5.0	5.8	5.4 ± 0.5
Bradykinin 1 ng	-	0	0	0	0	0
Bradykinin 10 ng	0	0	0	0	0	0
Bradykinin 5 µg	0	0	0	0	0	0
Bradykinin 10 µg	0	-	-	0	0	0
Bradykinin 20 µg	-	-	0	0	0	0

DRUG / EXPT. NO.	ΔHR 1	2	3	4	5	MEAN ± SEM
Adrenaline 1 µg	65	150	90	120	100	105.0 ± 14.3
Bradykinin 1 ng	-	0	0	0	0	0
Bradykinin 10 ng	0	0	0	0	0	0
Bradykinin 5 µg	0	0	0	0	0	0
Bradykinin 10 µg	0	-	-	0	0	0
Bradykinin 20 µg	-	-	0	0	0	0

TABLE 3.2

The effects of contralateral carotid sinus nerve section (see Methods: Chapter 3, Section 2) and bilateral carotid sinus nerve section on the responses of mean arterial blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during vertebral artery infusions of bradykinin.

DOG	CONTROL		CONTRALATERAL CSN SECTION		BILATERAL CSN SECTION	
	BP	HR	BP	HR	BP	HR
68	32	268	-12	53	16	163
69	72	123	48	83	44	131
70	92	84	23	72	-61	459
71	82	82	122	164	49	267
MEAN	69.5	139.3	45.3	93.0	12.0	255.0
SEM	13.1	43.9	28.4	24.5	25.4	73.9

TABLE 3.3

The effects of contralateral carotid sinus nerve section (see Methods: Chapter 3, Section 2) and bilateral carotid sinus nerve section on the responses of mean arterial blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during carotid artery infusions of bradykinin.

DOG	CONTROL		CONTRALATERAL CSN SECTION		BILATERAL CSN SECTION	
	BP	HR	BP	HR	BP	HR
68	75	130	65	232	0	77
69	35	87	44	295	110	343
70	48	85	60	89	-15	265
71	64	44	58	17	-11	0
MEAN	55.5	86.5	56.8	158.3	21.0	171.3
SEM	8.8	17.6	4.5	63.8	29.8	79.8

TABLE 3.4

The effects of contralateral carotid sinus nerve section (see Methods: Chapter 3, Section 2) and bilateral carotid sinus nerve section on the responses of mean arterial blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during intravenous infusions of bradykinin.

DOG	CONTROL		CONTRALATERAL CSN SECTION		BILATERAL CSN SECTION	
	BP	HR	BP	HR	BP	HR
68	-47	164	-45	156	-69	178
69	-25	146	-56	191	-133	269
70	-47	133	-54	115	-133	418
71	-19	155	-17	217	-129	302
MEAN	-34.5	149.5	-43.0	169.8	-116.0	291.8
SEM	7.3	6.6	9.0	22.1	15.7	49.6

TABLE 3.5

The relationship between dose of arginine-vasopressin and the change in mean blood pressure (BP - mm Hg x min) and heart rate (HR - beats) during 5 minute vertebral artery, carotid artery and intravenous infusions at the rate indicated.

ROUTE OF ADMINISTRATION	INFUSION RATE (mUnits/min)							
	5		20		50		100	
	BP	HR	BP	HR	BP	HR	BP	HR
VERTEBRAL ARTERY	0	0	70	0	67	-40	-	-
	-	-	28	-60	23	-83	0	-148
	-	-	0	-28	0	-127	78	-141
MEAN	0	0	32.6	-29.3	30.0	-83.3	39.0	-144.5
SEM	0	0	20.3	17.3	19.7	25.1	39.0	3.5
CAROTID ARTERY	0	0	21	0	59	-42	-	-
	-	-	0	-29	27	-79	0	-52
	-	-	23	-104	11	-127	14	-214
MEAN	0	0	14.7	-44.3	32.3	-82.7	7.0	-133.0
SEM	0	0	7.4	31.0	14.1	24.6	7.0	81.0
INTRAVENOUS	0	0	16	0	90	-34	-	-
	-	-	0	-75	63	-77	0	-147
	-	-	0	-88	108	-131	117	-131
MEAN	0	0	5.3	-54.3	87.0	-80.7	58.5	-139.0
SEM	0	0	5.3	27.4	13.1	28.1	58.5	8.0

TABLE 3.6

Responses of mean blood pressure (BP - mm Hg x min) and heart rate (HR - beats) to a 5 minute infusion of angiotensin II (32 ng/min) via a vertebral artery (AII), and 5 minute infusions of bradykinin (20 μg/min) via a vertebral artery (VA), carotid artery (CA) and intravenously (IV) before and after area postrema ablation (APA).

DOG	BP								HR							
	BEFORE APA				AFTER APA				BEFORE APA				AFTER APA			
	AII	VA	CA	IV	AII	VA	CA	IV	AII	VA	CA	IV	AII	VA	CA	IV
36	288	71	14	-28	40	-25	-62	-67	26	185	82	250	0	316	165	269
37	108	30	25	-20	0	-8	-100	-48	77	127	97	175	0	147	242	190
38	228	100	77	-32	0	-33	-17	-63	59	74	50	90	0	152	91	166
39	88	39	59	-21	0	-14	31	-33	83	165	119	102	0	109	107	194
40	120	178	35	-28	0	85	4	-9	49	347	145	179	0	225	134	175
41	68	33	24	-32	0	-2	17	-21	44	146	97	145	0	133	79	179
42	132	104	92	-18	0	29	37	-19	57	204	60	92	16	261	83	143
43	144	98	46	-56	14	29	34	-39	32	80	63	72	0	209	187	217
44	154	82	51	-25	0	-24	23	-42	94	263	122	95	0	195	140	111
MEAN	147.8	81.7	47.0	-28.9	6.0	4.1	-3.7	-37.9	57.9	176.8	92.8	133.3	1.8	194.1	136.4	182.7
SEM	23.2	15.5	8.6	3.8	4.5	12.5	16.0	6.6	7.7	29.0	10.7	19.5	1.8	22.2	18.1	14.8

TABLE 3.7

Responses of mean blood pressure (BP - mm Hg x min) and heart rate (HR - beats) to 5 minute infusions of bradykinin (20 µg/min) via a vertebral artery (VA), carotid artery (CA) and intravenously (IV) before and after a 5 minute infusion of physostigmine (eserine - 200 µg/min) via a vertebral artery.

	BP						HR					
	BEFORE ESERINE			AFTER ESERINE			BEFORE ESERINE			AFTER ESERINE		
	VA	CA	IV	VA	CA	IV	VA	CA	IV	VA	CA	IV
	93	49	-44	-11	24	-55	307	203	141	236	97	105
	80	40	-23	14	29	27	52	46	49	94	35	67
	62	39	-22	16	11	0	69	68	166	179	125	133
	51	22	-33	5	16	-30	71	133	260	82	91	205
MEAN	71.5	37.5	-30.5	6.0	20.0	-14.5	124.8	112.5	154.0	147.8	87.0	127.5
SEM	9.3	5.6	5.1	6.2	4.0	17.8	60.9	35.4	43.4	36.5	18.9	29.2

TABLE 3.8

Responses of mean blood pressure (BP - mm Hg x min) and heart rate (HR - beats) to a 5 minute infusion of angiotensin II (32 ng/min) via a vertebral artery (AII), and 5 minute infusions of bradykinin (20 μg/min) via a vertebral artery (VA), carotid artery (CA) and intravenously (IV) before and after a vertebral artery infusion of indomethacin (3 mg/min for 30 min).

DOG	BP								HR							
	BEFORE INDOMETH.				AFTER INDOMETH.				BEFORE INDOMETH.				AFTER INDOMETH.			
	AII	VA	CA	IV	AII	VA	CA	IV	AII	VA	CA	IV	AII	VA	CA	IV
56	94	89	63	-18	135	0	35	-16	37	168	103	107	0	65	51	191
58	186	75	68	-20	152	11	33	0	10	31	38	8	0	33	35	24
59	127	74	63	0	98	21	59	0	57	122	81	0	11	76	125	54
60	59	31	19	-33	55	-39	-8	-82	0	43	83	305	0	183	98	353
61	74	67	41	-48	71	-51	-25	-75	0	39	19	69	0	44	23	141
62	106	79	31	-23	84	-9	0	-23	24	103	57	121	0	66	34	149
63	40	116	114	-35	46	-9	33	-57	0	93	48	135	0	48	22	148
64	64	105	93	-26	103	25	14	-30	8	59	46	109	0	52	38	89
65	60	81	61	-23	102	28	21	-21	0	81	58	119	0	142	78	175
66	118	74	82	-17	126	32	68	20	0	53	84	101	17	57	87	137
MEAN	92.8	79.1	63.5	-24.3	97.2	0.9	23.0	-28.4	13.6	79.2	61.7	107.4	2.8	76.6	59.1	146.1
SEM	14.4	7.6	9.5	4.2	11.4	9.5	9.7	11.1	6.6	14.4	8.5	27.9	2.0	16.0	11.9	29.9

TABLE 4.1

The responses of blood pressure (mm Hg x min) during 5 minute infusions of angiotensin II at the doses indicated (ng/min) via a vertebral artery (VA) and intravenously (IV) and a 5 minute period of bilateral common carotid artery occlusion (BCO) before and after a 5 minute vertebral artery infusion of naloxone (1 µg/min) in morphine and chloralose anaesthetised dogs.

	BLOOD PRESSURE													
	BEFORE VA NALOXONE							AFTER VA NALOXONE						
ROUTE	VA	VA	VA	VA	IV	IV	BCO	VA	VA	VA	VA	IV	IV	BCO
DOSE	4	8	16	32	32	250	-	4	8	16	32	32	250	-
	6	31	65	141	-	175	-	0	34	91	187	-	192	-
	19	38	81	128	50	-	-	22	56	85	129	87	-	-
	11	14	54	145	39	185	-	27	17	67	165	22	145	-
	-	-	-	78	13	194	-	-	-	-	168	16	185	-
	33	42	64	89	10	175	204	37	63	68	82	41	163	360
	7	18	39	80	22	158	-	23	35	73	105	42	168	-
	32	56	106	140	33	164	204	35	85	131	169	32	186	214
	44	69	101	191	27	206	269	97	114	165	238	9	222	247
	16	48	82	125	14	122	241	32	55	104	133	18	132	301
	0	26	38	63	0	78	327	13	18	48	147	0	102	362
MEAN	18.7	38.0	70.0	118.0	23.1	161.9	249.0	31.8	53.0	92.4	152.3	29.7	166.1	296.8
SEM	5.2	6.3	8.7	13.2	5.6	14.0	25.7	9.6	11.2	12.8	14.6	9.1	12.7	33.2

TABLE 4.2

The responses of heart rate (beats) during 5 minute infusions of angiotensin II at the doses indicated (ng/min) via a vertebral artery (VA) and intravenously (IV) and a 5 minute period of bilateral common carotid artery occlusion (BCO) before and after a 5 minute vertebral artery infusion of naloxone (1 μg/min) in morphine and chloralose anaesthetised dogs.

	HEART RATE													
	BEFORE VA NALOXONE							AFTER VA NALOXONE						
ROUTE	VA	VA	VA	VA	IV	IV	BCO	VA	VA	VA	VA	IV	IV	BCO
DOSE	4	8	16	32	32	250	-	4	8	16	32	32	250	-
	0	0	0	16	-	-24	-	0	0	36	94	-	0	-
	4	8	25	38	0	-	-	7	13	25	41	13	-	-
	0	9	32	81	0	38	-	0	0	35	105	0	36	-
	-	-	-	8	0	-7	-	-	-	-	31	0	-9	-
	0	-13	0	0	-10	0	0	0	102	118	100	0	0	107
	0	0	0	0	0	-21	-	0	8	51	43	0	-21	-
	0	20	83	68	0	0	30	14	67	54	136	0	5	43
	0	27	47	89	-14	-6	22	44	92	176	277	-3	57	46
	0	11	24	26	0	-21	14	0	35	61	117	0	-26	62
	0	0	0	17	0	-14	60	0	0	16	32	0	-12	90
MEAN	0.4	6.9	23.4	34.3	-2.7	-6.1	25.2	7.2	35.2	63.6	97.6	1.1	3.3	69.6
SEM	0.4	4.2	10.0	11.2	1.9	6.7	11.2	5.2	14.6	18.2	24.6	1.6	9.5	14.0

TABLE 4.3

The responses of blood pressure (mm Hg x min) during 5 minute infusions of angiotensin II at the doses indicated (ng/min) via a vertebral artery (VA) and intravenously (IV) and a 5 minute period of bilateral common carotid artery occlusion (BCO) before and after a 5 minute intravenous infusion of naloxone (1 μg/min) in morphine and chloralose anaesthetised dogs.

	BLOOD PRESSURE													
	BEFORE IV NALOXONE							AFTER IV NALOXONE						
ROUTE	VA	VA	VA	VA	IV	IV	BCO	VA	VA	VA	VA	IV	IV	BCO
DOSE	4	8	16	32	32	250	-	4	8	16	32	32	250	-
	19	24	45	80	-	100	194	8	34	55	86	-	103	155
	10	24	43	92	17	191	172	12	20	36	124	23	210	200
	22	27	81	128	32	185	162	25	75	106	169	19	242	184
	48	106	167	192	30	171	239	58	136	171	226	21	190	237
	16	54	87	172	10	145	307	6	72	158	201	19	157	210
MEAN	23.0	47.0	84.6	132.8	22.3	158.4	214.8	21.8	67.4	105.2	161.2	20.5	180.4	197.2
SEM	7.3	17.7	25.1	24.4	6.1	18.6	29.7	10.8	22.6	30.0	28.4	1.1	26.6	15.2

TABLE 4.4

The responses of heart rate (beats) during 5 minute infusions of angiotensin II at the doses indicated (ng/min) via a vertebral artery (VA) and intravenously (IV) and a 5 minute period of bilateral common carotid artery occlusion (BCO) before and after a 5 minute intravenous infusion of naloxone (1 μg/min) in morphine and chloralose anaesthetised dogs.

	HEART RATE													
	BEFORE IV NALOXONE							AFTER IV NALOXONE						
ROUTE	VA	VA	VA	VA	IV	IV	BCO	VA	VA	VA	VA	IV	IV	BCO
DOSE	4	8	16	32	32	250	-	4	8	16	32	32	250	-
	0	11	9	37	-	0	23	0	20	26	68	-	19	16
	0	4	5	10	-14	-10	14	0	4	7	16	-6	-10	44
	0	7	26	45	0	-8	0	7	16	24	35	-9	3	0
	12	37	61	79	0	0	37	13	65	97	117	-11	21	48
	0	4	7	21	-6	-29	84	0	6	23	37	-4	-23	108
MEAN	2.4	12.6	21.6	38.4	-5.0	-9.4	31.6	4.0	22.2	35.4	54.6	-7.5	2.0	43.2
SEM	2.7	7.0	11.8	13.2	3.8	5.9	16.1	2.9	12.4	17.6	19.8	1.8	9.4	20.7

TABLE 4.5

The responses of blood pressure (mm Hg x min) during 5 minute infusions of angiotensin II at the doses indicated (ng/min) via a vertebral artery (VA) and intravenously (IV) and a 5 minute period of bilateral common carotid artery occlusion (BCO) before and after a 5 minute vertebral artery infusion of naloxone (1 μg/min) in chloralose anaesthetised dogs.

	BLOOD PRESSURE											
	BEFORE VA NALOXONE						AFTER VA NALOXONE					
ROUTE	VA	VA	VA	VA	IV	BCO	VA	VA	VA	VA	IV	BCO
DOSE	4	8	16	32	250	-	4	8	16	32	250	-
	21	46	119	161	-	276	14	46	98	161	-	178
	15	30	36	73	139	214	34	58	40	94	122	217
	0	31	59	51	95	363	15	36	39	57	194	322
	17	37	46	80	98	199	0	39	51	81	93	184
	0	13	15	38	70	415	0	15	25	41	70	330
MEAN	10.6	31.4	55.0	80.6	100.5	293.4	12.6	38.8	50.6	86.8	119.8	246.2
SEM	5.0	6.0	19.6	24.0	16.5	46.9	7.0	7.9	14.0	23.2	31.1	37.2

TABLE 4.6

The responses of heart rate (beats) during 5 minute infusions of angiotensin II at the doses indicated (ng/min) via a vertebral artery (VA) and intravenously (IV) and a 5 minute period of bilateral common carotid artery occlusion (BCO) before and after a 5 minute vertebral artery infusion of naloxone (1 μg/min) in chloralose anaesthetised dogs.

	HEART RATE											
	BEFORE VA NALOXONE						AFTER VA NALOXONE					
ROUTE	VA	VA	VA	VA	IV	BCO	VA	VA	VA	VA	IV	BCO
DOSE	4	8	16	32	250	-	4	8	16	32	250	-
	53	91	233	453	-	100	21	97	203	378	-	140
	76	79	108	116	-20	0	30	97	75	144	40	87
	6	32	54	51	-23	105	19	35	39	48	0	111
	0	19	41	41	-85	177	0	32	31	71	-66	179
	0	17	29	64	0	363	30	31	70	70	0	292
MEAN	27.0	47.6	93.0	145.0	-32.0	149.0	20.0	58.4	83.6	142.2	-6.5	161.8
SEM	17.6	17.4	41.9	87.3	21.2	67.6	6.1	17.6	34.7	68.4	25.4	40.2

B I B L I O G R A P H Y

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