CENTRAL ACTIONS OF BLOOD-BORNE ANGIOTENSIN II

by

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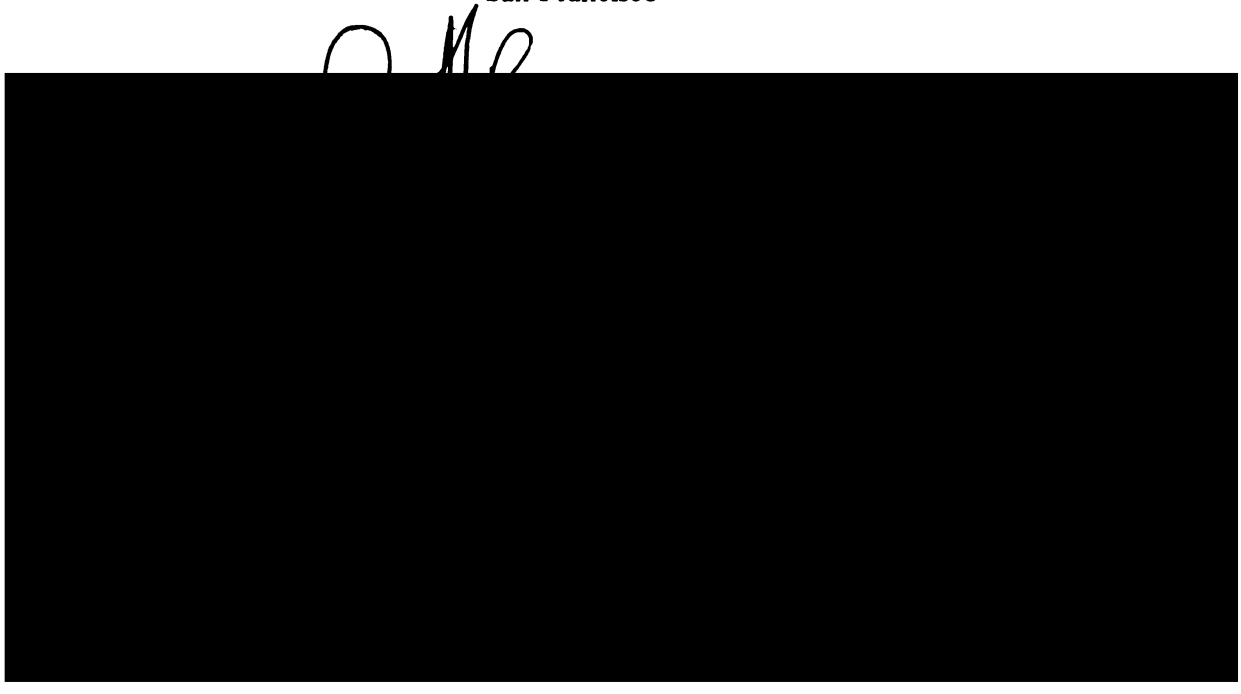

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Contents

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ABSTRACT

Centrally-mediated pressor and drinking responses to blood-borne angiotensin II (AII) are described in this dissertation. In chronically-catheterized conscious mongrel dogs, infusion of AII into both common carotid arteries at 1.0 and 2.0 ng/kg/min for 10 min caused a greater increase in arterial blood pressure than i.v. AII. Intracarotid AII at the higher dose reduced common carotid blood flow to half, presumably due to its vasoconstrictor action on the extracranial vascular beds. According to a theoretical analysis, this local vasoconstriction could lead to a pressor response large enough to account for that to intracarotid AII.

The mean blood flow in each internal carotid artery in conscious dogs determined by the radioactive microsphere method was 7.7 ml/min, and that in the

anastomotic artery between the external and internal carotid arteries was 3.3 ml/min. Since evidence exists that blood in both arteries mixes well before reaching the cerebral microcirculation, the brain would receive significantly lower concentration of AII when AII was infused into the external carotid arteries than when infused into the common carotid arteries. This was supported by the findings on plasma vasopressin response to AII. However, arterial pressure and plasma corticosteroid concentration, used as an index of ACTH secretion, increased to the same degree following both routes of AII. Therefore, even though some of the evidence suggests that the pressor effect of intracarotid AII is due to extracranial vasoconstriction, definite conclusions cannot be made.

Intraventricular saralasin, a competitive antagonist of AII, inhibited the pressor response to intravertebral, but not to intracarotid and i.v., AII. This result provides direct evidence that the pressor effect of intracarotid AII is mediated by different mechanism than that of intravertebral AII. It also suggests that the central pressor action of intravertebral AII is not essential in the pressor effect of circulating AII.

In Sprague-Dawley rats, intraventricular saralasin specifically inhibited drinking to AII i.v.; however,

it had no significant effect on drinking following 24- and 30-h water deprivation and abdominal vena caval ligation. Therefore, circulating AII appears not to play an essential role in drinking following these two stimuli.

CHAPTER I GENERAL INTRODUCTION

Renin was first named by Tigerstedt & Bergman (1898) to indicate a pressor substance found in saline extracts of rabbit kidney. Its physiological significance was not appreciated until after the pioneering studies of Cash (1926) and Goldblatt et al. (1934). Cash suggested that a pressor substance from the kidney played a role in the pathogenesis of experimental hypertension following bilateral ligation of the renal arteries in dogs. Goldblatt and his colleagues successfully developed persistent hypertension by constricting the main renal arteries in dogs. He subsequently showed that this type of hypertension is due primarily to a humoral mechanism initiated by the ischemia of the kidney (Goldblatt 1937). This important work of Goldblatt led immediately to the search for the pressor substance. It soon led to the discovery that renin is an enzyme and that the pressor action of renin is due to the formation of angiotensin, the true pressor substance, almost simultaneously by Page & Helmer (1940) and Braun-Menendez et al. (1940a). Angiotensin was later found to exist in two different forms, angiotensin I and II (Skeggs et al. 1954a). They were then purified (Skeggs et al. 1954b, Bumpus &

Page 1954), their structure determined (Elliott & Peart 1957), and synthesized (Rittel et al. 1957, Schwarz et al. 1957).

It is now known that renin is a very specific carboxyl protease (Skeggs et al. 1977). Although renin-like activity has been identified in many organs (Peach 1977), the enzyme studied most intensively and of most physiological significance is secreted into the bloodstream by the kidney. Renal renin is localized in the juxtaglomerular cells of the kidney (Cook 1971). These granular cells are located in the media of the afferent arterioles as they enter the glomeruli. Renin secretion is stimulated following systemic hypotension, increased activity of the renal sympathetic nerves and following a reduction in the sodium load to the distal tubule of the kidney (Davis & Freeman 1976).

After being secreted into the bloodstream, renin acts on an α-globulin, called renin substrate or angiotensinogen, to form angiotensin I. Most evidence indicates that the angiotensinogen in the circulation is produced by the liver (Reid et al. 1978). The plasma concentration of angiotensinogen is increased by administration of adrenocortical steroids, estrogens and angiotensin II (AII), and by nephrectomy, ureteral ligation, hemodilution and hypoxia (Reid et al. 1978).

Angiotensin I, a decapeptide, is converted to (AII), an octapeptide, after cleavage of the C-terminal dipeptide by converting enzyme. Although converting enzyme is ubiquitous (Erdös 1977), it has a very high activity in the lungs (Ng & Vane 1967). Most of the conversion from angiotensin I to II seems to occur during the passage of the blood through the lungs. Angiotensin II has a very short half-life in the blood, shorter than 1 min (Al-Merani et al. 1978). The enzymes that metabolize AII are generally termed angiotensinases. These include amino-, carboxy-, and endo- peptidases (Peach 1977). Angiotensinase activity is found in red blood cells and many tissues. One heptapeptide metabolite, [des-Asp^1] angiotensin II, is named angiotensin III and is biologically active.

There are various inhibitors of the renin-angiotensin system. Inhibitors of prostaglandin synthesis such as indomethacin and β-adrenergic blockers such as propranolol reduce renin secretion. The peptide pepstatin prevents renin from generating angiotensin I. Converting enzyme inhibitors such as SQ 20881 and SQ 14225 (catopril) inhibit conversion of angiotensin I to AII; however, they also inhibit the inactivation of bradykinin, a potent vasodilator. Analogs of AII such as saralasin are competitive inhibitors of AII

at the receptor level (Ganong 1981, Laragh et al. 1977).

Although angiotensin I appears to be as potent as AII in stimulating catecholamine secretion from the adrenal medulla (Peach 1977) and angiotensin III no less potent than AII in stimulating aldosterone secretion from the adrenal cortex (Freeman et al. 1977), the exact physiological roles of angiotensins I and III are not established.

On the other hand, angiotensin II has many well-established actions, peripherally and centrally. In the periphery, blood-borne AII is a potent stimulator of aldosterone secretion (Davis 1974). In cross-circulation experiments in dogs, Davis (1963) and his coworkers discovered that a humoral factor is responsible for the release of aldosterone from the adrenal cortex. This humoral substance was subsequently shown to be AII by Laragh et al. (1960) and Biron et al. (1961). Blood-borne AII has direct constrictor action on vascular smooth muscles; it also increases sympathetic activity by acting on the adrenal medulla to stimulate catecholamine secretion (Braun-Menendez et al. 1940b, Feldberg & Lewis 1964), on the sympathetic ganglia to facilitate ganglionic transmission (Lewis & Reit 1965), and on the post-ganglionic nerve terminals

to stimulate adrenergic transmitter release and to increase the responsiveness of smooth muscle to the transmitter (Zimmerman 1978). However, the vasoconstrictor effect of AII seems primarily to be due to its direct action on the vascular smooth muscle (Bohr 1974). Although AII has been called the most potent naturally occurring pressor agent, the maximum contraction produced by AII is generally less than that produced by α-adrenergic agonists (Bohr 1974). Angiotensin II constricts both pre- and post- capillary resistance vessels (Jarhult 1971), and its constrictor effect varies with different vascular beds.

Centrally, blood-borne AII stimulates secretion of vasopressin at least in some laboratories (Bonjour & Malvin 1970, Ramsay et al. 1978, Brooks et al. 1980) and ACTH (Maran & Yates 1977, Ramsay et al. 1978, Brooks et al. 1980). However, the physiological significance of these actions of AII is not established. Circulating AII also causes drinking and a centrally-mediated pressor response, which will be discussed in detail later. Angiotensin II administered into the lateral and third cerebroventricles also induces drinking and pressor response, and stimulates secretion of vasopressin (Severs et al. 1970, Mouw et al. 1971) and ACTH (Maran & Yates 1977). The sites of action of AII

for increased secretion of vasopressin and ACTH have not been established. However, for vasopressin, the supraoptic nucleus (Nicoll and Barker 1971), the posterior pituitary (Gagnon et al. 1973) and the subfornical organ (Simpson 1981) have been suggested. For ACTH, the median eminence (Gann 1969) and the anterior pituitary (Maran & Yates 1977) have been implicated.

Circulating AII does not gain access to the cerebroventricles (Ramsay & Reid 1975, van Houten et al. 1980), neither does intraventricular AII gain access to the blood (Brightman et al. 1970). Therefore, the sites of action of intraventricular AII are likely to be different from those of circulating AII. Although the brain renin-angiotensin system has been postulated (Ganten et al. 1971, Fischer-Ferraro et al. 1971), its physiological significance and even its existence remain debatable, Therefore, the actions of blood-borne rather than intraventricular AII are studied in this dissertation.

The central pressor action of circulating AII was first demonstrated by Bickerton & Buckley (1961) in cross-circulation experiments in dogs. Its physiological significance was not appreciated until Yu & Dickson (1965) showed that AII infused into the vertebral arteries of conscious rabbits increased blood pressure

in doses that were ineffective when given intravenously. This finding was later confirmed in greyhounds (Lowe & Scroop 1969), man (Ueda et al. 1969) and mongrel dogs (Ferrario et al. 1970). This pressor response is rapid in onset and usually accompanied by an increase in heart rate. It is mediated mainly by increased efferent sympathetic activity in mongrel dogs (Ferrario et al. 1972) and rabbits (Yu & Dickinson 1971), and by decreased vagal tone to the heart in greyhounds (Scroop & Lowe 1969).

By transecting the brainstem at different levels, the site responsible for the pressor action of intravertebral AII was shown to lie in the medulla in dogs (Joy & Lowe 1970a). Since AII is unlikely to cross the normal blood-brain barrier (Brightman et al. 1970, Ramsay & Reid 1975, van Houten et al. 1980), in order to exert a central action, circulating AII must act on some brain locus which is outside the blood-brain barrier. Circumventricular organs, located around the ventricular system, are different from typical brain tissue in that they are highly vascularized with fenestrated capillaries (Weindl 1973). Ablation of the area postrema, a circumventricular organ in the caudal medulla, in dogs abolished the pressor response to intravertebral AII (Joy & Lowe 1970b, Gildenberg et

al. 1973), reduced the pressor response to AII i.v. to half (Scroop et al. 1971), and impaired the ability to maintain blood pressure following hemorrhage (Katic et al. 1971) and the development of renal hypertension (Scroop et al. 1975). The area postrema was shown to be the most responsive site in the pressor effect to microinjection of AII in cats (Ueda et al. 1972). Electrical stimulation of the area postrema in dogs increased blood pressure and heart rate (Barnes et al. 1979). Therefore, the area postrema is generally taken as the site of action of the central pressor effect of circulating AII in dogs.

However, there is evidence that more rostral sites may also be involved in the central pressor action of AII. Intraventricular AII was shown to act on the subnucleus medialis in the midbrain to raise blood pressure in cats (Severs et al. 1967, Deuben & Buckley 1970). Transection of the midbrain abolished the pressor response to intraventricular, but not intravertebral, AII in dogs (Gildenberg et al. 1973). Microinjection of AII into the hypothalamus was reported to increase blood pressure in cats (Dutta et al. 1971). More recently, local injection of AII into the subfornical organ, a circumventricular organ adjacent to the dorsal third ventricle, has been shown to cause a pres-

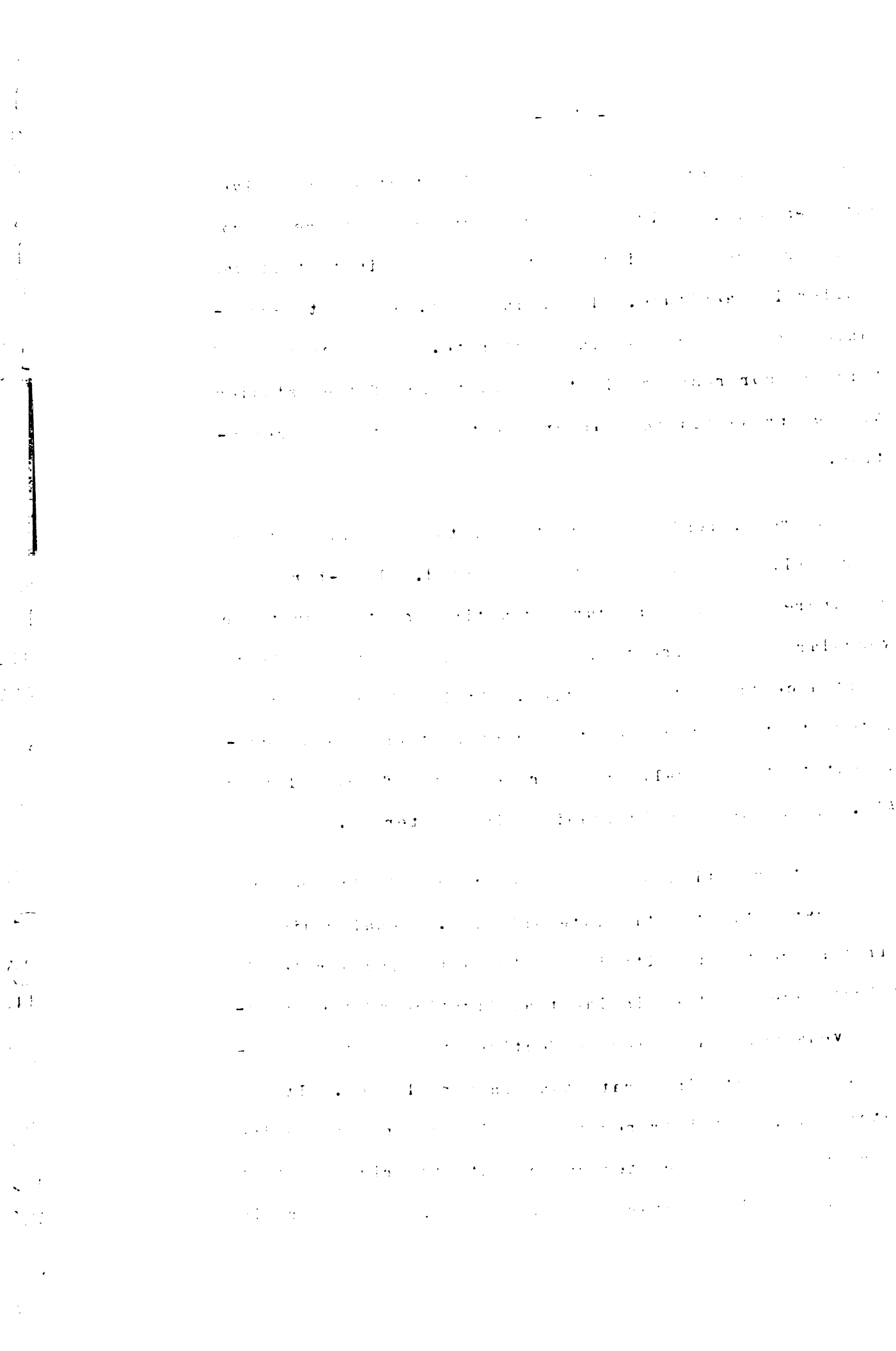
sor response in rats (Mangiapane & Simpson 1980). Angiotensin II infused into the carotid artery, rather than the vertebral artery, has been reported to elicit a greater increase in blood pressure than intra-aortic AII in rats (Haywood et al. 1980). This pressor effect was abolished by lesion of the anteroventral third ventricle (Fink et al. 1980), but not the area postrema (Haywood et al. 1980). The pressor response to AII i.v. was attenuated by subfornical organ lesion (Mangiapane & Simpson 1980), but not by area postrema lesion in rats (Zandberg et al. 1977, Buggy et al. 1977). Therefore, the area postrema does not seem to play an essential role in the central pressor action of circulating AII in rats.

Although intracarotid AII was generally reported to have no central pressor action in dogs (Severs & Daniels-Severs 1973), most of the studies were done on anesthetized preparations. Fitzsimons et al. (1978) reported that intracarotid AII increased blood pressure in 2 conscious dogs at a dose which was ineffective when given i.v. in 1 dog. However, they concluded, unjustifiably, that intracarotid AII induced no significant pressor response, despite of the small sample size. On the other hand, Reid (1980) recently showed that in conscious dogs intracarotid AII induced a pres-

sor response at doses that were ineffective when given intravenously. This pressor response differed from that produced by intravertebral AII in that it was smaller in magnitude, slower in onset, and not accompanied by an increase in heart rate. The mechanism of this pressor response is the main topic of the studies to be presented in Chapter III to V in this dissertation.

As renal renin is secreted into the bloodstream where AII is subsequently generated, blood-borne AII can increase blood pressure by acting on the systemic vascular beds directly, and by acting on the brain to exert a central pressor action. It is of considerable interest to determine the contribution of each component to the overall pressor effect of circulating AII. This question is examined in Chapter VI.

Another action of AII to be studied is its remarkable capacity to stimulate drinking. Renal renin was first suggested by Fitzsimons (1964) to play a role in thirst based on the finding that ligation of the abdominal vena cava was a less effective stimulus to drinking in nephrectomized rats than in normal ones. It was later found that intraperitoneal injection of saline extracts of renal cortex caused rats to drink and that the dipsogenic factor was inseparable from renin



(Fitzsimons 1969). As saralasin inhibits drinking induced by renin and angiotensin I, the true dipsogenic component was later shown to be AII (Fitzsimons, Epstein & Johnson 1978). Intracranial AII is the most potent dipsogen ever studied (Booth 1968, Epstein et al. 1970, Ramsay & Reid 1975, Fitzsimons & Kucharczyk 1978). Although systemic administration of AII is less potent than intracranial AII in eliciting drinking, blood-borne AII at concentrations within the physiological range has been reported to cause drinking in rats and dogs (Hsiao et al. 1977, Fitzsimons et al. 1978).

The subfornical organ has been suggested to contain receptors which mediate drinking produced by circulating AII in rats (Simpson et al. 1978). The evidence includes the observation that the subfornical organ is one of the most sensitive sites in the brain in eliciting drinking to local injection of AII, that ablation of the organ eliminated drinking elicited by AII i.v., and that infusion of saralasin directly into the organ selectively antagonized drinking induced by AII i.v. In addition, iontophoretic application of AII to individual neurons of the organ increased their firing rates (Felix & Akert 1974), and this effect could be antagonized by saralasin (Phillips & Felix 1976). However, the organum vasculosum of the lamina ter-

minalis, a circumventricular organ in the anteroventral third ventricle, has also been implicated as one of the receptor sites for the dipsogenic action of AII. Small amounts of AII applied to the region caused drinking in rats, and destruction of the region abolished drinking induced by intravenous or intraventricular AII (Phillips 1978). In addition, the preoptic area has been proposed to contain AII dipsogenic receptors (Mogenson et al. 1975). Further research is needed to determine the relative importance of these areas in the control of water intake.

Although studies on drinking following caval ligation in rats provided the first piece of evidence implicating the renal renin-angiotensin system in thirst, this conclusion has been questioned by several investigators (Lehr et al. 1975, Stricker 1977, Rolls & Wood 1977). The role of AII in drinking following water deprivation has also received much attention. Therefore, the role of the endogenous circulating AII in the control of drinking following water deprivation and caval ligation in rats is to be examined in Chapter VII.

CHAPTER II GENERAL METHODS

This chapter describes the general methods used in Chapter III to VI. To avoid the complications of anesthesia, all the experiments were carried out on conscious animals.

Animals

Trained adult mongrel dogs of either sex were used throughout the studies. They were housed individually in an air-conditioned room maintained at 22°C and 55% humidity and on a 6 A.M. - 6 P.M. light cycle. They were fed a ration of dry Purina dog chow daily at 2 P.M. and allowed water ad libitum. The average daily sodium intake was approximately 90 mEq. On the day of experiment, the dog was brought into a quiet room and was minimally restrained in a dog sling (Alice King Chatham, Los Angeles) during the experiment.

Angiotensin II

Synthetic angiotensin II ([Asp^1-Ile^5]angiotensin II, Bachem) was freshly prepared in a 0.9% NaCl solution to the concentration desired. It was infused at a

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rate of 0.3 - 0.5 ml/min into an artery or 0.5 - 1.0 ml/min into a femoral vein for 10 min. The order of infusions was randomized, and consecutive infusions were separated by at least 10 min apart.

Surgical Procedures

A small non-occluding catheter was made of two pieces of tygon tubing (Norton) glued together with cyclohexanone: a 5-cm-long smaller tubing (0.75 mm I.D., 1.2 mm O.D.) inserted into a 90-cm-long larger tubing (1.1 mm I.D., 1.8 mm O.D.). After the dog was anesthetized with pentobarbital, a carotid or vertebral artery was occluded temporarily for inserting the smaller end of a non-occluding catheter. The catheter was then secured in place by a purse-string suture in the arterial wall (Rudolph et al. 1956).

A tygon tubing (1.3 mm I.D., 2.3 mm O.D.) was inserted into one end of the femoral artery and vein and advanced into the aorta and vena cava, respectively. The other end of the vessel was tied off.

All the catheters were filled with heparin, passed subcutaneously and exteriorized at the back of the dog. A dog jacket (Alice King Chatham) was worn to protect

the catheters.

The dogs were allowed five to seven days to recover from the surgery. Their rectal temperatures were monitored daily and antibiotics (penicillin G and tylosin) were given if necessary.

Measurement of Cardiovascular Variables

The arterial blood pressure was measured by the femoral arterial catheter connected to a pressure transducer (P23Db, Statham). Mean arterial pressure was obtained by damping the arterial pressure electronically. Heart rate was measured by a tachometer, triggered by the arterial pulse pressure, and was checked by counting pulse waves in the arterial pressure tracing. All these variables were recorded on paper by a polygraph (Grass).

CHAPTER III PRESSOR RESPONSE TO INTRACAROTID INFUSION OF ANGIOTENSIN II

INTRODUCTION

In addition to the systemic vasoconstrictor effect, blood-borne angiotensin II has a central pressor component (Bickerton & Buckley 1961). Administration of AII into the vertebral arteries has been shown to cause a centrally-mediated pressor response in rabbits (Yu & Dickson 1965), dogs (Lowe & Scroop 1969, Ferrario et al. 1970) and man (Ueda et al. 1969). The area postrema, a circumventricular organ in the caudal medulla, is generally taken as the site of action of this pressor effect of AII in dogs (Joy & Lowe 1970b, Gildenberg et al. 1973).

However, there is evidence that more rostral sites may also be involved in the central pressor action of AII. Intraventricular AII was shown to act on the subnucleus medialis in the midbrain to increase blood pressure in cats (Deuben & Buckley 1970). Transection of the midbrain eliminated the pressor response to intraventricular, but not intravertebral, AII in dogs (Gildenberg et al. 1973). Microinjection of AII into the subfornical organ, a circumventricular organ adja-

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cent to the third ventricle, increased blood pressure in rats (Mangiapane & Simpson 1980). Infusion of AII into the carotid artery, but not the vertebral artery, elicited a greater increase in blood pressure than intra-aortic AII did in rats (Haywood et al. 1980). This pressor effect was abolished by lesion of the anteroventral third ventricle (Fink et al. 1980), but not the area postrema (Haywood et al. 1980).

Although intracarotid AII was generally reported to have no central pressor action in dogs (Severs & Daniels-Severs 1973), most of the studies were done on anesthetized dogs (Lowe & Scroop 1969, Ferrario et al. 1970a, Joy & Lowe 1970). More recently Reid (1980) showed that infusion of AII into the common carotid arteries in conscious dogs induced a pressor response at doses that were ineffective when given intravenously. This pressor response differed from that produced by intravertebral AII in that it was smaller in magnitude, slower in onset, and not accompanied by an increase in heart rate. It could be mediated centrally; however, there exists at least one alternative.

Most of the blood flow in the common carotid artery perfuses the extracranial vascular beds via the external carotid artery in dogs. Being a potent

vasoconstrictor, AII infused into the carotid artery is likely to significantly reduce the blood flow through the external carotid artery. This reduction in local blood flow could cause a pressor response. The possibility then exists that the pressor response to intracarotid AII might be simply due to extracranial vasoconstriction.

To test this hypothesis, carotid blood flow was monitored during intracarotid AII infusion. Then the pressor effect of AII infused into the common carotid arteries was compared with that of AII infused into the external carotid arteries. A theoretical analysis is presented in the Appendix to examine the quantitative repationship between the reduction in local blood flow and the pressor effect.

METHODS

1. Measurement of Carotid Blood Flow

A non-occluding catheter was chronically implanted in both common carotid arteries in 4 dogs (19-29 kg) for AII infusion. A pre-calibrated electromagnetic flow transducer (3-4 mm I.D., Biotronex) and a hydraulic silastic cuff (IVM, Healdsburg, CA) were implanted around the right common carotid artery for flow measurement. The cuff was positioned more distal

from the heart than the flow transducer and was used to occlude the carotid blood flow for zero-flow determination.

After recovering from the surgery, the dog was brought into the laboratory and the flow transducer connected to a flowmeter (BL-10, Biotronex). After the dog had calmed down, AII (2.0 ng/kg/min) was infused bilaterally into common carotid arteries for 10 min. Carotid blood flow was monitored throughout the experiment.

2. Pressor Responses to Angiotensin II

A non-occluding catheter was chronically implanted in both common and both external carotid arteries in 7 dogs (17-25 kg) for AII infusions. To cannulate the external carotid artery, a catheter was inserted into the common carotid artery just below the carotid sinus bifurcation, and its tip was advanced beyond the bifurcation of the lingual artery from the external carotid artery. A femoral artery and vein were also cannulated for blood pressure measurement and AII infusion, respectively.

After recovering from the surgery, the dog was infused with AII into both common or both external carotid arteries or a femoral vein in a random order.

Two doses of AII were infused: 1.0 and 2.0 ng/kg/min. Arterial blood pressure and heart rate were monitored throughout the experiment.

3. Data Analysis

Student's t-test for paired data was used to analyze carotid flow data. Analysis of variance for repeated measurements (Winer 1971) followed by Newman-Keuls post-hoc comparisons (Zar 1974) was used to analyze the pressor responses to different routes of AII infusion. The level of significance was set at 0.05. Data are expressed as mean±standard deviation.

RESULTS

The mean blood flow in the common carotid arteries of 4 dogs was 139±26 ml/min during the control pre-infusion period. It decreased gradually and reached a mean of 71±14 ml/min during intracarotid infusion of AII (Fig.III.1 & 2). This decrease was significant ($p < 0.05$).

The mean arterial pressure during the control pre-infusion period was approximately 100 mmHg. The increase in mean arterial pressure during AII infusion into the common carotid arteries was similar to that during AII infusion into the external carotid arteries,

and both were greater than that during intravenous AII at two different doses (Fig.III.3 & Table III.1). Heart rate did not change significantly during any routes of AII infusion (Table III.2).

Based on the assumption that the cardiac output and the total resistance of other vascular beds are unchanged, the pressor response induced by intracarotid AII can be predicted from Fig.III.4. For a typical ratio of cardiac output to bilateral carotid flows (r) of 10, halving bilateral carotid flows will cause a pressor effect of approximately 5 mmHg, provided the control pre-infusion mean aortic pressure is 100 mmHg.

DISCUSSION

Reid's (1980) recent finding that intracarotid AII induced a greater pressor response than AII i.v. in conscious dogs was confirmed in this study (Fig.III.3). However, previous studies on anesthetized dogs reported that the pressor response to intracarotid AII was no greater than that to AII i.v. (Lowe & Scroop 1969, Ferrario et al. 1970, Joy & Lowe 1970a). It is difficult to explain this discrepancy. However, different animal preparations could account for it. Sweet et al. (1971) reported that infusion of AII at a rate of 10 ng/kg/min into the vertebral artery, but not the common carotid

artery, increased blood pressure in conscious dogs. However, the same dose of AII was also ineffective when given i.v. Their dogs appeared to be insensitive to AII since this dose of AII i.v. has otherwise been shown to be pressor in conscious dogs (Chapter VI, Fukiyama et al. 1971, Ramsay et al. 1978).

On the other hand, Fitzsimons et al. (1978) reported that intracarotid AII at a dose of 2-3 ng/kg/min increased blood pressure by approximately 10 mmHg in 2 conscious dogs, and that the same dose given i.v. was ineffective in 1 dog. In spite of the small sample size, they did statistics on these data. It is therefore unjustified for them to conclude that intracarotid AII induced no significant pressor response.

Angiotensin II infused into the renal artery and mesenteric vein has been shown to cause smaller pressor responses than AII i.v. (Akinkugbe et al. 1966) as the kidney and liver inactivate significant amounts of AII (Hodge et al. 1967). In contrast, even though as much as 50% of the AII administered into the carotid arteries was inactivated in its first passage through the head (Hodge et al. 1967, Reid, unpublished observation), intracarotid AII elicited a greater pressor response than AII i.v. (Fig.III.3). The question arises then: what is the mechanism of the pressor

action of intracarotid AII ?

Intracarotid AII could act on the brain to cause a centrally-mediated pressor effect. Since the carotid blood does not perfuse the hindbrain and AII does not cross the blood-brain barrier, AII must be acting on some circumventricular organ rostral to the area postrema. The subfornical organ (Mangiapane & Simpson 1980) and the organum vasculosum of the lamina terminalis (Fink et al. 1980), both adjacent to the third ventricle, are likely candidates since both have been proposed to contain receptors for pressor action of AII in rats.

Another alternative is that intracarotid AII could constrict the extracranial vascular beds to increase the systemic vascular resistance and result in a pressor response. When AII was infused at a rate of 1 ng/kg/min into each common carotid artery, the plasma AII concentration in the artery could reach over 200 pg/ml, approximately ten times normal concentration (Ramsay et al. 1978). Since AII is a potent vasoconstrictor and 90% of the common carotid blood flow perfuses the extracranial vascular beds (Chapter IV), it is not surprising to see that the blood flow in the common carotid artery halved during intracarotid AII infusion (Fig.III.2). This is in agreement with

Greenfield & Tindall (1968), who reported that the external carotid blood flow reduced to two thirds of its control value shortly after an AII injection into the external carotid artery in one man. According to the theoretical analysis presented in the Appendix, this reduction in flow could result in a pressor response of 5 mmHg (Fig. III.4). which happened to be the difference in the pressor effects of intracarotid and intravenous AII (Fig. III.3). Therefore, the extracranial vasoconstriction hypothesis appears to be able to account for most of the pressor response to intracarotid AII, at least theoretically.

The extracranial vasoconstriction hypothesis would gain strong support from the result that external carotid infusion of AII increased blood pressure to the same degree as common carotid infusion did (Fig. III.3), provided that the external carotid blood did not perfuse the brain. However, Jewell (1952) has provided anatomic evidence that anastomoses exist between the external and internal carotid circulations in dogs. Before the functional significance of these anastomoses in conscious dogs is understood, which is the topic of the following chapter, the results of the present study do not permit definite conclusion concerning the mechanism of the pressor response to intracarotid AII.

APPENDIX

To derive the relationship between the change in mean aortic blood pressure and that in mean blood flow in some systemic artery,

let Pa = mean aortic blood pressure

Pv = mean vena caval blood pressure

CO = mean cardiac output

Qc = mean blood flow in some systemic artery

TPR = mean total peripheral resistance

= (Pa - Pv) / CO

Rc = total resistance of the local vascular beds perfused by the artery

= (Pa - Pv) / Qc

Ro = total resistance of all other systemic vascular beds

= (Pa - Pv) / (CO - Qc)

By definition,

$$Pa - Pv = CO \times TPR \qquad \langle III.1 \rangle$$

$$1/Rc + 1/Ro = 1/TPR \qquad \langle III.2 \rangle$$

In a new state, these equations become

$$Pa' - Pv' = CO' \times TPR' \qquad \langle III.1a \rangle$$

$$1/Rc' + 1/Ro' = 1/TPR' \qquad \langle III.2a \rangle$$

<III.2> - <III.2a>

$$(1/Rc - 1/Rc') + (1/Ro - 1/Ro') = (1/TPR - 1/TPR')$$

or

$$(1 - Rc/Rc') + (1/Ro - 1/Ro')Rc = (Rc/TPR)(TPR'-TPR)/TPR'$$

$$= rX/(1+X) \qquad \langle III.3 \rangle$$

where $TPR' = (1+X)TPR$

and $r = Rc/TPR = CO/Qc$

Assume $Ro' \cong Ro$,

i.e., change in total resistance of all other systemic vascular beds is negligible,

then <III.3> becomes

$$1 - Rc/Rc' \cong rX/(1+X)$$

or

$$Rc'/Rc \cong (1+X)/[1-(r-1)X] \qquad \langle III.4 \rangle$$

Assume $CO' \cong CO$,

i.e., change in cardiac output is negligible,

then <III.1a>/<III.1> becomes

$$(Pa'-Pv')/(Pa-Pv) \cong TPR'/TPR$$

$$\cong 1+X \qquad \langle III.5 \rangle$$

By definition,

$$Qc = (Pa-Pv)/Rc$$

$$Qc' = (Pa'-Pv')/Rc'$$

so $Qc'/Qc \cong (1+X)(Rc/Rc')$

<III.4> becomes

$$Qc'/Qc \cong 1-(r-1)X \qquad \text{<III.4a>}$$

Since Pv, Pv' << Pa, Pa',

<III.5> becomes

$$Pa'/Pa \cong (Pa'-Pv')/(Pa-Pv) \cong 1+X$$

or

$$p/Pa \cong X$$

where

$$p = Pa'- Pa$$

<III.4a> becomes

$$Qc'/Qc \cong 1-(r-1)(p/Pa)$$

or

$$p/Pa \cong [1-(Qc'/Qc)]/(r-1) \qquad \text{<III.6>}$$

Therefore, when Qc is changed to Qc', without concurrent changes in Ro and CO, the corresponding change in mean aortic pressure (p) can be predicted from <III.6>. The straight lines plotted in Fig.III.4 represent Eq.<III.6> with different values of r.

Typically, Pa $\cong$ 100 mmHg, and for both carotid arteries, r $\cong$ 10, to induce a pressor response of 5 mmHg, Qc' $\cong$ 0.55 Qc, i.e., one can increase blood pressure by 5 mmHg by reducing carotid blood flows to half.

The above derivation was only based on two assumptions, i.e., Ro' ≅ Ro and CO' ≅ CO. When a vasoconstrictor such as AII is infused into the artery, Ro' and CO' are more likely to be less than Ro and CO, respectively, due to the baroreceptor reflex.

Then Eq.<III.5> becomes

$$Pa'/Pa \leq TPR'/TPR = 1+X$$

or

$$p/Pa \leq X$$

Eqs.<III.4a> and <III.6> become

$$Qc'/Qc \leq 1-(r-1)X$$

and

$$p/Pa \leq X \leq [1-(Qc'/Qc)]/(r-1)$$

Therefore, Eq.<III.6> overestimates p/Pa; the actual lines in Fig. III.4 will shift downward. However, if peripheral spill-over of the vasoconstrictor occurs, i.e., the vasoconstrictor acts on other systemic beds as well, then the lines will shift upward.

Finally, Eq.<III.6> applies to not only vasoconstrictors but also vasodilators, where Qc' > Qc and p < 0.

Table III.1 Mean arterial blood pressure (mmHg) during the control pre-infusion periods and its changes during infusions of angiotensin II (AII) at two doses into both common carotid arteries (CC), both external carotid arteries (EC), and the femoral vein (iv).

(a) AII (2 x 1.0 ng/kg/min)

Dog	Control	CC AII	Control	EC AII	Control	iv AII
1	109	+11	106	+10	112	+5
2	125	+15	118	+18	125	+5
3	125	+16	101	+14	107	+11
4	88	+14	97	+8	104	+6
5	90	+8	89	+8	91	+6
6	83	+9	83	+10	87	+4
7	78	+5	78	+3	80	+8
mean	96	+11	96	+10	101	+6
SD	16	4	14	5	16	2

(b) AII (2 x 0.5 ng/kg/min)

1	100	+6	100	+5	100	+3
2	130	+8	125	+11	124	+2
3	105	+10	107	+8	109	+4
4	103	+7	97	+10	104	+3
5	85	+4	85	+5	83	+2
6	83	+3	83	+4	82	+1
7	80	+2	80	+3	82	+5
mean	98	+6	97	+7	98	+3
SD	17	3	16	3	16	1

Table III.2 Mean heart rate (beats/min) during control pre-infusion periods and its changes during infusions of AII at two doses into both common carotid arteries, both external carotid arteries, and the femoral vein.

(a) AII (2 x 1.0 ng/kg/min)

Dog	Control	CC AII	Control	EC AII	Control	iv AII
1	55	-1	55	-4	52	+2
2	55	0	70	-5	55	-5
3	45	-7	48	-10	45	0
4	72	-7	69	-7	66	0
5	67	-1	58	-1	56	+1
6	83	+3	83	+4	82	+1
7	89	-9	94	-14	91	-1
mean	67	-3	68	-5	64	0
SD	16	4	16	6	17	2

(b) AII (2 x 0.5 ng/kg/min)

1	56	0	56	-3	54	-2
2	68	+2	60	+7	63	-9
3	45	-5	55	-5	45	-2
4	63	+5	60	0	52	+5
5	77	+3	78	-2	78	0
6	55	-1	58	-3	65	-6
7	80	+1	90	-4	97	+3
mean	63	+1	65	-1	65	-2
SD	13	3	13	4	18	5

Fig. III.1 A typical example of the effects of intracarotid AII on arterial blood pressure (mmHg) and mean blood flow (ml/min) in the right common carotid artery (CC) of a conscious dog. Angiotensin was infused at 1 ng/kg/min into each artery. The left arrow indicates the beginning of AII infusion, and the right one the end of infusion. MAP: mean arterial blood pressure. BP: arterial blood pressure.

Fig. III.1

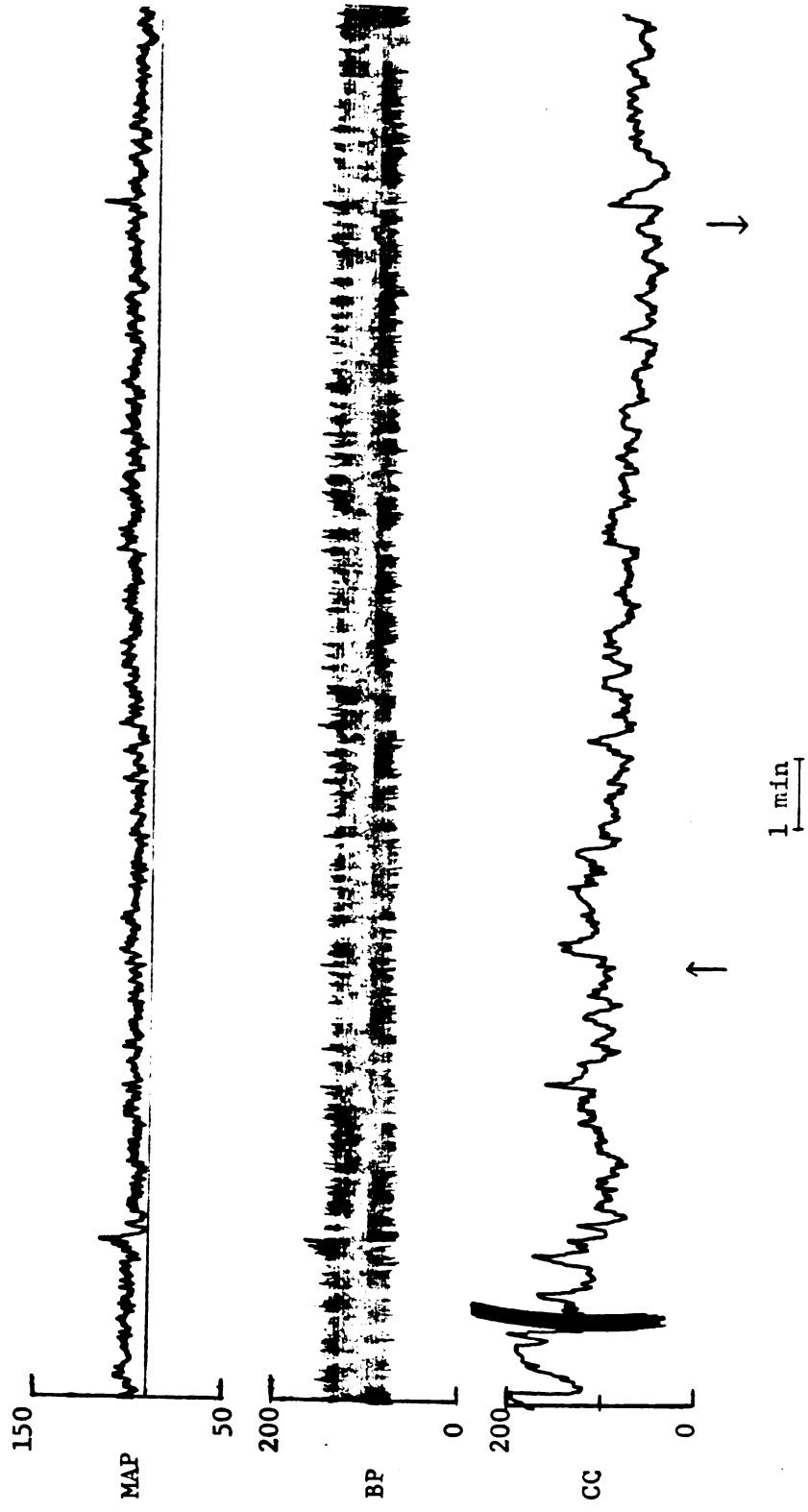


Fig. III.2 Mean blood flow (ml/min) in the right common carotid artery during the control pre-infusion period and intracarotid AII infusion (C.C.AII) in 4 dogs. Different symbols represent different dogs. The dose of AII is explained in the previous figure.

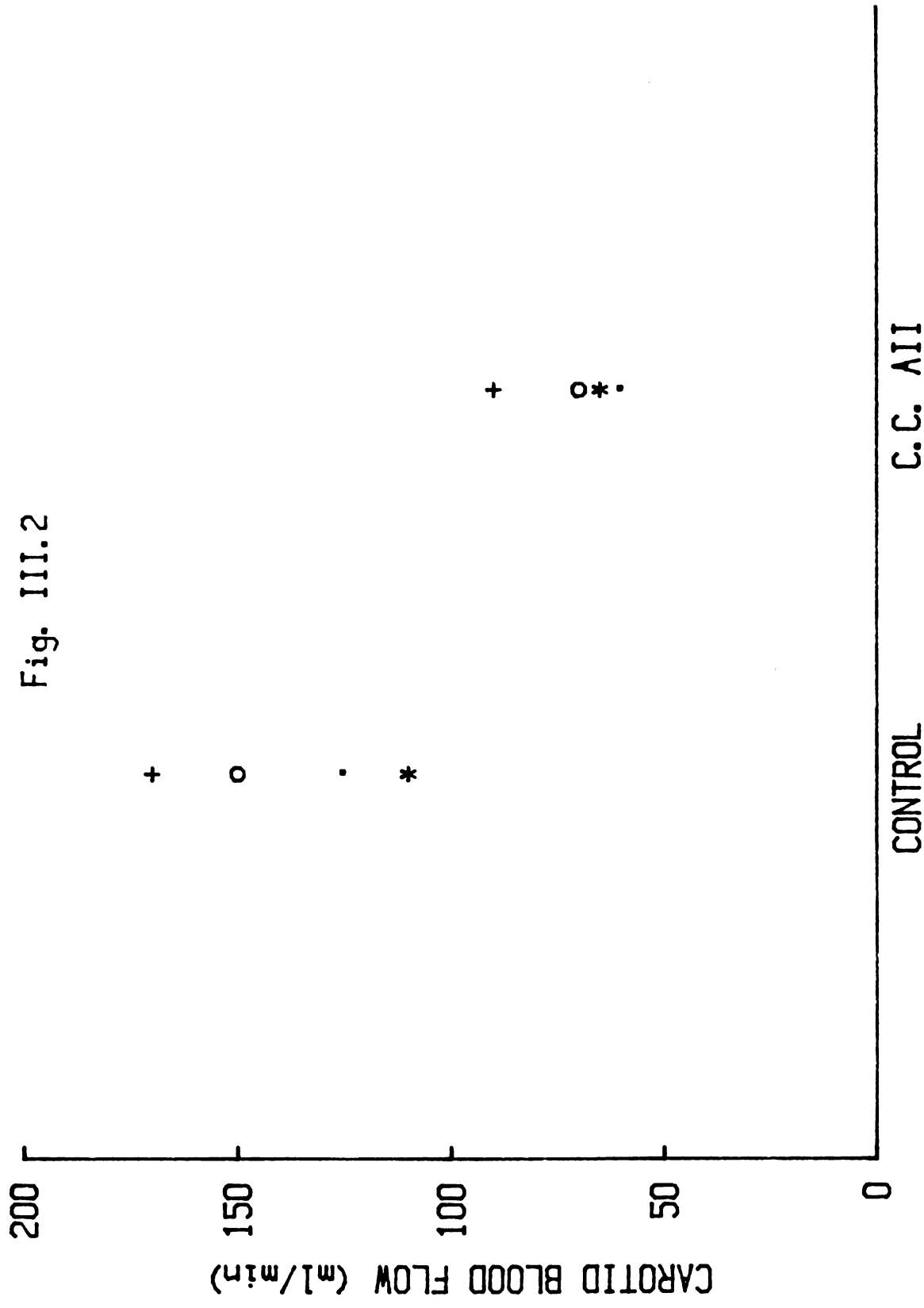


Fig. III.2

Fig. III.3 Mean pressor response during AII infusions into both common carotid arteries (CC), both external carotid arteries (EC) and the femoral vein (i.v.) at two doses in 7 dogs. * significantly different form i.v. ($p < 0.05$). mean$\pm$SEM are shown.

Fig. III.3

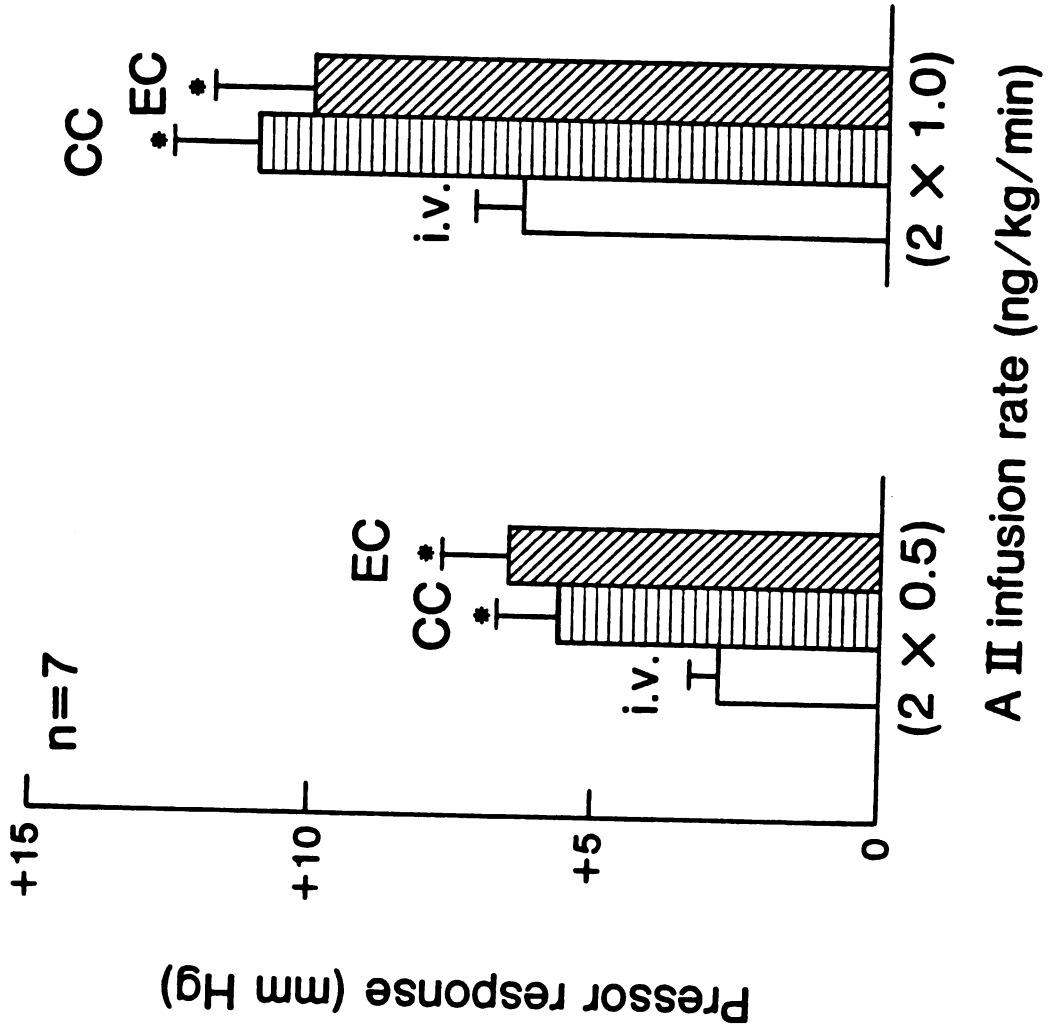

Pressor response (mm Hg)
+15
+10
+5
0
n=7
CC
EC
i.v.
(2 X 0.5)
(2 X 1.0)
A II infusion rate (ng/kg/min)

Fig. III.4 Change in mean aortic pressure (p) vs blood flow in some systemic artery (Qc') as predicted from Eq.<III.6>. Different lines represent different ratios of cardiac output to the control blood flow in the artery (r). Pa: control mean aortic pressure. Qc: control mean blood flow in the artery.

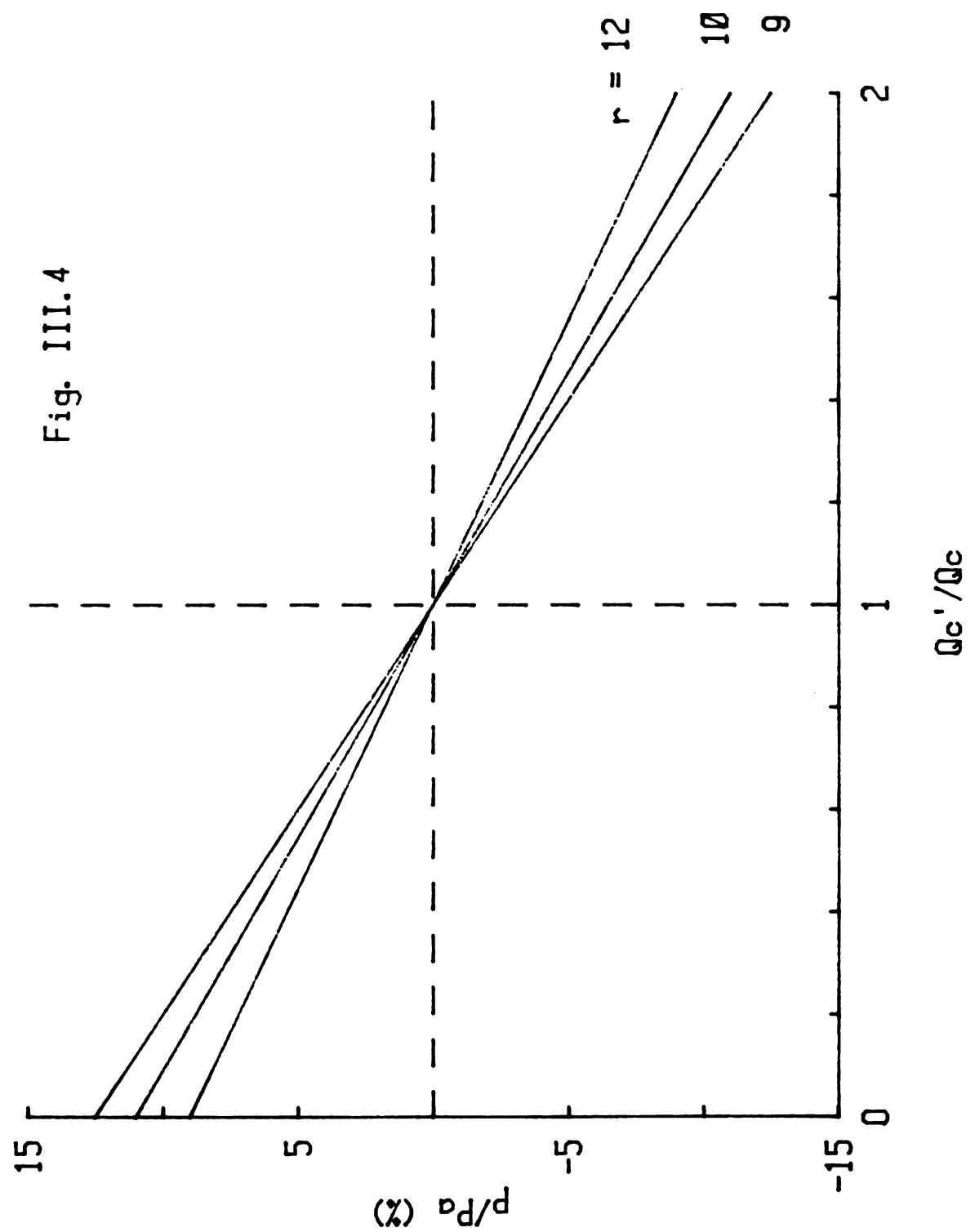


Fig. III.4

CHAPTER IV ANASTOMOSES BETWEEN THE EXTERNAL CAROTID AND INTRACRANIAL CIRCULATIONS

INTRODUCTION

Several anastomoses exist between the external carotid and intracranial circulations in dogs, through which external carotid blood can perfuse the brain. The anastomotic artery running between the external ophthalmic and internal carotid arteries was described long ago by Ellenberger & Baum (1891) and its functional importance was studied by Bouckaert & Heymans (1935). Anatomic evidence on other anastomoses between the external carotid and intracranial circulations in dogs was described in detail by Jewell (1952). Angiographic studies on these anastomoses were reported by de la Torre et al. (1959). However, these studies provided only qualitative descriptions on the anastomoses; moreover, none were done on conscious dogs.

To provide quantitative data on these anastomoses, in the present study radioactive microspheres were injected into the common and external carotid arteries of conscious dogs. By comparing microsphere distributions in the brain and extracranial vasculature, the blood flows in the anastomoses and internal carotid

artery were determined. To study the functional significance of the anastomoses, the effects of infusions of angiotensin II into the common and external carotid arteries on vasopressin and corticosteroid secretion were compared.

METHODS

Microsphere Studies

Four dogs (17-24 kg) were chronically prepared with non-occluding catheters in common and external carotid arteries on both sides for microsphere injections. The tip of the common carotid catheter pointed toward the heart to assure a good mixing of the injected spheres within the blood stream.

Fifteen μm radioactive spheres (3M) labeled with Gd^{153}, Co^{57}, Sn^{113}, Sr^{85}, Mn^{54}, and Zn^{65} were used in this study. The spheres were suspended in a 5% dextran solution with 0.05% polyoxyethylene 80 sorbitan monooleate (Tween 80) to prevent aggregation (Heymann et al. 1977). Approximately half a million spheres were placed in a 0.5-ml injector vial. After vigorous shaking of the vial, 2.5 ml of saline was flushed through the vial into the catheter over 30 s. The number of spheres injected was determined by counting the radioactivity of the vial before and after the

injection.

At least two different isotopes were injected into both common and both external carotid arteries of the dog. The isotopes and the order of injections were randomized.

During common carotid injections, a roller pump (Cole-Parmer, Chicago) was used to withdraw blood, at a rate of 1.5-2.5 ml/min, from the external carotid artery to estimate the blood flow in the common carotid artery. The pump was turned on 1 min before and remained on until 3 min after the sphere injection.

After the experiment, the dog was killed with an overdose of sodium pentobarbital. The brain as well as the masseter muscles was removed and fixed in 4% formaldehyde. Three to five days later, the brain was dissected into pieces and weighed. After carbonization, all the samples were counted for the radioactivity of each isotope by an Ultima/II multichannel pulse-height analyzer (Ino-Tech, Fort Atkinson, Wisconsin).

Blood Flow Estimation

Blood flow in the common carotid artery was estimated by the reference sample method (Heymann et

al. 1977), i.e., Flow = (reference sample withdrawal rate)/(fraction of injected spheres in the reference sample).

To estimate the anastomotic and internal carotid blood flows, let

CC = Common carotid blood flow

EC' = External carotid blood flow beyond the bifurcation of lingual artery

IC = Internal carotid blood flow

A = Anastomotic flow between the intracranial and external carotid circulations beyond the bifurcation of lingual artery

B, B' = Fractions of injected spheres trapped in the brain when injected into the common and external carotid arteries, respectively

T, T' = Fractions of injected spheres trapped in the masseter muscles when injected into the common and external carotid arteries, respectively

For tissues which are perfused by the external carotid artery, such as the masseter muscles (Evans & Christensen 1979),

EC' x T' = Blood flow to masseter muscles

= CC x T

or

$$EC' = CC \times T/T' \qquad \text{<IV.1>}$$

When spheres were injected into the external carotid artery, the only routes that the spheres could reach the brain were via the anastomoses (Fig.IV.1), hence

$$B' = A / EC' \qquad \text{<IV.2>}$$

When spheres were injected into the common carotid artery, they could reach the brain via both the internal carotid artery and the anastomoses, hence

$$B = (A + IC) / CC \qquad \text{<IV.3>}$$

Since T, T', B, and B' were measured and CC was estimated by the reference sample method, EC' could be calculated from Eq.<IV.1>, anastomotic flow (A) from Eq.<IV.2>, and the internal carotid flow (IC) from Eq.<IV.3>.

Humoral Responses to Angiotensin II Infusion

Angiotensin II was infused into both common or both external carotid arteries or femoral vein in 7 dogs (17-25 kg) at a rate of 5.0 ng/kg/min for 10 min. Blood samples were taken just before and at the end of infusion for arginine vasopressin assay, and in 5 dogs for 11-hydroxycorticosteroids assay. Changes in the plasma concentration of corticosteroids were used as an index of changes in ACTH secretion.

Plasma vasopressin concentration was determined by radioimmunoassay (Keil & Severs 1977). Plasma 11-hydroxycorticosteroids were determined by a competitive protein binding radioassay (Murphy 1967). In the dog, these consist mainly of cortisol plus some corticosterone.

To analyze the humoral responses to different routes of AII infusion, analysis of variance for repeated measurements followed by Newman-Keuls post-hoc comparisons was used. The level of significance was set at 0.05. Data are expressed as mean±standard deviation.

RESULTS

Microsphere Studies

During microsphere injections, the dog remained calm and the arterial blood pressure and heart rate were constant. When injected into the common carotid arteries, 8.3±2.6% of the injected spheres reached the brain; so did 4.5±1.3% of the spheres injected into the external carotid arteries (Tables IV.1 & 2). Very few spheres reached the brainstem and cerebellum, while more than 95% of the brain spheres were located in the telecephalon.

The mean blood flow in each common carotid artery estimated by the reference sample method was 140±32 ml/min (Table IV.3). The calculated internal carotid blood flow ranged from 6.1 to 9.3 with a mean of 7.7 ml/min/artery, and the anastomotic flow ranged from 2.4 to 4.9 with a mean of 3.3 ml/min. The ratio of the anastomotic to internal carotid flow ranged from 0.3 to 0.6.

To study the sphere distributions in the brain, the telecephalons of 3 dogs were grossly divided into 3 parts of roughly equal weight: anterior, middle and posterior. The percentage of total spheres in the brain located in each part of the telecephalon when the spheres were injected into the external carotid artery was almost identical to that when injected into the common carotid artery (Fig.IV.2). In other words, microsphere distributions in the telecephalon were similar whether the spheres were injected into the external carotid or into the common carotid artery.

Humoral Responses to Angiotensin II

Plasma arginine vasopressin concentration in 7 dogs increased from a mean of 2.0 to 3.8 pg/ml during common carotid infusions of AII (Table IV.4). The vasopressin concentration increased only slightly from

2.1 to 2.9 pg/ml after external carotid AII, and this increase was significantly less than that after common carotid AII ($p < 0.05$). Vasopressin concentration was unchanged after intravenous AII.

Plasma 11-hydroxycorticosteroids concentration in 5 dogs increased significantly from a mean of 2.2 to 4.9 and 2.3 to 4.9 µg/dl after AII infusions into the common and external carotid arteries, respectively (Table IV.5). It decreased slightly from 2.6 to 2.0 ug/dl after intravenous AII.

DISCUSSION

The present study confirmed the existence of and, more importantly, provided quantitative data on the anastomoses between the intracranial and external carotid circulations in conscious dogs.

The microsphere method used in this study is very reliable in estimating blood flows so long as all the requirements are met (Heymann et al. 1977).

Firstly, the injected microspheres must be well-mixed within the blood stream. Since the common carotid catheters pointed against the blood flow, the spheres injected through these catheters were likely to mix well within the blood stream. This view was sup-

ported by the result that the common carotid blood flow estimated by the reference sample technique (Table IV.3) was so close to that measured by an electromagnetic flowmeter as described in Chapter III (Fig.III.2). However, the external carotid injections of microspheres posed a problem since these catheters pointed along the flow. It was hoped that the presence of the catheter itself might be able to generate turbulence and that the tortuosity and length of the external carotid artery could result in adequate mixing of the spheres within the blood stream. The small variabilities in the estimated values of EC'/CC (Table IV.3) and in the brain microsphere distributions (Table IV.2) supported this view.

Secondly, the spheres must be large enough to be trapped in the microvessels in their first circulation after injection; however, they must be small enough to distribute evenly within the blood stream to reflect the actual blood flow pattern. Fifteen μm spheres used in this study have been generally thought to fit these criteria and recommended for studies in the cerebral blood flow (Marcus et al. 1976 & Fan et al. 1979). Although this might not be the case for studies in blood flow to the facial muscles due to the possible existence of large arteriovenous shunts, the ratio of

EC'/CC (or T/T') is unaffected by these shuntings because the same correction factor would appear in both T and T' and cancel out each other (Eq.<IV.1>).

Finally, the number of spheres injected must be large enough to give reliable counts (Buckberg et al. 1971); however, it must not be too large to affect general and local circulations. A million spheres were injected at a time, which result in more than a thousand spheres in each sample, more than enough to give reliable counts. On the other hand, these injections appeared not to affect circulations since the dogs remained calm and their blood pressures and heart rates were unchanged during the experiments. Therefore, all the requirements for microsphere studies seem well met in this study.

According to Jewell (1952), there are five anastomoses between the external carotid and intracranial circulations: the occipital-vertebral anastomosis, the ascending pharyngeal-internal carotid anastomosis, and the three anastomoses between the internal maxillary artery and intracranial vessels -- the anastomotic artery, the ophthalmic anastomosis, and the ethmoidal anastomosis.

In the analysis of anastomotic flows (Fig.IV.1),

the occipital-vertebral and ascending pharyngeal-internal carotid anastomoses were ignored for two reasons: Firstly, if the occipital-vertebral anastomosis is functionally important, one should expect to see substantial number of spheres in the brainstem and cerebellum when the spheres are injected into the common carotid artery. However, this is not so (Table IV.1), It is therefore concluded that this anastomosis is unlikely to be functionally important in normal conscious dogs. Secondly, although this study provided no information about the functional significance of the ascending pharyngeal - internal carotid anastomosis, Jewell(1952) and others concluded that in dogs this anastomosis was of little or no functional significance. Hence it seemed justified to ignore this anastomosis too.

Of the three anastomoses between the internal maxillary artery and intracranial vessels, the anastomotic artery is generally thought to be the most important one (Jewell 1952). Therefore, although the anastomotic flow (A) estimated in this study theoretically is the total flow of all the anastomoses beyond the bifurcation of the lingual artery, practically it could be taken as primarily the flow through the anastomotic artery alone. This view is supported by the finding

that the microsphere distributions in the telecephalon were similar whether the spheres were injected into the common or external carotid artery (Fig.IV.2). This finding suggests that the anastomotic flow mixes well with the internal carotid flow before reaching the cerebral microcirculations. Of the above three anastomoses only the blood in the anastomotic artery is able to do so because only it joins the internal carotid artery before reaching the circle of Willis (Jewell 1952).

The blood flows in the internal carotid and anastomotic arteries estimated in this study seem reasonable when compared with previous reports. The internal carotid blood flow measured by an electromagnetic flowmeter when the external carotid artery was clamped in anesthetized dogs was 20 ml/min (Vidrio & Hong 1976), more than twice the present estimate (7.7 ml/min). This difference is expected since Schneider & Schneider (1934) reported that internal carotid blood flow doubled when the external carotid artery was ligated because of the existence of anastomoses between intra- and extra- cranial circulations (Bouckaert & Heymann 1935). The mean telecephalon blood flow calculated from dividing the sum of the internal carotid and anastomotic flows by the telecephalon weight (65 ± 6 g)

was 35±10 ml/100 g/min in the present study. This flow rate is in agreement with previous studies (Roth et al. 1970, Fan et al. 1979, Marcus et al. 1981) although it underestimates the actual telecephalon blood flow since the basilar artery is believed to perfuse part of the telecephalon via the posterior cerebral artery (Jewell & Verney 1957, de la Torre et al. 1962, Wellens et al. 1975).

According to the present estimation, the anastomotic flow is about half of the internal carotid flow (Table IV.3), therefore the external carotid artery contributes significantly to the perfusion of the brain in normal conscious dogs. This point was supported by the result that external carotid infusion of AII increased plasma 11-hydroxycorticosteroids concentration, used as an index of ACTH secretion, to the same extent as common carotid infusion did (Table IV.5). However, 8.3% of the spheres injected into the common carotid artery reached the brain while only 4.5% did when injected into the external carotid artery (Tables IV.1 & 2). Hence, the brain received higher concentration of AII when AII was infused into the common carotid artery than when same dose of AII was infused into the external carotid artery. This probably explains why plasma vasopressin concentration increased more

when AII was infused into the common carotid artery than when AII was infused into the external carotid artery (Table IV.4). However, it is difficult to explain the difference in the vasopressin and corticosteroids responses to external carotid AII; further research is in order.

In conclusion, the anastomoses between the external carotid and intracranial circulations are functionally important in conscious dogs. Therefore, the possibility still exists that the pressor response to external carotid AII described in Chapter III is due to a central action of AII.

Table IV.1 Distribution of microspheres in the brain when injected into the common carotid artery. Data are expressed as percentage of injected spheres.

Dog #	1	2	3	4	mean	SD
medulla	0.002	0.009	0.001	0.001	0.003	0.004
pons	0.002	0.002	0	0	0.001	0.001
midbrain	0.002	0.001	0.001	0	0.001	0.001
cerebellum	0.001	0.020	0.002	0.002	0.006	0.009
pituitary	0.052	0.018	0.014	0.050	0.034	0.020
diencephalon	0.080	0.71	0.038	0.058	0.22	0.33
telecephalon	8.18	7.52	5.03	11.26	8.00	2.56
whole brain	8.32	8.28	5.09	11.3	8.26	2.56

Table IV.2 Distribution of microspheres in the brain when injected into the external carotid artery. Data are expressed as percentage of injected spheres.

Dog #	1	2	3	4	mean	SD
medulla	0.001	0.002	0	0	0.001	0.001
pons	0.002	0.002	0	0.002	0.002	0.001
midbrain	0.001	0.006	0	0	0.002	0.003
cerebellum	0.001	0.002	0.001	0	0.001	0.001
pituitary	0.027	0.020	0	0.001	0.012	0.014
diencephalon	0.080	0.35	0.013	0.002	0.11	0.16
telecephalon	5.69	3.68	2.85	5.23	4.36	1.32
whole brain	5.80	4.06	2.86	5.24	4.49	1.31

Table IV.3 Estimation of blood flows (ml/min) in each common carotid artery (CC), internal carotid artery and anastomoses between the external carotid and intracranial circulations. EC': blood flow in the external carotid artery beyond the bifurcation of the lingual artery.

Dog #	1	2	3	4	mean	SD
common carotid	162	115	173	109	140	32
EC'/CC	0.53	0.51	0.54	0.54	0.53	0.01
anasto-moses	4.9	2.4	2.7	3.1	3.3	1.1
internal carotid	8.4	7.1	6.1	9.3	7.7	1.4

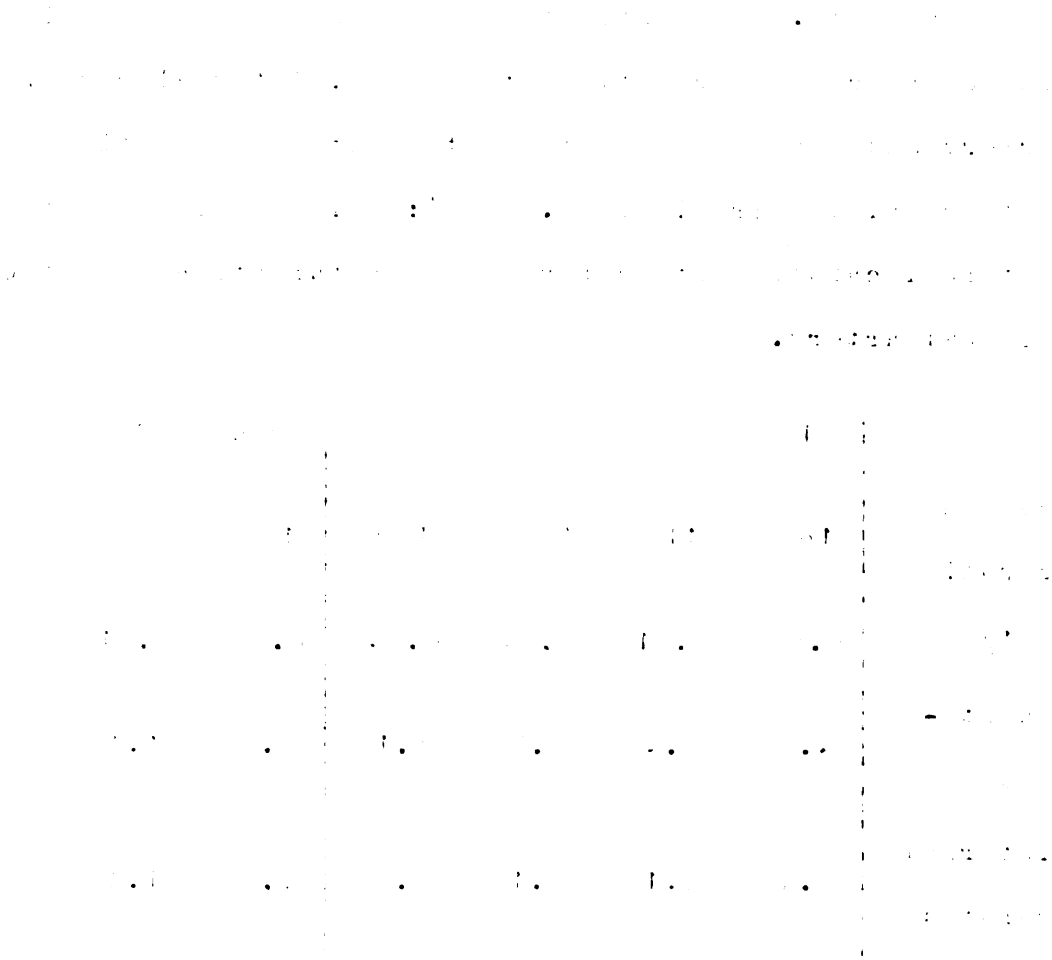


Table IV.4 Plasma arginine vasopressin concentration (pg/ml) during control pre-infusion periods and during infusions of AII at 5.0 ng/kg/min into both common carotid arteries (CC), both external carotid arteries (EC), and the femoral vein (iv) for 10 min.

Dog #	Control	CC AII	Control	EC AII	Control	iv AII
1	1.7	4.2	1.0	1.0	3.0	3.4
2	0.9	3.8	0.8	1.5	1.7	2.5
3	0.8	1.5	1.2	1.6	0.5	1.4
4	1.8	3.3	1.6	3.2	1.7	1.8
5	2.4	2.7	2.6	3.9	3.3	2.5
6	2.5	3.6	2.8	2.9	3.4	2.7
7	4.1	7.4	4.7	6.4	5.6	5.8
mean	2.0	3.8	2.1	2.9	2.7	2.9
SD	1.1	1.8	1.4	1.8	1.6	1.4

Table IV.5 Plasma 11-hydroxycorticosteroids concentration (μg/ml) during control pre-infusion periods and during infusions of AII at 5.0 ng/kg/min into both common carotid arteries (CC), both external carotid arteries (EC), and the femoral vein (iv) for 10 min.

Dog #	Control	CC AII	Control	EC AII	Control	iv AII
1	1.7	6.3	2.3	8.0	3.3	2.7
2	3.6	6.8	3.4	5.5	2.7	2.0
3	2.8	4.4	2.2	3.5	2.0	1.4
4	1.1	2.9	1.8	3.9	2.8	2.2
5	1.9	4.1	2.0	3.4	2.2	1.9
mean	2.2	4.9	2.3	4.9	2.6	2.0
SD	1.0	1.6	0.6	2.0	0.5	0.5

Fig.IV.1 Schematic drawing of the carotid arteries and the anastomoses (A) between the external carotid and intracranial circulations. CC: common carotid artery. IC: internal carotid artery. EC': external carotid artery beyond the bifurcation of the lingual artery.

Fig. IV.1

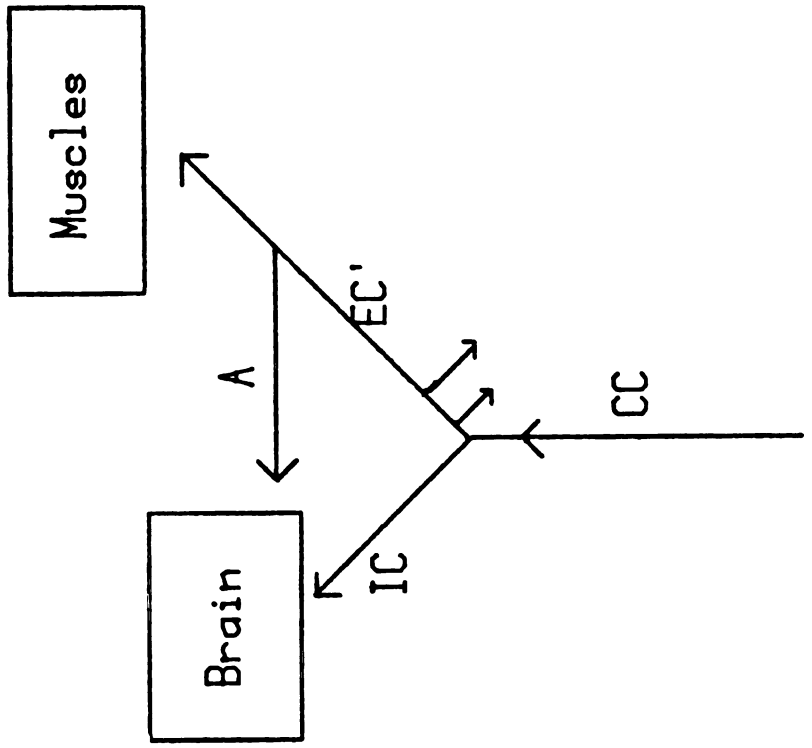
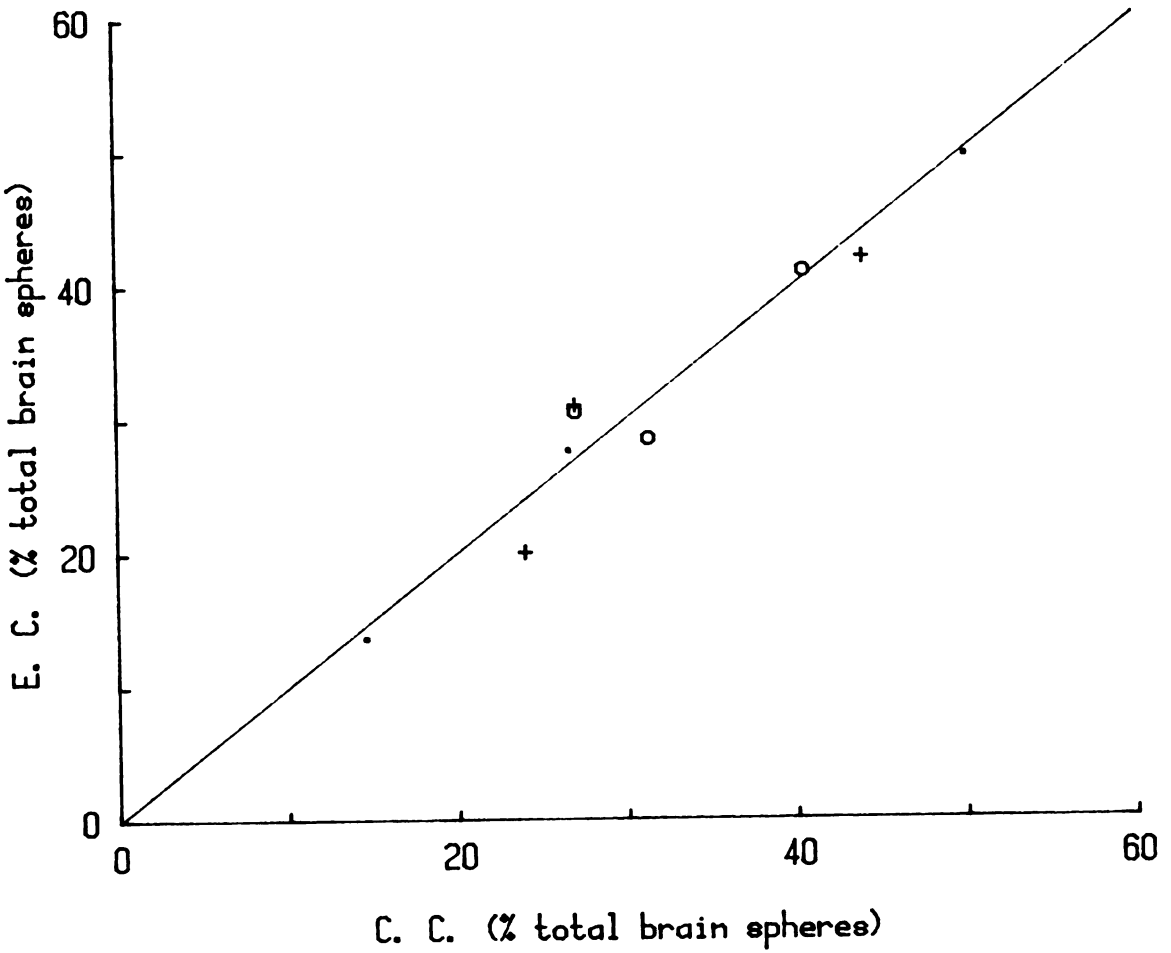


Fig.IV.2 Comparison of the distribution of microspheres in three parts of the telecephalon when injected into the common carotid artery (C.C.) vs that when injected into the external carotid artery (E.C.) in 3 dogs. The telecephalon was grossly divided into 3 parts: anterior, middle and posterior. The data are expressed as percentage of total spheres in the brain. Different symbols represent different dogs.

Fig. IV.2


60
40
20
0
0
20
40
60
E. C. (% total brain spheres)
C. C. (% total brain spheres)

CHAPTER V PRESSOR RESPONSES TO INTRACAROTID INFUSIONS OF PHENYLEPHRINE AND VASOPRESSIN

As discussed earlier, the possibility exists that the pressor response to low doses of intracarotid AII is due to its extracranial vasoconstrictor effect. If this is true, intracarotid infusion of any other agents which cause extracranial vasoconstriction should also increase blood pressure more than intravenous infusion. To further test this hypothesis, the pressor responses to intracarotid infusions of two well-known vasoconstrictors, phenylephrine and vasopressin, were studied.

Phenylephrine, an α_1-adrenergic agonist, was infused into the common carotid arteries on both sides or femoral vein at a rate of 1.0 μg/kg/min for 10 min in 5 conscious dogs (19-22 kg). Synthetic arginine vasopressin (Bachem) was infused at a rate of 2.0 ng/kg/min in 3 dogs (20-22 kg).

RESULTS

Intracarotid phenylephrine slightly increased mean arterial pressure from 95±4 to 99±7 mmHg (mean±standard deviation), whereas intravenous phenylephrine increased blood pressure from 95±4 to 110±8 mmHg (Fig.

V.1). The pressor response induced by intravenous phenylephrine was significantly greater than that induced by intracarotid phenylephrine ($p < 0.05$, paired t-test). Intracarotid phenylephrine seemed to decrease heart rate more than intravenous phenylephrine did in 4 dogs (-15 ± 5 vs -7 ± 6 beats/min, Fig. V.2). Statistical analysis was not done because of the small sample size.

Intracarotid vasopressin did not consistently alter the mean arterial pressure (mean change -2 ± 5 mmHg, Fig. V.3), whereas intravenous vasopressin increased blood pressure in all 3 dogs (mean increase 6 ± 1 mmHg). Both routes decreased heart rates in all 3 dogs (-9 ± 2 and -12 ± 7 beats/min, Fig. V.4).

DISCUSSION

If the vasoconstriction hypothesis is true, i.e., if extracranial vasoconstriction is responsible for the pressor response to intracarotid AII, intracarotid infusion of any other vasoconstrictor should increase blood pressure too. Both phenylephrine and vasopressin are potent vasoconstrictors. In sharp contrast to AII, however, intracarotid phenylephrine and vasopressin increased blood pressure to a less extent than intravenous infusions did (Figs. V.1 & 3). One possible explanation is that the vasoconstriction hypothesis

is not true and that both phenylephrine and vasopressin are cleared significantly through the cranial circulation. Therefore, smaller amounts of the vasoconstrictors reach general circulation and result in a smaller pressor response when they are infused into the carotid artery than when infused intravenously. However, since vasopressin is inactivated largely in the liver and kidneys and phenylephrine is metabolized mainly in the liver (Goodman & Gilman 1980), they are unlikely to be cleared significantly through the cranial circulation. Hence this explanation is probably not true.

Another possibility is that intracarotid phenylephrine and vasopressin could act on the brain to cause a depressor response. Although intraventricular α-agonists have been shown to lower blood pressure (Kaneko et al. 1960), systemically-administered phenylephrine has minimal central action (Goodman & Gilman 1980). Injection of norepinephrine and epinephrine into the internal carotid artery was reported not to decrease blood pressure in man (Greenfield & Tindall 1968). Furthermore, vasopressin has never been reported to have a central depressor action. Therefore, this possibility is unlikely to be true.

Finally, intracarotid phenylephrine and vasopressin could act on the carotid sinus to increase

baroreceptor activity and hence cause a depressor effect. Intracarotid infusions delivered a much higher concentration of drugs to the carotid sinus than intravenous infusions did. Heymans & Delaunois (1951) showed that constriction of the carotid sinus wall was responsible for the increased baroreceptor stimulation leading to hypotension and diminution of the carotid occlusion reflex when epinephrine or norepinephrine was applied locally. Vasopressin and other vasoconstrictors other than AII have also been shown to have the similar effect (Heymans 1955). This view is supported by the present finding that even though it caused a smaller pressor response, intracarotid phenylephrine, presumably acting on the carotid sinus, induced a greater fall in heart rate than intravenous infusion did (Fig. V.2). On the other hand, McCubbin et al. (1957) showed that AII had no effect on the carotid sinus. This point is supported by the finding presented in Chapter III that external carotid infusion of AII increased blood pressure to the same degree as common carotid infusion did and that both routes of infusion did not alter heart rate significantly. By recording the action potentials in single baroreceptor fibers, Lumbers et al. (1979) also concluded that AII had no effect on the sensitivity of carotid sinus baroreceptor. Therefore, it is possible that the

pressor effects of intracarotid phenylephrine and vasopressin caused by extracranial vasoconstriction, unlike AII, might be masked by their depressor effects induced by their direct action on the carotid sinus.

In conclusion, due to the possible direct actions of phenylephrine and vasopressin on the carotid sinus, this study does not rule out the possibility that extracranial vasoconstriction is responsible for the pressor response to intracarotid AII.

Fig.V.1 The pressor response to intravenous (iv) phenylephrine (PhE) was greater than that to common carotid infusion (CC) in 5 conscious dogs. Phenylephrine was infused at 1.0 μg/kg/min for 10 min. Different symbols represent different dogs. Control: pre-infusion control period.

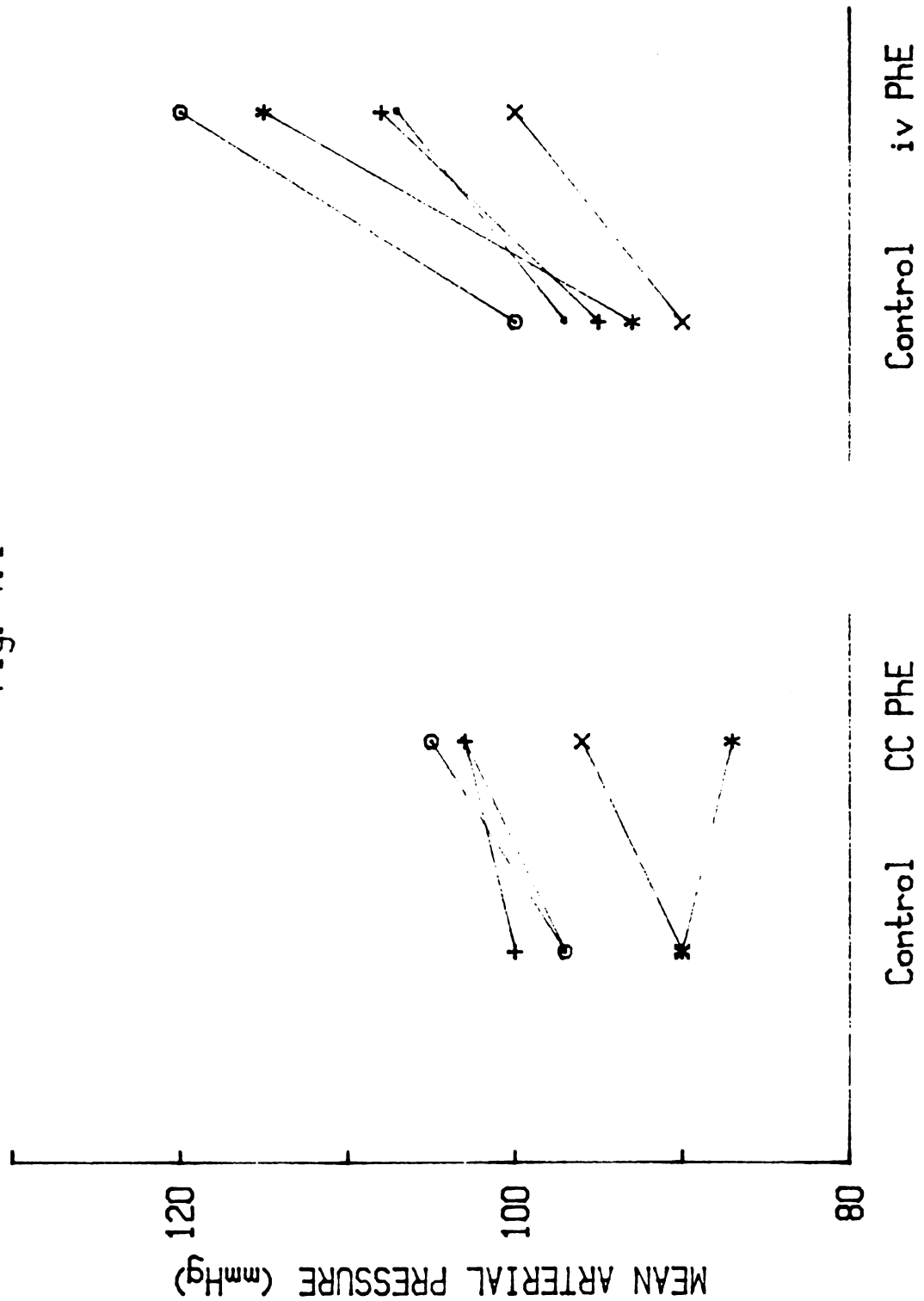

MEAN ARTERIAL PRESSURE (mmHg)
120
100
80
Control
CC PhE
Control
iv PhE

Fig. V.1

Fig.V.2 Mean heart rate during control pre-infusion periods and during intracarotid and intravenous phenylephrine in 4 dogs. Intracarotid phenylephrine appeared to decrease heart rate more than i.v. infusion.

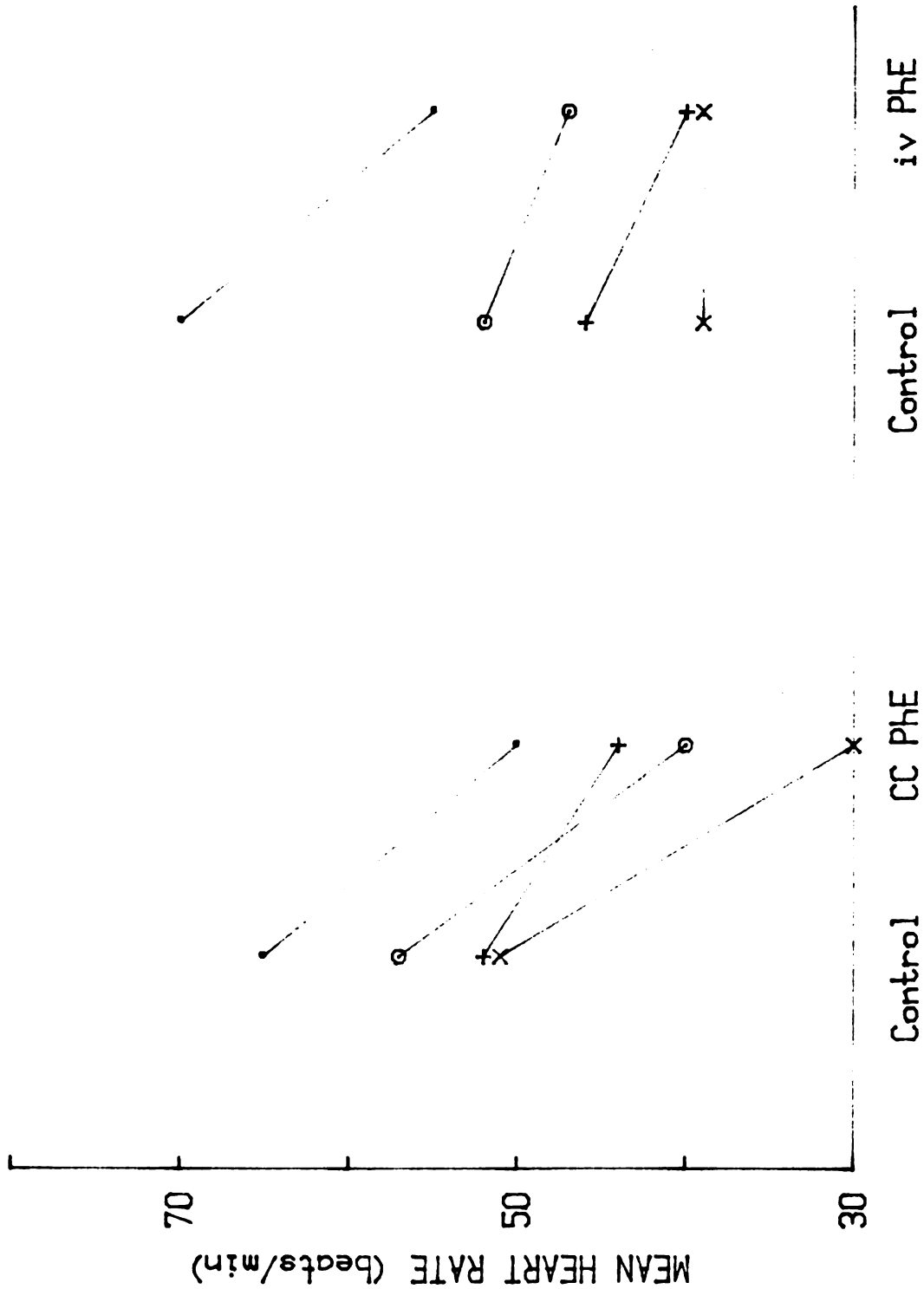

MEAN HEART RATE (beats/min)
70
50
30
Control
CC PhE
Control
iv PhE

Fig. V.2

Fig.V.3 Mean arterial pressure during control pre-infusion periods and during intracarotid and intravenous infusion of arginine vasopressin (AVP) in 3 dogs. Vasopressin was infused at 2.0 ng/kg/min for 10 min.

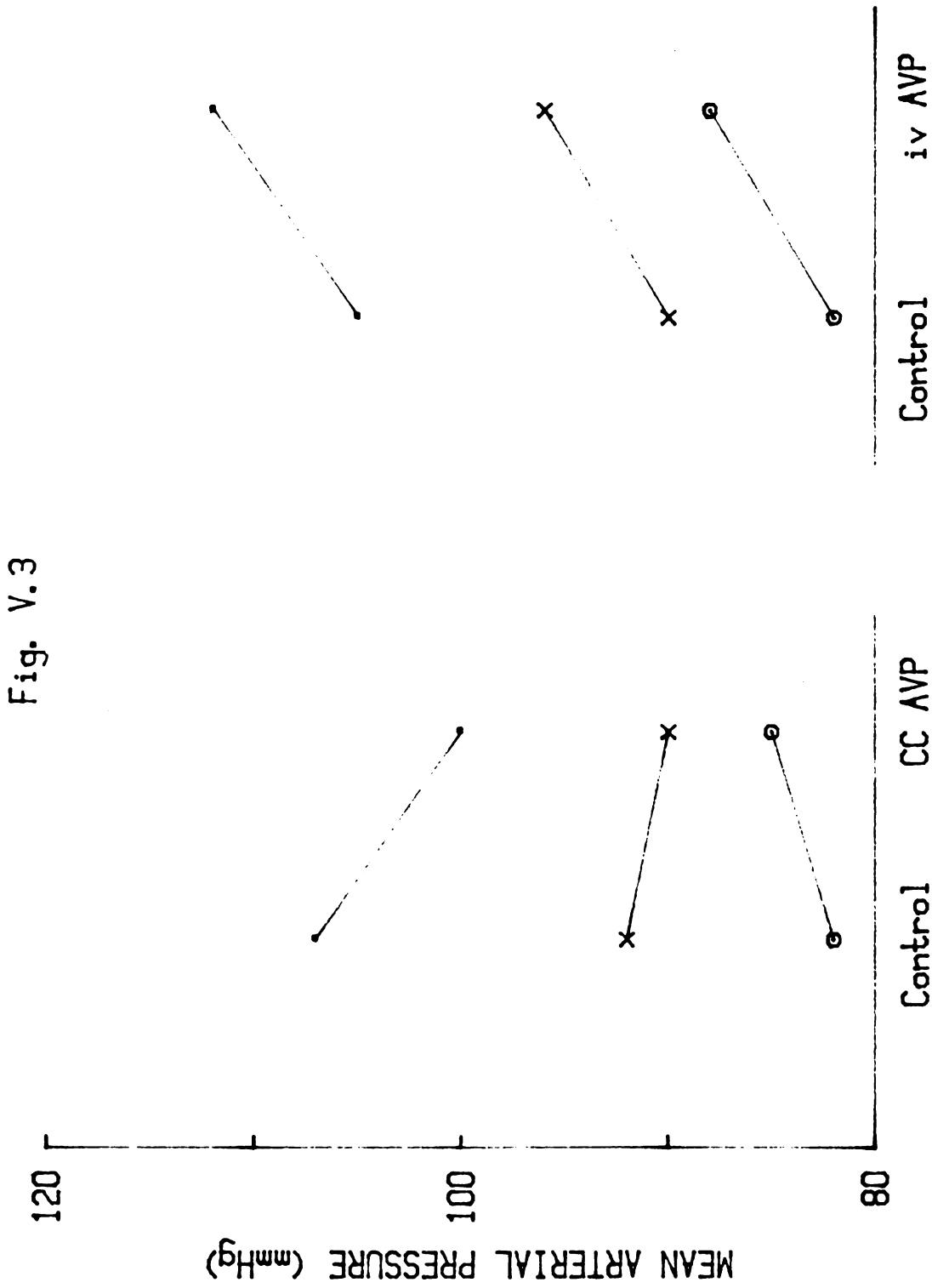

MEAN ARTERIAL PRESSURE (mmHg)
120
100
80
Control
CC AVP
Control
iv AVP

Fig. V.3

Fig.V.4 Mean heart rate during control pre-infusion periods and during intracarotid and intravenous infusion of arginine vasopressin in 3 dogs.

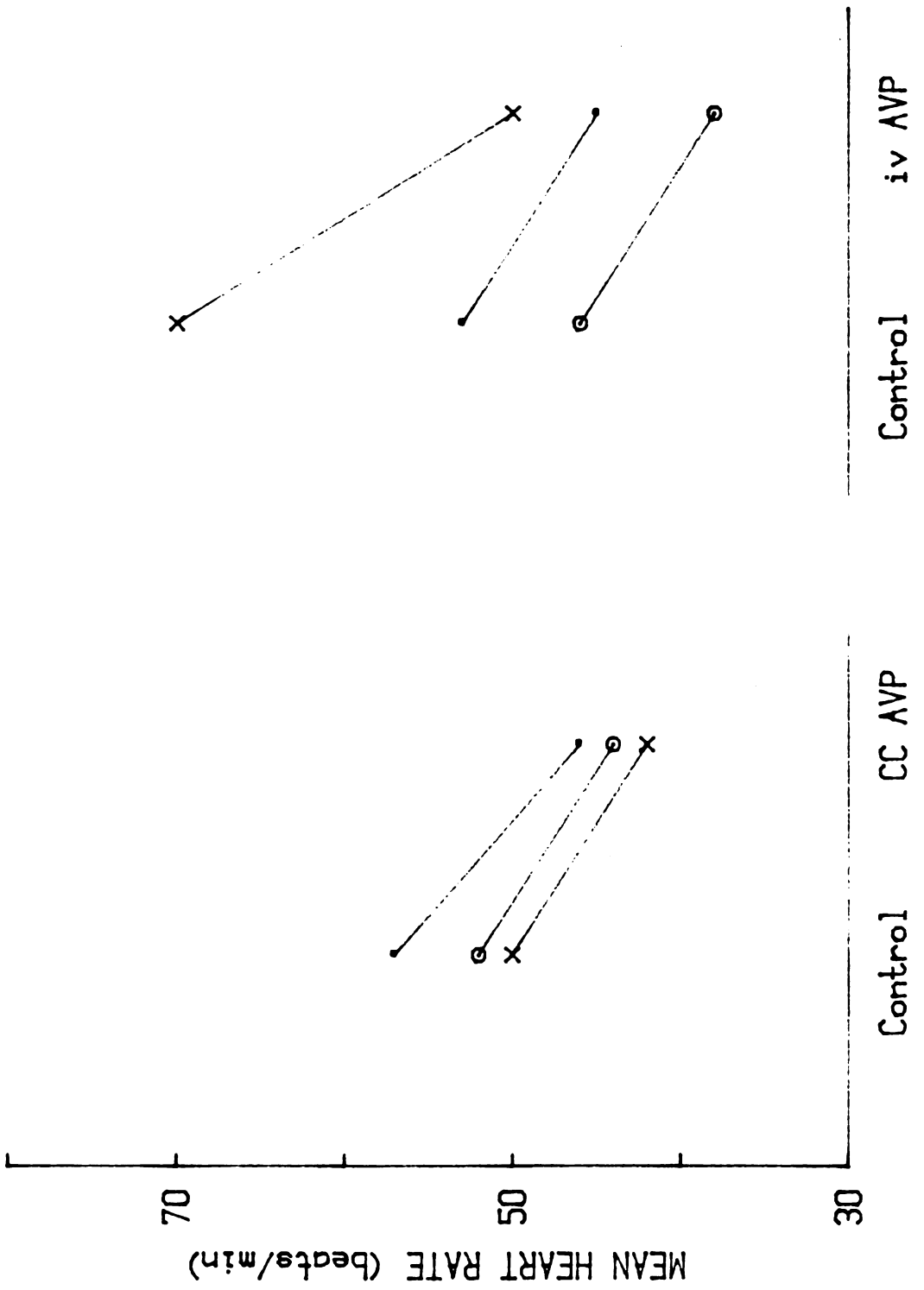

MEAN HEART RATE (beats/min)
70
50
30
Control
CC AVP
Control
iv AVP

Fig. V.4

CHAPTER VI EFFECTS OF INTRAVENTRICULAR SARALASIN ON THE PRESSOR RESPONSE TO BLOOD-BORNE ANGIOTENSIN II

INTRODUCTION

As discussed previously, administration of AII into the vertebral arteries has been demonstrated to elicit a centrally mediated pressor response in dogs (Lowe & Scroop 1969, Ferrario et al. 1970), rabbits (Yu & Dickinson 1965) and man (Ueda et al. 1969). Blood-borne AII can therefore act not only on the vascular smooth muscles but also on the brain to increase arterial blood pressure. However, to what extent the central pressor component contributes to the pressor response to blood-borne AII is still unsettled.

Ablation of the area postrema in the greyhound not only abolished the pressor action of intravertebral AII (Joy & Lowe 1970b) but also reduced that of intravenous AII (Scroop et al. 1971). This lesion also lowered blood pressure following hemorrhage (Katic et al. 1971) and impaired the development of acute renal hypertension (Scroop et al. 1975). Hence, the central pressor component of circulating AII appeared to be important in greyhounds.

However, the area postrema is very close to the

nucleus tractus solitarii, an important medullary site of blood pressure regulation (Crill & Reis 1968). Ablation of the area postrema might damage this adjacent nucleus as well. Furthermore, these studies were done on anesthetized greyhounds. As the complications of anesthesia are well-known, there seems to be a need to examine this question in conscious dogs by using different approach.

In the present study, saralasin, a competitive antagonist of AII, was infused into the third cerebroventricle of conscious dogs. Its effects on the pressor responses to intravertebral, intracarotid and intravenous AII were then compared. The pressor response to intravenous AII was to mimic the overall pressor effect of circulating AII, and that to intravertebral and intracarotid AII to mimic its central pressor component.

METHODS

A cannula was chronically implanted in the third cerebroventricle of 5 mongrel dogs (20-25 kg). The cannula was a 20-gauge stainless steel tubing (Small Parts, Miami) bent at a 120^{o}-angle in one end. The cannula was placed stereotaxically through a hole drilled in the skull and was held in place by dental

cement with 4 stainless steel screws fixed in the skull. The placement of the cannula was determined by X-ray photography after injecting 0.3 ml meglumine iothalamate (Conray) through the cannula (Thrasher et al. 1980). A tygon tubing was connected to the end of the cannula, passed subcutaneously and exteriorized at the back of the dog.

One week later, the second surgery was performed. Small, non-occluding catheters were implanted in vertebral and common carotid arteries on both sides for AII infusion. Two other catheters were placed in a femoral artery and vein for blood pressure recording and AII infusion, respectively.

The dogs were allowed to recover from the surgery for another week. On the day of the experiment, the dog was brought into the laboratory. Saralasin or artificial cerebrospinal fluid (CSF) was infused into the third ventricle for 60 min, AII was then infused into both carotid arteries, both vertebral arteries or femoral vein in random order. Arterial blood pressure and heart rate were monitored throughout the experiment. Consecutive experiments were separated by at least one day apart to avoid any carry-over effects.

Saralasin ($[Sar^1\text{-}Ile^5\text{-}Ala^8]$ angiotensin II,

Bachem) was freshly prepared in artificial CSF. The composition of the artificial CSF was described by Thrasher et al. (1980). Its pH was approximately 7.4 and was buffered by bicarbonate and phosphate. Saralasin was infused at a rate of 0.1 μg/kg/min (in 5 μl/min). Angiotensin II was infused at a rate of 1.0 ng/kg/min into a carotid artery, 0.5 ng/kg/min into a vertebral artery, or 2, 5 and 10 ng/kg/min into a femoral vein.

Student's paired t-test was used to analyze the effects of saralasin on the blood pressure and heart rate changes during AII infusions into the vertebral or carotid artery. Analysis of variance for repeated measurements was used to analyze those during intravenous infusion. Data are expressed as mean±standard deviation.

RESULTS

The control mean arterial pressure and heart rate during intraventricular infusion of artificial CSF were 115±9 mmHg and 82±16 beats/min, respectively. They were 115±12 mmHg and 90±8 beats/min, respectively, during intraventricular saralasin infusion. When AII was infused into the vertebral arteries, the mean blood pressure and heart rate increased promptly by 15±6 mmHg

and 19±12 beats/min, respectively, during intraventricular artificial CSF infusion (Fig.VI.1, 2 & 3). Intraventricular saralasin significantly inhibited these increases; the mean changes in blood pressure and heart rate were 4±6 mmHg and -2±5 beats/min, respectively, to intravertebral AII (Fig.VI.1, 2 & 3).

On the other hand, intraventricular saralasin had no effect on the pressor response to intracarotid AII (10±5 vs 10±4 mmHg, Fig.VI.2). Saralasin also had no effect on the pressor responses to intravenous AII at three different doses (Fig.VI.4, Table VI.1). Heart rate did not change significantly to intracarotid (Fig.VI.3) and intravenous AII (Table VI.2) during intraventricular infusion of either artificial CSF or saralasin.

DISCUSSION

When AII is infused intravenously, it acts peripherally to cause systemic vasoconstriction; however, it also has access to the brain where it can exert a central pressor action. Since intraventricular saralasin inhibited the pressor action of intravertebral AII (Fig.VI.2), one would expect that saralasin would also reduce the pressor response to AII i.v. if the central pressor action of intravertebral AII was

important. However, this was not so (Fig.VI.4). It is therefore concluded that the central pressor action of intravertebral AII is not essential in the pressor response to blood-borne AII.

Another finding in keeping with this conclusion is the heart rate response. Intraventricular saralasin inhibited the tachycardia produced by intravertebral AII (Fig.VI.3). This central action of intravertebral AII appeared not to be essential in the maintenance of heart rate during i.v. AII, because saralasin did not lower heart rate when AII was given intravenously (Table VI.2).

The present conclusion, however, is contrary to previous reports on greyhounds. The central pressor action of intravertebral AII was shown to be important in the pressor response to AII i.v. (Scroop et al. 1971), in the maintenance of blood pressure following hemorrhage (Katic et al. 1971) and in the development of acute renal hypertension (Scroop et al. 1975).

It is difficult to explain this discrepancy. However, species difference is one possible explanation. Mongrel dogs and greyhounds are different in that the pressor response to intravertebral AII is mediated mainly by withdrawal of the vagal tone to the heart in

greyhounds (Scroop & Lowe 1969), whereas by increased efferent sympathetic activity in mongrel dogs (Ferrario et al. 1972). In addition, studies on the greyhounds were all done during morphine-chloralose anesthesia, whereas the present study was carried out in conscious dogs. In addition to the effects of anesthesia, the trauma caused by extensive surgery can only be avoided by working on conscious animals fully recovered from surgery.

Furthermore, ablation of the area postrema might also damage adjacent nuclei such as the nucleus tractus solitarii, the site of the first medullary relay in the baroreceptor reflex arc (Crill & Reis 1968). In some of the studies on greyhounds, either the control heart rate (Scroop et al. 1971) or blood pressure (Scroop et al. 1975) appeared to be elevated after the lesion. This could be due to the unexpected damage of this adjacent nucleus (Zandberg et al. 1977).

On the other hand, the present finding is in agreement with some other studies. Ramsay et al. (1978) reported that intraventricular saralasin had no effect on the pressor response to AII i.v. in pentobarbital-anesthetized mongrel dogs. Reid (1976) found that intraventricular saralasin had no effect on the maintenance of blood pressure in sodium-depleted

mongrel dogs, whose plasma AII concentrations were elevated. Most recently, by comparing the effects of saralasin infusions into the vertebral artery, carotid artery and femoral vein on the blood pressure, Brooks & Reid (1981) also concluded that the effect of AII on the maintenance of blood pressure in sodium-depleted dogs was not mediated via the brain. In the rat, intraventricular saralasin had no effect on the blood pressure following ligation of the abdominal vena cava (to be presented in Chapter VII), a procedure that increased the plasma AII concentration.

Sweet et al. (1977) showed that intraventricular administration of the antagonist, [Sar^1-Ile^8] AII, did not affect the pressor response to AII i.v. in rats, which is in keeping with the present study. Although the same treatment lowered blood pressure in experimental renal hypertensive rats, this finding did not lead to the conclusion that the central pressor component was important. The reason is that the antagonist also lowered blood pressure in the spontaneously hypertensive rats, whose plasma AII concentrations were not elevated (Mann et al. 1978); thus this treatment did not specifically inhibit the pressor action of circulating AII. Similar results were obtained by using saralasin (Mann et al. 1978).

Lesions of the anteroventral third ventricle have been reported to depress the pressor response to AII i.v., and to lower blood pressure in renal hypertensive rats (Buggy et al. 1977). However, the same lesion also lowered blood pressure in several forms of low-renin hypertension, such as deoxycorticosterone-salt and spontaneous hypertension (Brody et al. 1978). Therefore, this lesion did not specifically inhibit the pressor action of circulating AII, and these observations did not provide further information on the significance of the central pressor component of circulating AII.

There was a recent report that subfornical organ lesion reduced the pressor effect of i.v. AII in rats (Mangiapane & Simpson 1980). However, since this lesion also inhibited the dipsogenic effect of i.v. AII (Simpson et al. 1978), the possibility exists that the central pressor component inhibited by the lesion is due to the arousal effect of AII. More research is needed to clarify the specificity of this lesion.

Since the pressor response to intravertebral, but not intracarotid, AII was inhibited by saralasin (Fig.VI.2), these results thus provide direct evidence that the mechanism of the pressor response to intracarotid AII is different from that to intravertebral

AII. This is in keeping with the result of the microsphere study presented in Chapter IV, where carotid blood was shown not to perfuse the medulla. So the site of action of intracarotid AII must not lie in the medulla where the intravertebral AII acts to raise blood pressure. In addition, intraventricular saralasin also inhibited drinking and vasopressin secretion to AII i.v. (Thrasher & Ramsay, unpublished observation), hence the sites of these AII actions must be different from that of the pressor action of intracarotid AII.

In the rat, intracarotid rather than intravertebral AII was recently reported to cause a greater pressor response than intra-aortic AII did (Haywood et al. 1980). The pressor response to intracarotid AII was inhibited by intraventricular saralasin. Rats thus appear to be different from dogs in the central pressor actions of circulating AII. The subfornical organ (Mangiapane & Simpson 1980) and the organum vasculosum of the lamina terminalis (Fink et al. 1980) have been implicated to contain receptors for the pressor effect of AII in rats.

In conclusion, the central pressor action of intravertebral AII appears to be not essential in the pressor response to blood-borne AII in conscious

mongrel dogs. In addition, the sites of the pressor actions of intracarotid and intravertebral AII are different.

Table VI.1 Lack of effect of intraventricular saralasin on the mean arterial blood pressure (mmHg) during i.v. AII at 2.0, 5.0 and 10 ng/kg/min for 10 min. (a) control intraventricular artificial CSF infusion, (b) intraventricular saralasin infusion (0.1 µg/kg/min).

(a) Artificial CSF

Dog #	Control	AII (2.0)	Control	AII (5.0)	Control	AII (10)
1	97	103	95	120	100	140
2	95	100	107	117	100	117
3	115	123	113	127	110	130
4	105	110	110	120	108	123
5	103	108	102	115	105	120
mean	103	109	105	120	105	126
SD	8	9	7	5	5	9

(b) Saralasin

1	102	110	100	122	100	135
2	94	98	93	101	93	120
3	113	118	115	127	110	128
4	110	117	115	125	120	132
5	102	105	103	112	102	117
mean	104	110	105	117	105	126
SD	7	8	10	11	10	8

Table VI.2 Lack of effect of intraventricular saralasin on the mean heart rate (beats/min) during i.v. AII at 2.0, 5.0 and 10 ng/kg/min for 10 min. (a) control intraventricular artificial CSF infusion, (b) intraventricular saralasin infusion (0.1 μg/kg/min).

(a) Artificial CSF

Dog #	Control	AII (2.0)	Control	AII (5.0)	Control	AII (10)
1	70	60	60	55	0	85
2	60	50	57	45	62	48
3	55	40	50	50	45	35
4	65	61	61	66	62	64
5	83	83	83	77	80	80
mean	67	59	62	59	64	62
SD	11	16	12	13	13	21

(b) Saralasin

1	55	50	70	65	67	55
2	65	58	62	60	60	65
3	70	65	65	55	65	60
4	70	66	80	82	70	80
5	83	83	80	75	78	75
mean	69	64	71	67	68	67
SD	10	12	8	11	7	10

Fig.VI.1 A typical example of the inhibition effects of intraventricular saralasin on the pressor and tachycardia responses to intravertebral AII. Upper panel: intraventricular control infusion of artificial CSF (aCSF). Lower panel: intraventricular infusion of saralasin (0.1 μg/kg/min). MAP: mean arterial blood pressure (mmHg), HR: heart rate (beats/min). Angiotensin was infused at 0.5 ng/kg/min into each artery between the arrows.

Fig. VI.1

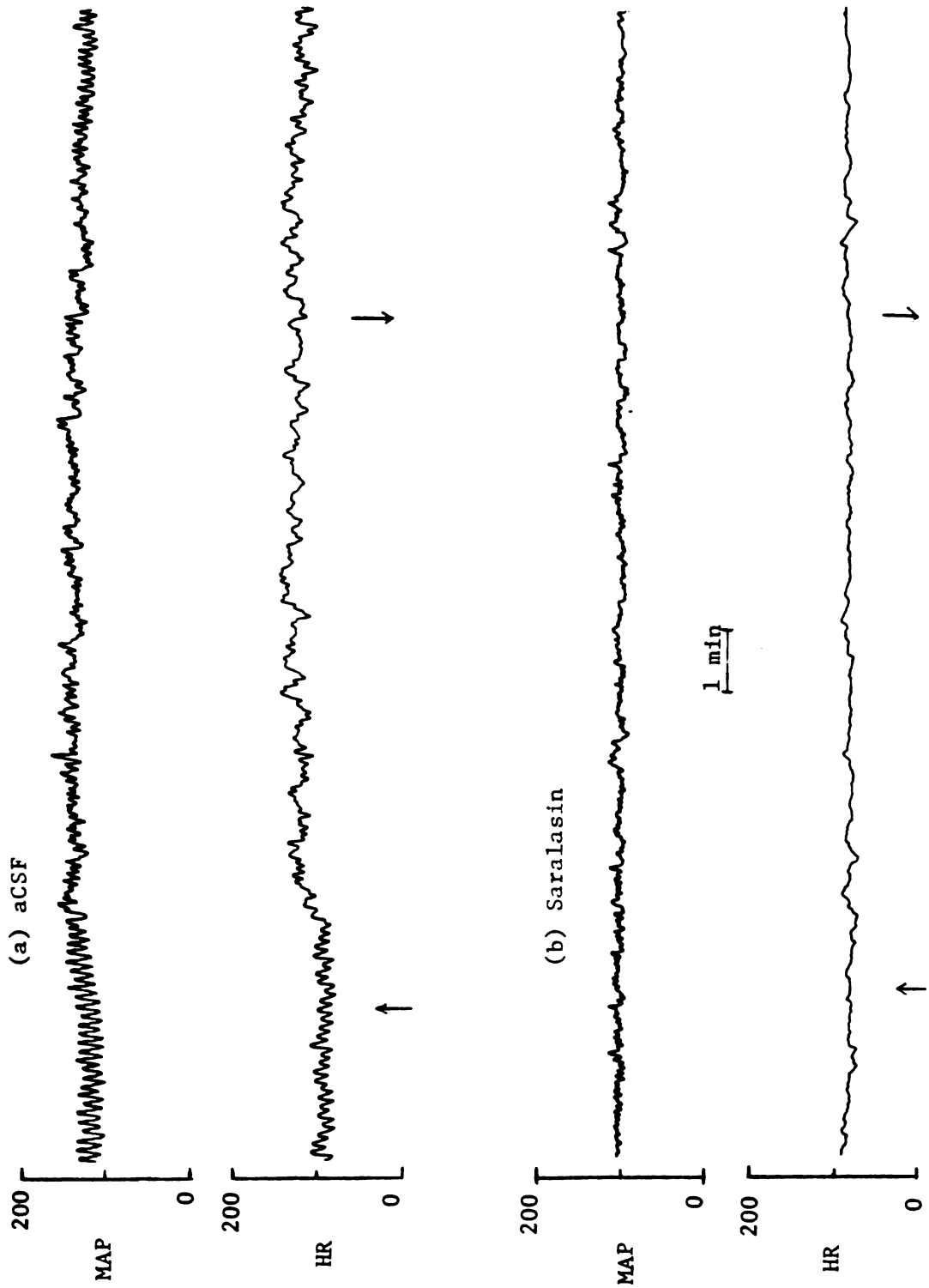


Fig.VI.2 Effects of intraventricular saralasin (Sar) on the pressor responses to intravertebral (left) and intracarotid AII in 5 dogs. Saralasin significantly reduced the pressor effect of intravertebral AII ($p < 0.05$). The AII infusion rates were 1.0 and 2.0 ng/kg/min for the vertebral and common carotid arteries, respectively. aCSF: intraventricular control infusion of artificial CSF. Different symbols represent different dogs.

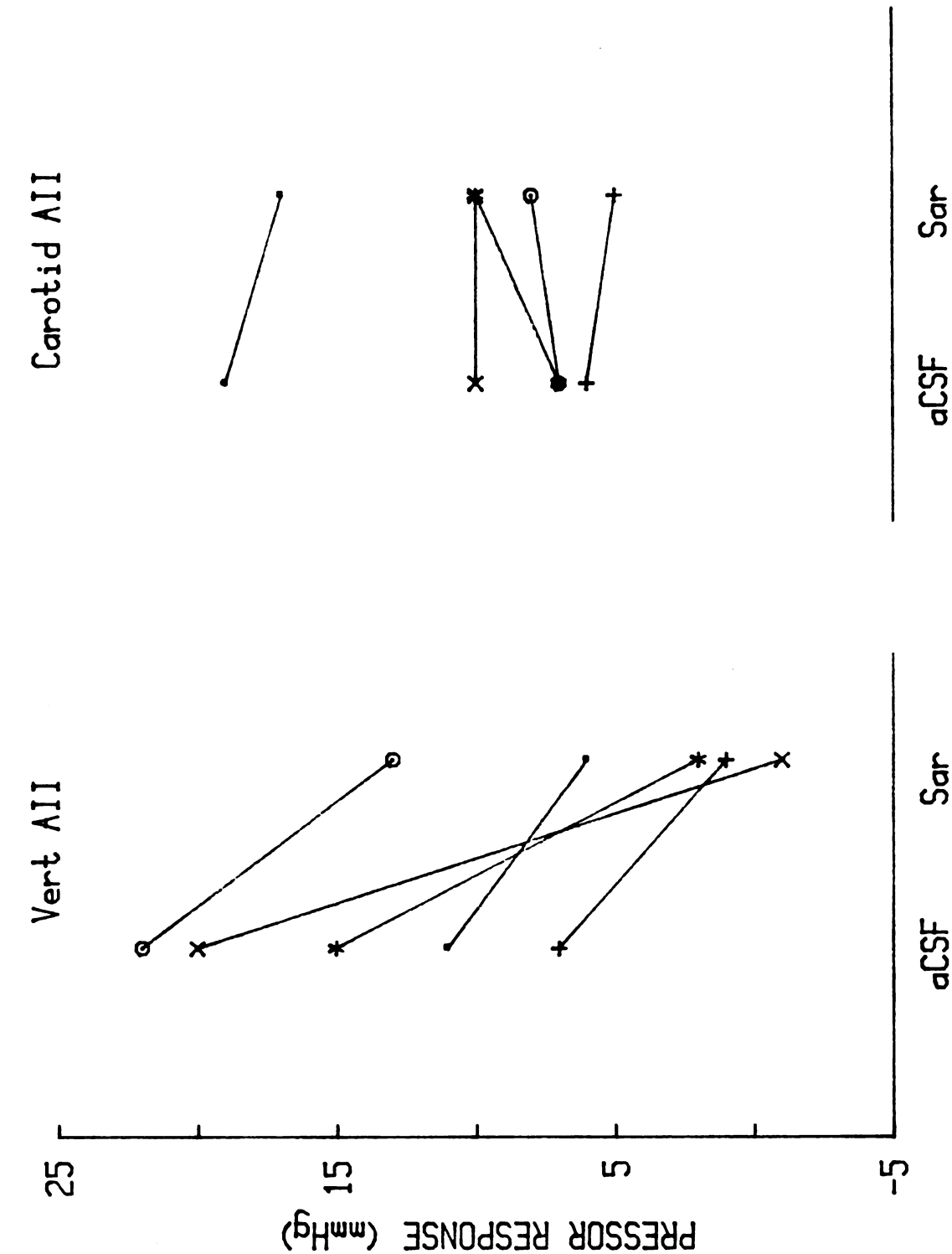

Vert AII
Carotid AII
PRESSOR RESPONSE (mmHg)
25
15
5
-5
aCSF
Sar
aCSF
Sar

Fig. VI.2

Fig.VI.3 Effects of intraventricular saralasin on the heart rate responses to intravertebral (left) and intracarotid AII in 5 dogs. Saralasin significantly reduced the increase in heart rate following intravertebral AII ($p < 0.05$). The doses of AII were the same as those in the previous figure. The symbols of two dogs are overlapped in the right panel.

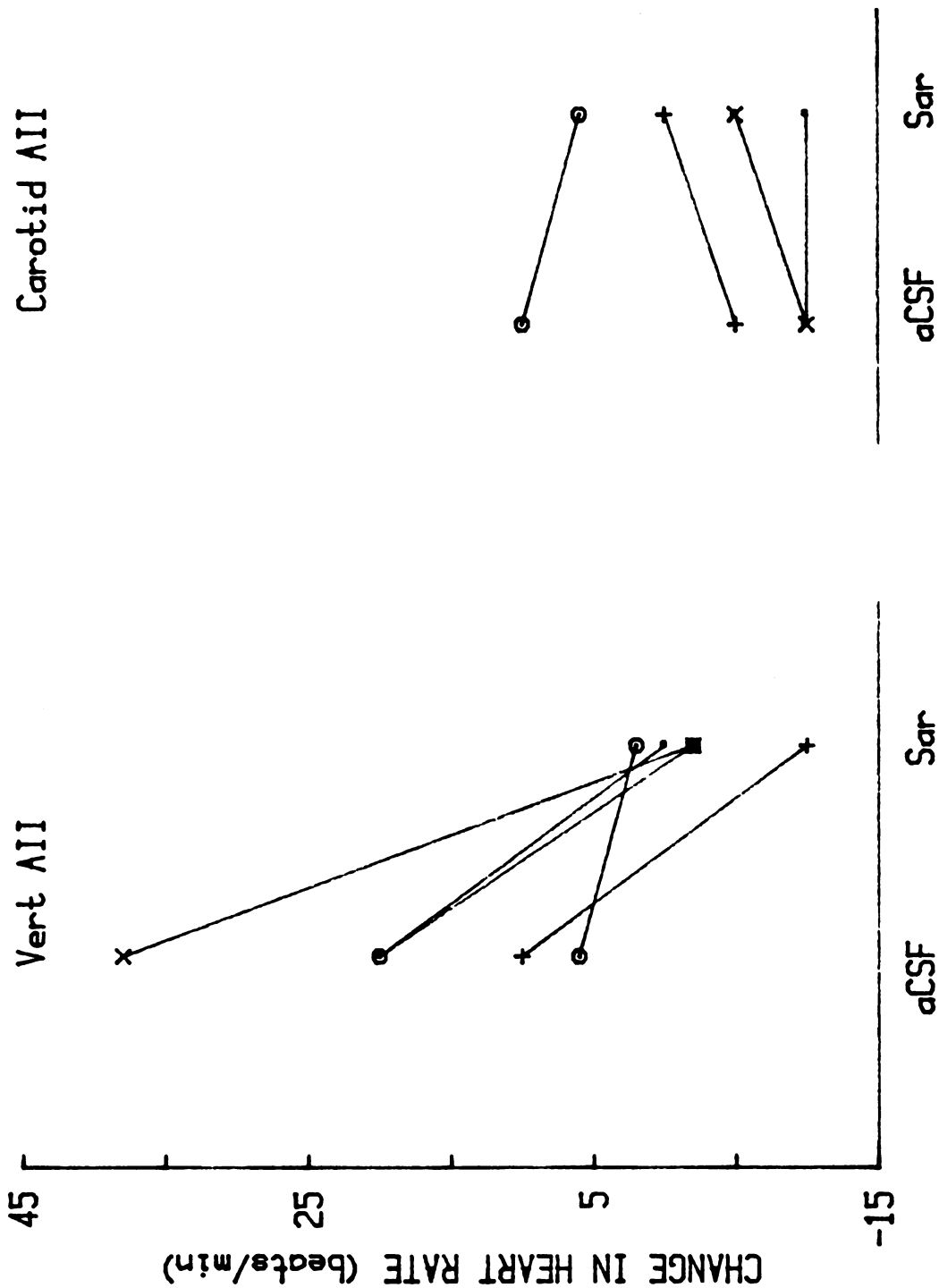

Vert AII
Carotid AII
aCSF
Sar
aCSF
Sar
45
25
5
-15
CHANGE IN HEART RATE (beats/min)

Fig. VI.3

Fig.VI.4 Lack of effect of intraventricular saralasin on the pressor response to i.v. AII. Mean and standard error of mean are shown.

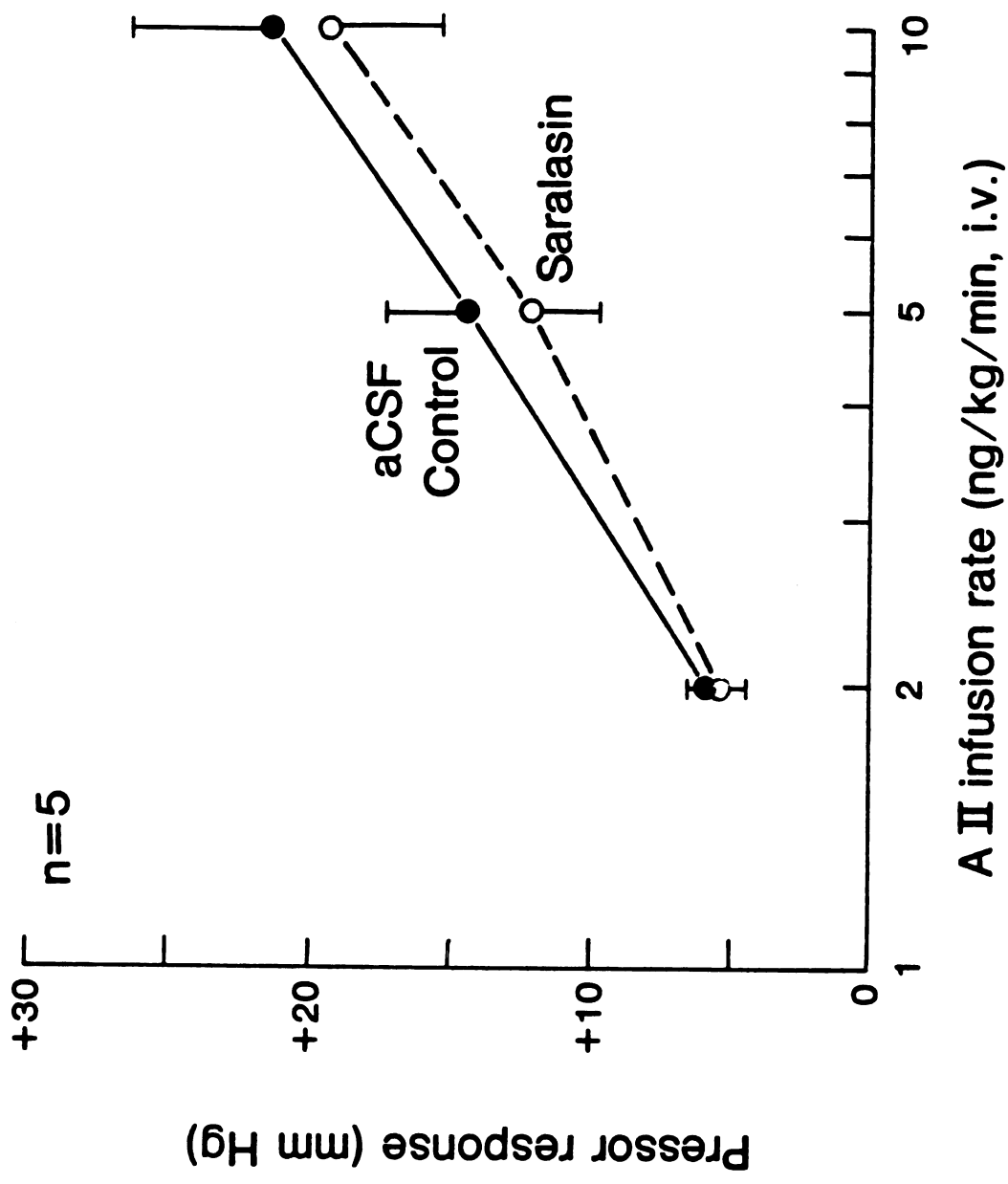

Pressor response (mm Hg)
+30
+20
+10
0
n=5
aCSF
Control
Saralasin
1
2
5
10
A II infusion rate (ng/kg/min, i.v.)

Fig. VI.4

CHAPTER VII IS ANGIOTENSIN ESSENTIAL IN DRINKING INDUCED BY WATER DEPRIVATION AND CAVAL LIGATION?*

INTRODUCTION

Angiotensin II has been shown to be a potent dipsogen (Epstein 1978, Fitzsimons 1978), however the role of endogenous angiotensin in the control of drinking is still not established. Ligation of the inferior vena cava was first demonstrated by Fitzsimons (1969) to elicit drinking in the rat. Because this drinking response was inhibited by bilateral nephrectomy (Fitzsimons 1969) and restored by intravenous infusion of AII (Fitzsimons & Simons 1969), he proposed that the renal renin-angiotensin system played a crucial role in the control of drinking following caval ligation. Nevertheless, this hypothesis has been subsequently questioned by several investigators (Lehr et al. 1975, Rolls & Wood 1977, Stricker 1977). Water deprivation is another thirst stimulus that has received much attention. Intracerebroventricular (ICV) administration of saralasin failed to show any inhibition of drinking induced by water deprivation in rats, dogs, sheep, and goats (Abraham et al. 1976, Olsson 1975,

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Ramsay & Reid 1975, Severs et al. 1977). However, controversy remains. Malvin, Mouw, and Vander (1977) reported that prolonged infusion of saralasin ICV increased the latency of water-deprived rats to drink, and this has been interpreted as evidence for the participation of the renin-angiotensin system in the control of drinking following water deprivation.

In the present study, we first determined a dose of saralasin ICV that inhibited the drinking responses to AII but not to hypertonic saline. We then used this dose of saralasin to investigate the role of the renin-angiotensin system in the control of drinking following water deprivation and caval ligation.

METHODS

General Methods

Subjects. Male Sprague-Dawley rats (body wt 250-400 g) were used. Except during experiments, animals were housed individually in a room maintained at 20-25°C; lights were on from 5 A.M. to 7 P.M. Water and food (Purina rat chow) were available ad libitum. At 8 A.M. on the day of an experiment, animals were transferred to plastic cages (10 in. x 18 in. x 8 in.) where they had previous experience of all the necessary experimental maneuvers. By 5 P.M. the experiment was

finished, and animals were moved back to their home cages. Consecutive experiments on the same animal were usually separated 2 days apart to minimize any possible carry-over effect.

Chemicals and plasma renin activity assay. Saralasin and angiotensin II were freshly prepared in isotonic saline to the concentration desired, then divided into small volumes and stored at -25°C until needed.

Blood sample were collected by decapitation of the rats. Plasma renin activity (PRA) was measured with a radioimmunoassay for angiotensin I (Reid et al. 1972, Stockigt et al. 1971) and expressed as nanograms of angiotensin I formed per milliliter of plasma during a 3-h incubation.

Intracranial cannulation and infusion. The implantation of lateral ventricular cannula was made according to the procedures of Simpson et al. (1978). At least 4 days were allowed for animals to recover from the surgery. The placement of the cannula was checked by the drinking response to AII ICV (10 ng in 1 µl solution) and rechecked at postmortem by the presence of bromphenol blue inside the ventricle following ICV injection of the dye (1 µl).

The following regime of administration of saralasin ICV was adopted throughout the study unless otherwise noted. One microliter of saralasin (4 mg/ml) was given ICV as a bolus and followed by infusion at a rate of 1.3 µl/h for 75 min without any water or food present. The thirst stimulus was then administered with water, but no food, available. Saralasin ICV infusion was continued throughout the rest of the experiment. Animals were unrestrained during the infusion.

Evaluation of Blockade of Angiotensin with Central Saralasin Technique

To verify the capability of saralasin ICV to specifically inhibit drinking to AII, three procedures were carried out. First, drinking was measured for 30 min following an injection of 10 ng AII (10 ng/µl) ICV in a group of eight rats treated with saralasin or saline vehicle ICV. Four rats received saralasin first, the other four, saline first. Second, drinking was measured for 30 min following a 20-min i.v. infusion of AII (5 ng/µl), via an indwelling jugular catheter (Hsiao et al. 1977), at 100 ng/min (n = 11) in the presence of saralasin or saline ICV. Finally, drinking was measured for 3 h following an intraperitoneal (ip) injection of 1% body weight of 1 M NaCl in

the presence of saralasin (n = 10) or saline vehicle ICV (n = 7). The hypertonic saline was injected under light ether anesthesia.

Water Deprivation

Twenty-four-hour water deprivation. The effect of saralasin ICV on drinking following 24-h water deprivation was studied on 15 rats. They were deprived of water for 24 h with food available. The animals were then randomly divided into two groups, the experimental group (n = 8) received saralasin ICV treatment and the control group (n = 7) received saline vehicle ICV. After the preinfusion of saralasin or saline ICV, animals were allowed free access to water but not food. Water intake was monitored for the following 3 h.

After being deprived of water for 24 h an additional group of nine rats was killed and blood collected for PRA assay. Blood samples were also obtained from a further eight rats for control measurements.

Thirty-hour water deprivation. Fourteen rats were deprived of water for 30 h and were randomly divided into two equal groups. The experimental group received treatment of saralasin ICV and the control group received artificial cerebrospinal fluid (CSF). The composition of the artificial cerebrospinal fluid was

described by Malvin et al. (1977). Saralasin was prepared in artificial CSF; the concentration was 20 ng/µl. No initial ICV injection was given, and the ICV infusion rate was 200 µl/h or 4 µg/h. Seventy-five minutes after the commencement of ICV infusion, water but not food was made available to the animal. Water intake was monitored for the following 90 min.

An additional 16 rats were also deprived of water for 30 h and were randomly divided into two equal groups. The experimental group received treatment with saralasin ICV, and the control group received saline vehicle ICV. The intracranial infusion protocol for 24-h water deprivation was followed, except that the concentration of saralasin was 10 times greater, i.e., 40 µg/µl. Water intake was recorded for 90 min.

Caval Ligation

The abdominal vena cava of a rat was exposed through a small loin incision during ether anesthesia, the cava was then ligated with a silk ligature just above the entry of the right renal vein as described by Fitzsimons (1969). After the incision had been closed, the animal was returned to its cage and the ICV infusion was started immediately. The whole procedure took 10-15 min to complete. Animals were assigned randomly

to receive ICV treatment of either saralasin or saline vehicle. One microliter of the solution was administered as a bolus followed by infusion at a rate of 1.3 µl/h for 5.5 or 6 h. In the first series of experiments, 20 animals (body wt 309±7 g) received saralasin (4 ug/ul) and the other 14 (320±11 g) received saline. In the second series, 9 rats (311±11 g) received saralasin (8 µg/µl) and the other 10 (328±13 g) received saline. Water intake was recorded every 30 min throughout the experiment.

The left carotid arteries of an additional group of rats were cannulated with polyethylene tubing during ether anesthesia. At least 1 day was allowed for animals to recover from the surgery. On the day of the experiment, the arterial catheter was connected to a transducer (Statham, P23Db) and the blood pressure recorded on a polygraph (Grass, model 5). After the blood pressure had remained stable for 10 min, the inferior vena cava of the animal was then ligated and ICV infusion started as described above. Four rats received saralasin (4 µg/µl), and the other three received saline vehicle treatment. Blood pressure was monitored continuously for the following 4 h.

The inferior vena cavae of a group of nine rats were ligated, and another group of five were sham

operated. The sham-operated animal received the same surgical procedures as the ligated one except that the silk ligature around the vena cava was tightened. Two hours after the surgery, animals were killed by decapitation and blood samples collected for PRA assay.

Data Analysis

Student's t-test was employed to analyze the data of the hypertonic saline injection experiment and of plasma renin activity. Two-factor analysis of variance for repeated measurements (Bostrom 1979, Winer 1971) was performed on all other data. A value of 0.05 was taken as the level of significance. Data are expressed as mean±SE.

RESULTS

Evaluation of Blockade of Angiotensin with Central Saralasin Technique

Injection of 10 ng AII into the lateral ventricle stimulated drinking promptly, and the response lasted a maximum of 15 min. As shown in Table VII.1, saralasin ICV inhibited drinking elicited by AII ICV, whereas the order of presentation of ICV treatments had no significant effect on the drinking response ($P > 0.25$).

Intravenous infusion of AII (100 ng/min) for 20

min reliably induced drinking. The latency to drink and amount of water consumed varied from animal to animal, but remained rather constant from experiment to experiment for the same animal. Shortly after the commencement of AII i.v. infusion, animals showed some sign of distress, presumably due to the hypertensive effect of AII. After 5-15 min the animals appeared to be aroused and began to drink. Saralasin ICV abolished the drinking response in all but one animal tested, and the effect was highly significant (Table VII.1). The order of presentation of ICV treatments had no effect on drinking ($P > 0.50$).

Animals recovered very rapidly for the light ether anesthesia, during which 1% body weight of 1 M NaCl was injected ip. They started to drink within 30 min after the injection and in general finished drinking within 90 min. Water intake in the saralasin group was not significantly different from that in the saline controls (Table VII.1).

In summary, saralasin ICV at the dose we used inhibited drinking to AII ICV and i.v., but not to hypertonic saline ip. These results suggest that saralasin ICV specifically antagonizes drinking induced by AII and thus appears to a useful tool to assess the involvement of endogenous AII in drinking following

water deprivation and caval ligation.

Water Deprivation

Twenty-four-hour water deprivation. The initial weight was 325±5 g for the saralasin group and 326± 9 g for the controls, and the weight losses due to 24-h water deprivation were 27±1 and 23±1 g, respectively. Immediately after water was made available to the water-deprived animals, they started to drink promptly. Most of the drinking was finished in 30 min. As shown in Fig.VII.1, treatment with saralasin ICV had no significant effect on the drinking response ($P > 0.10$).

Plasma renin activity was 14.0±1.4 ng/ml/3h in rats deprived of water for 24 h ($n = 9$) and 7.1±0.7 in controls ($n = 8$). The difference is significant ($P < 0.001$).

Thirty-hour water deprivation. The initial weight was 308±5 g for the saralasin group and 309±6 g for the control group, and the weight losses due to 30-h deprivation of water were 39±1 and 34±2 g, respectively. Saralasin-treated animals behaved no differently from controls with regard to either the latency to drink or the amount of water consumed (Fig.VII.2, $P > 0.10$).

Even when treated with a much larger dose of saralasin (52 μg/h), another group of animals showed no evidence of suppression of water intake after being deprived of water for 30 h (Fig.VII.3, $P > 0.10$).

Caval Ligation

As shown in Fig.VII.4, for the saline vehicle controls the mean arterial pressure (MAP) dropped to 37±7 mmHg immediately after ligation of the abdominal vena cava. The blood pressure recovered rapidly and reached 90 mmHg in 30 min, by that time animals had already recovered from ether. MAP remained around 100 mmHg for the next 3 h. Treatment with saralasin ICV had no significant effect on the recovery of MAP.

Two hours after caval ligation, plasma renin activity was 34.6±2.9 ng/ml/3h ($n = 9$), whereas it was 14.8±2.1 in the sham-operated group ($n = 5$). The difference is significant ($P < 0.001$).

As shown in Fig.VII.5, most of the drinking occurred in the first 3 h following caval ligation. Treatment with saralasin ICV did not significantly affect water intake in the 5.5-h period following the ligation ($P > 0.10$). When the dose was doubled, saralasin had no significant effect on this drinking response either (Fig.VII.6, $P > 0.25$).

DISCUSSION

As shown in Table VII.1, we have demonstrated that infusion of saralasin inhibited drinking to either AII ICV or i.v., but not to hypertonic saline ip. Because hypertonic saline loading is believed to cause cellular dehydration thirst, which is not mediated by the renin-angiotensin system (Abdelaal et al. 1976, Fitzsimons 1972, Simpson et al. 1978, Summy-Long & Severs 1974), our observations thus suggest that saralasin ICV at the dose we chose (5.2 μg/h) is capable of specifically antagonizing drinking induced by AII. As the CSF volume of a rat is no greater than 1 ml, the CSF AII concentration in the ventricle would reach at least 10,000 pg/ml following the injection of 10 ng AII ICV. According to Mann et al. (1980), the plsama AII level would reach 2,000 pg/ml following AII i.v. infusion at a rate of 100 ng/min in a 300-g rat as in this study. This concentration of plasma AII is much higher than those that would have occurred with the other thirst stimuli investigated in the present study, i.e., water deprivation and caval ligation (Abdelaal et al. 1976, Johnson et al. 1981). Thus, if the renin-angiotensin system is involved, treatment with saralasin ICV should show inhibition in water intake following water deprivation and caval ligation.

Saralasin ICV however neither increased the latency to drink nor decreased the amount of water consumed in rats deprived of water for 24 h (Fig.VII.1). Malvin et al. (1977), in contrast, reported that infusion of saralasin ICV significantly affected the latency to drink but not the total amount of water drunk in rats deprived of water for 30 h. This has been interpreted as evidence for the participation of the renin-angiotensin system in the control of drinking following water deprivation. As the differences between our results and Malvin et al. were so striking, we tried to repeat their protocol, and the result was again negative (Fig.VII.2), as was the result of another experiment using an even higher dose of saralasin (Fig.VII.3). Whereas the discrepancy between the finding of Malvin et al. and ours is difficult to reconcile, our observations are in line with other reports in rats (Severs et al. 1977), dogs (Ramsay & Reid 1975), goats (Olsson 1975), and sheep (Abraham et al. 1976). The amount of water drunk by the control group in the present study was similar to those reported elsewhere (Hoffman et al. 1978, Ramsay et al. 1977a, Severs et al. 1977) suggesting that the ICV infusion had no deleterious effects on drinking behavior.

As demonstrated in both dogs and rats by Ramsay et al. (1977a & b), approximately two-thirds of water intake following 24-h water deprivation is due to cellular dehydration and the other third due to extracellular dehydration. Because the renin-angiotensin system is not involved in cellular dehydration thirst, it could contribute to, at most, one-third of the drinking induced by water deprivation. Our three sets of consistent observations further indicate that the renin-angiotensin system alone does not play an essential role in the control of drinking following 24- or 30-h water deprivation in rats. However, the possibility that angiotensin combined with another system, e.g., cholinergic system, might subserve this drinking response is not ruled out (Hoffman et al. 1978).

Based primarily on the finding that water intake following ligation of the inferior vena cava was reduced by bilateral nephrectomy (Fitzsimons 1969) and restored by AII i.v. (Fitzsimons & Simons 1969) in the rat, Fitzsimons proposed that the renin-angiotensin system played an important role in the control of drinking following caval ligation. Historically this was the first piece of evidence implicating the renin-angiotensin system in extracellular dehydration thirst (Fitzsimons 1978). However, Stricker (1977, 1978)

reported that nephrectomized rats were in hypotensive shock after caval ligation, presumably due to the absence of the pressor action of AII. He therefore argued that it was the general debility, rather than the removal of the dipsogenic action of AII per se, that rendered the reduction in water intake following caval ligation in nephrectomized rats.

A definitive way to resolve this controversy seems to utilize a treatment that inhibits the dipsogenic action of AII while leaving its pressor effect intact. As shown in Fig.VII.4, saralasin ICV did not affect the recovery of blood pressure following caval ligation. In contrast, as discussed earlier, this dose of saralasin specifically antagonized drinking stimulated by high titers of AII. When applied to caval-ligated rats, however, saralasin ICV did not significantly affect the drinking response (Fig.VII.5). The amount of water drunk by the saline controls is compatible with other studies (Lehr et al. 1975, Stricker 1977), therefore ICV infusion did not affect the drinking response. Even with a larger dose, saralasin still had no significant effect on this drinking (Fig.VII.6). In agreement with Stricker (1977, 1978), Rolls and Wood (1977), and Lehr et al. (1975), these results suggest that the renin-angiotensin system is not essential in

the control of drinking following caval ligation in rats.

The regulation of blood pressure after ligation of the inferior vena cava poses an intriguing question. The mean arterial pressure recovered from 40 to reach 90 mmHg in 30 min following the ligation (Fig.VII.4). However Stricker (1978) reported that it took 4 h for the blood pressure of caval-ligated rats to get to the same level. The difference is probably due to anesthesia; our observations were made on animals which had recovered from ether by 30 min after the ligation, whereas Stricker's were made on rats during pentobarbital anesthesia throughout the entire experimental period.

In summary, infusion of saralasin ICV at a proper dose specifically antagonized drinking induced by AII, however it did not inhibit water intake following water deprivation or caval ligation. These findings indicate that the renin-angiotensin system alone does not play an essential role in the control of drinking following water deprivation or caval ligation in rats.

Table VII.1 Evaluation of blockade of angiotensin with central saralasin technique

stimulus	n	Saralasin ICV	Saline ICV	P
AII ICV	8	0.5 ± 0.3*	6.9 ± 1.3*	<0.005
AII i.v.	11	0.09 ± 0.09*	3.3 9+- 0.5*	<0.001
1 M NaCl ip	17	2.7 ± 0.3# (n = 10)	2.2 0.5# (n = 7)	>0.40

*Water intake (ml) in 30 min following the challenge of the thirst stimulus. #Water intake (ml/100 g body wt) in 90 min following the challenge of the thirst stimulus.

Fig.VII.1 Effect of administration of saralasin ICV (5.2 μg/h) on water intake following 24-h water deprivation. There was no significant difference between saralasin group (n = 8) and saline vehicle controls (n = 7) ($P > 0.10$). Water was made available at t = 0. Mean and SE are shown.

Fig. VII.1

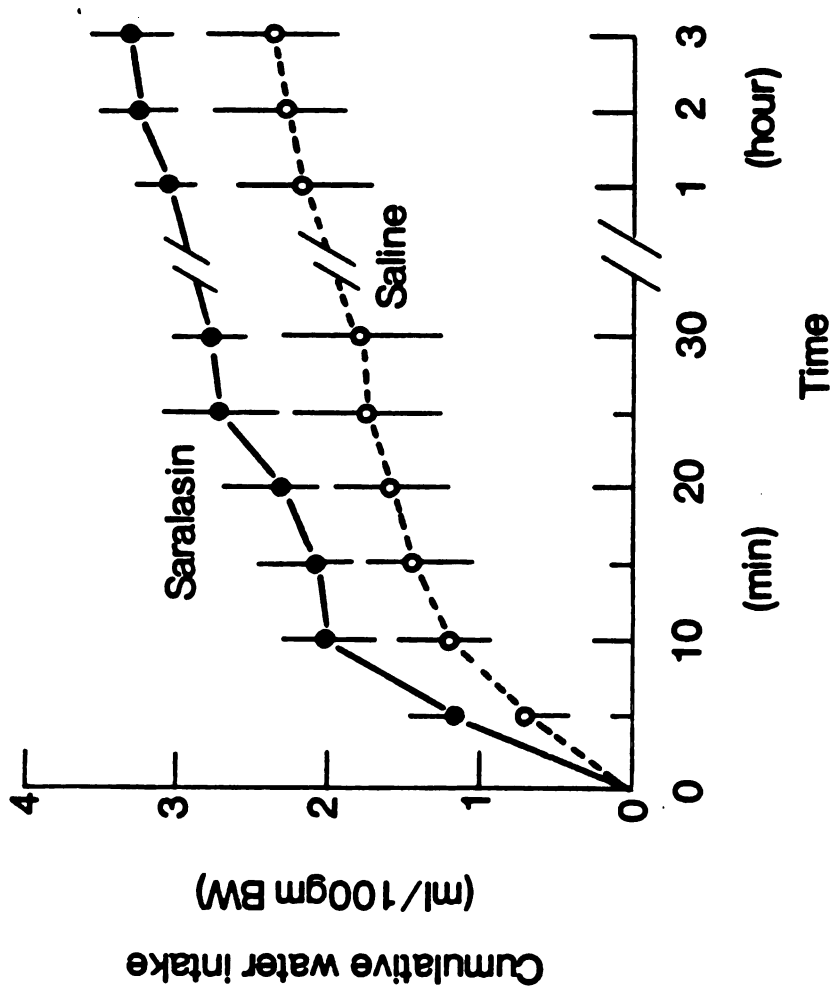
Cumulative water intake
(ml/100gm BW)
4
3
2
1
0
Saralasin
Saline
0
10
20
30
(min)
1
2
3
(hour)
Time

Fig.VII.2 Effect of administration of saralasin ICV (4.0 μg/h) on water intake following 30-h water deprivation. Protocol of Malvin et al. (1977) for saralasin treatment was followed. There was no significant difference between saralasin group and CSF controls ($P > 0.10$). Water was made available at t = 0. Mean and SE are shown. n = 7 in both groups.

Fig. VII.2

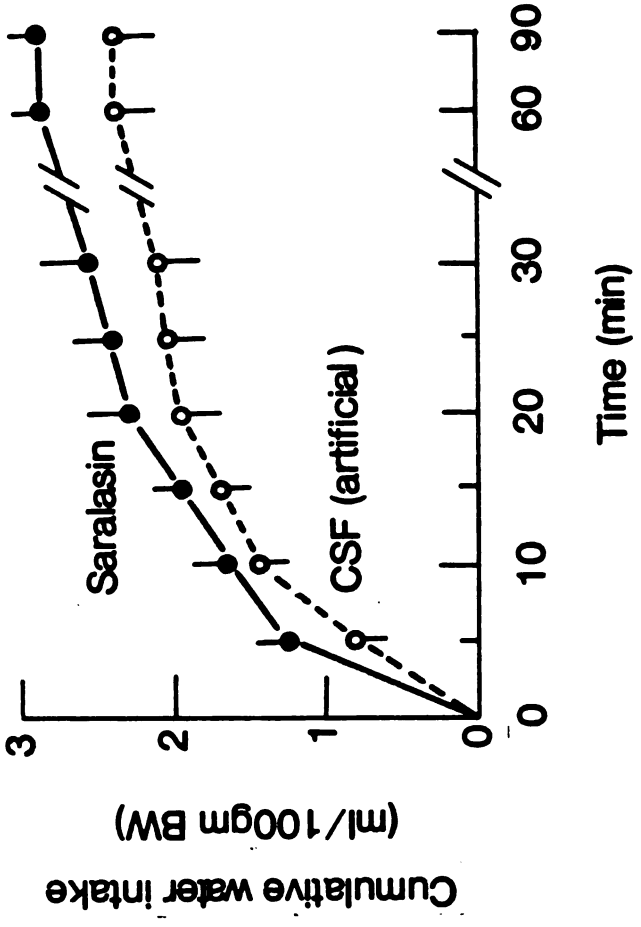
Cumulative water intake
(ml/100gm BW)
3
2
1
0
Saralasin
CSF (artificial)
0
10
20
30
60
90
Time (min)

Fig.VII.3 Effect of administration of saralasin ICV (52 µg/h) on water intake following 30-h water deprivation. Difference between saralasin group and saline vehicle controls was not significant ($P > 0.10$). Water was made available at t = 0. Mean and SE are shown. n = 8 in both groups.

Fig. VII.3

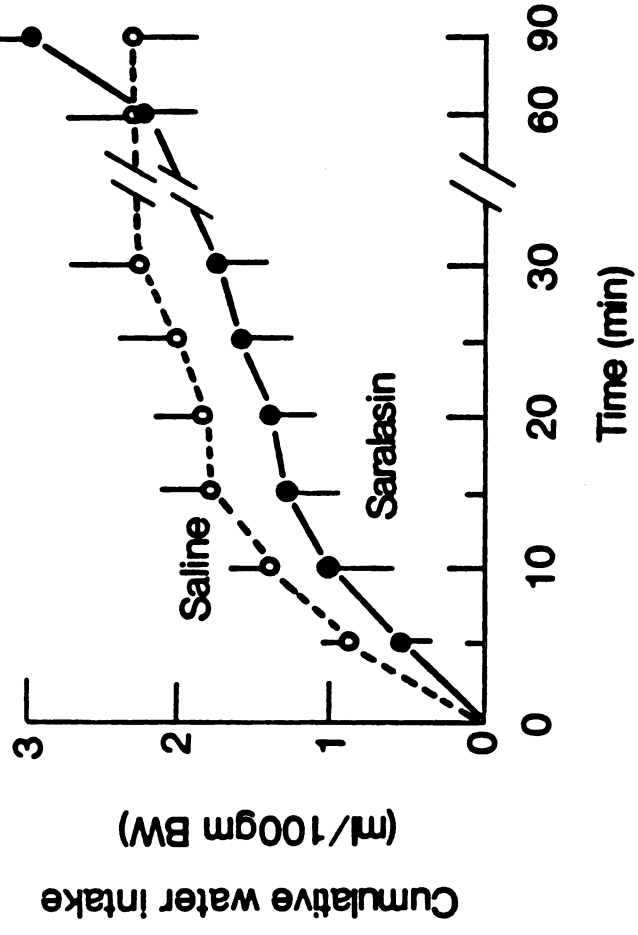
Cumulative water intake
(ml/100gm BW)
3
2
1
0
Saline
Saralasin
0
10
20
30
60
90
Time (min)

Fig.VII.4 Effect of saralasin ICV (5.2 μg/h) on recovery of blood pressure following caval ligation (t = 0). No significant difference existed between saralasin group (n = 4) and saline vehicle controls (n = 3). Mean and SE are shown.

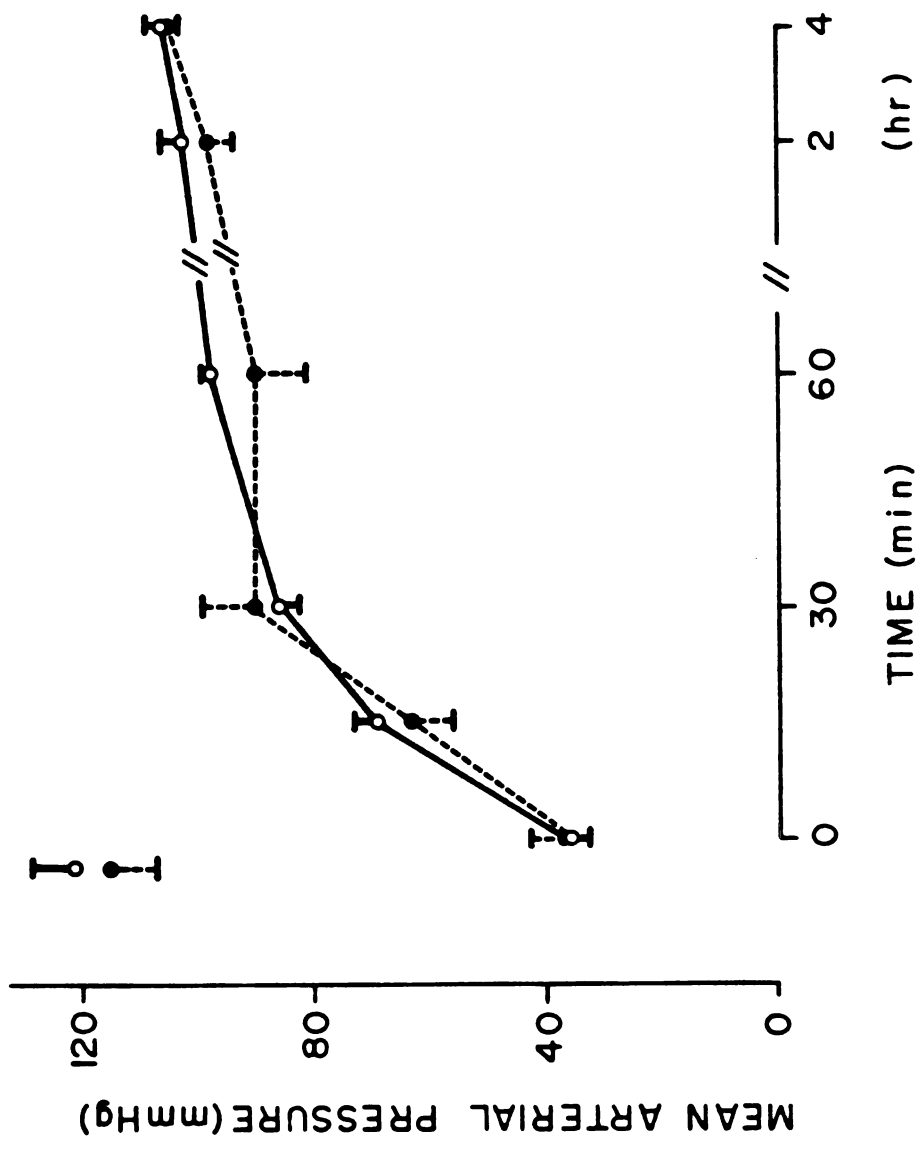
SARALASIN (5.2μg/hr)
SALINE
MEAN ARTERIAL PRESSURE (mmHg)
120
80
40
0
0
30
60
2
4
TIME (min)
(hr)

Fig. VII.4

Fig.VII.5 Effect of administration of saralasin ICV (5.2 μg/h) on water intake following caval ligation (t = 0). Difference between saralasin group (n = 20) and saline vehicle controls (n = 14) was not significant (P > 0.10). Mean and SE are shown.

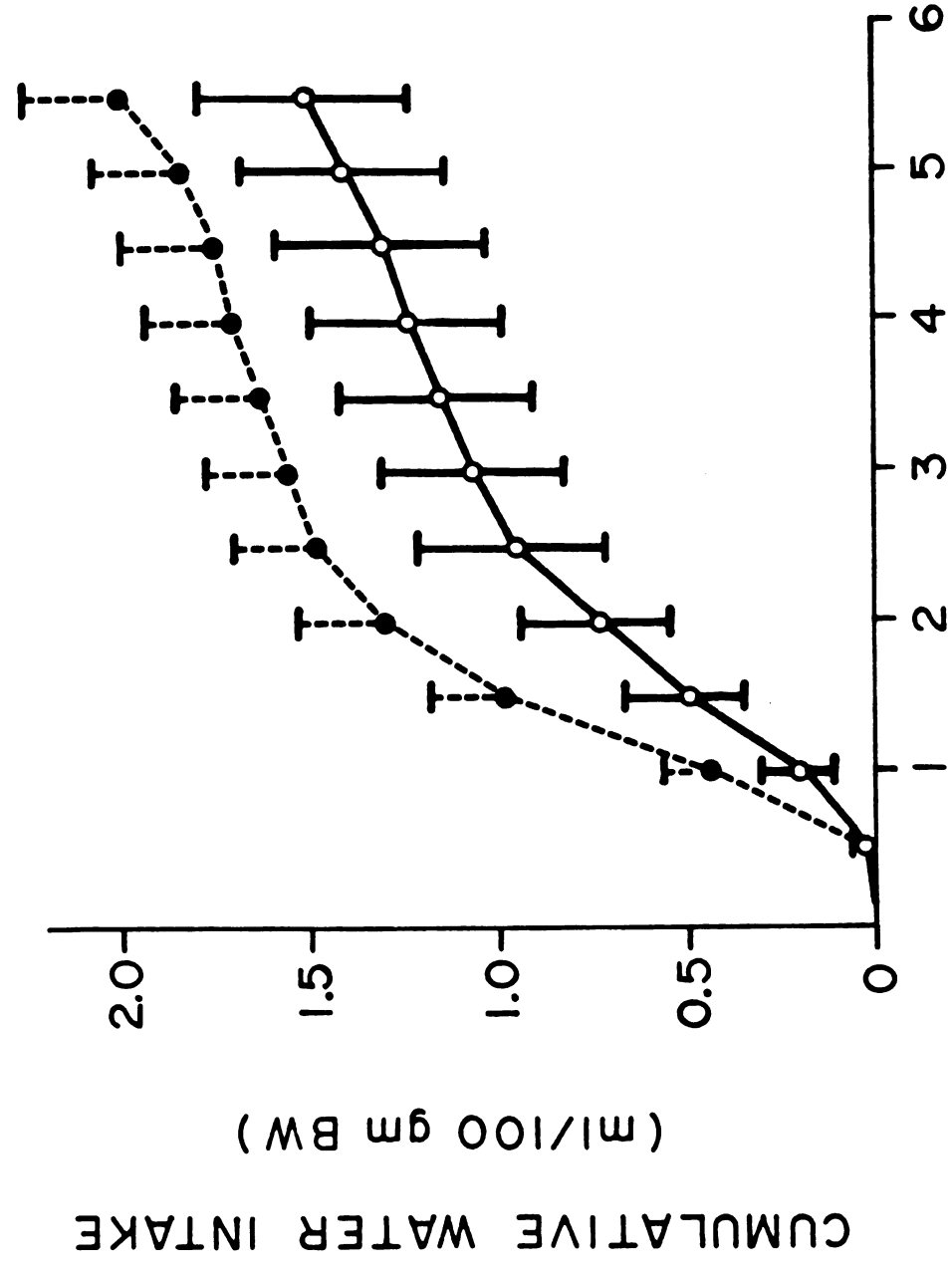
SARALASIN (5.2µg/hr)
SALINE
CUMULATIVE WATER INTAKE
(ml/100 gm BW)
2.0
1.5
1.0
0.5
0
1
2
3
4
5
6
TIME (hour)

Fig. VII.5

Fig.VII.6 Effect of administration of saralasin ICV (10.4 µg/h) on water intake following caval ligation (t = 0). Difference between saralasin group (n = 9) and saline vehicle controls (n = 10) was not significant (P > 0.25). Mean and SE are shown.

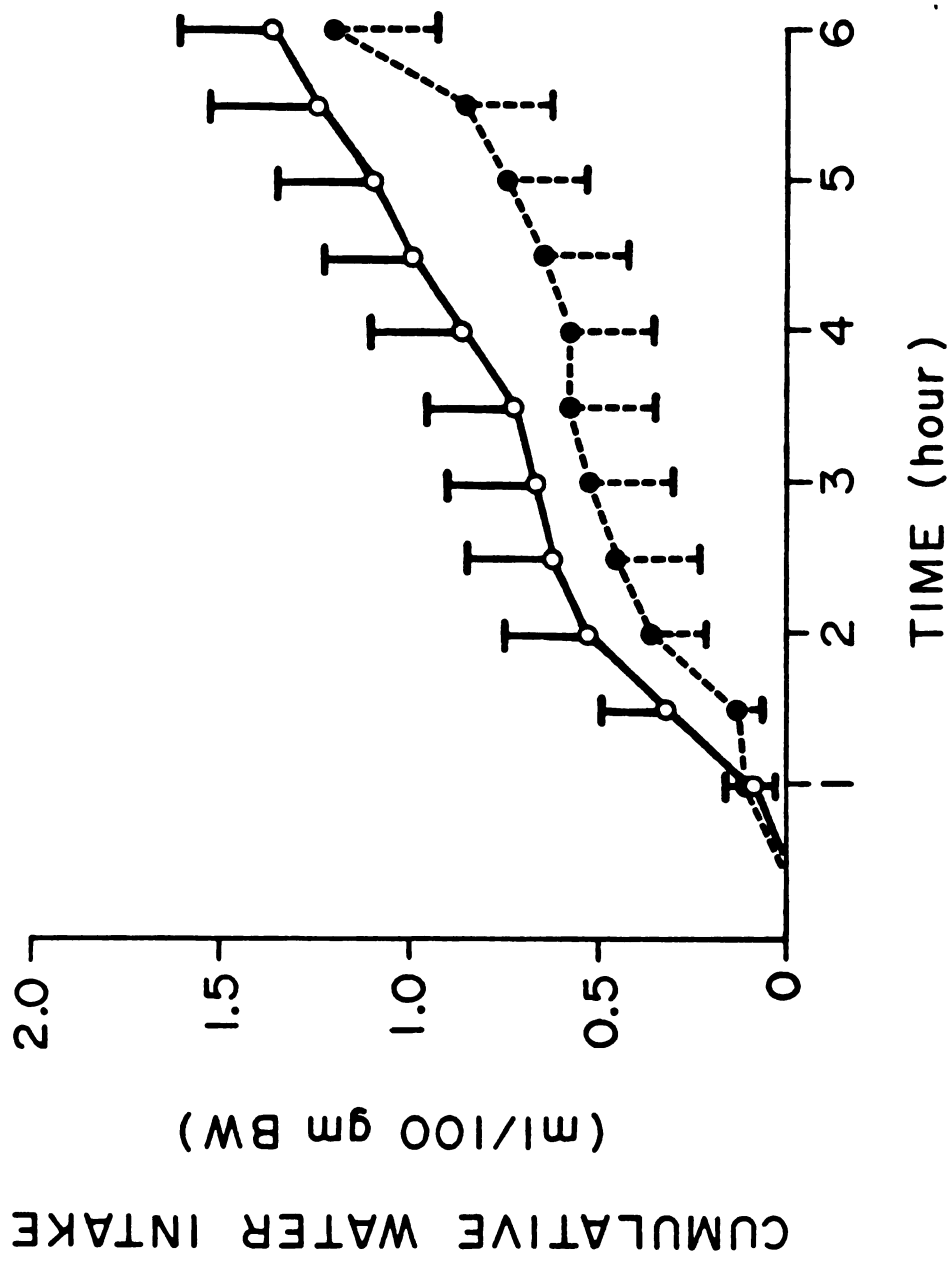
SARALASIN (10.4 µg/hr)
SALINE
CUMULATIVE WATER INTAKE
(ml/100 gm BW)
2.0
1.5
1.0
0.5
0
1
2
3
4
5
6
TIME (hour)

Fig. VII.6

CHAPTER VIII GENERAL DISCUSSION AND CONCLUSIONS

The pressor response to infusion of AII into the common carotid arteries in conscious mongrel dogs is clearly different from that to intravertebral AII. Firstly, the pressor response to intracarotid AII, unlike that to intravertebral AII, is slow in onset and not accompanied by an increase in heart rate. Secondly, the area postrema, located in the caudal medulla, is generally considered to be the site of pressor action of intravertebral AII. Since the carotid blood does not perfuse the medulla (Table IV.1), the site of action of intracarotid AII cannot not be the same as that of intravertebral AII. Finally, intraventricular saralasin, a competitive antagonist of AII, inhibited the pressor response to intravertebral, but not to intracarotid, AII (Fig.VI.2). This provides direct evidence that the site of action of intracarotid AII is different from that of intravertebral AII.

However, the mechanism responsible for the pressor effect of intracarotid AII remains unclear. Extracranial vasoconstriction could possibly account for this pressor effect. Intracarotid AII at 1.0 ng/kg/min reduced the carotid blood flow to half (Fig.III.1), which could result in a pressor response of 5 mmHg

according to the theoretical analysis (Fig.III.4). The difference between the pressor effect of intracarotid AII and that of AII i.v. in these experiments happened to be 5 mmHg (Fig.III.3).

The mean blood flow in each internal carotid artery in conscious dogs determined by the radioactive microsphere method was 7.7 ml/min, and that in the anastomotic artery between the external and internal carotid arteries was 3.3 ml/min. Additional evidence exists that anastomotic blood mixed well with internal carotid blood before reaching the cerebral microcirculation (Fig.IV.2). Therefore, although the external carotid artery contributes significantly to the perfusion of the brain, the brain would receive significantly higher concentration of AII when AII was infused into the common carotid artery than when infused into the external carotid artery (Tables IV.1 & 2). This argument is supported by the observation that plasma vasopressin concentration increased more when AII was infused into the common carotid artery than when infused into the external carotid artery (Table IV.4). However, the magnitude of the pressor effect of AII infused into the common carotid artery was the same as that infused into the external carotid artery. These findings therefore argue against the hypothesis that

the pressor response to intracarotid AII is centrally mediated.

However, it is difficult to explain the finding that plasma corticosteroid concentration increased to the same degree when AII was infused into either common or external carotid artery (Table IV.5). Plasma corticosteroid concentration was used as an index of ACTH secretion. Although it is not very likely, the possibility exists that plasma corticosteroid concentration might not accurately reflect ACTH secretion.

The finding that intracarotid infusions of phenylephrine and vasopressin caused smaller increase in blood pressure than intravenous infusions (Fig.V.1 & 3) seem to be at odds with the extracranial vasoconstriction hypothesis. However, evidence exists that intracarotid vasopressin and phenylephrine could act directly on the carotid sinus to increase baroreceptor activity and therefore result in a smaller pressor response. In contrast, AII appears not to have such action on the carotid sinus since both common and external carotid infusions of AII induced same degree of pressor response (Fig.III.3). Therefore, these findings do not rule out the extracranial vasoconstriction hypothesis.

In conclusion, although some of the evidence suggests that the pressor action of intracarotid AII is due to extracranial vasoconstriction, definite conclusions are not possible.

The central pressor action of intravertebral AII appears not to be essential for the pressor response to blood-borne AII. This is because that intraventricular saralasin inhibited the pressor effect of intravertebral, but not of intravenous, AII (Figs.VI.2 & 4). If the pressor action of intravertebral AII is essential, saralasin should reduce the pressor effect of AII i.v. as well.

Blood-borne AII appears not to play an essential role in drinking following water deprivation and caval ligation in rats. This is because that intraventricular saralasin specifically inhibited drinking to AII i.v.; however, it had no significant effect on drinking following the above two stimuli.

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