STUDIES ON THE INTERACTION BETWEEN ANGIOTENSIN AND THE SYMPATHETIC NERVOUS SYSTEM.

A thesis presented by David Andrew Arlwydd Owen for the degree of DOCTOR OF PHILOSOPHY in the UNIVERSITY OF ASTON IN BIRMINGHAM.

Department of Pharmacy,
The University of Aston in Birmingham.

August 1969.

ABSTRACT.

The interactions between angiotensin and the sympathetic nervous system in the mammalian cardiovascular system have been studied.

In the isolated central artery from the rabbit ear the constrictor action of angiotensin was enhanced in the presence of endogenous noradrenaline but unaltered by exogenous noradrenaline. In pithed rats the responses to each of three procedures causing the release of noradrenaline from sympathetic nerve endings were increased by angiotensin. The responses to injected noradrenaline were unaltered by angiotensin. The possible significance of these results is discussed.

Using a technique developed from existing methods the action of a variety of drugs on the cardiovascular system of conscious cats has been studied. It was shown that the extent of the pressor effect of angiotensin was determined, in part, by the noradrenaline content of the neuronal stores. The responses were reduced by depletion of noradrenaline and restored when the stores were replenished. The responses to angiotensin were increased after inhibition of monoamine oxidase. The role of endogenous noradrenaline in the pressor responses to angiotensin remains uncertain but is independent of post-synaptic adrenergic receptor sites.

A series of metal ion chelating agents have been examined for specific anti-angiotensin activity. Of these

only sodium diethyl-dithiocarbamate possessed specific anti-angiotensin activity. This could be readily demonstrated in pithed rats but in anaesthetised cats was complicated by the ability of sodium diethyl-dithiocarbamate to potentiate the responses to noradrenaline and therefore to potentiate the adrenal medullary component of the angiotensin response. The failure of the other chelating agents to block the pressor responses to angiotensin suggests that sodium diethyl-dithiocarbamate may act independently of metal chelation. It is suggested that sodium diethyl-dithiocarbamate might be a suitable prototype from which to develop a specific anti-angiotensin agent. No such agent exists at present and its discovery might facilitate investigations into the role of the renin-angiotensin system in the aetiology of hypertension.

CONTENTS

HISTORICAL INTRODUCTION.

In 1628 William Harvey first described the anatomical structure of the circulatory system as it is understood to-day. Although diseases of the heart had been recognised centuries before the classical work of Harvey an understanding of circulatory disorders had been hindered by the lack of understanding of the circulation of the blood. The diagnostic value of blood pressure measurement was first appreciated in the early 19th century when Potain in Paris and Sir Thomas Allbutt in Cambridge began regular routine use of the syphgmomanometer in their clinical examinations. This routine use of blood pressure measurement soon established the dangers of hypertension but all treatment techniques remained inadequate for a further hundred years. The delay in the development of adequate treatment for hypertension was probably due at least in part, to the fear of physicans that lowering the blood pressure would lead to renal failure and poor tissue perfusion thereby further reducing the poor life expectancy of hypertensives. Only when Page & Heuer (1937a,b) found that partial surgical sympathectomy in fact increased the life expectancy of hypertensive patients did the search for less drastic anti-hypertensive techniques gain its current important place in modern medicine.

Although the precise aetiology of hypertension remains obscure there is considerable evidence implicating both renal and neurogenic mechanisms in some forms of the disease.

1. The role of the kidney in hypertension.

"It has been well established that renal diseases can cause hypertension, however hypertension can occur without renal disease and renal disease without hypertension" (Glenn & Anderson, 1967). It has been estimated that between 5-10% of patients presenting themselves for antihypertensive therapy have definite renal disease and many more are suspected of having non-detectable renal disease (see for example Kennedy, Luke, Briggs, & Barr-Stirling, 1965; Luke, Kennedy, Briggs, Struthers, Watt, Short & Barr-Stirling, 1968).

The role of the kidney in those hypertensive diseases in which it is implicated (usually termed renal or renovascular hypertension) is complex and investigation has been hindered by species variation e.g. man and rats appear to differ from other species in that interference with the blood supply to only one kidney in these species can cause marked hypertension in the presence of the other normal kidney (Peart, 1959).

Although a number of possible roles for the kidney in renal hypertension have been examined both experimentally and clinically the most favoured mechanism is that the kidney initiates hypertension by the production and liberation of a pressor substance into the circulation.

The evidence supporting this hypothesis has been based on a wide range of observations.

In 1898 Tigerstedt & Bergman first reported that saline extracts of kidney tissue contained a pressor substance which they called renin. Renin is now known to be contained within the juxtaglomerular cells of the kidney from which it can be released by a variety of stimuli (see later). When released into the circulation renin, which is a proteolytic enzyme, reacts with a non-pressor globulin in the plasma to yield angiotensin I which undergoes further enzymatic change to the active pressor principle angiotensin II (see review by Page & McCubbin, 1968).

There remains controversy as to whether the blood levels of renin or angiotensin are consistently elevated during renal hypertension.

In experimental animals made hypertensive by techniques similar to that first described by Goldblatt, Lynch, Hanzal & Summerville (1934) increased levels of circulating pressor substance are usually reported during the acute stages of the hypertension (for example Braun-Menendez, Fasciolo, Leloir & Munoz, 1940; Houssay & Braun-Menendez, 1942; Warter, Schwartz, Bloch, Velly, Imbs & Desaulles, 1967). In clinical hypertension and chronic experimental hypertension however both increased (for example Page, 1940; Khan, Skeggs, Shumway & Wisenbaugh, 1952; Judson & Helmer, 1965) and normal (for example Dexter

& Haynes, 1942; Taquini & Fasciolo, 1946; Pickens, Dunstan, Bumpus & Page, 1965) levels of circulating pressor substance have been measured.

The question of whether the circulating levels of angiotensin are elevated in renal hypertension is highly pertinent to the hypothesis that renal hypertension is due to increased plasma levels of angiotensin. Clarification of this problem requires the development of more sensitive and more specific angiotensin assay techniques.

If renal hypertension is caused by elevated plasma levels of angiotensin then continuous intravenous infusion of angiotensin might be expected to elicit hypertension.

Infusions of angiotensin in large quantities however only cause transient hypertension (Gross, Bock & Turrian, 1961; Brown, Chapius & Robertson, 1963; Day, McCubbin & Page, 1965) probably because of the development of tachyphylaxis. The continuous infusion of smaller doses however leads to a well sustained hypertension in both experimental animals (Page & Olmsted, 1961; Brown et al, 1963; Dickinson & Lawrence, 1963; McCubbin, de Moura, Page & Olmsted, 1965; Yu & Dickinson, 1965; Dickinson & Yu, 1967a,b) and in man (Laragh, Cannon & Ames, 1964) although there may be delay in the onset of hypertension. The hypertension associated with long term infusion of small quantities of angiotensin is dependent on an intact peripheral sympathetic nervous system (McCubbin et al, 1965;

Dickinson & Yu, 1967b) and the contribution of the sympathetic nervous system to the developing hypertension steadily increases (Dickinson & Yu, 1967b) although there is no simultaneous increase in the urinary excretion of catecholamines or their metabolites in rabbits (Dickinson, de Swiet & Schaepdryver, 1968). The prsistence of the hypertension following the completion of the angiotensin infusions has been reported variously as only a few minutes (Dickinson & Yu, 1967a) to a few days (Dickinson & Lawrence, 1963: McCubbin et al, 1965). The experiments where the onset of hypertension was most delayed were usually those in which it persisted longest on completion of the infusion (McCubbin et al, 1965).

The production of antibodies to renin and to angiotensin added to the evidence of a role for the renin/angiotensin system in renal hypertension although until chemically pure renin becomes available doubt must persist as to the specificity of antirenin serum. Antirenin serum has been prepared from kidney extracts of a number of species and it specifically reduces the cardiovascular sensitivity to renin (Johnson & Wakerlin, 1940: Wakerlin, 1958) and to angiotensin (Hedwall, 1968; Oken & Biber, 1968). Intramuscular injections of dog or hog antirenin reverse chronic renal hypertension in dogs (Helmer, 1958: Wakerlin, 1958; Deodhar, Haas & Goldblatt, 1964) but rabbit antirenin although blocking the acute effects of injected angiotensin does not reverse chronic renal hypertension in rats (Hedwall, 1968).

Inhibition of the renin/renin substrate reaction by a phospholipid substance isolated from the kidneys, plasma and red blood cells of both man and dogs has been reported (Sen, Smeby & Bumpus, 1967:1969; Ostrovsky, Sen, Smeby & Bumpus, 1967; Meabashi, Miura & Yoshinga, 1968; Devaux, Alexandre, Menard, Meyer & Milliez, 1968). This phospholipid is present in greater quantities in the plasma of uraemic patients than in normal persons (Maebashi et al, 1968) and lowers the blood pressure in chronic experimental renal hypertensive rats (Sen et al, 1967;1969). The enormous potential value of this substance clinically remains undetermined.

2. <u>The role of the sympathetic nervous system in renal hypertension</u>.

The precise role of the sympathetic nervous system in renal hypertension remains undetermined.

Complete or almost complete surgical sympathectomy in dogs does not prevent the onset of hypertension following the production of renal ischemia (Freeman & Page, 1937; Heymans, Bouckhaert, Elaut, Bayless & Saaman, 1937; Verney & Vogt, 1938; Grimson, 1941) nor does immunosympathectomy in rats (Dorr & Brody, 1966). Destruction of the spinal cord below C_5 does reverse chronic renal hypertension in dogs although this is not permanent and the hypertension slowly returns (Glenn, Child & Page, 1938). In rats although immunosympathectomy does not prevent the onset of renal hypertension the

hypertension does not persist (Dorr & Brody, 1966). Complete destruction of the entire central nervous system (Dock, 1940; Taquini, Blaquier & Bohr, 1961; Taquini, 1963) or blockade of the peripheral autonomic nervous system by ganglion blockers (Conway, 1955; Doyle & Smirk, 1955) lowers the blood pressure in both normotensive and renal hypertensive subjects. The reduction is always greatest in the hypertensive subjects indicating the contribution of the autonomic nervous system to renal hypertension.

The development of a wide range of effective anti-hypertensive drugs which impair peripheral sympathetic nerve function has given much weight to the hypothesis that the neurogenic component of hypertensive diseases (including renal hypertension) is an overactivity of the peripheral sympathetic nervous system. Drugs which have been successfully used to lower blood pressure include those which impair sympathetic function by ganglion blockade, transmitter depletion, adrenergic neurone blockade, α- receptor blockade, β-receptor blockade (although these drugs also possess adrenergic neuron blocking activity (Day, Owen & Warren, 1968; Dunlop & Shanks, 1969)) and those which lead to synthesis of false transmitters (see reviews by Boura & Green, 1964: Green & Boura, 1964: Gross, 1966: Schlittler, 1967).

The nature of the overactivity of the sympathetic nervous system would seem to be due, at least in part, to

an alteration of the function of the baroreceptor system which usually helps in the maintenance of a normal and stable blood pressure. McCubbin, Green & Page (1956) first showed that in experimental renal hypertension there is an upwards resetting of the baroreceptor system. The change in function of the baroreceptor system has been confirmed by other workers (McCubbin, 1958; Kezdi, 1962; Kezdi & Wennemark, 1964; Krieger & Marseillan, 1966; Alexander & de Cuir, 1967; Aars, 1968a,b). The resetting of the baroreceptor system follows the hypertension and although it helps to maintain the high blood pressure it does not appear to contribute to its onset.

Increased responsiveness of the cardiovascular system to sympathetically mediated pressor stimuli have been frequently reported (see for example Verney & Vogt, 1938; Hines, 1940; Conway, 1958). It has been suggested that the increased responsiveness is due entirely to the increased tone of the vasculature in hypertension (Conway, 1958). However Doyle & Black (1955) found that hypertensive patients remained more sensitive than normotensives to pressor agents even after the administration of ganglion blocking drugs to reduce vascular tone.

There is confusion as to the possibility of increased sensitivity of isolated cardiovascular tissues taken from renal hypertensive subjects. Gordon & Nogueira (1962) found increased responsiveness to noradrenaline in rat aortic strips from renal hypertensive rats and McGregor

& Smirk (1968) made a similar observation using the mesenteric vessels of hypertensive rats However Baum & Shropshire (1967) and Baum (1969) found that isolated atria and perfused hind-limb preparations taken from renal hypertensive rats were not more sensitive than controls to sympathetic stimulation.

Measurement of tissue levels of noradrenaline and noradrenaline turnover rates have not added much to the understanding of the neurogenic component of renal hypertension. Increased tissue levels of noradrenaline have been measured by Zussman (1967) and by Henning (1969) in rats but Robertson, Hodge, Laverty & Smirk (1968) found normal or decreased noradrenaline levels in their rats as did Taquini (1963) in dogs. Increased cardiac noradrenaline turnover has been measured in renal hypertensive rats (Volicier, Scheer, Hilse & Visweswaran, 1968) but turnover was normal in a group of essential hypertensive patients (DeQuattro & Sjoerdsma, 1968).

INTERACTIONS BETWEEN ANGIOTENSIN AND THE SYMPATHETIC NERVOUS SYSTEM.

1. Release of renin.

The control of renin release from the juxtaglomerular cells of the kidney is complex (See reviews by Vander, 1967; Page & McCubbin, 1968). These authors have suggested four mechanisms which might control the release of renin although interactions between these mechanisms seem probable.

The mechanisms suggested are:-

(a) Baroreceptor control within the kidney where the mechanism responds to changes in the mean renal arterial pressure.

(b) Macula densa control where the mechanism responds to changes in the sodium load to the macula densa.

(c) Hormonal control involving one or more feedback mechanisms. It has been well established that both angiotensin and aldosterone can independently exert negative feedback control over the release of renin. Vander (1967) has suggested that there is also a third, as yet, unidentified hormone which influences renin release.

(d) Sympathetic nerve control It is known that the juxtaglomerular cells are richly innervated with sympathetic nerves (DeMylder, 1952; Barajas, 1964; Wagermark, Ungerstedt & Ljundquist, 1968) and many of these nerves terminate in areas of high renin content (Wagermark et al, 1968).

Direct or indirect activation of the renal sympathetic nerves (Vander, 1965; Bunag, Page & McCubbin, 1966a) or infusions of catecholamines into the renal artery (Vander, 1965; Wathan, Kingsbury, Stouder, Schneider & Rostorfer, 1965; Bunag et al, 1966a) cause an increase in the renin released into the renal vein. The quantity of renin released by sympathetic nerve stimulation is however also determined by dietary sodium being greatest when sodium intake is most restricted (Bunag, Page & McCubbin, 1966b).

These experimental studies have established that the renal nerves can contribute to the release of renin but Vander (1967) has expressed doubts as to the physiological importance of this.

2. Central pressor action of angiotensin.

Bickerton & Buckley (1961) first showed that angiotensin elicited a systemic pressor response when administered directly into the brain. The central stimulant action of angiotensin has been frequently confirmed (Halliday & Buckley, 1962; Nashold, Mannarino & Wunderlich, 1962; Dickinson & Lawrence, 1963; Ikeda, Fujii, Murata, Terasawa, Osawka, Hosoda, Kurihara, Kimata & Okinaka, 1963; Benetato, Haulica, Uluita, Bubuiana, Mocodean, Stefanescu & Suhacui, 1964; Smookler & Buckley, 1965; Yu & Dickinson, 1965; Severs, Daniels, Smookler, Kinnard & Buckley, 1966; Smooker, Severs, Kinnard & Buckley, 1966; Severs, Daniels & Buckley, 1967; Bourdois & Panisset, 1968; Cranston, Laverty, Lowe & Rosendorff, 1968; Daniels & Buckley, 1968; Scroop & Lowe, 1968). However, Kaneko, McCubbin & Page (1960) and Zimmerman (1967) could detect no central action of angiotensin. Intracisternal injections of angiotensin are not pressor in dogs (Bianchi, de Schaepdryver, de Vleeschhower & Preziosi, 1959).

The site of the central action of angiotensin has not been definitely established but the suprapontine levels have been suggested (Nashold et al, 1962; Severs et al, 1966), although Cranston et al (1968) favour more than one discreet

area. The pressor effect of centrally administered angiotensin is reduced by section of the spinal cord (Smookler & Buckley, 1965; Severs et al, 1966), autonomic ganglion blockade (Yu & Dickinson, 1965); noradrenaline depletion (Benetato et al, 1964; Smookler et al, 1966), increasing the brain noradrenaline levels (Smookler et al, 1966), α- and β- adrenergic receptor blockade (Bickerton & Buckley, 1961; Severs et al, 1966; Bourdois & Panisset, 1968), reduction of brain calcium levels (Daniels & Buckley, 1968), pretreatment with atropine (Benetato et al, 1964; Scroop & Lowe, 1968) and by bilateral vagotomy (Scroop & Lowe, 1968). The bulk of the evidence suggests that the central pressor action of angiotensin is mediated via the peripheral sympathetic nervous system although the finding of Scroop & Lowe (1968) that angiotensin exerted its central pressor action by inhibition of vagal tone to the heart is important as this occurs using doses of angiotensin more in keeping with physiological angiotensin levels than those used by other workers.

3. Effects of angiotensin on the adrenal medulla.

Angiotensin shares with many other polypeptides the ability to stimulate the release of catecholamines from the adrenal medulla both in vivo and in vitro (Braun-Menendez et al, 1940; Kaneko, McCubbin & Page, 1961; Cession & Cession-Fossion, 1963; Benelli, Della Bella & Gandini, 1964; Cession, 1964a; Feldberg & Lewis, 1964,1965; Poisner & Douglas, 1965; Robinson, 1965,1967; Vogt, 1965; Lewis &

Reit, 1966; Peach, Cline & Watts, 1966: Ross & White, 1966; White & Ross, 1966; Ajzen & Woods, 1967; Piper & Vane, 1967; Staszewska-Barczak & Konopka-Rogatko, 1967; Staszewska-Barczak & Vane, 1967; Aiken & Reit, 1968). The potency of angiotensin as an adreno-medullary stimulant varies from species to species (Vane, 1969). Release of amines from the adrenal medulla was found to make little or no contribution to the pressor response in man (Scroop & Whelan, 1968) or in rats (Hughes, 1968a; Schmitt & Schmitt, 1968a). In dogs angiotensin has been reported as a very potent adrenal medullary stimulant (Robinson, 1965;1967) and as a weak stimulant (Kaneko et al, 1961; Staszewska-Barczak & Vane, 1967).

The action of angiotensin on the adrenal medulla in vitro is dependent on the presence of calcium ions (Poisner & Douglas, 1965). The action is not blocked by either hexamethonium or by pempidine (Feldberg & Lewis, 1965; Staszewska -Barczak & Konopka-Rogatko, 1967; Staszewska-Barczak & Vane, 1967) but is subject to tachyphylaxis (Feldberg & Lewis, 1965).

It has been suggested that angiotensin acts directly on the medullary cells (Feldberg & Lewis, 1965) although there is recent evidence of a baroreceptor control of amine release from the adrenal medulla (Niijima & Winter, 1968) through which angiotensin might exert its effects.

4. Action of angiotensin on autonomic ganglia.

The action of angiotensin on the ganglia of the autonomic nervous system is more complex than its action at

the adrenal medulla. In very small quantities angiotensin inhibits transmission across the superior cervical ganglion of the cat (Haefely, Hurlimann & Thoenen, 1965; Panisset, Biron & Beaulnes, 1966; Panisset & Bourdois, 1968a,b). This inhibitory action of angiotensin is blocked by pretreatment with dihydroergotamine (Panisset et al, 1966; Panisset & Bourdois, 1968a).

Larger doses of angiotensin, however, directly stimulate the ganglia and facilitate ganglionnic transmission across both sympathetic and parasympathetic ganglia (Khairallah & Page, 1961; Robertson & Rubin, 1962; Lewis & Reit, 1965: 1966; Godfraind, Kaba & Polster, 1966; Panisset et al, 1966; Trendelenberg, 1966; Machova & Boska, 1967; Panisset, 1967; Turker & Kayaalp, 1967; Aiken & Reit, 1968; Panisset & Bourdois, 1968a,b). This stimulant action of angiotensin is not blocked by non-depolarising ganglion blockers (Robertson & Rubin, 1962; Panisset et al, 1966; Trendelenberg, 1966; Aiken & Reit, 1968) or sympatholytic agents (Machova & Boska, 1967) but is abolished by depolarisation of the ganglia (Khairallah & Page, 1961; Trendelenberg, 1966).

The contribution of autonomic ganglion stimulation to the pressor response following intravenous injection of angiotensin is probably insignificant. Drugs which impair the function of the sympathetic nervous system peripheral to the ganglia e.g. adrenergic neuron blockers do not decrease the pressor action of intravenous angiotensin (Laurence & Nagle, 1963).

5. Action of angiotensin on the heart.

The action of angiotensin on cardiac function in whole animals is not very marked but may be masked by cardiovascular reflexes.

In whole animals angiotensin is usually reported to cause cardiac stimulation which is mediated, at least in part, via the sympathetic nerves and the adrenergic β-receptors of the heart (Beaulnes, Brodeur & Gariepy, 1964; James, 1965; Krasney, Paudler, Smith, Davis & Youmans, 1965; Marchetti, Merlo, & Noseda, 1965; Rosas, Montague, Gross & Bohr, 1965; Farr & Grupp, 1966;1967; Krasney, Paudler, Hogan, Lowe & Youmans, 1966; Ross & White, 1966; Krasney, Thompson & Lowe, 1967; Ross, 1967). The contribution of the adrenal medulla to this cardiac stimulant action of angiotensin is uncertain. Ross & White (1966) found that adrenalectomy modified, but did not abolish, the angiotensin induced cardiac stimulation in cats but Farr & Grupp (1967) found no change after adrenalectomy in dogs. It has been suggested that the effect may be partly of central origin (Nishith, Davis & Youmans, 1962; Nishith, Ganguly, Ramanathan & Sreepathi Rao, 1966) but this is unlikely as it is not reduced by ganglion blocking drugs (Farr & Grupp, 1967).

6. The effect of angiotensin at sympathetic nerve endings.

Interactions between angiotensin and the sympathetic nervous system at the level of the nerve endings are frequently reported. Despite this the nature of the

interaction at this level is poorly understood and awaits an adequate explanation.

Intravenous infusions of angiotensin enhance the responses of the cardiovascular system of dogs (McCubbin, & Page, 1963a,b; McCubbin et al, 1965; Page, Kaneko & McCubbin, 1966) man (Kaneko, Takeda, Nakajima & Ueda, 1966) and pithed rats (Day & Owen, 1969) to endogenous noradrenaline released by sympathetic nerve stimulation, ganglion stimulants and by indirect sympathomimetics but does not change the responses to exogenous noradrenaline. There are also reports of enhancement of the responses to both endogenous and exogenous noradrenaline during angiotensin infusions in pithed rats (Schmitt & Schmitt, 1967a,b) and Pals, Fulton & Masucci (1968) found that infusion of angiotensin for a short period in pithed rats did not potentiate the responses to endogenous noradrenaline.

The sympathetically mediated vasoconstrictor responses of isolated vascular muscle are also enhanced in the presence of, or after angiotensin. This has been observed in the mesenteric vessels of the rat (McGregor, 1965) and of the cat (Panisset & Bourdois, 1967;1968a,b.c), the pulmonary artery of the rabbit (Su, 1965), the dog isolated hind limb (Zimmerman & Gomez, 1965; Zimmerman, 1967; Zimmerman & Whitmore, 1967), the renal artery of the dog (Zimmerman & Gisslen, 1968) and the vessels of the whole rabbit ear (Sakuri & Hashimoto, 1965) but angiotensin

causes a reduction of the vasoconstriction caused by noradrenaline, sympathetic nerve stimulation and by tyramine in the isolated central artery from the rabbit ear (Day & Owen, 1968a,b). Angiotensin also increases the responses of non-vascular smooth muscle to sympathetic nerve stimulation. Benelli et al (1964) and Graham & Al Katib (1967) observed potentiation of the sympathetically mediated contractions of the guinea-pig vas deferens in the presence of angiotensin and the responses of the cat spleen to splenic nerve stimulation are increased by angiotensin (Benelli et al, 1964; Thoenen, Hurlimann & Haefely, 1965; Hertting & Suko, 1966). Hughes (1968b) found no potentiation of the sympathetic nerve stimulation responses of a variety of non-vascular smooth muscles in the presence of angiotensin.

Angiotensin causes an increased sympathetic discharge in anaesthetised rabbits (Aars & Akre, 1968) and in anaesthetised dogs (Schmitt & Schmitt, 1968b).

The vasoconstrictor responses of the vasculature of the human hand (Johnsson, Henning & Ablad, 1965; Scroop & Whelan, 1966:1968: Henning & Johnsson, 1967) and the dog renal artery (McGiff & Fasy, 1964;1965) to angiotensin are mediated via the sympathetic nervous system and can be abolished by impairment of these nerves. The overall pressor response to intravenous angiotensin however cannot be due to increased sympathetic discharge as drugs which impair the peripheral sympathetic function and block the

vasoconstriction reported above do not reduce the overall angiotensin pressor response (for example Laurence & Nagle, 1963).

Infusion of noradrenaline or procedures which release noradrenaline from the sympathetic nerve endings e.g. ganglion stimulants, indirect sympathomimetics, nerve stimulation or reserpine are reported to enhance the responses of the cardiovascular system of a number of species to angiotensin (Haas & Goldblatt, 1959; Schmitt & Schmitt, 1967a,b; Pals & Fulton, 1968).

Isolated or denervated vascular muscle, with the exception of the aorta, is very insensitive to angiotensin (Zimmerman, 1962; Laverty, 1963; de la Lande & Rand, 1965; McGiff & Fasy, 1964;1965; McGregor,1965; Somlyo, Woo & Somlyo, 1965; Bohr & Uchida, 1967; Day & Owen, 1968). The responses to angiotensin can however be partially restored by mobilization of the endogenous noradrenaline by nerve stimulation, where possible, or by indirect sympathomimetic amines (Zimmerman, 1962; Laverty, 1963; Day & Owen, 1968) but not by exogenous noradrenaline (Day & Owen, 1968). The report of Gokhale, Gulati, Kelkan & Kelkan (1966) that the non-innervated human umbilical artery was very insensitive to angiotensin has provided further support for the hypothesis that the complete vasoconstrictor action of angiotensin requires a functional sympathetic innervation. Lewis (1968) however has reported that both human and sheep umbilical arteries are very responsive to angiotensin.

On the aortic strip preparation taken from many species angiotensin causes a contraction which is mediated, in part, by the release of noradrenaline from stores within the wall of the aorta (Distler, Liebau & Wolff, 1965a,b; Liebau, Distler & Wolff, 1965:1966: Suzuki & Matsumoto, 1966: Schumann & Guther, 1967) although Turker & Karahneyinoglu (1968) found that cocaine did not interfere with the response of the rabbit aortic strip to angiotensin.

Although these observations have established that angiotensin acts at the sympathetic nerve endings no adequate explanation of this action has been forthcoming.

Paliac & Khairallah (1967a,b) have shown that massive quantities of angiotensin can inhibit the uptake of noradrenaline into neuronal storage sites and they have suggested that this could explain the ability of angiotensin to enhance the responses of many preparations to sympathetic nerve stimulation. Panisset & Bourdois (1967:1968,a,b,c) have confirmed that much smaller concentrations of angiotensin can reduce uptake of noradrenaline in the perfused cat mesenteric vasculature. In pithed rats angiotensin possesses no activity as an inhibitor of noradrenaline uptake (Pals & Masucci, 1968; Pals et al, 1968) and Zimmerman & Gisslen (1968) found that although cocaine and angiotensin both enhanced the sympathetically mediated constrictor response of the dog perfused renal artery they did so by different mechanisms. They found that the increased responses in the presence of angiotensin were associated with increased release of transmitter. The hypothesis

that the effects of angiotensin at the sympathetic nerve endings are due to inhibition of noradrenaline uptake fails to explain the differential effect of angiotensin on endogenous noradrenaline whilst not affecting exogenous noradrenaline nor does it explain the ability of angiotensin to potentiate the responses to indirect sympathomimetics (see back).

Pals & Fulton (1968) have suggested that there is a synergistic interaction between angiotensin and noradrenaline at the adrenergic α-receptors. They developed this hypothesis to explain the ability of infusion of noradrenaline to potentiate the responses to angiotensin but this hypothesis also fails to account for the differential effects of angiotensin on endogenous and exogenous noradrenaline.

The most likely action of angiotensin is that it can, under some conditions, cause a release of noradrenaline from its neuronal storage sites. This has been established for the aortic strip where it occurs readily and it may be that angiotensin can increase the amount of noradrenaline released in response to procedures which themselves release noradrenaline and the enhancement of the responses to those procedures which release noradrenaline is due to the increased release of transmitter. In 1965 Sakuri & Hashimoto found that although infusions of angiotensin alone did not increase the urinary excretion of catecholamines in rabbits they did greatly increase the excretion caused by tyramine. Beaullnes, Nantel & Panisset (1966) observed

that angiotensin and tyramine both released noradrenaline from isolated rabbit atria. Zimmerman & Whitmore (1967) found that angiotensin caused a small increase in the quantity of noradrenaline they were able to collect after stimulation of the sympathetic supply to the dog hind limb and Zimmerman & Gisslen (1968) found that this increased release of noradrenaline also occurred in the dog perfused renal artery in the presence of angiotensin. This increased release was more readily detected at 5 pulses per second than at 2 pulses per second although the responses were enhanced at both frequencies of stimulation. Although angiotensin also increases the responses of the cat spleen to splenic nerve stimulation this is not associated with increased release of noradrenaline (Thoenen et al, 1965; Hertting & Suko, 1966). Clarification of this question of increased release of transmitter is important but the minute quantities of noradrenaline released and its subsequent rapid metabolism have so far prevented this.

7. Effect of drugs which modify sympathetic nerve function.

The hypertension which results from long term infusion of small quantities of angiotensin is prevented or reversed by adrenergic neuron blocking drugs (McCubbin et al, 1965; Yu & Dickinson, 1965) and by ganglion blockade (Yu & Dickinson, 1965).

The use of drugs which modify sympathetic function has not added greatly to the understanding of the interaction between

the acute pressor effect of angiotensin and the sympathetic nervous system. In whole animals drugs which impair the function of the sympathetic nervous system do not usually reduce the responses to angiotensin and in fact are frequently reported to increase them There is however some doubt as to the effect of reserpine treatment on the pressor responses to angiotensin. Baum (1963) reported that reserpine reduced the responses to angiotensin in dogs but subsequently Cessi & Bano (1964a) using rabbits and Louis & Doyle (1966) using dogs found no change in the responses to angiotensin after reserpine.

The acute responses to angiotensin are not reduced by immunosympathectomy in the rat (Brody, 1964), by adrenergic neuron blockade in man (Laurence & Nagle, 1963) or in rats (Hughes, 1968a; Pals, 1968; Schmitt & Schmitt, 1968), α- or β- blocking drugs in rabbits (Cessi & Bano, 1964b) or in rats (Hughes, 1968a; Schmitt & Schmitt, 1968), autonomic ganglion blockade in dogs (Zimmerman, 1964) or rats (Laverty, 1962; Gordon & Stephenson, 1967) or inhibition of uptake into neuronal noradrenaline stores in dogs (Kaumann, Zuberbuhler & Taquini, 1964; Graham, Aboud & Eckstein, 1965) or in cats (Miele, 1966).

DRUGS WHICH SPECIFICALLY BLOCK THE ACTIONS OF ANGIOTENSIN

Although the enormous potential value, both clinically and experimentally, of a specific anti-angiotensin drug has long been recognised no such drug exists and the search continues.

Although the production of anti-renin sera has been successfully achieved in the past (see back) this has not been of any clinical value probably because of the difficulty of production and the lack of pure renin for sera production.

A number of substances have been suggested as specific angiotensin antagonists. Cinnarazine has been reported as a specific anti-angiotensin agent (Schaper, Jageneau, Zhonneux, Van Nueton & Janssen, 1963; Cession, 1964b, Van Nueton, Dresse & Dony, 1964; Godfraind, 1968) despite its known anti-histamine action for which it has been used clinically.

Lidoflazine has also been reported as a specific anti-angiotensin substance (Godfraind, 1968) but there is considerable doubt as to its specificity (Turker & Kayaalp, 1967; Ellis & Reit 1969).

In 1966 Gascon & Walaszek found that a flavonoid, osajin, specifically blocked the action of angiotensin on guinea-pig ileum. They suggested that osajin might act by chelation of copper or zinc ions. Osajin however is inactive as an angiotensin antagonist <u>in vivo</u> (Walaszek personal communication).

Thus, no specific anti-angiotensin drug has been found to date.

EXPERIMENTAL METHODS.

METHODS.

1. Perfused isolated artery preparation.

Rabbits were stunned by a blow on the back of the neck and bled out. Both ears were removed and cannulae inserted into the central artery of each ear as near the base of the ear as possible. The blood within the vascular bed was flushed out with the perfusion fluid and one ear was stored in a beaker of perfusion fluid at 4°C. This artery was used, if required, if storage had not exceeded 4 hours. The artery removed from the second ear was dissected free of surrounding tissues and a length of 4-5 cm taken for perfusion. The preparation was set up for perfusion as described by de la Lande & Rand (1965), except that the artery was suspended in air instead of in an organ-bath containing the perfusion solution. In this way drugs injected or infused into the lumen of the vessel could not produce an effect on the outside of the artery. The preparation was perfused with Kreb's bicarbonate solution containing (g/l): NaCl 7.7, KCl 0.34, $CaCl_2$ 0.3, KH_2PO_4 0.16, $MgSO_4$ 0.29, $NaHCO_3$ 2.1 and dextrose 2.0. The fluid was maintained at 37°C and continuously gassed with 95% oxygen and 5% carbon dioxide. Perfusion pressures were recorded by means of a mercury manometer (rat blood pressure type) or by using a blood pressure transducer (Devices/C.E.C. type 4-327-L221) and a Devices M4 electronic recorder.

Drugs were dissolved in the perfusion solution and injected into the arterial cannula in a volume not exceeding 0.1 ml. or were added to the resevoir containing the perfusion fluid.

The vascular sympathetic nerves were stimulated by threading the artery through bipolar platinum electrodes (Burn & Rand, 1960) and delivering rectangular pulses from an electronic stimulator (Scientific & Research Instruments); details of stimulation parameters are given in the experimental results section. In general, pulses of supramaximal strength (20-50 volts) of 1 millisecond duration and frequencies of 1-50 pulses per second were used. Stimulation was usually applied for periods of 40 seconds in every 4 minutes.

In those experiments where blood taken from the rabbit was used for perfusion the set up was slightly modified. Rabbits were anaesthetised with urethane (1.5g/kg) dissolved as a 25% solution in tap water and injected into a marginal ear vein. The right carotid artery was cannulated and blood was collected into a vessel containing 5000 units of heparin. The blood was collected by allowing it to flow from the cannulated artery until the flow rate declined at which time the artery was clamped for about two minutes after which a further collection was made until the flow stopped completely. The central artery from one of the ears was then cannulated, removed from the ear and set up for perfusion in the normal way. The blood was placed in the resevoir and after perfusion through the artery was collected, replaced in the resevoir and re-used.

2. Measurement of pressor responses in anaesthetised rabbits.

Rabbits were anaesthetised with urethane (1.5g/kg) injected into a marginal ear vein. When the rabbit was anaesthetised the trachea was cannulated to facilitate

breathing. The right femoral vein was cannulated for the administration of drugs and 1000 units of heparin injected intravenously. The injection of heparin was repeated at intervals of one hour. The right carotid artery was cannulated and blood pressure recorded using a mercury manometer and a kymograph.

Pressor agents were dissolved in saline and administered in a volume of 0.1-1.0 ml. and washed in with a further 2.0 ml. of saline.

3. The pithed rat preparation.

Male Wistar rats weighing 250-350 g were anaesthetised with pentobarbitone (Nembutal) 60mg/kg injected intraperitoneally; atropine sulphate 1mg/kg was administered by the same route immediately after the pentobarbitone. The trachea was cannulated, artificial respiration started and the animal pithed by the method of Shipley & Tilden (1947) using a copper pithing rod prepared as described by Gillespie & Muir (1967). Both jugular veins were then cannulated with polyethylene or nylon tubing, PP25 size. One was used for drug injections and the other for continuous infusions. The left common carotid artery was cannulated with polyethylene or nylon tubing, PP30 size, and the blood pressure recorded by means of a mercury manometer or by a blood pressure transducer (Devices/C.E.C. type 4-327-L221) connected to a Devices M4 electronic recorder. Before commencing the experiment each rat received intravenous injections of heparin 1000 units/kg, atropine sulphate 1mg/kg and d-tubocurarine 3mg/kg.

Sympathetic stimulation. Electrical stimulation of the spinal sympathetic outflow was carried out as described by Gillespie & Muir (1967). A length of copper wire was attached firmly under the skin of one leg and connected to one pole of a constant voltage square wave stimulator (Scientific & Research Instruments) and the other pole was connected to the pithing rod.

The sympathetic outflow was stimulated with supramaximal strength pulses (usually 80 volts) of 1 millisecond duration at frequencies ranging from 0.1-8.0 pulses per second applied for periods of 40 seconds and repeated at intervals of not less than 4 minutes.

Adrenalectomy. Bilateral adrenalectomy was performed under pentobarbitone anaesthesia via retroperitoneal incisions. The wounds were closed with metal clips and the animals left for 30 minutes before pithing.

Heart rate measurement. In a few experiments the effect of stimulation of the spinal sympathetic outflow on heart rate was measured. This was carried out by use of a Neilson tachygraph connected to a Devices M4 electronic recorder and triggered from the E.C.G. signal.

Drugs were dissolved in normal saline and were injected in volumes not exceeding 0.2 ml. and flushed in with a further 0.1 ml. of saline. Infusions were made using a Palmer automatic injection apparatus at rates up to 0.05 ml./minute.

4. Measurement of blood pressure in anaesthetised cats.

Anaesthesia was induced with halothane 3.5% in a mixture of oxygen 20% and nitrous oxide 80%. The right femoral vein was then cannulated and chloralose 80mg/kg (as a 1% solution in saline) injected slowly to maintain anaesthesia. During the injection of chloralose the halothane content of the gaseous anaesthesia was gradually reduced and when about half the chloralose had been given the gaseous anaesthesia was withdrawn. The trachea was located and a metal cannula inserted to facilitate respiration. The right common carotid artery was cannulated with PP 90 nylon tubing and the cannula connected to a blood pressure transducer (Devices/C.E.C. type 4-327-L221) and a Devices M4 electronic recorder. Heparin, 1000 units/kg, was given intravenously.

Adrenalectomy. In some experiments the adrenal glands were removed, tied off, or prepared to allow clamping of the adrenal blood supply when required. The adrenal glands were located through a mid-line incision in the abdominal muscle wall and the surrounding tissues dissected away with as little damage as possible. When the blood vessels to and from the glands had been freed of surrounding tissues they were either tied tightly and the adrenals glands left untouched or tied so as to permit the removal of the glands, or left so that artery forceps could be applied when required.

Drugs were administered via the cannulated femoral vein. All drugs were dissolved in saline and given in a volume not exceeding 1 ml. and washed in with 2 ml. of saline.

5. Measurement of blood pressure and heart rate in conscious, unrestrained cats.

Thuransky (1966) first described a technique for the direct measurement of blood pressure in conscious cats. This method however provided no adequate means of closing the arterial cannula. Hall, Gomersall & Heneage (1968) improved the method of closing the arterial cannula by use of a one way valve which could be screwed into the skull. This technique, although an improvement on the method of Thuransky (1966) had the big disadvantage of possible brain damage resulting from the pressure used when fixing the valve into the skull.

A technique has been devised in which it was hoped to overcome some of the disadvantages of the methods of both Thuransky (1966) and Hall et al (1968).

Female cats weighing 2.4-3.2 kg were used in these experiments. For at least ten days prior to the operation the cats were subject to considerable individual attention and familiarised with the room and cage which was to be used for experiments. These efforts to gain the confidence of the animals were found to help their recovery from the operation.

Anaesthesia was induced using halothane 3.5% in a mixture of 20% oxygen and 80% nitrous oxide. The anaesthesia was maintained by halothane 1.5% in the same mixture of oxygen and nitrous oxide.

The cat was shaved along the back of the neck and from the base of the mouth to the top of the chest. A small incision was made at the back of the neck and an incision of about 3 inches length at the front. The arterial and venous cannulae, filled with heparinised saline and sealed, were passed beneath the skin from the back of the neck to the front A branch of the right jugular vein was located and the venous cannula, polyethylene tubing PP 30 size, was inserted and fed into the main jugular vein to a depth of about 3 inches. The cannula was then tied tightly into the branch of the vein with at least five ties. By tying the cannula into a branch of the vein there was minimal interference with the normal blood flow through the main jugular vein. The venous cannula at the back of the neck was then cut so that about 3 inches was permitted to hang freely. The cannula was closed with a pin.

The arterial cannula, polyvinylchoride tubing PP 90 size, was connected to a blood pressure transducer (Devices/ C.E.C. type 4-327-L221) and the transducer to a Devices M4 electronic recorder. The right carotid artery was located and dissected free of surrounding tissues with care so as to minimise the damage done to these tissues. The artery was tied off craniad and the cannula inserted and loosely tied into the artery. The bull-dog clip was removed and the cannula slowly fed into the artery whilst the blood pressure was continuously monitored. When the cannula reached the end of the inominate artery it either passed into the required position in the aorta or into the heart. When the cannula

travelled into the aorta the normal blood pressure pulse was recorded on the Devices recorder but when the cannula entered the left ventricle the pulses became very wide as the recorder monitored the pressure within the left ventricle. When the cannula entered the ventricle it was withdrawn and fed back into the artery until it entered the aorta. In some operations the cannula became kinked and the pulse recording was lost. On these occasions the cannula was withdrawn and the process of feeding the cannula into the artery repeated until the cannula was lying as required. The cause of the kinking of the cannula was not determined but may have been due to the cannula being pushed against the wall of the aorta. When the tip of the cannula was thought to lie in the aorta in the required position the cannula was tied tightly into the carotid artery with at least five ties. The cannula was closed by means of rubber tipped artery clamps and disconnected from the transducer. The cannula was cut off close to the back of the neck and attached to a valve of the type described by Hall et al (1968). The valve was itself fixed to a small rectangular piece of perspex, about $1\frac{1}{2}$ inches long, $\frac{1}{4}$ inch wide and $\frac{1}{8}^{th}$ of an inch thick. Four small holes were drilled in the perspex, one at each corner. The perspex was stitched firmly beneath the skin at the back of the neck by means of the four holes in the perspex and the wound closed with thread stitches. When finally stitched only the top of the valve appeared above the skin at the back of the neck. Fig.1. shows the position of the valve at the back of the neck. The valve was covered with a dust-cap.

Fig.1 Conscious cat blood pressure. This photograph illustrates the position of the one way valve at the back of the neck of a cat. In this photograph the dust cap has been removed from the valve and replaced by the valve top which connects the valve to the Devices Electronic recorder by means of the soft polyethylene tubing shown.

During the operative procedure penicillin and sulphathiazole dusting powder was liberally sprinkled onto all open wounds at frequent intervals and a further dusting was made just prior to closing the wounds. The incisions were closed with cotton thread sutures and lightly bandaged.

Just before the end of the anaesthesia chlorpromazine (1mg/kg) was injected into the muscle of one leg. The chlorpromazine slowed the recovery from anaesthesia and reduced the tendency of the cats to scratch their wounds when first they regained consciousness.

During the first seven post-operative days the arterial cannula was flushed out daily with 2 ml. of heparinised saline (50 units/ml.). Subsequently the arterial cannula was flushed out with 2 ml. of heparinised saline on alternate days and before starting each experiment.

The cats were allowed at least 3 days for recovery after which the process of training them to sit quietly during experiments and to accept intravenous injections of solutions at room temperature without alarm began. In most cases the cats became sufficiently well trained to start experiments after a further 3 days.

The cage in which the animals were placed for experiments permitted free movement although after a few minutes exploration each day it was usual for the cats to sit quietly for periods of 2-3 hours or more. Fig.2. shows a cat sitting quietly in the cage during an experiment. The valve dust-cap was removed for experiments and replaced by the valve top which opened it and which was connected to a blood

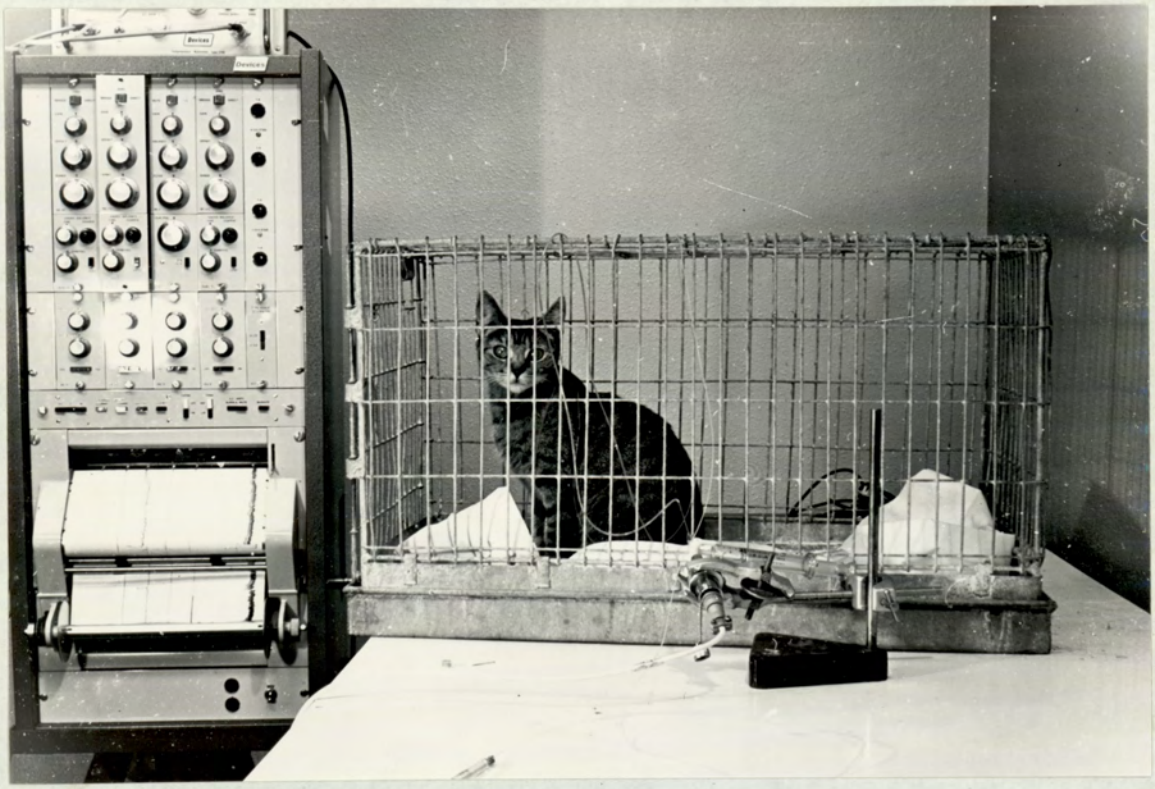

Fig.2 Conscious cat blood pressure. This photograph was taken during a typical experiment in which the aortic blood pressure of a conscious cat was continuously monitored.

pressure transducer (Devices/C.E.C. type 4-327-L221) which was itself connected to an M4 Devices electronic recorder. The heart rate was recorded by means of a Neilson tachygraph unit connected to the Devices recorder and triggered from the blood pressure pulse.

Intravenous injections were made into the implanted venous cannula. The cannula was connected to a long length of polyethylene tubing, PP30 size, by means of a broken off hypodermic needle and drugs dissolved in normal saline were administered in a volume of less than 0.4 ml. and flushed in with 1 ml. of saline By using a dose volume of less than 0.4 ml. the complete drug solution was retained in the tubing until flushed in and the injection could be delayed on the few occasions when the cat was disturbed at the start of the injection.

DRUGS USED.

Adrenaline bitartrate (B.D.H.)

α-methyl dopa (Aldomet, Merck, Sharp & Dohme)

Angiotensinamide (Hypertensin, Ciba)

Ascorbic acid (B.D.H.)

Atropine sulphate (B.D.H.)

Bethanidine sulphate (Burrough's Wellcome)

Chloralose (B.D.H.)

Chlorpromazine hydrochloride (Largactil, May and Baker)

Cocaine Hydrochloride (B.D.H.)

Colchicine (B.D.H.)

Desipramine hydrochloride (Pertofran, Geigy)

Dexamphetamine sulphate (Sigma)

d,l-Dopa (Sigma)

Dopamine hydrochloride (Sigma)

Guanethidine monosulphate (Ismelin, Ciba)

Heparin (Evans Medical)

5-hydroxytryptamine creatinine sulphate (Sigma)

McN-A-343 (McNeil)

Nialamide (Pfizer)

Noradrenaline bitartrate (B.D.H.)

Noradrenaline hydrochloride (Sigma)

Pempidine tartrate (May & Baker)

Penicillamine hydrochloride (Dista)

Phentolamine methansulphonate (Rogitine, Ciba)

Phenylethylamine hydrochloride (Sigma)

Propranolol hydrochloride (Inderal, I.C.I.)

Reserpine (Serpasil, Ciba: and Halewood Chemical Company)

Sodium diethyl-dithiocarbamate (B.D.H.)

Syrosingopine (Aspro-Nicholas)

Tetrabenazine (Hoffman La-Roche)

Tranylcypromine sulphate (Smith, Kline and French)

d-Tubocurarine (Duncan, Flockhart and Evans)

Tyramine hydrochloride (Sigma)

Urethane (B.D.H.)

The doses of noradrenaline quoted in this thesis are expressed as base: all other doses are expressed in terms of the salts listed above.

In experiments in the isolated ear artery preparation drugs were dissolved in the perfusion fluid. In all other experiments drugs were dissolved in normal saline except syrosingopine and tetrabenazine. These two substances were prepared for injection by dissolving the solid in 3 drops of lactic acid and 0.8 ml. of 100% ethanol and then made up to half the final volume with distilled water. This gave a solution of pH about 2.5 the pH of the solution was then adjusted to 4.5 by the addition in drops of a saturated aqueous solution of sodium bicarbonate. When the solution was at pH 4.5 it was made up to the final volume with further distilled water.

EXPERIMENTAL RESULTS.

RESULTS.

SECTION 1. THE INTERACTION BETWEEN ANGIOTENSIN AND THE SYMPATHETIC NERVOUS SYSTEM IN THE ISOLATED EAR ARTERY OF THE RABBIT

Chapter 1.

The vasoconstrictor action of angiotensin; and the effect of angiotensin on the responses to sympathetic nerve stimulation.

McCubbin & Page (1963a,b) found that in anaesthetised dogs the pressor responses to procedures which release noradrenaline from sympathetic nerve endings were usually enhanced during intravenous infusions of angiotensin but the responses to injected noradrenaline were unaltered.

This ability of angiotensin to enhance the responses to sympathetic nerve stimulation has been observed in a number of other preparations including isolated vascular muscle (McCubbin & Page, 1963a; Su, 1965), the guinea-pig isolated vas deferens (Benelli et al, 1964; Graham & Al Katib, 1967) and the cat isolated spleen (Benelli et al, 1964; Thoenen et al, 1965; Hertting & Suko, 1966). The interation between angiotensin and the sympathetic nervous system is unexplained although Benelli et al (1964) have suggested that angiotensin may promote the release of noradrenaline from sympathetic nerve endings.

The isolated central artery from the rabbit ear, as described by de la Lande & Rand (1965), has been used to study this interaction between angiotensin and the sympathetic nervous system as it affords a convenient and simple *in vitro*

system suitable for this study.

Results.

1. Effect of angiotensin on perfusion pressure.

Injections of angiotensin from 50ng to 1mg were required to cause a measurable increase in perfusion pressure. One in every seven or eight preparations was however insensitive even to doses of 1mg. If the dose of angiotensin was repeated at intervals of not less than 10 min the responses to the second and subsequent injections was constant and smaller than the response to the first injection. Compared with noradrenaline angiotensin was less potent, on a weight basis, and gave a far less steep dose/response curve as is shown in Fig.3. The relative potencies of angiotensin and noradrenaline in the isolated artery preparation contrast sharply with their relative pressor potencies in anaesthetised rabbits where angiotensin was four (one experiment) or ten (one experiment) times more potent than noradrenaline and the dose/response curves were almost parallel.

Infusions of angiotensin in concentrations up to 500ng/ml. produced either a small transient increase in perfusion pressure or were without effect. Infusions of noradrenaline in excess of 1ng/ml. caused a dose dependent vasoconstriction which was well maintained.

2. Effect of angiotensin on the responses to sympathetic nerve stimulation.

The addition of angiotensin to the perfusion fluid in concentrations of less than 1ng/ml. had no effect on perfusion

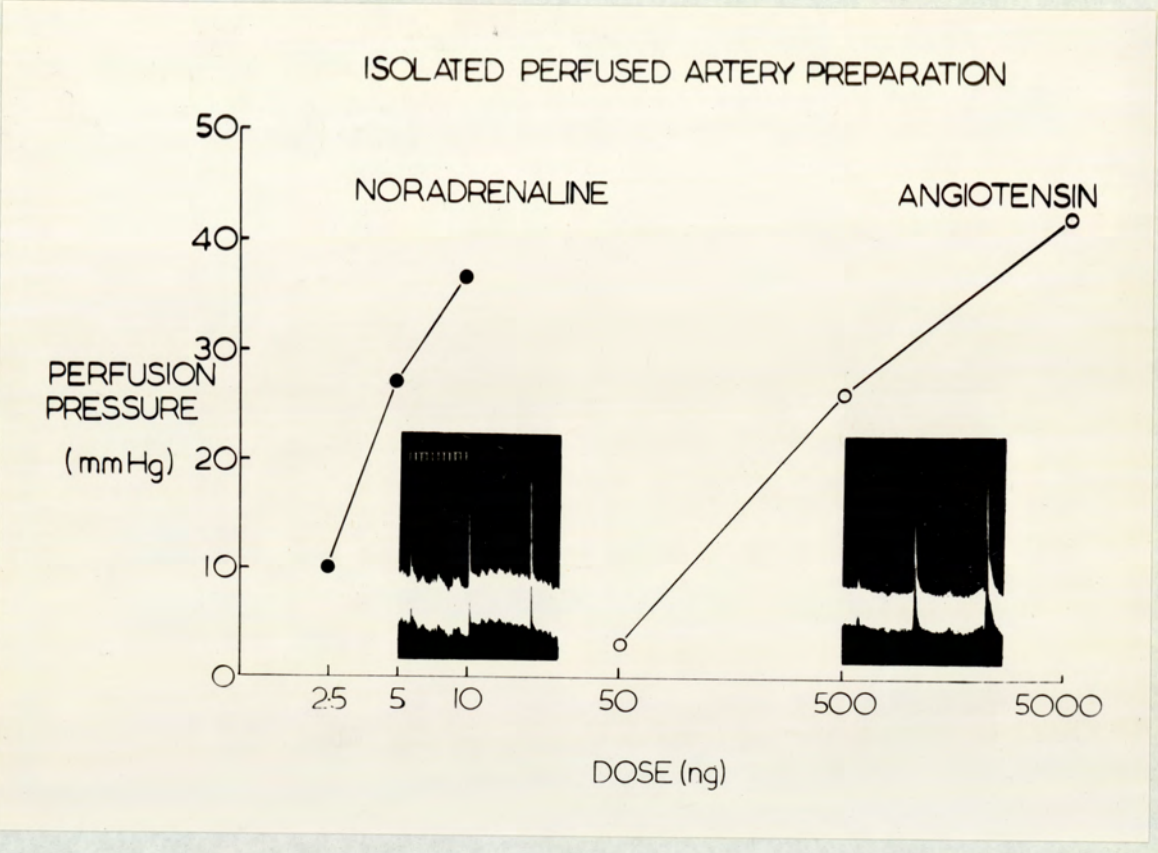

Fig.3 Isolated artery preparation. Vasoconstrictor responses to angiotensin (50,500 and 5000 ng) and to noradrenaline (2.5, 5 and 10 ng). The graph illustrates these responses as dose/response curves. Time scale in minutes.

pressure or on the responses to sympathetic stimulation. In concentrations from 1-500ng/ml. in the perfusing fluid or by intra-arterial injection of 1μg-1mg. the predominant effect of angiotensin was to impair the responses to sympathetic stimulation. The effect of a single dose of 5μg of angiotensin on the responses to sympathetic stimulation is illustrated in Fig.4.

In a few preparations although the predominant effect of angiotensin was to impair the responses to sympathetic stimulation the impairment was preceded by a short lived enhancement of the sympathetic constriction as shown in Fig.5.

The anti-sympathetic action of angiotensin was most marked at the lower frequencies of stimulation. Fig.6. illustrates an experiment where the addition of angiotensin to the perfusion fluid virtually abolished the responses to the lower frequencies of stimulation but was almost without effect on the responses to the higher frequencies.

3. Effect of angiotensin on the responses to tyramine and to noradrenaline.

Angiotensin caused a reduction in the responses of the artery to both tyramine and to noradrenaline. This action of angiotensin occurred at those dose levels which caused a reduction in the responses to sympathetic nerve stimulation.

4. Comparison of the anti-sympathetic action of angiotensin with other anti-sympathetic drugs.

The anti-sympathetic action of angiotensin was not altered by the inclusion of cocaine 5μg/ml. in the perfusion fluid although in other experiments cocaine prevented the

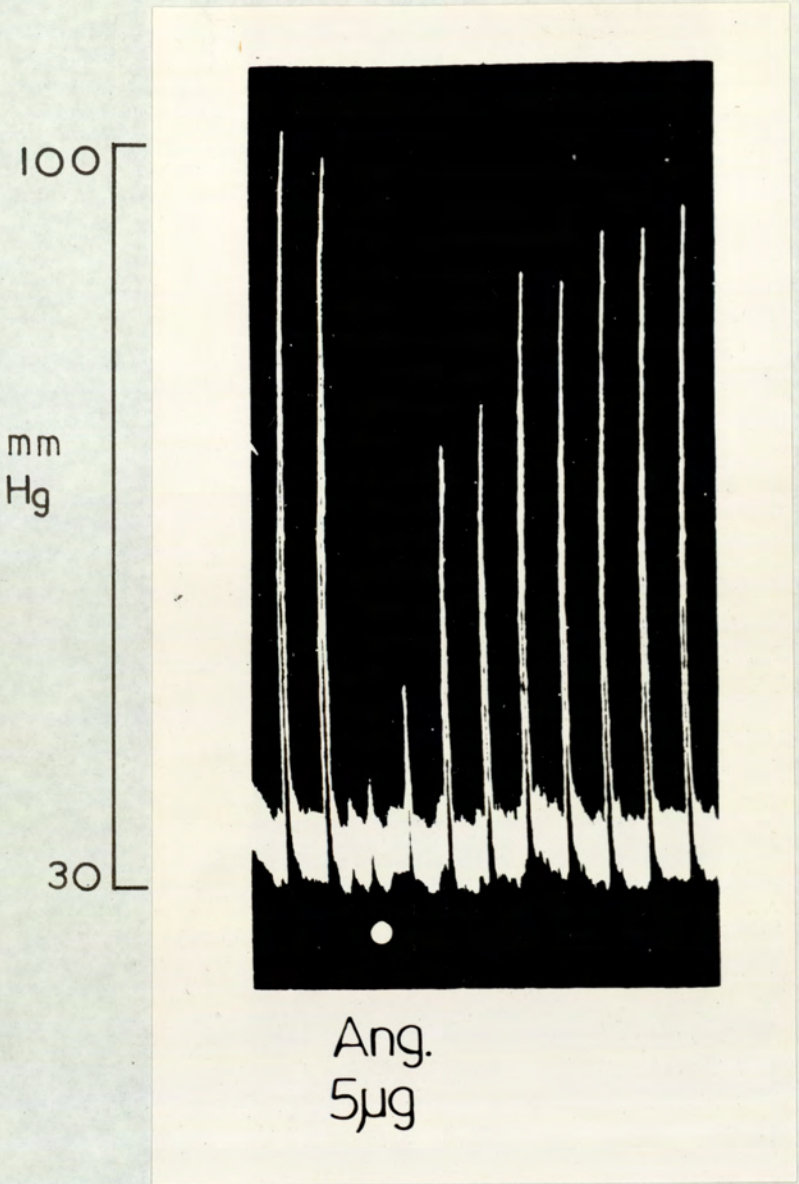

Fig.4 Isolated artery preparation. Responses to sympathetic nerve stimulation at 5 pulses per second, supramaximal strength, before and after an intra-arterial injection of angiotensin (5µg).

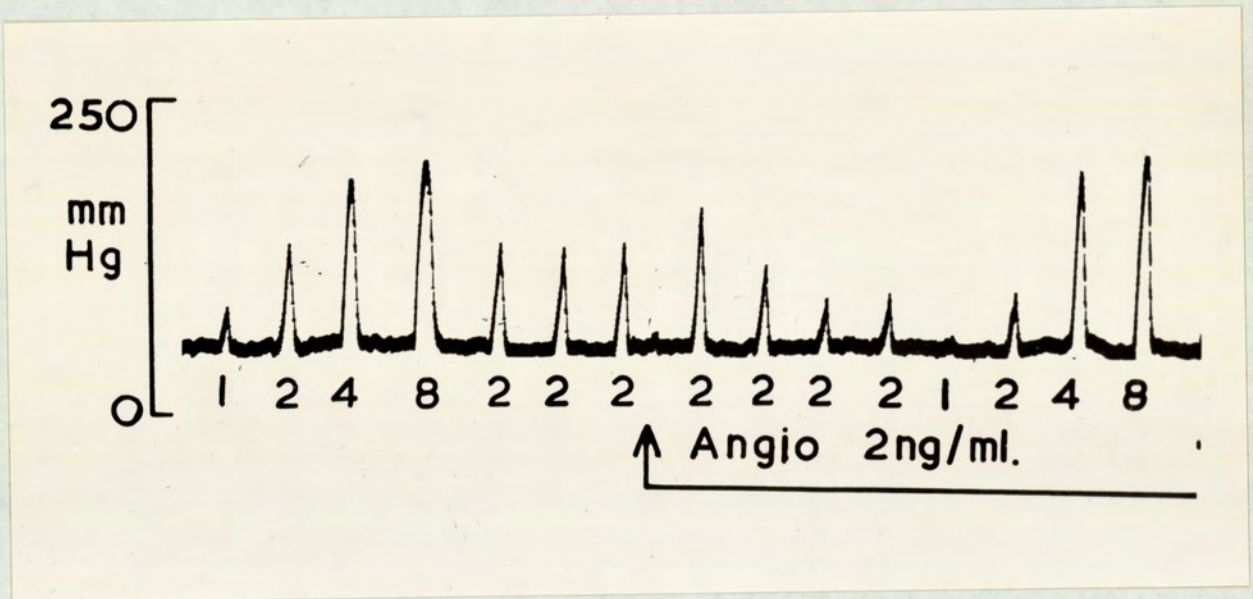

Fig.5 Isolated artery preparation. Responses to sympathetic nerve stimulation at 1, 2, 4 and 8 pulses per second, supramaximal strength. The responses to stimulation at 2 pulses per second were increased and then decreased by an infusion of angiotensin (2ng/ml.). Time scale in minutes.

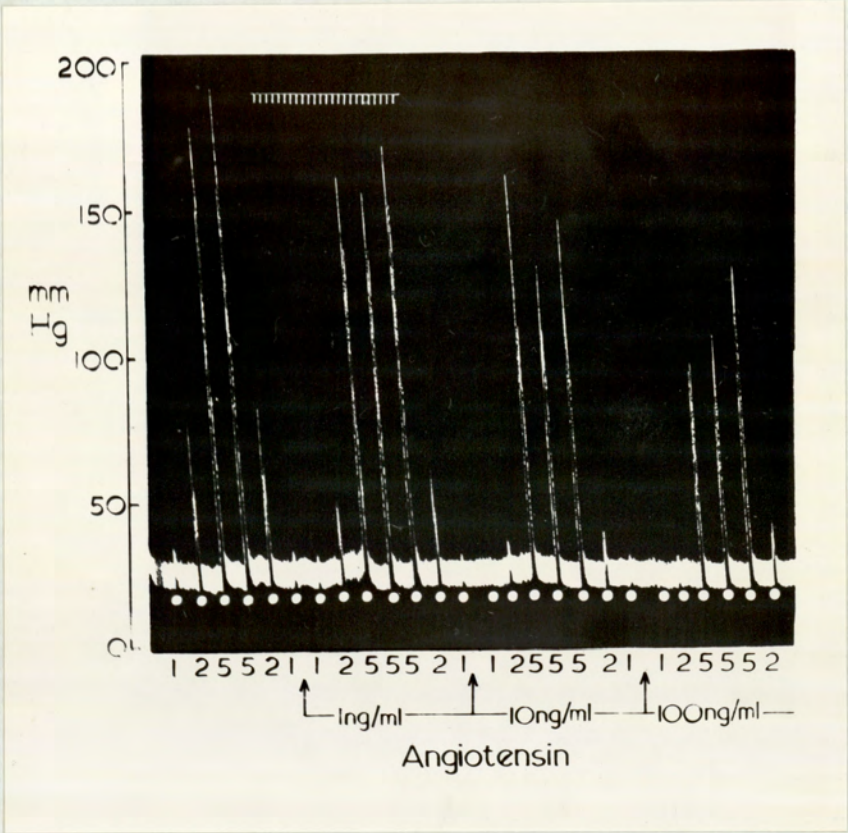

Fig.6 Isolated artery preparation. Responses to sympathetic nerve stimulation at 1, 2 and 5 pulses per second, supramaximal strength. Infusions of angiotensin at 1, 10 and 100 ng/ml. reduced the responses at all frequencies but the reduction was most marked at the lower frequencies of stimulation. Time scale in minutes.

anti-sympathetic action of guanethidine 2µg/ml. Unlike guanethidine which increased the responses to noradrenaline angiotensin reduced these responses. The responses to sympathetic nerve stimulation which were blocked by guanethidine recovered after the inclusion of dexamphetamine sulphate (500ng/ml.) in the perfusion fluid. Dexamphetamine sulphate (500ng/ml.) failed to reverse the block caused by angiotensin.

Pretreatment of rabbits with reserpine (0.25mg/kg/day intravenously) for 1-4 days reduced or abolished the responses to sympathetic stimulation in isolated ear artery preparations but unlike the block caused by angiotensin there was little or no change in the responses to noradrenaline in these preparations. Inclusion of reserpine 50-500ng/ml. in the perfusion fluid caused an initial increase in the responses to sympathetic stimulation but this was short lived and followed by a gradual reduction of the responses until they were abolished after 2-3 hours. The inclusion of reserpine in the perfusion fluid did not alter the responses to injected noradrenaline.

Infusion of noradrenaline (100ng/ml.) which caused a marked increase in the perfusion pressure, or of d,l-dopa (500ng/ml.), which caused no vasoconstriction, did not reverse the anti-sympathetic actions of angiotensin or reserpine.

The addition of phentolamine 250-500ng/ml. to the perfusion fluid reduced the responses to all frequencies of nerve stimulation as well as those to noradrenaline and tyramine.

Discussion.

Despite its potent *in vivo* vasoconstrictor action angiotensin is a weak *in vitro* vasoconstrictor (see for example Zimmerman,

1962; Laverty, 1963). This feeble vasoconstrictor action of angiotensin in the isolated ear artery of the rabbit was observed by de la Lande & Rand (1965) and has been confirmed in these experiments. In anaesthetised rabbits noradrenaline was less potent than angiotensin as a pressor agent whereas in this preparation it is far more potent than angiotensin. In addition to its weak action the dose/response curve to angiotensin is very shallow and the biggest vasoconstrictor response which could be achieved after injection of angiotensin was always much less than the maximal noradrenaline response. The next chapters include an investigation into the factors necessary for the complete angiotensin response which are apparently absent in the isolated preparation.

Angiotensin enhances sympathetically mediated responses in many smooth muscle preparations (see for example McCubbin & Page, 1963a; Su, 1965) however in this preparation the predominant action of angiotensin was to impair the responses to sympathetic nerve stimulation as well as those to noradrenaline and tyramine. In a small number of experiments however the impairment of the responses to sympathetic stimulation was preceded by a transient enhancement. As the ability of angiotensin to increase sympathetically mediated responses has been established and occurs in a variety of both vascular and non-vascular preparations the anti-sympathetic action of angiotensin was suprising. The occasionnal enhancement of the responses caused by angiotensin followed by the more frequent reduction of responses suggested that

angiotensin might exert a dual effect in this tissue, the usual enhancement of responses to sympathetic stimulation and also an anti-sympathetic action which is dominant.

The anti-sympathetic action of angiotensin is not specific and both noradrenaline and tyramine responses were similarly reduced. The anti-sympathetic action of angiotensin differs from that of guanethidine in that responses to noradrenaline are increased by guanethidine rather than decreased as occurs with angiotensin and the blocking action of angiotensin could not be prevented by cocaine or reversed by dexamphetamine.

The action of angiotensin also differed from that of reserpine. Angiotensin reduced the responses to both nerve stimulation and to noradrenaline whereas reserpine reduced only the responses to nerve stimulation. Infusions of d,l-dopa or noradrenaline failed to reverse the anti-sympathetic effects of either angiotensin or reserpine However, it is unlikely that the mechanism of the block caused by these two substances is the same since the angiotensin block was of rapid onset whilst that of reserpine occurred over a period of 2-3 hours.

The action of angiotensin was similar to that caused by phentolamine in that both caused a reduction in the responses to sympathetic stimulation, noradrenaline and tyramine but whereas the anti-sympathetic action of phentolamine was marked at all frequencies of stimulation the action of angiotensin was marked at the lower frequencies only.

The anti-sympathetic action of angiotensin would appear to differ from the anti-sympathetic actions of guanethidine, reserpine or phentolamine and may be due to a non-specific effect on vascular smooth muscle. The importance of this anti-sympathetic action of angiotensin is not clear. The effect seen in this preparation may resemble the effect observed by McCubbin & Page (1963a) in anaesthetised dogs. These workers found that whereas infusions of angiotensin usually increased the responses to carotid occlusion in a minority of experiments angiotensin reduced the carotid occlusion pressor response.

Chapter 2.

Effect of endogenous noradrenaline release on the vasoconstrictor action of angiotensin.

Although the aortic strip preparation of many species is very sensitive to angiotensin (see for example Distler et al, 1965a,b) most isolated vascular preparations are highly insensitive to angiotensin (see for example de la Lande & Rand, 1965; Bohr & Uchida, 1967). Zimmerman (1962) and Laverty (1963) have shown that the vasoconstrictor action of angiotensin is dependent on a functional sympathetic nervous system. The absence of sympathetic tone might explain the feeble activity of angiotensin in isolated preparations.

In this chapter the role of the sympathetic nerves in the vasoconstrictor response to angiotensin has been examined in the isolated ear artery of the rabbit.

Results.

1. Effect of angiotensin administered during sympathetic stimulation. (61 expts.)

In about 75% of experiments the constrictor response to angiotensin (50-500ng) was markedly potentiated either in height, duration or in both these parameters during sympathetic stimulation. In about 10% of preparations the response was not increased in height but showed two peaks of vasoconstriction which could become quite separate. In the remaining experiments, including those which were insensitive to 1mg of angiotensin, sympathetic stimulation did not potentiate the response to angiotensin.

The responses to intra-arterial noradrenaline were unaffected or reduced during sympathetic stimulation. Fig.7. illustrates an experiment in which angiotensin and noradrenaline were given before, during and after sympathetic stimulation at two different frequencies. The responses to angiotensin were markedly increased during each period of sympathetic stimulation whereas those to noradrenaline were reduced. The extent of the enhancement of the angiotensin response during sympathetic stimulation varied in different experiments and at different frequencies of stimulation. In four experiments in which a two point dose/response curve to angiotensin was constructed before and during sympathetic stimulation it was found that the dose/response curves were parallel and that the enhancement of the responses was equivalent to increasing the angiotensin dose 50-100 times.

The enhancement of the angiotensin vasoconstrictions during sympathetic stimulation could be demonstrated several times in one experiment but usually the potentiation became less pronounced possibly as a result of the sympathetic blocking action of angiotensin described in the previous chapter.

Effect of tyramine administered during sympathetic stimulation.

In non-stimulated preparations intra-arterial injections of tyramine (10-50μg) caused dose dependent vasoconstrictions. During sympathetic stimulation the responses to tyramine were potentiated when compared with the response prior to stimulation.

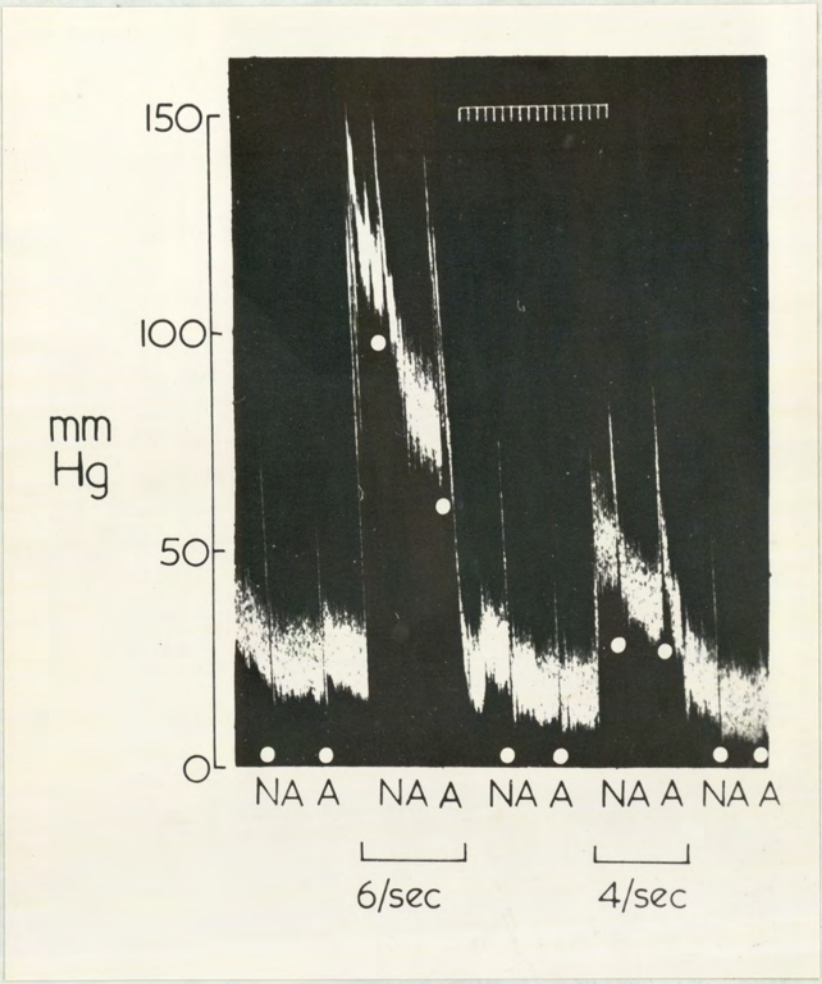

Fig.7 Isolated artery preparation. Responses of the isolated ear artery preparation to intra-arterial injections of noradrenaline (2.5 ng) at NA and to angiotensin (500 ng) at A. The response to angiotensin but not that to noradrenaline, was enhanced during continuous sympathetic stimulation at 6 pulses per second and at 4 pulses per second. Stimulation with supramaximal strength pulses. Time scale in minutes.

Fig.8. illustrates an experiment in which the response to 15µg. of tyramine injected prior to sympathetic stimulation and which caused only a small vasoconstriction was markedly enhanced during stimulation.

Effect of angiotensin administered during noradrenaline infusion.

Infusion of noradrenaline (1-5ng/ml.) raised the perfusion pressure to similar levels to those produced by sympathetic stimulation at 5-10 pulses per sec. In most experiments the responses to angiotensin were unchanged during these infusions but in a few experiments the responses were slightly increased or decreased. In all these experiments injections of noradrenaline produced a smaller effect during noradrenaline infusions.

Effect of tyramine during noradrenaline infusions.

During infusions of noradrenaline (1-5ng/ml.) the responses to intra-arterial tyramine were slightly larger than the responses prior to infusion of the noradrenaline. The enhancement of the tyramine responses during noradrenaline infusion was always less than the enhancement associated with sympathetic nerve stimulation.

Effect of angiotensin during infusions of indirectly acting sympathomimetics.

The responses to intra-arterial injections of angiotensin were increased during infusions of both phenylethylamine and tyramine (1-6µg/ml.). The responses were usually increased in height but in two experiments the angiotensin responses during

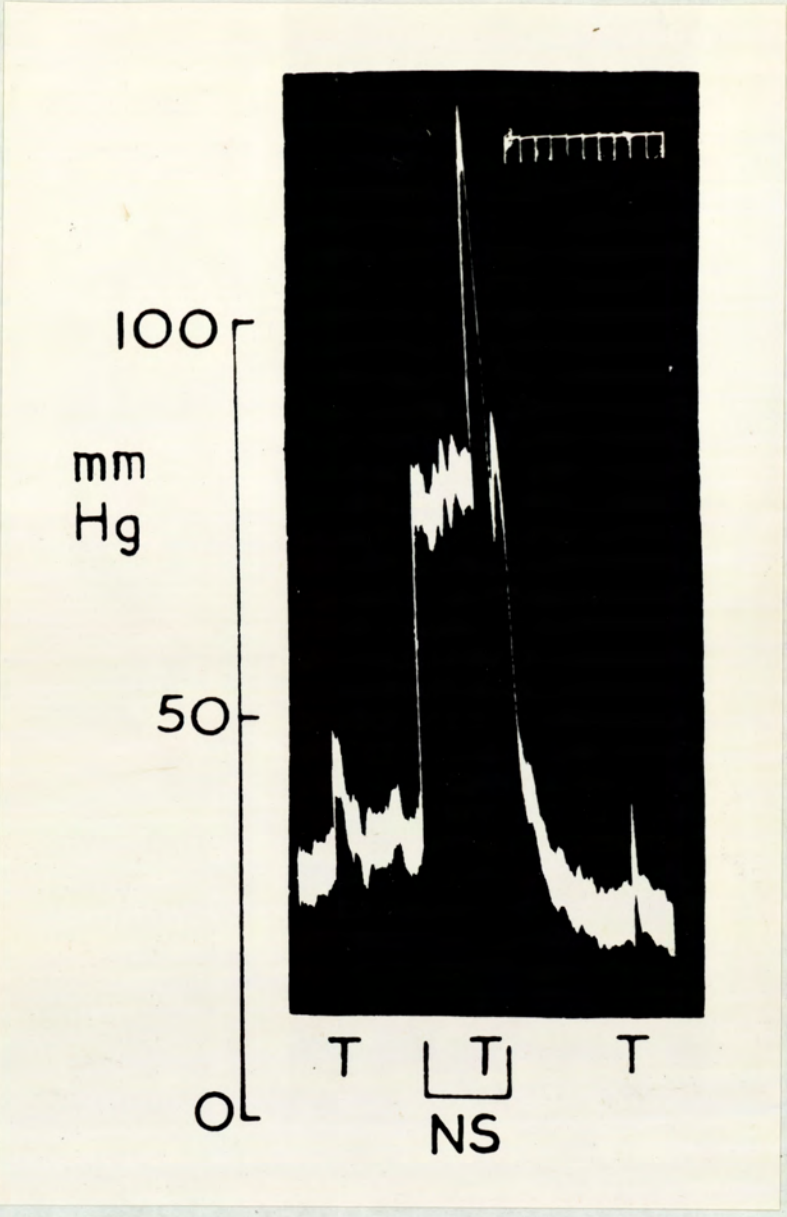

Fig.8 Isolated artery preparation. Experiment showing the enhanced vasoconstriction to tyramine (15μg) at T during continuous sympathetic stimulation, at the bar, at 2 pulses per second, supramaximal strength. Time scale in minutes.

these infusions became biphasic and resembled the biphasic response to angiotensin occasionally seen during sympathetic stimulation. Fig.9. shows an experiment in which the responses to angiotensin were greatly enhanced during an infusion of phenylethylamine (6µg/ml.) but return to their pre-infusion size on completion of the infusion.

Effect of angiotensin during infusions of 5-Hydroxytryptamine.

This substance has been shown to increase the responses to sympathetic stimulation and to vasoconstrictor agents in this preparation (de la Lande, Cannell & Waterson, 1966). Infusions of 5-hydroxytryptamine (20ng/ml.) increased the responses to sympathetic stimulation, noradrenaline and angiotensin, thus confirming its non-specific sensitising effect in this tissue.

Effect of increasing perfusion rate on vasoconstrictor agents.

The perfusion pressure was raised from 30-40 mm.Hg. to 80-100 mm Hg by increasing the rate of flow of the perfusion fluid from about 8 ml./min to about 20ml./min. The vasoconstrictor responses to angiotensin, noradrenaline and tyramine were decreased but returned to control levels when the flow rate was returned to normal.

Action of drugs affecting sympathetic mechanisms.

Reserpine

(a) Pretreatment

Reserpine (0.25mg/kg/day intravenously) was administered to seven rabbits for 1-4 days. Four preparations taken from these rabbits were completely unresponsive to sympathetic

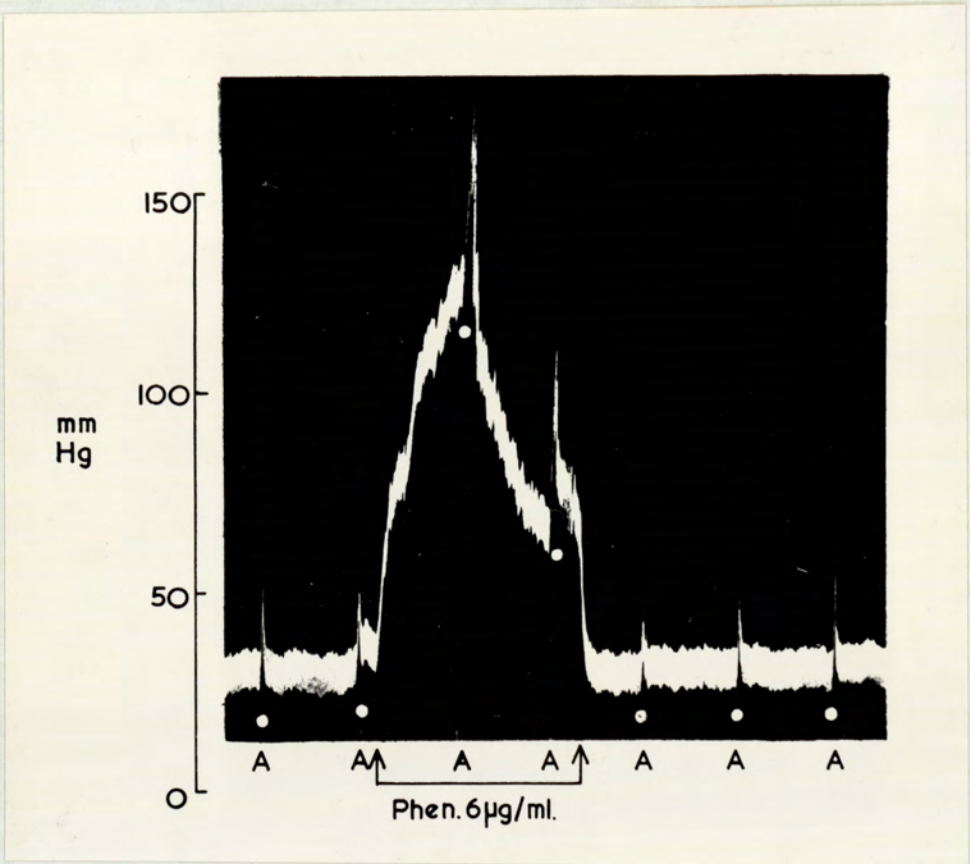

Fig.9 Isolated artery preparation. Responses to 500 ng of angiotensin injected into the artery at 10 minute intervals, at the dots, before, during and after adding phenylethylamine (6µg/ml.) to the perfusion fluid. Time scale in minutes.

stimulation, although they showed a normal sensitivity to angiotensin. In none of these preparations was the vasoconstrictor activity of angiotensin increased during sympathetic stimulation. In five other preparations taken from these rabbits, however, a small sympathetic vasoconstriction remained and the vasoconstrictor response to angiotensin was markedly increased during sympathetic stimulation.

(b) Acute effects.

Addition of reserpine (50-500ng/ml.) to the perfusion fluid caused an initial enhancement of the responses to sympathetic stimulation. This enhancement was short-lived and followed by a gradual reduction of the responses until they were abolished, usually 2-3 hours after the addition of the reserpine. At the time when the responses to stimulation were abolished the responses to intra-arterial angiotensin and noradrenaline were unaltered. The vasoconstrictor response to angiotensin was not increased in these preparations during sympathetic stimulation.

Guanethidine.

Guanethidine (2-6μg/ml.) added to the perfusion fluid completely abolished the responses to sympathetic stimulation and prevented the potentiation of the angiotensin responses during stimulation. The responses to angiotensin in the absence of stimulation were, however, consistently increased in the presence of guanethidine. Fig.10 illustrates an experiment in which guanethidine (4μg/ml.) added to the

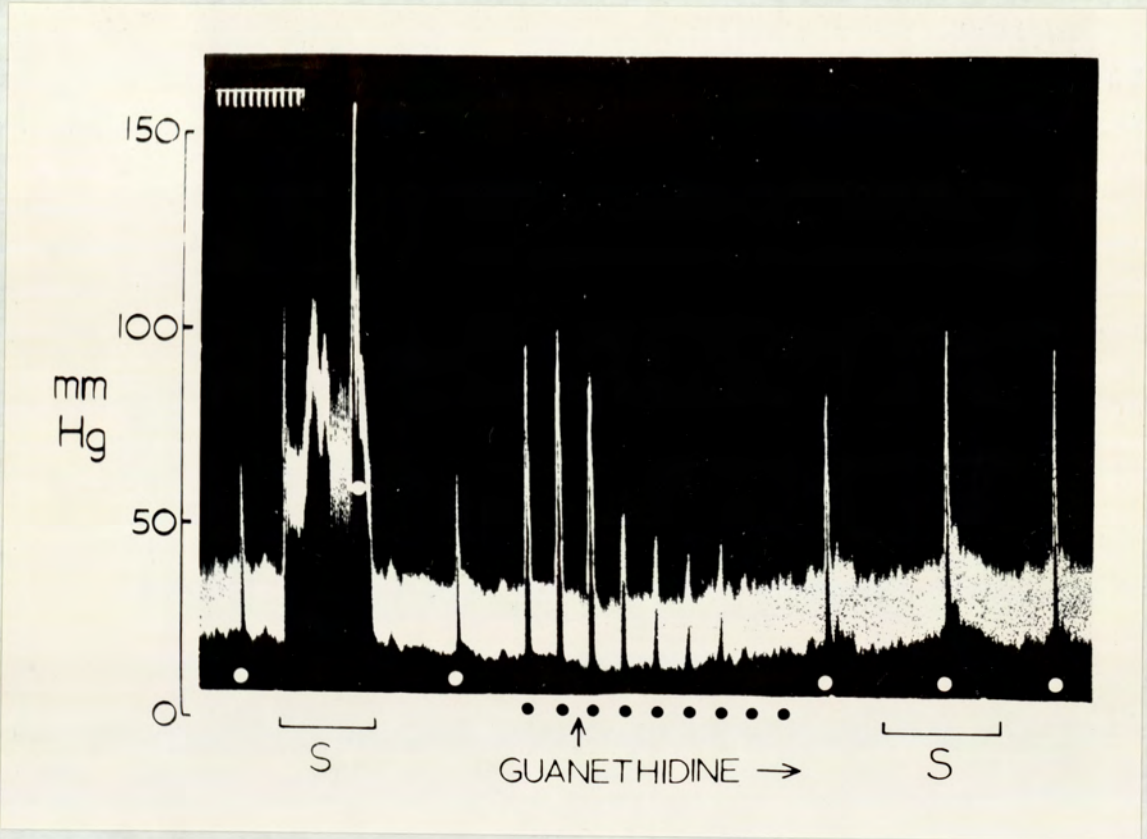

Fig.10 Isolated artery preparation. Angiotensin (250 ng) was injected intra-arterially at the white dots and its constrictor effect was enhanced during continuous sympathetic stimulation with supramaximal strength pulses at 4 pulses per second, at S. At the black dots intermittent sympathetic stimulation was applied for 40 seconds at the same frequency. Guanethidine (4µg/ml.) was added to the perfusion fluid (at the arrow) and abolished the sympathetic constriction and also the enhancement of the angiotensin response during continuous stimulation. Time scale in minutes.

perfusing fluid increased the vasoconstrictor response to angiotensin but prevented any further enhancement during sympathetic stimulation. The potentiation of the angiotensin responses by guanethidine in the absence of sympathetic stimulation occurred in preparations taken from animals pretreated with reserpine and was therefore apparently independent of its blocking action on adrenergic neurons.

Phentolamine.

Addition of phentolamine (50-250ng/ml.) to the perfusing fluid abolished the responses to sympathetic stimulation and to noradrenaline but did not alter those to angiotensin. Phentolamine did however prevent the enhancement of the angiotensin vasoconstriction by sympathetic stimulation.

Cocaine and desipramine.

The results obtained with both cocaine and with desipramine were essentially similar. Addition of cocaine (5µg/ml.) or desipramine (2µg/ml.) to the perfusion fluid caused a small increase in the responses to both sympathetic stimulation and to noradrenaline but did not alter the responses to angiotensin. The potentiation of the vasoconstrictor action of angiotensin during sympathetic stimulation was similar to or slightly less in the presence of cocaine or desipramine than in their absence.

Discussion.

Angiotensin seems to interact with the sympathetic nervous system in several ways. Thus there is evidence which

suggests that at least part of the pressor activity of angiotensin is mediated by the central nervous system (Bickerton & Buckley, 1961). Second, angiotensin increases the responses of tissues to noradrenaline released from peripheral nerves both in whole animals (McCubbin & Page, 1963a; Zimmerman & Gomez, 1965) and in isolated tissues (Benelli et al, 1964; Thoenen et al, 1965). Finally in some vascular beds the vasoconstrictor action of angiotensin is dependent on an intact sympathetic innervation and is greatly reduced by procedures abolishing sympathetic tone (Zimmerman, 1962; Laverty, 1963).

These experiments have confirmed that the vasoconstrictor action of angiotensin in this isolated vascular tissue is weak but could be increased by administration during sympathetic nerve stimulation. Zimmerman (1962) has made a similar observation in the dog's perfused hind-limb preparation; he showed that acute sympathectomy greatly reduced the vasoconstrictor activity of angiotensin but that sensitivity could be partly restored by administering angiotensin during sympathetic stimulation.

The responses to angiotensin were not regularly increased by infusing noradrenaline and were diminished by increasing the perfusion pressure by increasing the perfusion rate. The effect of sympathetic nerve stimulation cannot therefore be attributable to the increased level of perfusion pressure. The angiotensin responses were, however, increased in the presence of amines which liberate noradrenaline suggesting that endogenously released noradrenaline is

necessary for the enhancement of responses to angiotensin. The results using drugs which modify sympathetic activity support this view; abolition of the responses to sympathetic nerve stimulation by complete reserpinization, by guanethidine or by phentolamine prevented the enhancement of the angiotensin responses during sympathetic stimulation.

The mechanism whereby angiotensin responses are increased during release of noradrenaline is not certain. One possibility is that the vascular sensitivity to angiotensin is increased in the presence of endogenous noradrenaline. A more attractive explanation is suggested by Benelli et al (1964) that angiotensin normally releases noradrenaline and owes part of its vasoconstrictor activity to this. The release of noradrenaline by other agents such as sympathetic nerve stimulation may be facilitated by angiotensin. This suggestion is supported by the fact that the response to tyramine, a substance known to produce its effect through release of endogenous noradrenaline, is also potentiated during sympathetic stimulation. There are also other reasons which favour this explanation. First, it accounts for the failure of exogenous noradrenaline to enhance the angiotensin vasoconstriction. Second, in some preparations the response to angiotensin during sympathetic stimulation consisted of two separate peaks; presumably the first was due to angiotensin itself and the second to the noradrenaline released by angiotensin. The enhanced single response seen in other experiments are likely to have been due to the superimposition of the two phases. Third,

phentolamine, which acts by blocking α- receptors, prevented the sympathetic enhancement of the response to angiotensin. The simplest interpretation of these observations is that phentolamine prevented the action of the noradrenaline released by angiotensin.

These results suggest that angiotensin resembles tyramine in causing release of endogenous noradrenaline. Angiotensin (but not tyramine) has also a direct action of its own, however, which is not dependent on intact noradrenaline stores and is therefore not abolished by cocaine or desipramine or by depletion of endogenous noradrenaline stores by reserpine.

It has not proved possible to test this hypothesis directly. de la Lande, Paton & Waud (1968) have tried to measure the quantities of noradrenaline released by this preparation in response to stimulation of the sympathetic nerves. The quantities released were very small and frequently they were unable to detect any noradrenaline in the perfusion fluid after nerve stimulation. The maximum quantities detected were 27 picograms per impulse. An attempt to measure an increased release of noradrenaline by nerve stimulation following injection of angiotensin was not therefore made.

Chapter 3.

Effect of modification of the perfusion fluid on the responses to angiotensin.

Many isolated and denervated vascular preparations are known to be highly insensitive to angiotensin (see for example de la Lande & Rand, 1965; McGregor, 1965; Bohr & Uchida, 1967). The lack of sensitivity of these preparations to angiotensin is partly due to lack of sympathetic tone (Zimmerman, 1962: Laverty, 1963). Activation of the sympathetic nerves supplying the vascular muscle was shown by these workers to increase the vasoconstrictor responses to angiotensin. However, Zimmerman (1962) showed that the vasoconstrictor responses to angiotensin were only partially restored during sympathetic stimulation in the vessels of the dog isolated perfused hind-limb. In the previous chapter it was shown that the angiotensin induced vasoconstriction in the isolated ear artery from the rabbit was increased by stimulation of the sympathetic nerves but it appeared that further factors were necessary to reveal the complete vasoconstrictor action of angiotensin.

This chapter describes some experiments carried out in an attempt to identify the missing factor(s).

Results.

1. Perfusion of the ear artery with rabbit blood.

Blood was taken from the same rabbit as the ear artery preparation. The rabbits were anaesthetised with urethane and as much blood as possible was collected from the right

carotid artery into heparin. When the rabbit yielded no further blood the central artery from one ear was removed and set up as usual but the blood, was used for perfusion.

In these experiments using the usual flow rate the perfusion pressure was far higher than in those experiments where Kreb's bicarbonate solution was used for perfusion. The responses of the artery to angiotensin, noradrenaline, tyramine and sympathetic nerve stimulation were considerably smaller than those seen in ~~ringer~~ Ringer perfused preparations and were completely absent in one experiment.

When the flow rate was reduced so as to lower the perfusion pressure to 30-40 mm Hg the responses to all four vasoconstrictor procedures remained poor. Although the perfusion pressure could be reduced to 30-40 mm Hg by reduction of the flow rate the perfusion pressure rapidly increased until the preparation became very difficult to work with.

2. Addition of horse serum, rabbit plasma or cow plasma to the perfusion fluid. (12 expts.)

In a series of experiments responses to angiotensin, noradrenaline, tyramine and nerve stimulation were recorded using Kreb's bicarbonate solution for perfusion as usual. Horse serum, rabbit plasma or cow plasma were then added to the perfusion fluid to a maximum concentration of 20%. The results obtained with each of these three blood products were essentially identical. In each case there was a small increase in the resting perfusion pressure of the artery but there was no change in the responses of the artery to angiotensin

noradrenaline, tyramine or nerve stimulation. There was also no increase in the degree of enhancement of the responses to angiotensin by sympathetic nerve stimulation.

3. Alteration of some electrolyte constituents of the perfusion fluid.

Blair-West, Harding & McKenzie (1968) have shown that both increasing and decreasing the sodium content of the perfusion fluid reduces the weak vasoconstrictor action of angiotensin in the whole isolated ear of the rabbit.

The effect of changing the concentration of calcium and magnesium in the perfusion fluid has been examined.

(a) Calcium.

Increasing the calcium in the perfusion fluid to double its normal concentration had little or no effect on the responses to angiotensin, noradrenaline or tyramine but caused an increase in the responses to nerve stimulation. Administration of angiotensin during sympathetic stimulation in these experiments caused the usual enhancement of the angiotensin responses.

Decreasing the calcium in the perfusion fluid to one half of the normal level caused a reduction in the responses to angiotensin, noradrenaline, tyramine and sympathetic nerve stimulation.

(b) Magnesium.

Increasing the concentration of magnesium in the perfusion fluid to double its normal level reduced the responses to angiotensin, noradrenaline, tyramine and nerve stimulation.

The complete absence of magnesium from the perfusion fluid caused a slight increase in the responses to all four vasoconstrictor techniques. As the concentration of magnesium was gradually returned to normal the responses to angiotensin, noradrenaline, tyramine and sympathetic nerve stimulation returned to normal.

4. Effect of changing the concentration of glucose in the perfusion fluid.

In the complete absence of glucose the responses of the artery to angiotensin, noradrenaline, tyramine and nerve stimulation were initially normal but showed a rapid decline and if glucose was not subsequently added to the perfusion fluid the responses to all four procedures were abolished.

In concentrations of from one half to eight times normal glucose the responses to all procedures were unchanged but in concentrations greater than eight times normal the responses to all procedures declined.

Discussion.

It was thought possible that the poor vasoconstrictor activity of angiotensin in the isolated artery from the rabbit ear might be due, in part, to the absence of a necessary factor normally present in blood. Perfusion of the artery with blood taken from the same rabbit however did not reveal any factor although this may have been masked by the difficulty of carrying out blood perfusion experiments. The perfusion pressure in these experiments was always high

and although initially it could be reduced by reduction of the perfusion fluid it gradually rose until further reduction of the perfusion fluid was not possible yet the pressure became too great to permit injection into the artery. The gradual increase in the perfusion pressure was possibly caused by the rupture of the blood cells by the perfusion pump as it was necessary to continuously recirculate the blood. The breakdown of the cells probably released 5-hydroxytrytamine and possibly other vasoactive substances which caused the vasoconstriction.

The experiments using blood products without cells to avoid the difficulty of cell breakdown did not reveal any factor necessary for the vasoconstrictor action of angiotensin. Plasma from the blood of the rabbit from which the artery was taken, cow plasma and horse serum caused no increase in the vasoconstrictor response to angiotensin.

Blair-West et al (1968) found that in the isolated whole ear of the rabbit alteration of the sodium content of the perfusion fluid reduced the vasoconstrictor responses to angiotensin. Farmer & Campbell (1967) have examined the effect of alteration of the contents of the perfusion fluid on the responses to noradrenaline, tyramine and nerve stimulation. The present experiments have confirmed the results of these workers and have shown that changing the content of calcium, magnesium, and glucose in the

perfusion fluid had no marked effect on the responses to angiotensin.

The results described in this chapter suggest that the poor vasoconstrictor action of angiotensin in this isolated tissue is not likely to be due to the lack of any factor normally present in rabbit blood but absent in the perfusion fluid nor is it likely to be due to an incorrect electrolyte content of the perfusion fluid.

SECTION 2. A STUDY OF THE INTERACTION BETWEEN ANGIOTENSIN AND THE SYMPATHETIC NERVOUS SYSTEM IN THE PITHED RAT.

Chapter 1.

Effect of infusions of angiotensin on the pressor responses to endogenous and exogenous noradrenaline.

The mechanism of the interaction between angiotensin and the sympathetic nervous system is poorly understood.

McCubbin & Page (1963a,b) reported that in anaesthetised dogs infusions of angiotensin increased the pressor responses to endogenous noradrenaline released by tyramine, by activation of the sympathetic ganglia with autonomic ganglion stimulants and by reflex activation of the sympathetic nervous system by carotid artery occlusion. It was postulated that such an interaction may be implicated in human hypertensive diseases and might account for the "neurogenic component" of renal hypertension.

The site of the interaction of angiotensin with the sympathetic nervous system has not been clearly determined.

It has been well established that angiotensin can cause a systemic pressor response when injected directly into the central nervous system (see for example Bickerton & Buckley, 1961). This systemic pressor response can be abolished by blocking the peripheral sympathetic nervous system with drugs such as reserpine, guanethidine or adrenergic receptor blocking drugs. The possibility that

this central action of angiotensin accounted for the results of McCubbin & Page (1963a,b) was supported when Pals et al (1968) failed to repeat some of McCubbin & Page's observations in the pithed rat preparation.

It is well known that angiotensin can release amines from the adrenal medulla (see for example Feldberg & Lewis, 1964;1965). It would seem possible that the interaction between angiotensin and the sympathetic nerves could take place at the adrenal medulla.

The evidence from isolated tissues is confusing. Angiotensin enhances the responses to sympathetic nerve stimulation in a number of sympathetically innervated tissues (for example Benelli et al, 1964; Su, 1965) which suggests that the action of angiotensin must be at the sympathetic nerve endings. However, other workers have reported that angiotensin did not enhance responses to sympathetic stimulation and tyramine in isolated tissues (Day & Owen, 1968b; Hughes, 1968b).

In the present experiments the pithed rat preparation, as described by Gillespie & Muir (1967), has been used to try to determine the site and mechanism of the action of angiotensin on the sympathetic nervous system. This preparation has been used as it enables the interaction between angiotensin and the sympathetic nervous system to be studied on the intact cardiovascular system free from the influence of possible effects on the central nervous system.

Results.

Pressor response to intravenous infusion of angiotensin.

Infusions of angiotensin at dose levels exceeding 25ng/kg/min caused a sustained and dose-dependent increase in the blood pressure. The maximal sustained increase in the pressure was usually achieved with a dose level of about 200ng/kg/min whilst doses of less than 25ng/kg/min were without significant pressor effect. In about half of the animals infused with angiotensin at the 200ng/kg/min level the blood pressure returned rapidly to the pre-infusion level on discontinuing the infusion whilst in the remainder the pressure fell below the control level and did not subsequently recover

Effect of angiotensin infusions on the responses to endogenously released noradrenaline. (20 expts.)

Sympathetic stimulation. Electrical stimulation of the spinal sympathetic outflow for 40 seconds in every 4 minutes over a wide range of frequencies (0.1 to 4 pulses per second) caused reproducible pressor responses for periods up to several hours. Infusions of angiotensin markedly enhanced the responses, the effect being most marked at the lower stimulus frequencies. The potentiation of the responses occurred soon after the start of the infusion and the maximal effect at a particular dose level was usually produced after about 10-20 minutes. Occasionally, some enhancement was noticed with infusions of angiotensin (10ng/kg/min) too small to increase the blood pressure but the most marked effect was obtained with doses of the

order of 200ng/kg/min which produced the highest sustained pressor effect. This effect is illustrated in Fig.11. In panel A are shown the control responses to sympathetic stimulation at three stimulus frequencies. These responses were not altered (panel B) during the continuous infusion of normal saline (0.05ml/min.) but were considerably increased (panel C) during the infusion of angiotensin 200ng/kg/min. dissolved in the same volume of saline. In panel D the responses are shown 30 minutes after discontinuing the angiotensin infusion at which time the response to the lowest stimulus frequency was still enhanced whilst the responses to the other two frequencies had returned to control values. The persistence of the angiotensin induced potentiation of the sympathetic responses at the lower frequencies of stimulation occurred in a majority of the experiments in which the blood pressure returned to the control level after the angiotensin infusions. In those experiments in which the blood pressure fell below control levels after angiotensin the sympathetic responses were also reduced below the control values.

Indirectly-acting sympathomimetic amines The pressor responses to injections of tyramine or dexamphetamine were potentiated during infusions of angiotensin. The onset of the potentiation was rather slower than that to sympathetic stimulation but the extent of the enhancement was often greater. The responses to tyramine slowly declined over 2-3 hours after discontinuing the angiotensin infusion.

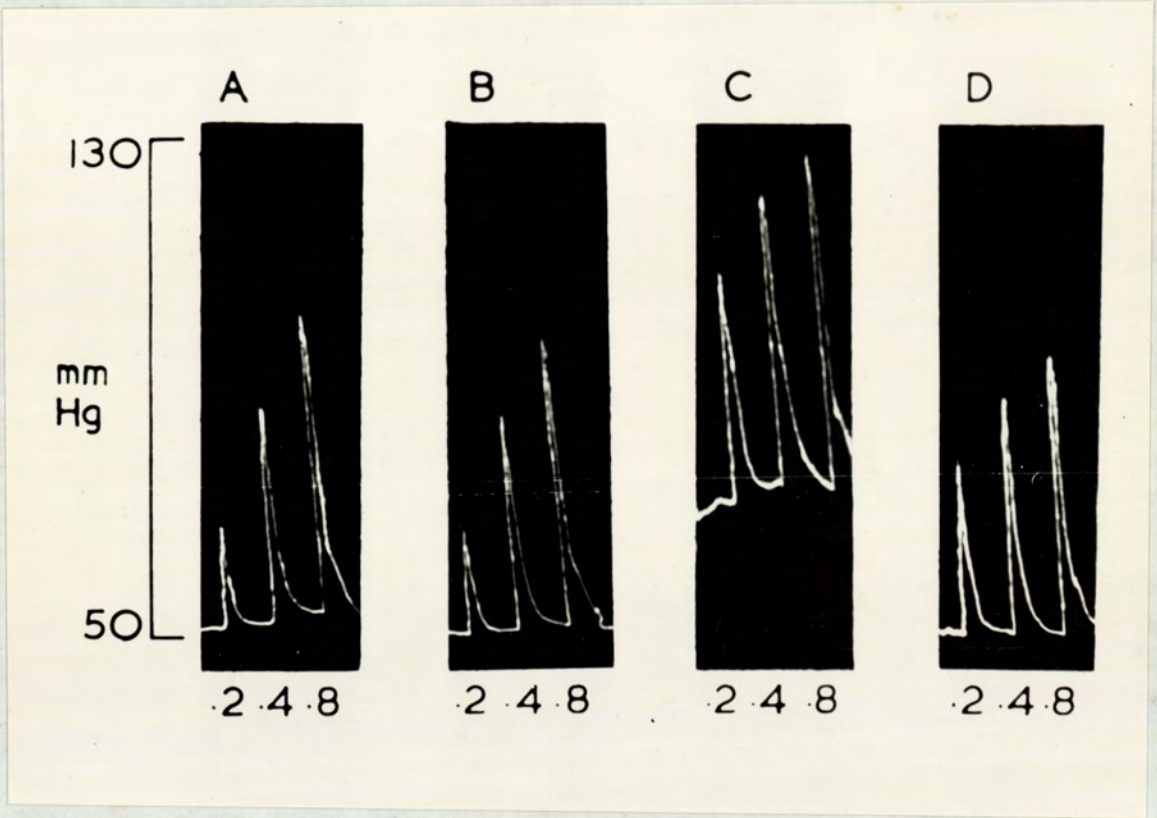

Fig.11 Pithed rat blood pressure preparation. Electrical stimulation of the spinal sympathetic outflow at three stimulus frequencies (0.2, 0.4 and 0.8 pulses per second) for 40 second periods. In A, control responses at the start of the experiment repeated in B 60 minutes later during the intravenous infusion of saline (0.05 ml./minute). In C the responses were repeated during the intravenous infusion of angiotensin (200ng/kg/minute) and in D control responses 30 minutes after discontinuing the angiotensin infusion.

This effect is shown in Fig.12 where the responses to successive injections of tyramine were progressively increased during an angiotensin infusion and were maintained at the elevated level after the infusion.

Autonomic ganglion stimulation Injections of tetramethylammonium (TMA) caused a biphasic pressor response consisting of a primary sharp peak followed by a secondary rise which was usually less pronounced in height but more prolonged than the primary rise (see Fig.13). It appeared likely that the primary peak was due to sympathetic ganglion stimulation whilst the secondary component was due to release of amines from the adrenal medulla since this component was absent in adrenalectomised rats. Angiotensin infusions increased both components of the TMA pressor response although the effect was usually more marked on the adrenal component which became more pronounced than the primary response. Fig.13 illustrates an experiment in which the pressor responses to TMA were markedly enhanced during an infusion of angiotensin at two dose levels. After the infusion the blood pressure fell below the control level and the responses to TMA were initially depressed, but later recovered and were still much larger than the control responses at the end of the experiment. Unlike the potentiation of the responses to sympathetic stimulation caused by angiotensin which was maximal at infusion rates of the order of 200ng/kg/min the responses to TMA continue to increase as the infusion of angiotensin was increased up to an infusion rate of about 1µg/kg/min. See Fig.13

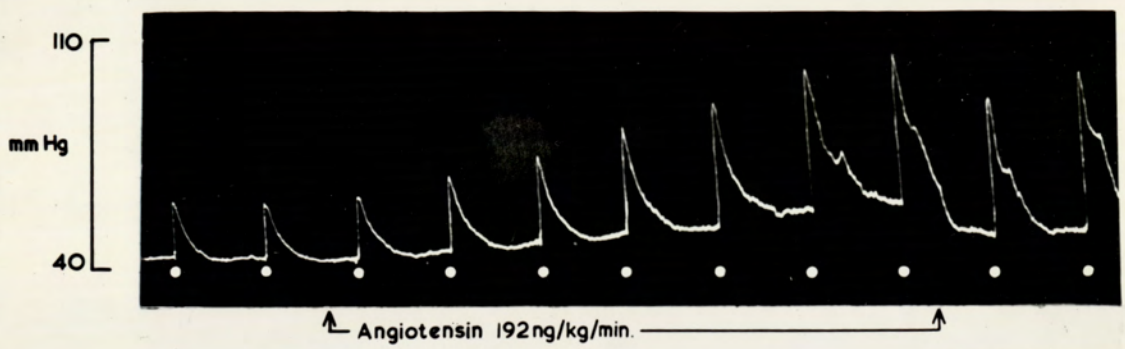

Fig.12 Pithed rat blood pressure preparation. Responses to 20μg intravenous doses of tyramine (at white dots). Between the arrows angiotensin (192ng/kg/minute) was infused intravenously. Injections at 8 minute intervals.

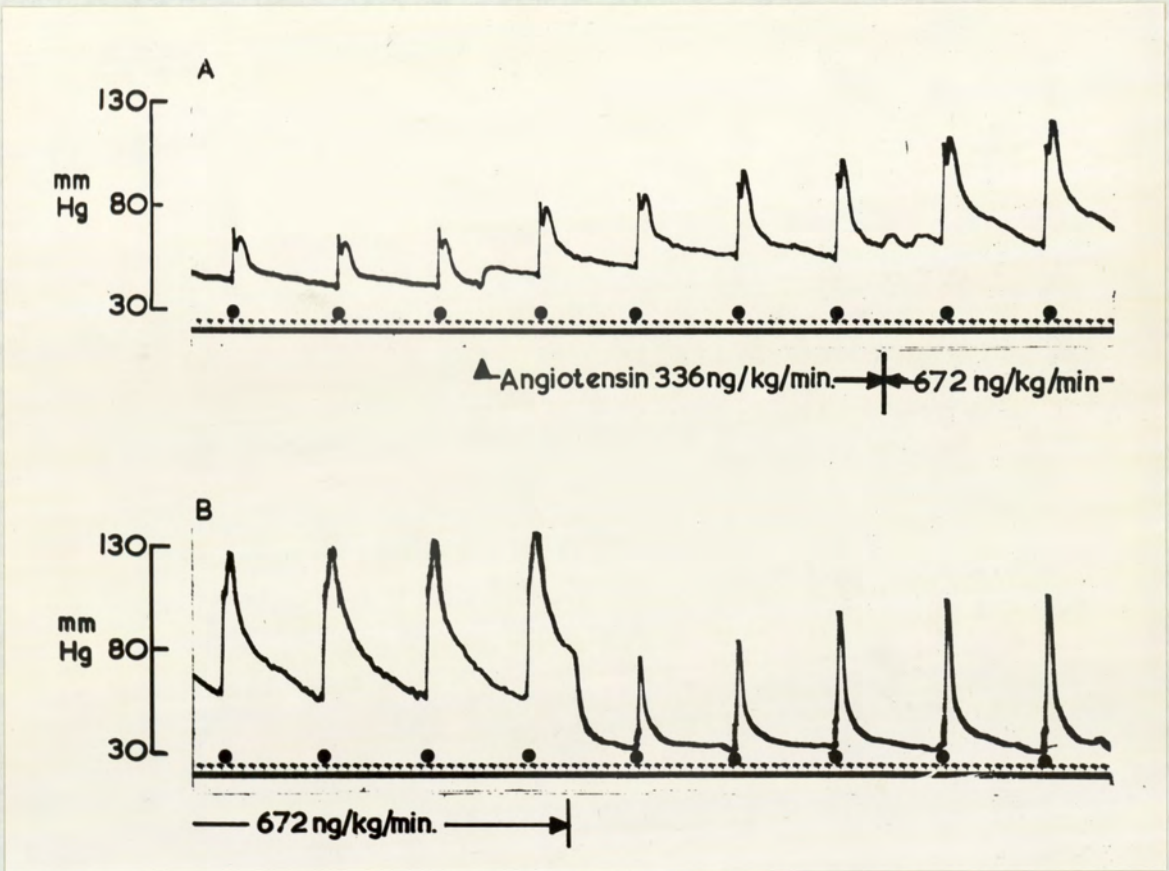

Fig.13 Pithed rat blood pressure. Responses to 15μg doses of TMA (at dots) before, during and after intravenous infusion of angiotensin at two dose levels. A and B are consecutive traces. Time scale in minutes.

Angiotensin infusion	Responses mm Hg	Mean	S.E.	P
0 ng/kg/minute	24, 25, 27	25.3	± 1.0	
336 ng/kg/minute	36, 36, 43, 50	41.25	± 3.4	< 0.05
672 ng/kg/minute	57,61,71.5,71.5 75,79	69.2	± 3.5	< 0.05

Effect of angiotensin infusions on responses to exogenous noradrenaline.

The pressor responses to injections of noradrenaline were usually unaltered by infusions of angiotensin at dose levels which markedly increased the responses to sympathetic stimulation. In some experiments there was a slight increase or a slight decrease in the responses to injected noradrenaline. Fig.14 illustrates an experiment in which an infusion of angiotensin caused a potentiation of the responses to sympathetic stimulation but caused a slight reduction in the responses to injected noradrenaline.

Mechanism of the angiotensin potentiation of the responses to procedures releasing neuronal noradrenaline.

An attempt was made to elucidate the mechanism by which angiotensin increased the responses to endogenous noradrenaline by comparing its effects with those of three other substances.

(a) Noradrenaline. Noradrenaline infusions have been shown to increase the vasoconstrictor effects of both sympathetic stimulation and tyramine in the cat (Burn & Rand, 1960). For this reason the effect of angiotensin and noradrenaline infusions have been compared in the pithed rat preparation.

In these experiments noradrenaline was found to be about half as potent as angiotensin in causing a sustained increase in the blood pressure. However, in equipressor amounts noradrenaline was without effect on the responses to sympathetic stimulation in a majority of experiments and caused only a small potentiation of the responses in

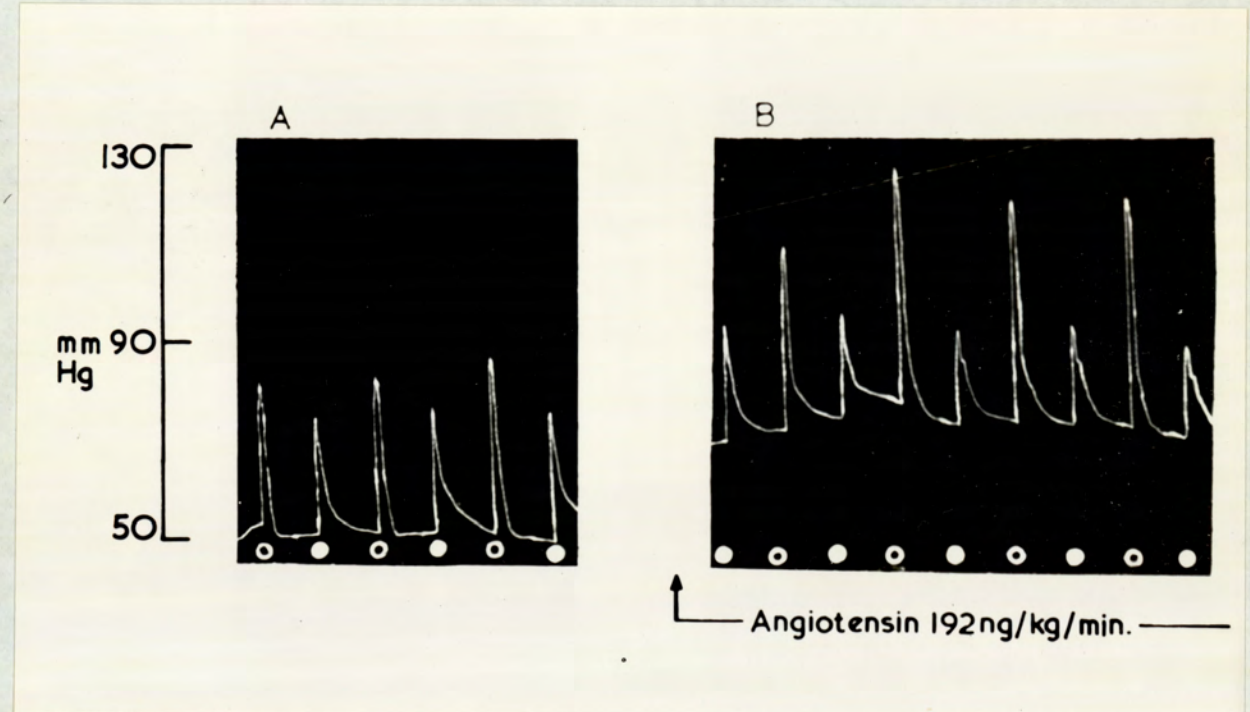

Fig.14 Pithed rat blood pressure preparation. In A, alternate sympathetic stimulations at 0.5 pulses per second for 40 second periods (open dots) and intravenous noradrenaline in 20 ng doses (closed dots). In B, the sequence was repeated during the intravenous infusion of angiotensin (192ng/kg/minute).

the remainder. The responses to tyramine were regularly increased by infusions of noradrenaline.

In rats pretreated 30-60 minutes before the experiment with desipramine (5mg/kg intraperitoneally) the pressor action of tyramine was reduced or abolished whilst the responses to sympathetic stimulation and noradrenaline were increased. Infusion of noradrenaline (400ng/kg/min) into these animals produced no enhancement of the responses to sympathetic stimulation.

In other experiments using desipramine pretreated rats infusions of angiotensin caused a further large enhancement of the sympathetic responses, a slight recovery of the tyramine responses, and did not affect the noradrenaline responses.

(b) Desipramine. This drug is known to reduce the uptake of noradrenaline by sympathetic nerves and this results in an increase in the responses to sympathetic stimulation and to noradrenaline (Axelrod, Whitby & Hertting, 1961; Sigg, Soffer & Gyermek, 1963). Recent evidence suggests that angiotensin may increase sympathetic responses by a similar mechanism (Palaic & Khairallah, 1967a,b). In the present experiments it was confirmed that desipramine (5mg/kg) markedly increased the responses to sympathetic stimulation. In contrast to the action of angiotensin however, desipramine reduced or abolished the responses to tyramine and enhanced the responses to noradrenaline.

(c) Tyramine. This substance is known to increase the release of noradrenaline from sympathetic nerve endings (Burn & Rand, 1958) and a similar action has been proposed for angiotensin to account for its action of increasing the responses to endogenous but not exogenous noradrenaline (McCubbin & Page, 1963a: Benelli et al, 1964: Day & Owen, 1968b).

In these experiments infusions of tyramine in pressor amounts (16-128μg/kg/min) markedly reduced the responses to both noradrenaline and sympathetic stimulation during the infusion period. However, after the infusion was discontinued both responses increased until they were usually larger than the control responses and remained enhanced for up to three hours thereafter. Fig.15 illustrates the effect of infusion increasing amounts of tyramine on the responses to sympathetic stimulation. The pressor response to infusions of tyramine and the post-infusion enhancement of the responses to noradrenaline and sympathetic stimulation were absent in rats pretreated with desipramine.

Investigation of the heart and adrenal medulla as possible sites for the interaction between angiotensin and the sympathetic nervous system.

Effect of adrenalectomy. In bilaterally adrenalectomised rats the basal blood pressure was lower than in control rats and the sensitivity to infusions of angiotensin was decreased. An infusion of about 500ng/kg/min of angiotensin

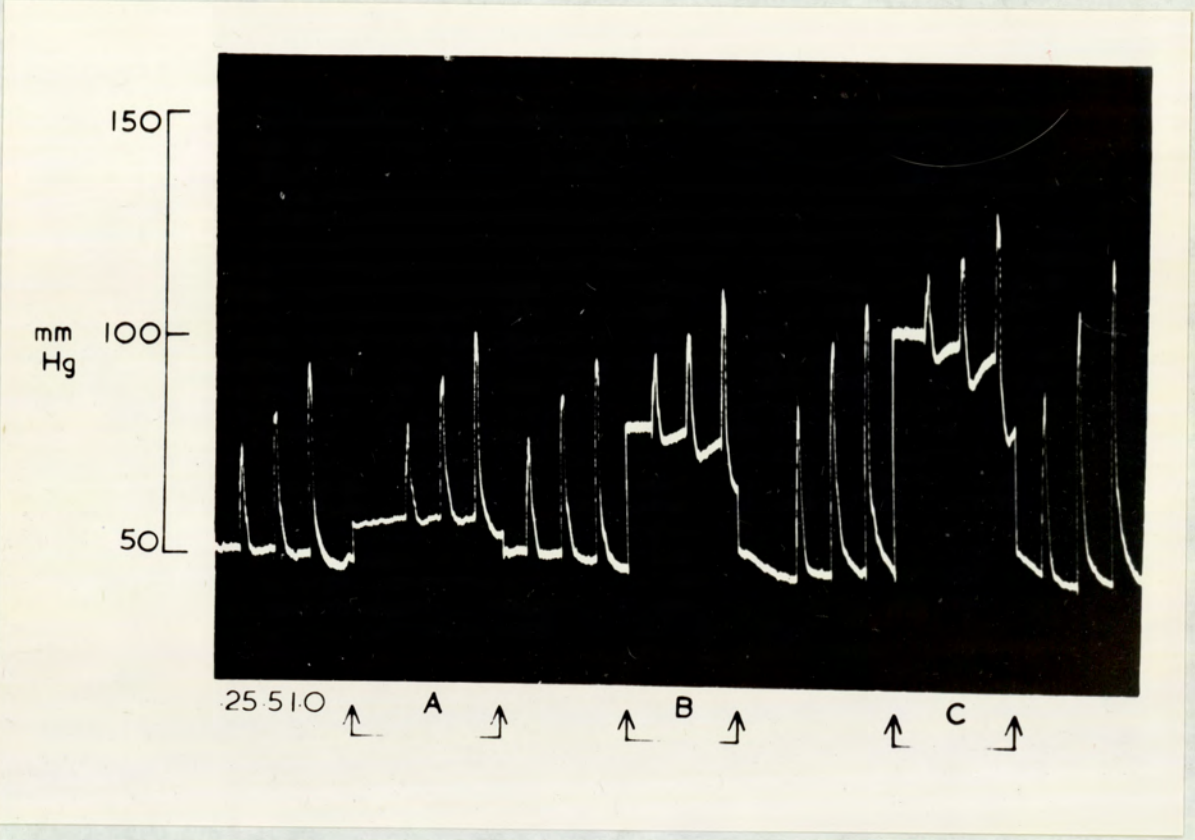

Fig.15 Pithed rat blood pressure preparation. Responses to stimulation of the spinal sympathetic outflow at three stimulus frequencies (0.25, 0.5 and 1 pulses per second). During A, B and C tyramine was infused intravenously at 16, 32 and 64μg/kg/minute respectively. Responses are also shown after discontinuing the infusion at each of the infusion rates.

was usually required to produce a maximal sustained pressor in these rats and the blood pressure invariably fell below the control level after discontinuing the infusion.

Adrenalectomised rats were sometimes slightly less responsive to sympathetic stimulation than control animals. Angiotensin infused in pressor quantities potentiated the responses to sympathetic stimulation but to a more variable and usually smaller extent than in control rats.

The pressor response to TMA in adrenalectomised rats consisted of a single sharp peak which was abolished by guanethidine (2.5mg/kg intravenously). Infusions of angiotensin in adrenalectomised rats caused some increase in the responses to TMA although this was never as great as the potentiation seen with control rats. This is shown in Fig.15a. In none of the experiments using adrenalectomised animals did the enhancement of the responses to either sympathetic stimulation or TMA persist after the angiotensin infusion.

Effect of propranolol. Although it seemed unlikely that the potentiation of the responses to endogenous noradrenaline in the presence of angiotensin was due solely to increasing cardiac output it was considered necessary to check this using the β-adrenergic receptor blocking drug propranolol. Stimulation of the spinal sympathetic outflow caused an increase in the heart rate of up to 50 pulses per minute which was prevented by pretreatment with propranolol (1mg/kg intravenously). However, the pressor responses to sympathetic stimulation were slightly increased rather than decreased after propranolol and the responses were further enhanced during an angiotensin infusion as in untreated rats.

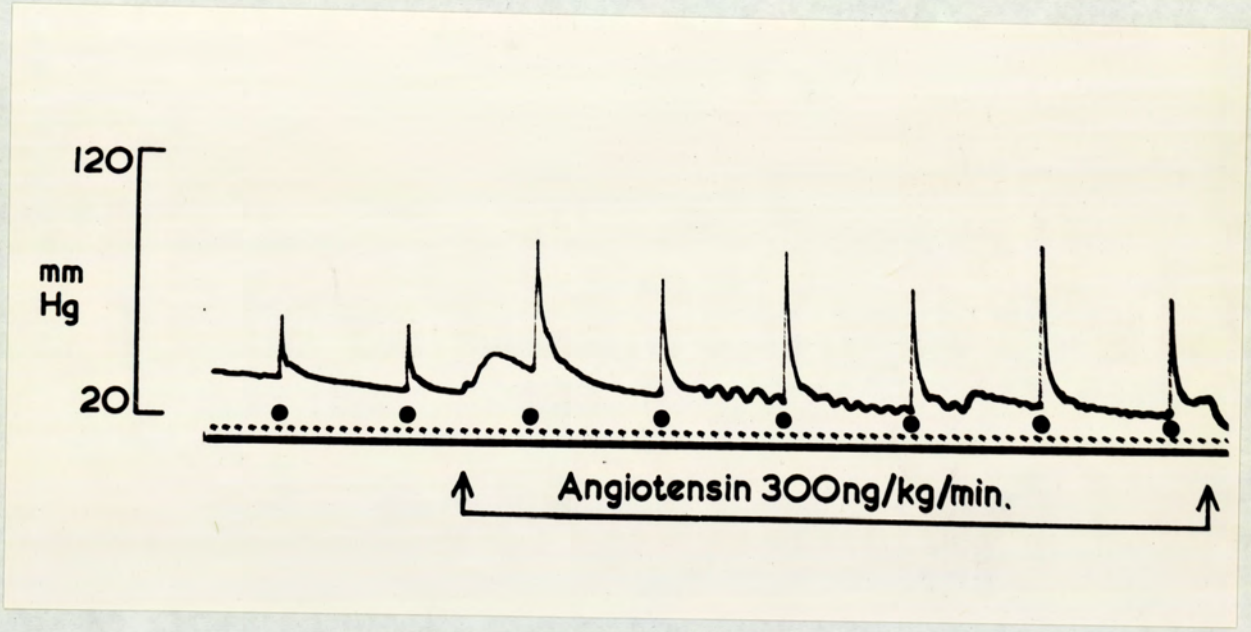

Fig.15a Pithed rat blood pressure preparation. Single sharp pressor responses to TMA (15μg) in an adrenalectomised, pithed rat enhanced during an intravenous infusion of angiotensin. Time scale in minutes.

Discussion.

McCubbin & Page (1963a,b) reported that infusions of angiotensin increased the pressor responses to procedures causing noradrenaline release from its neuronal stores. Pals et al (1968), however, found that angiotensin had no such effect in pithed rats. The differing results obtained by these two groups of workers could be accounted for by species variation or by the presence of an intact central nervous system in the experiments of McCubbin & Page. The latter possibility is perhaps the more likely in view of the centrally mediated pressor effect of angiotensin first reported by Bickerton & Buckley (1961).

However, in the present experiments it was shown that neither possibility is correct since angiotensin increased the responses to all procedures causing release of neuronal noradrenaline in the pithed rat. The major difference between the present experiments and those of Pals et al (1968) using tyramine and autonomic ganglion stimulants is that these workers infused angiotensin for only 15 minutes whereas in the present experiments longer infusion periods were usually necessary to show a marked effect with angiotensin.

Angiotensin releases catecholamines from the adrenal medulla (see for example Feldberg & Lewis, 1964; 1965). Experiments using adrenalectomised rats indicated that the potentiation of the responses to endogenous noradrenaline by angiotensin in normal rats included an adrenal component..

The adrenal contribution to the responses to TMA was considerable but smaller in the case of the responses to

sympathetic stimulation. The main interaction between angiotensin and the sympathetic nerves would seem to be independent of the adrenal medulla occuring probably at the sympathetic nerve endings.

The mechanism of the action whereby angiotensin increases the responses to endogenous noradrenaline released from the sympathetic nerve endings but does not increase the responses to injected noradrenaline has been frequently discussed. It is unlikely that angiotensin increases the sensitivity of the cardiovascular system to the sympathetic transmitter as the responses to injected noradrenaline are not increased by angiotensin. The same evidence would also exclude the possibility of angiotensin inhibiting the action of the enzymes concerned with the degradation of noradrenaline. It has been suggested that angiotensin may inhibit the uptake of noradrenaline into sympathetic nerve terminals (Palaic & Khairallah, 1967a,b). Such a mechanism is consistent with the observation that sympathetic nerve responses were increased but is inconsistent with the potentiation of the responses to tyramine and the lack of potentiation of the responses to injected noradrenaline. The action of desipramine, a potent inhibitor of noradrenaline uptake (Axelrod et al, 1961; Sigg et al, 1963) also argues against this mechanism to account for the action of angiotensin in these experiments.

Desipramine caused a similar enhancement of the responses to both sympathetic stimulation and to noradrenaline but reduced or abolished the responses to tyramine. Pals &

Masucci (1968) and Pals et al (1968) found that angiotensin did not prevent noradrenaline uptake into the sympathetic nerve terminals in the pithed rat.

It would appear that the most likely explanation of these results is that angiotensin in some way increases the release of noradrenaline from its storage sites in response to procedures such as nerve stimulation and tyramine. The direct evidence at present available on this hypothesis is contradictory. Although angiotensin increases the contraction of the spleen following splenic nerve stimulation this is not associated with an increased release of noradrenaline (Thoenen et al, 1965; Hertting & Suko, 1966). The enhancement of the sympathetic vasoconstriction in the dog hind-limb (Zimmerman & Whitmore, 1967) and the dog renal artery (Zimmerman & Gisslen, 1968) however, is associated with a small increase in the release of transmitter. The difference between the experiments of Zimmerman and his co-workers and Thoenen et al (1965) and Hertting & Suko (1966) might be due to the different rates of stimulation of the sympathetic nerves. In the vascular preparations studied by Zimmerman and co-workers frequencies of 2-10 pulses per second were employed whereas higher frequencies of stimulation were necessary to contract the splenic muscle. Zimmerman & Gisslen (1968) found that although angiotensin enhanced sympathetically mediated vasoconstriction in the dog renal artery at all frequencies tested this was only associated with an increased release of noradrenaline at 5 pulses per second and not at 2 or 10 pulses per second. In the experiments reported by

Thoenen et al (1965) and Hertting & Suko (1966) the splenic nerves were stimulates at frequencies in excess of 5 pulses per second.

Chapter 2.

Effect of infusions of noradrenaline on the pressor responses to angiotensin and tyramine in the pithed rat.

The pressor action of angiotensin in the pithed rat has been shown to be independent of noradrenaline release from the adrenal medulla or from sympathetic nerve endings (Schmitt & Schmitt, 1968a). Despite this infusions of noradrenaline have been shown to potentiate the pressor responses to angiotensin in the pithed rats (Schmitt & Schmitt, 1967b; Pals & Fulton, 1968). The nature of the potentiation is not understood. This chapter describes some experiments carried out in order to study this interaction between angiotensin and noradrenaline.

Results.

Effect of infusions of noradrenaline on the responses to angiotensin and tyramine.

Infusions of noradrenaline in excess of about 50ng/kg/min caused a sustained pressor response. During such infusions the pressor responses to injections of both angiotensin and tyramine were increased. Fig.16 illustrates an experiment in which the responses to angiotensin and tyramine, each at two dose levels, were enhanced during an infusion of noradrenaline. On completion of the infusion of noradrenaline the responses to both angiotensin and tyramine declined to their pre-infusion size or in some experiments to less than this.

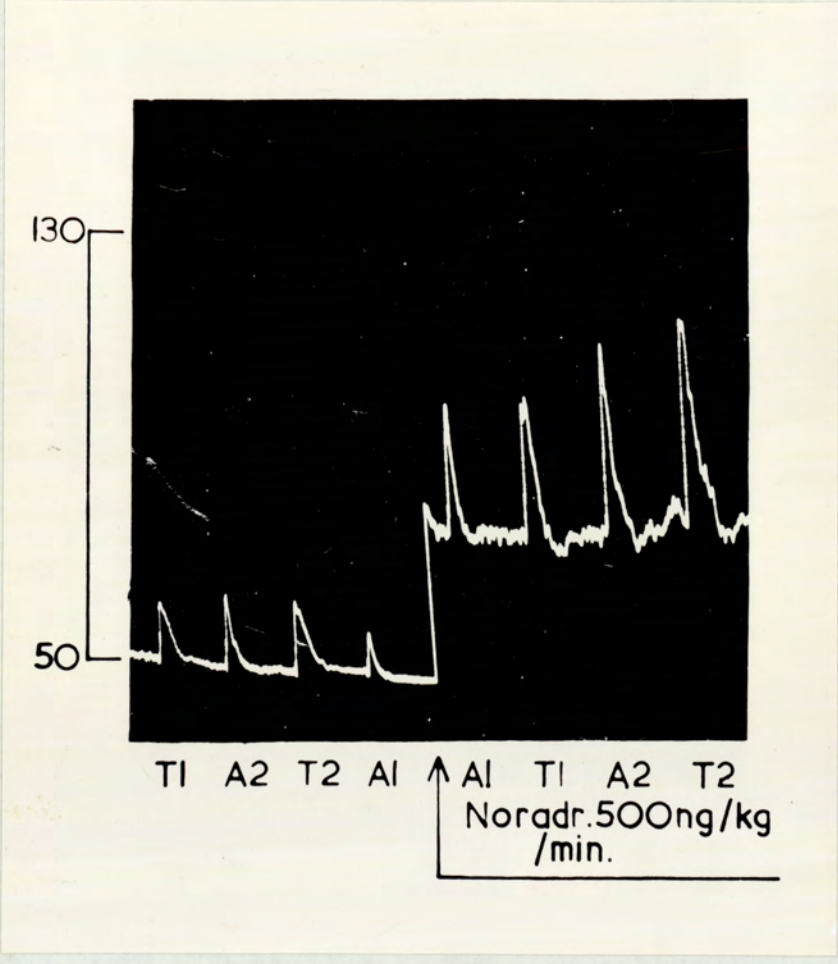

Fig.16 Pithed rat blood pressure preparation. Responses to angiotensin at two dose levels, 2 ng at A1 and 4 ng at A2, and to tyramine at two dose levels, 10µg at T1 and 20 µg at T2 before and during an intravenous infusion of noradrenaline (500 ng/kg/minute). During the infusion of noradrenaline the pressor responses to angiotensin and to tyramine were enhanced.

The extent of the enhancement of the responses to both angiotensin and tyramine was time-dependent. Fig.17 shows the gradual increase of the responses to angiotensin during an infusion of noradrenaline The responses increased steadily with time and the maximal enhancement was usually seen after about 2 hours.

Effect of phentolamine on the responses to angiotensin during infusions of noradrenaline.

In some experiments where infusions of noradrenaline caused an increase in the responses to angiotensin and tyramine administration of phentolamine during the infusion reduced the responses to both angiotensin and tyramine. This is illustrated by Fig.18. The responses to angiotensin and tyramine were increased during the infusion of noradrenaline but 30 minutes after phentolamine (0.25mg/kg intravenously) the pressor response to the noradrenaline infusion was reduced as were the responses to injections of angiotensin and tyramine. After a further 30 minutes the resting blood pressure had returned to its pre-infusion level despite the continuation of the noradrenaline infusion and the responses to angiotensin and tyramine were smaller than their respective controls. The responses to angiotensin showed no further decline over the next 30 minutes whereas the responses to tyramine were abolished.

Pretreatment with phentolamine (0.5mg/kg intraperitoneally) 30 minutes before pithing prevented the pressor response to both infusions of noradrenaline and injections of tyramine

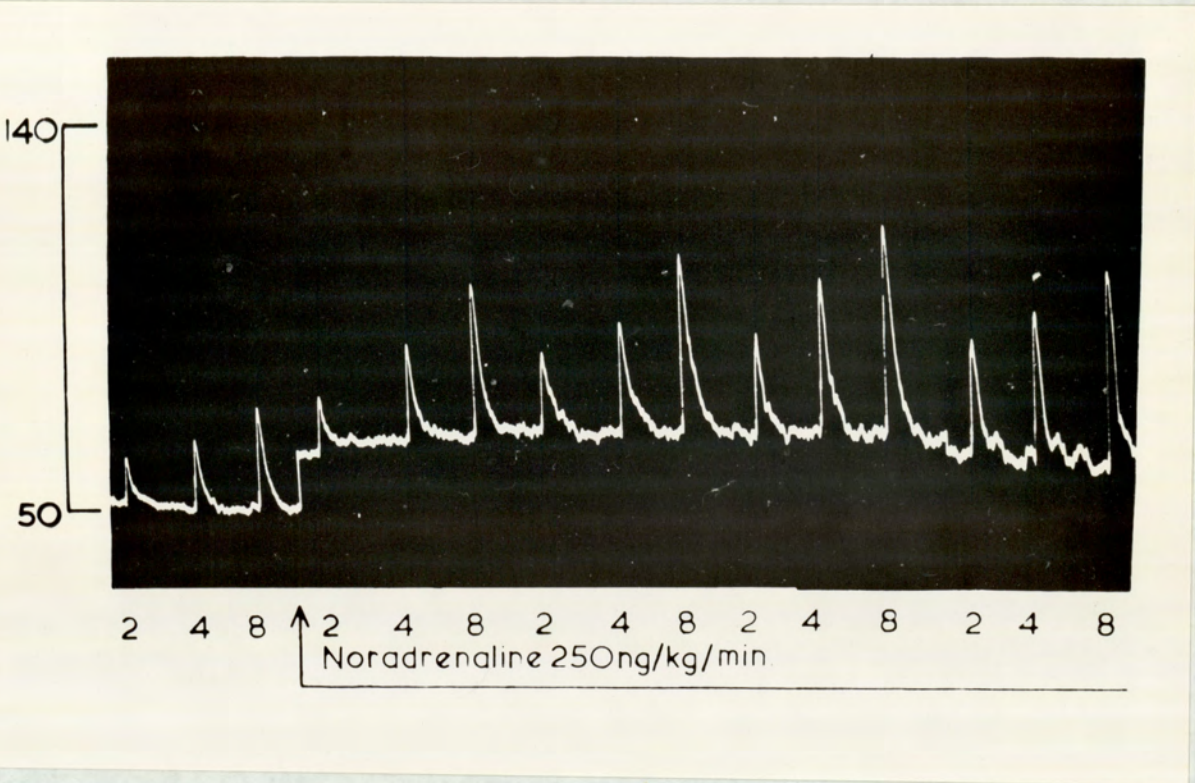

Fig.17 Pithed rat blood pressure preparation. Responses to angiotensin 2, 4 and 8 ng before an intravenous infusion of noradrenaline. During the infusion of noradrenaline the responses to all three dose levels of angiotensin were increased. The kymograph was stopped for intervals of 20 minutes between each consecutive group of three responses.

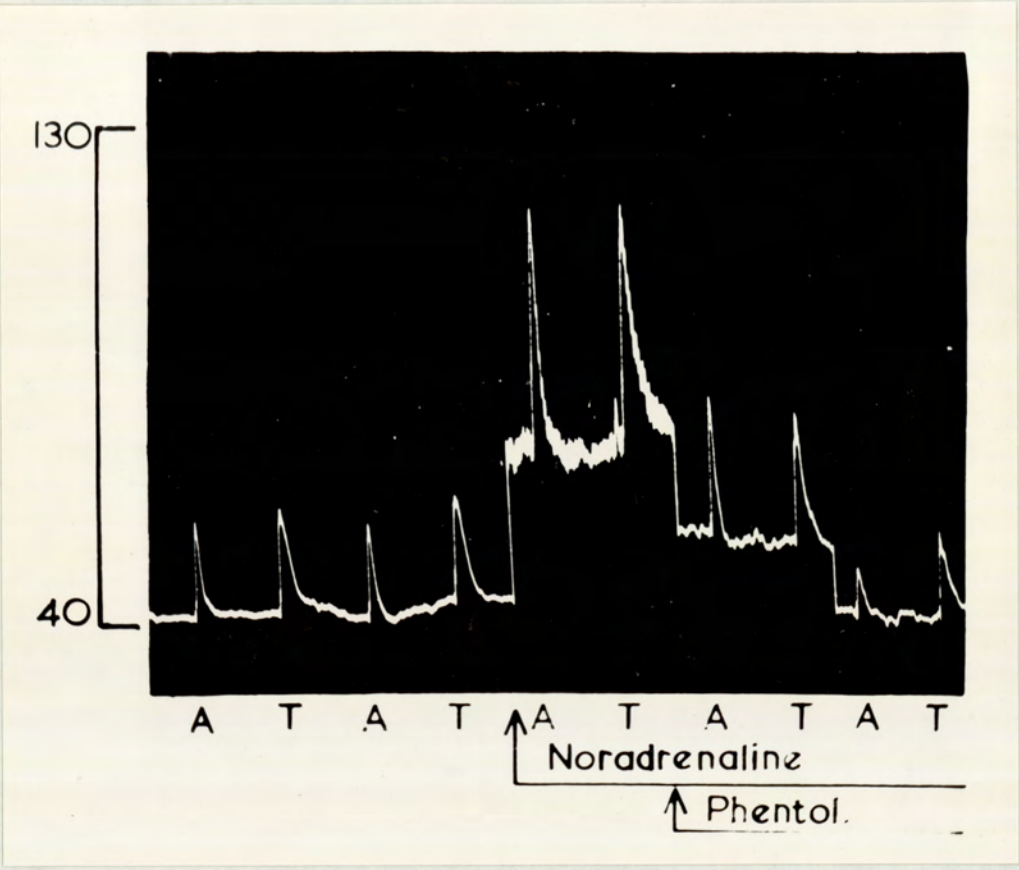

Fig.18 Pithed rat blood pressure preparation. Responses to angiotensin (5 ng) at A and to tyramine (15µg) at T. The responses to both pressor agents were enhanced during an infusion of noradrenaline (500ng/kg/minute) but this enhancement was reversed after phentolamine (0.25 mg/kg intravenously) despite the continuation of the noradrenaline infusion. The kymograph was stopped for periods of 30 minutes between each group of two responses.

but did not alter the control responses to angiotensin. However, the enhancement of the angiotensin responses during the infusion of noradrenaline was prevented.

Effect of desipramine on the responses to angiotensin during infusions of noradrenaline.

Pretreatment with desipramine 5mg/kg intraperitoneally 90 minutes before pithing increased the pressor response to infusions of noradrenaline and abolished the responses to tyramine. This pretreatment caused a slight increase in the control responses to angiotensin and during infusions of noradrenaline the responses to angiotensin were enhanced as usual.

Discussion.

Interactions between angiotensin and the sympathetic nervous system have been frequently reported in many preparations including the pithed rat (Day & Owen, 1969). Reports of interactions between angiotensin and exogenous noradrenaline are however, less widely recorded. Schmitt & Schmitt (1967b) and Pals & Fulton (1968) have shown that the responses to angiotensin in the pithed rat are increased during infusions of noradrenaline and this observation has been confirmed in the present experiments. However, the extent of the enhancement observed in these experiments was greater than that reported by the previous groups of workers. The enhancement was found to increase with time and in these experiments the infusions were continued for

longer periods than in the experiments of Schmitt & Schmitt (1967b) and Pals & Fulton (1968); the difference is therefore probably due to the time factor alone.

Schmitt & Schmitt (1967b) made no attempt to explain their findings but Pals & Fulton (1968) thought that the potentiation might be due to synergism between angiotensin and noradrenaline at the adrenergic α- receptors. The results obtained with phentolamine in these experiments support this hypothesis.

Although the idea of synergism between angiotensin and noradrenaline explains the results reported in this chapter it fails to explain the results of the previous chapter in which it was shown that angiotensin enhanced the pressor responses to endogenous noradrenaline but did not alter the responses to exogenous noradrenaline. If the interaction between angiotensin and the sympathetic nervous system was due simply to synergism between angiotensin and noradrenaline at the adrenergic receptors then potentiation of the responses to both endogenous and exogenous noradrenaline would be expected.

The responses to tyramine showed a parallel enhancement with the angiotensin responses. Tyramine is known to exert its pressor action by releasing noradrenaline from neuronal storage sites (see for example Burn & Rand, 1958). It would seem possible that the increased responses to tyramine are due to increasing the noradrenaline content of the sympathetic

neurones. This hypothesis is supported by the gradual development of the enhancement of the tyramine responses although the rapid decline of the responses on completion of the infusion is suprising. If the responses to tyramine were increased by increasing the level of noradrenaline in the neuronal stores it is possible that this mechanism could also account for the enhancement of the responses to angiotensin. The results obtained with desipramine, however, argue against this hypothesis. Pretreatment with desipramine might be expected to prevent the accumulation of noradrenaline in the sympathetic neurons and consequently prevent the potentiation of angiotensin responses during infusions of noradrenaline. Desipramine did not, however, interfere with the potentiation of angiotensin responses during noradrenaline infusions.

SECTION 3. A STUDY OF THE ACTION OF SOME DRUGS ON THE CARDIOVASCULAR SYSTEM OF CONSCIOUS, UNRESTRAINED CATS.

Chapter 1.

A preliminary investigation into the reproducibility of pressor responses.

In order to study the action of angiotensin on the cardiovascular system of conscious cats, thereby eliminating the possibility of artefacts due to anaesthesia, a technique for the continuous, direct measurement of aortic blood pressure in conscious, unrestrained cats was developed.

Although the main aim of the technique was to avoid the complications imposed by anaesthesia it seemed probable that the method might have other advantages and some disadvantages over cardiovascular studies in anaesthetised animals. This chapter describes the preliminary experiments carried out which were designed to determine the value of conscious cats for cardiovascular studies and also to determine the limitations imposed by the technique.

Results.

Reproducibility of pressor responses to intravenous injections of a variety of pressor substances.

1. Acute experiments. The responses to intravenous injection of a variety of pressor substances were similarly reproducible as those from experiments in anaesthetised animals. Fig.19

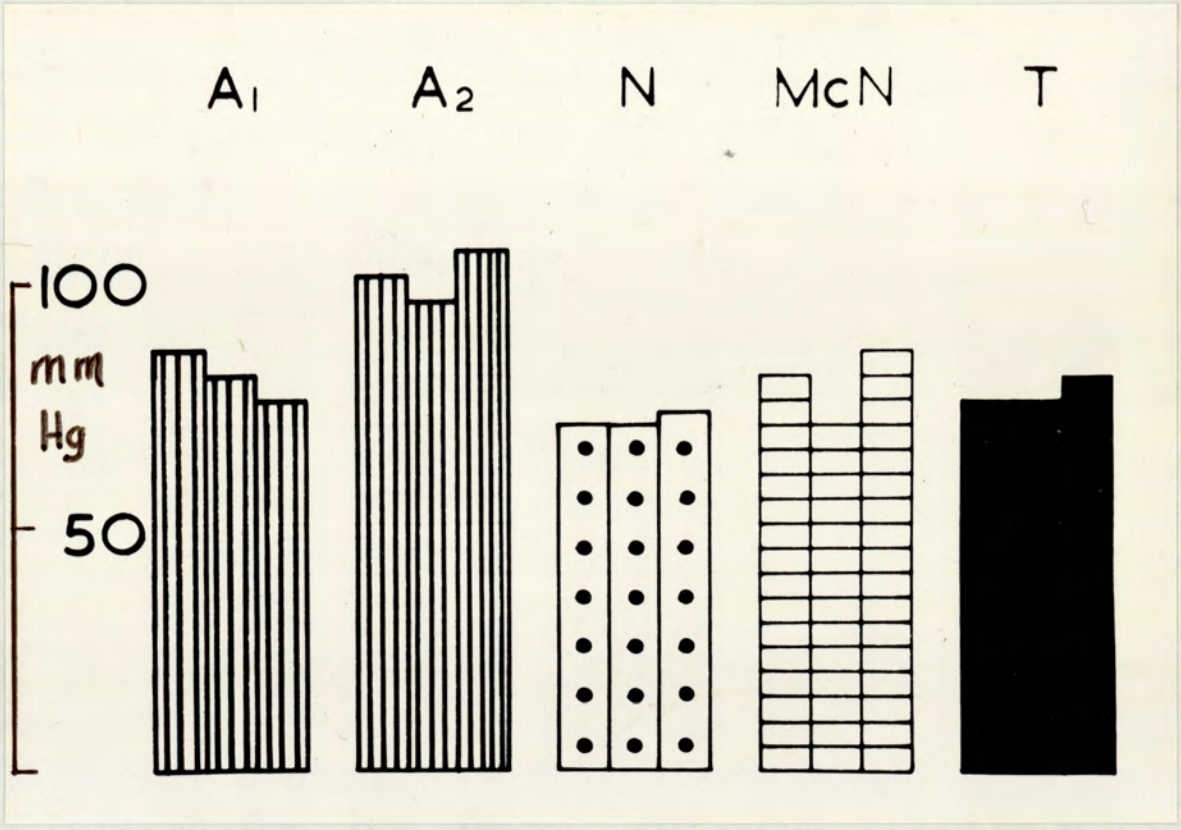

Fig.19 Conscious cat blood pressure. Histograms showing the reproducibility of consecutive responses to pressor agents in conscious cats. Each histogram represents the response to each of three consecutive responses to angiotensin (50ng/kg) at A_1, angiotensin (100ng/kg) at A_2, noradrenaline (200ng/kg) at N, McN-A-343 (15µg/kg) at McN, and tyramine (50µg/kg) at T.

PRESSOR AGENT	DOSE	RESPONSES	MEAN	STANDARD ERROR
ANGIOTENSIN	50ng/kg	85, 80, 75	80.0	±2.89
ANGIOTENSIN	100ng/kg	100, 95, 105	100.0	±2.89
NORADRENALINE	200ng/kg	70, 70, 72.5	70.83	±0.83
MCNEIL-A-343	15µg/kg	80, 70, 85	78.33	±4.55
TYRAMINE	50µg/kg	75, 75, 80	76.66	±1.74

is a histogram showing the pressor responses to three consecutive injections of angiotensin (50ng/kg and 100ng/kg), noradrenaline (200ng/kg), McN-A-343 (15µg/kg) and tyramine (50µg/kg). The responses shown in Fig.19 are typical of the responses obtained on repeated injection of each of these four pressor substances.

2. Chronic experiments. One advantage which might be derived from the use of conscious animals rather than anaesthetised ones is that long-term experiments, which could not be carried out in anaesthetised animals, might be possible. This possibility was tested by comparison of the responses to intravenous pressor agents over a period of days.

The responses to a given dose of any of the pressor agents showed only a small variation from day to day. Each dose of a particular drug was repeated three times and the mean of these responses showed only a very small change from day to day. Fig.20 shows the mean of three responses to each of the pressor substances on three consecutive days. The responses shown are to angiotensin (50ng/kg and 100ng/kg), noradrenaline (200ng/kg), McN-A-343 (15µg/kg) and tyramine (50µg/kg). The mean of these three responses to each of the drugs was always within 10% of the mean response obtained on other days and usually the variation fell within a 5% range. Fig.20 is typical of the results obtained from all five cats tested. Experiments to determine the reproducibility of the pressor responses were carried out

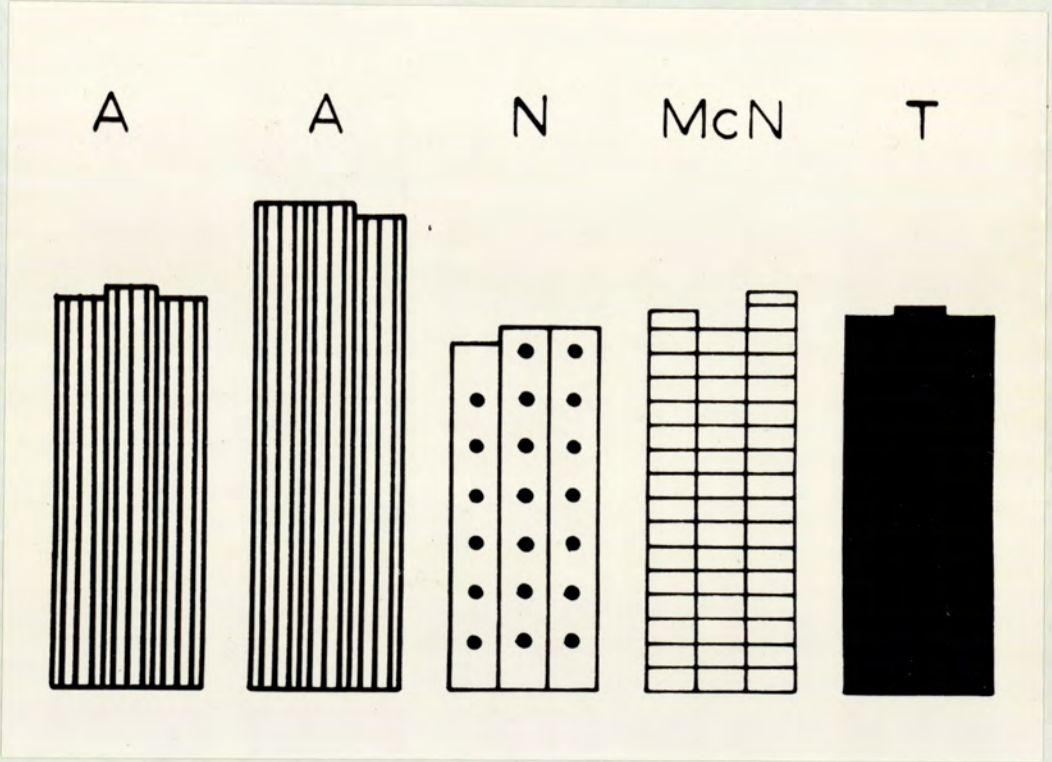

Fig.20 Conscious cat blood pressure. Histograms showing the reproducibility of responses to pressor substances from day to day. Each histogram represents the mean of three consecutive responses on each of three consecutive days. The histograms represent responses to angiotensin (50 and 100ng/kg) at A, noradrenaline (200ng/kg) at N, McN-A-343 (15µg/kg) at McN, and tyramine (50µg/kg) at T.

Doses of angiotensin, MCNeil-A-343 and tyramine repeated at 8 minute intervals. Doses of noradrenaline repeated at 5 minutes.

PRESSOR AGENT	DOSE	RESPONSES	MEAN	STANDARD ERROR
ANGIOTENSIN	50ng/kg	80, 82·5, 80	80·83	±0·84
ANGIOTENSIN	100ng/kg	100, 100, 97·5	99·16	±0·83
NORADRENALINE	200ng/kg	72·5, 75, 75	74·16	±0·85
MCNEIL-A-343	15µg/kg	80, 75, 82·5	79·16	±2·21
TYRAMINE	50µg/kg	77·5, 80, 77·5	78·33	±0·84

for three consecutive days in four of the cats and for five days in the remaining animal. Fig.20 is typical of the results recorded in all five cats used in these experiments.

Heart rate

The resting heart rate was determined daily in each cat for three days. Although the resting heart rate showed greater daily variation than did the responses to pressor substances the rate from day to day in any one cat was always within 10-20 beats per minute of the rate on other days.

Resting blood pressure

The resting blood pressure, both systolic and diastolic pressures, did not vary by more than 5-10 mm Hg from day to day.

Discussion.

The experiments described in this chapter were designed to determine the value of cardiovascular studies in conscious animals as compared with similar studies in anaesthetised animals.

The experiments described show that the technique has advantages other than the removal of possible artefacts due to anaesthesia. These preliminary experiments showed that these conscious cats are suitable for studies over a number of days or weeks as the responses to pressor agents show little daily variation. Studies in anaesthetised animals have always been restricted in terms of time and have required the use of two or more groups of animals so that one group can act as controls for the other. Using

conscious cats long-term studies can be made in which animals act as their own controls both before and after drug treatment and the recovery from drug treatment can be studied. Experiments can also be repeated in the same animals after allowing time for recovery or animals can be treated with a number of different drugs.

Other advantages of this technique include the ease with which daily measurement of aortic blood pressure and heart rate can be made.

The disadvantages of this technique appear minimal. The expected disadvantage was that activity and movement of the animals would cause considerable variation in the resting blood pressure making measurement of pressor responses difficult and lead to poorly reproducible responses. Although movement of the cats did lead to variation in the resting blood pressure after a few days training the cats usually settled very quickly after being placed in the cage used for experiments and did not subsequently move much once they had found a comfortable resting position. On the few occasions that the cats became restless injections of pressor substances were delayed until the animals became quiet. Under these conditions the responses to pressor substances were as regularly reproducible in these animals as in anaesthetised ones.

Chapter 2.

The effect of depletion of neuronal noradrenaline stores on the responses to angiotensin, noradrenaline, tyramine and McN-A-343.

Although angiotensin is known to exert some of its actions via nervous pathways and to have an, as yet unclarified, action at sympathetic nerve endings a sympathetic component has not been identified in the acute pressor response to injections of angiotensin in either human or experimental animal studies (see for example Laurence & Nagle, 1963: Schmitt & Schmitt, 1968a). Baum (1963) and Farr & Grupp (1967) did, however, find some reduction in angiotensin potency in reserpine treated dogs.

There are a number of reports of anaesthesia impairing the pressor effectiveness of angiotensin and renin (see review by Page & McCubbin, 1968). It would seem possible that anaesthetic agents could reduce the action of angiotensin by a reduction in sympathetic activity associated with anaesthesia. This would imply that a sympathetic component is involved in the acute pressor response to angiotensin and the failure of many workers to detect this may be caused by the use of anaesthetised, pithed or in some studies ganglion-blocked anaesthetised animals.

The possibility that a sympathetic component might be involved in the acute pressor response to angiotensin in conscious cats has been studied. In order to determine

the changes in the functionnal state of the sympathetic nervous system after drug treatment the responses to angiotensin were compared with the responses to three other pressor agents of known action. These were noradrenaline the sympathetic transmitter, tyramine which acts by release of neuronal noradrenaline (Burn & Rand, 1958), and McN-A-343 which stimulates the ganglia of the sympathetic nervous system (Roszkowski,1961; Levy & Ahlquist,1962).

In this chapter the experiments described are those in which the functionnal state of the sympathetic nervous system was modified by the depletion of neuronal noradrenaline using reserpine which depletes peripheral and central neuronal stores of noradrenaline, tetrabenazine which causes the depletion of central noradrenaline only and syrosingopine which depletes only peripheral noradrenaline (Brodie, 1960).

Results.

Effect of reserpine in conscious cats

Reserpine was administered intravenously in doses ranging from 10-250μg/kg. At a dose of 10μg/kg there was no detectable change in the resting blood pressure or resting heart rate At a dose of 50μg/kg daily for seven days there was a reduction of 20-30 mm Hg in the resting blood pressure after 2-3 days. The blood pressure then became stable at the new lower level. This dose of reserpine also caused a reduction of 10-20 beats per minute in the resting heart rate and depression of the central nervous system. The cats became immobile and appeared to

lose interest in their surroundings. At a dose of 50μg/kg reserpine caused mild diarrhoea in about half of the experiments but in none of the experiments was there relaxation of the nictitating membranes nor was there any change of body weight. Doses in excess of 50μg/kg up to a maximum of 250μg/kg caused a greater reduction in resting blood pressure (up to 50 mm Hg in both systolic and diastolic pressures), resting heart rate (up to 50 beats per minute), relaxation of the nictitating membranes, loss of body weight of up to 10% of the control weight, severe diarrhoea and very marked depression of the central nervous system.

Reserpine depressed the responses to intravenous injections of angiotensin, noradrenaline, tyramine and McN-A-343. At the lowest dose level of reserpine used (10μg/kg) only the responses to tyramine were affected whereas at 50μg/kg there was a reduction in the responses to all four pressor agents. Fig.21 shows the effect of reserpine (50μg/kg) on the responses to angiotensin (50ng/kg and 100ng/kg). In panel A the responses are shown prior to reserpine and panel B shows the reduction of the responses to angiotensin 24 hours after treatment with reserpine. Fig.22 illustrates the effect of treatment with reserpine (50μg/kg daily) for seven days on the pressor responses to angiotensin, noradrenaline, tyramine and McN-A-343. The responses to noradrenaline were initially depressed but after the first day made an almost complete recovery. After the second day the responses to all pressor substances

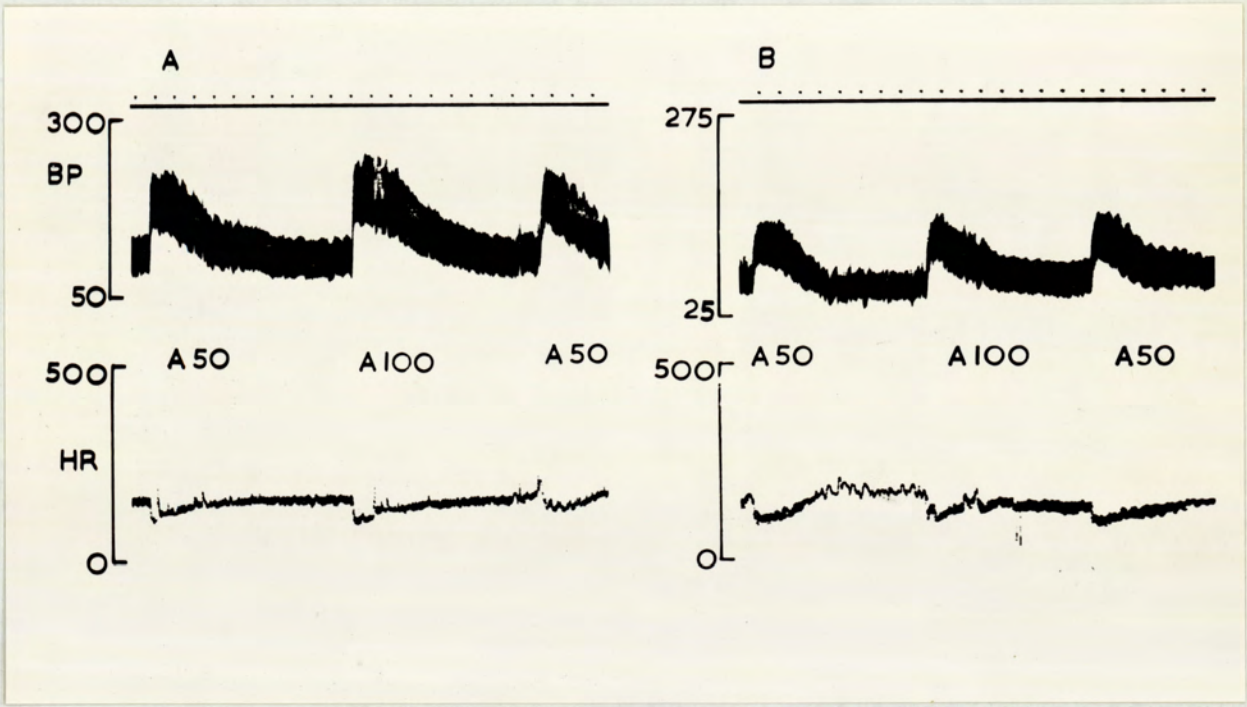

Fig.21 Conscious cat blood pressure. Effect of reserpine (50μg/kg intravenously) on the pressor responses to angiotensin (50ng/kg) at A50 and (100ng/kg) at A100. Panel A shows the responses to angiotensin at both dose levels prior to the administration of reserpine and panel B shows the responses 24 hours after reserpine. Time scale in minutes.

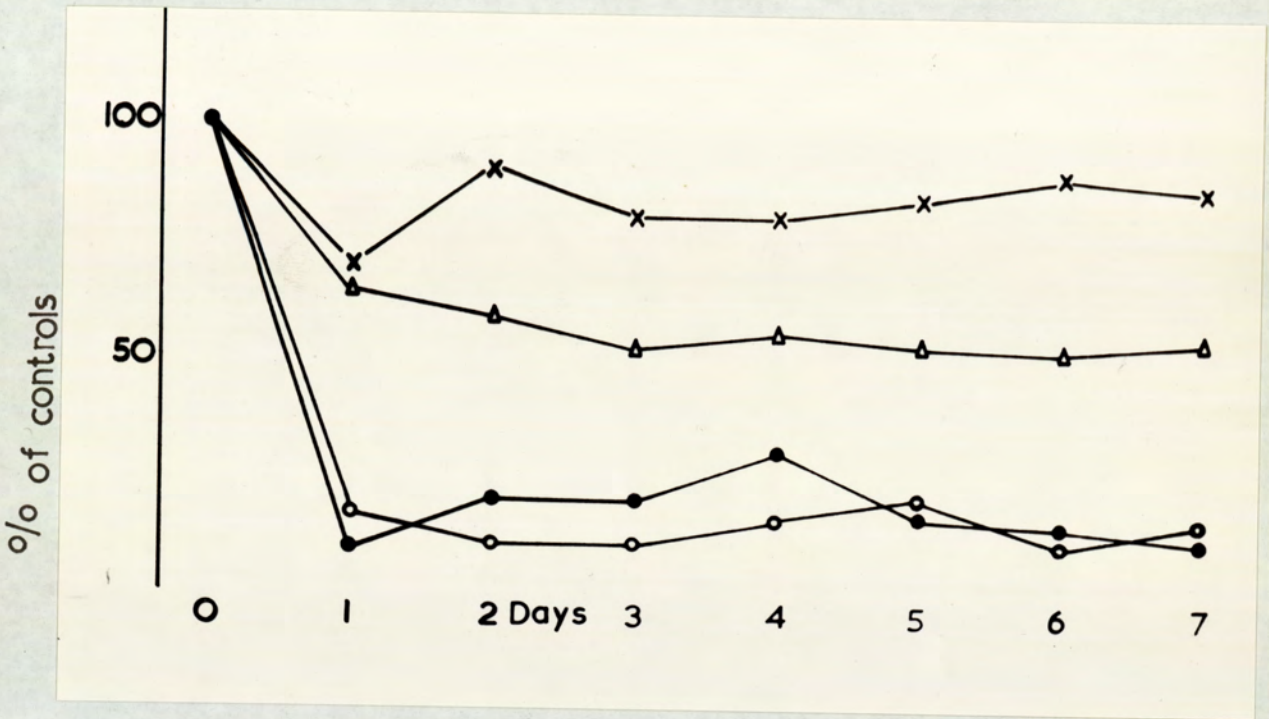

Fig.22 Conscious cat blood pressure. Effect of reserpine (50μg/kg/day intravenously for seven days) on the pressor responses to angiotensin, 50ng/kg (triangles), noradrenaline 200ng/kg (crosses), McN-A-343, 15μg/kg (closed circles), and tyramine, 50μg/kg (open circles). All points plotted are the mean of three consecutive responses expressed as a percentage of the control responses.

Results taken from three cats.

became almost constant. The responses to angiotensin were reduced to about one half of their control size whereas the responses to both tyramine and McN-A-343 were almost abolished. Fig.23 shows the effect of a single dose of reserpine (250μg/kg) on the responses to the pressor agents. Twenty four hours after the reserpine the responses to all four pressor substances were reduced, the effect being most pronounced on the responses to McN-A-343 and tyramine. Although the responses to noradrenaline, tyramine and McN-A-343 were reduced more by this larger dose of reserpine the responses to angiotensin were reduced by a similar amount as after a 50μg/kg dose of reserpine. The responses to all four pressor substances showed some recovery after 48 hours and recovery was complete for angiotensin, noradrenaline and McN-A-343 after 72 hours but the responses to tyramine were reduced for up to 9 days. The reduction of the responses to angiotensin by reserpine was similar for all doses of angiotensin tested over the range 25-200ng/kg. Fig.24 shows the effect of a single dose of reserpine (100μg/kg) on the responses to tyramine (50μg/kg), noradrenaline (200ng/kg) and angiotensin (25ng/kg and 200 ng/kg). At the higher dose of angiotensin (200ng/kg) the pressor response changed from the normal monophasic response and became biphasic consisting of two peaks of about equal height the second one being of greater duration. This second peak was abolished by the intravenous administration of a mixture of phentolamine (3.5mg/kg) and propranolol (1mg/kg) suggesting that catecholamines from the adrenal

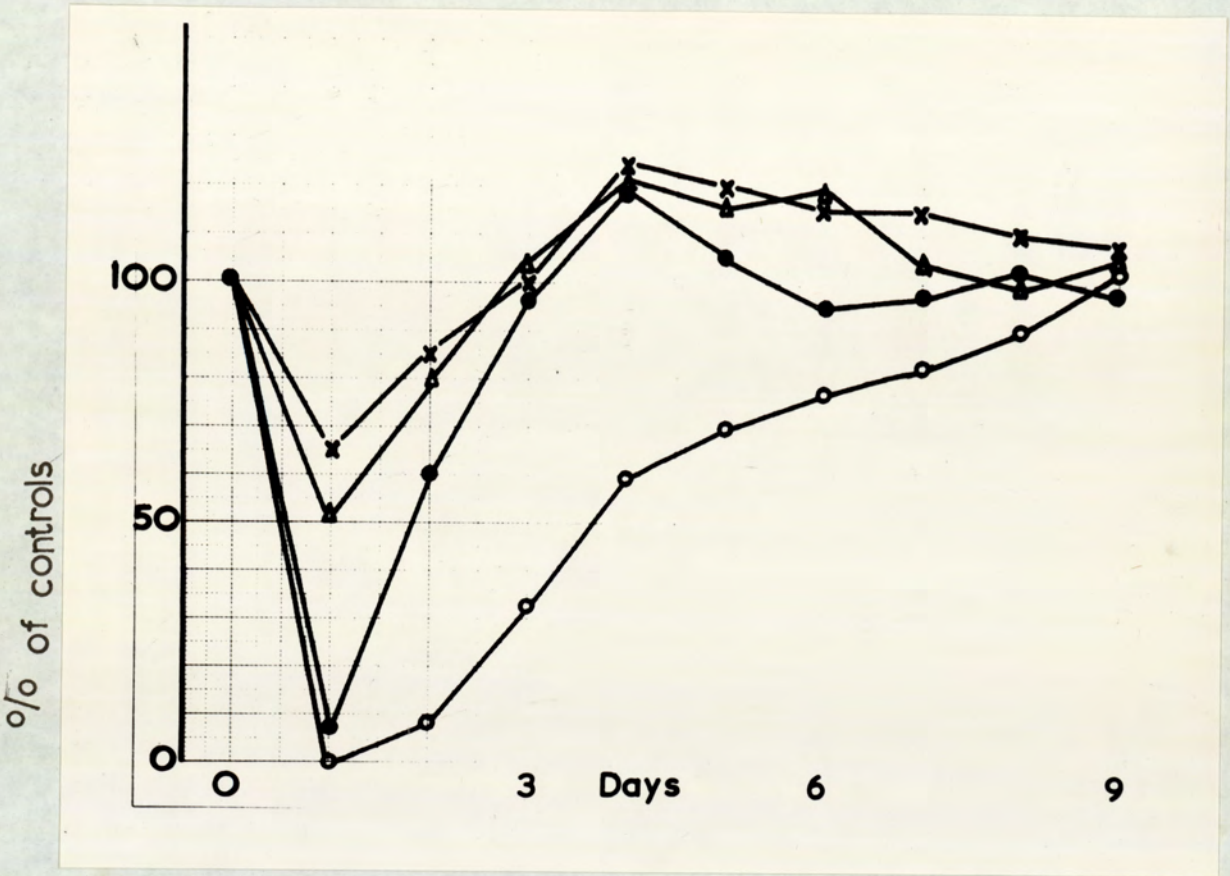

Fig.23 Conscious cat blood pressure. Effect of a single dose of reserpine (250μg/kg intravenously) on the pressor responses to angiotensin, 50ng/kg (triangles), noradrenaline, 200ng/kg (crosses), McN-A-343, 15μg/kg (closed circles), and tyramine, 50μg/kg (open circles). All points plotted are the mean of three consecutive responses expressed as a percentage of the control responses.

Results taken from three cats

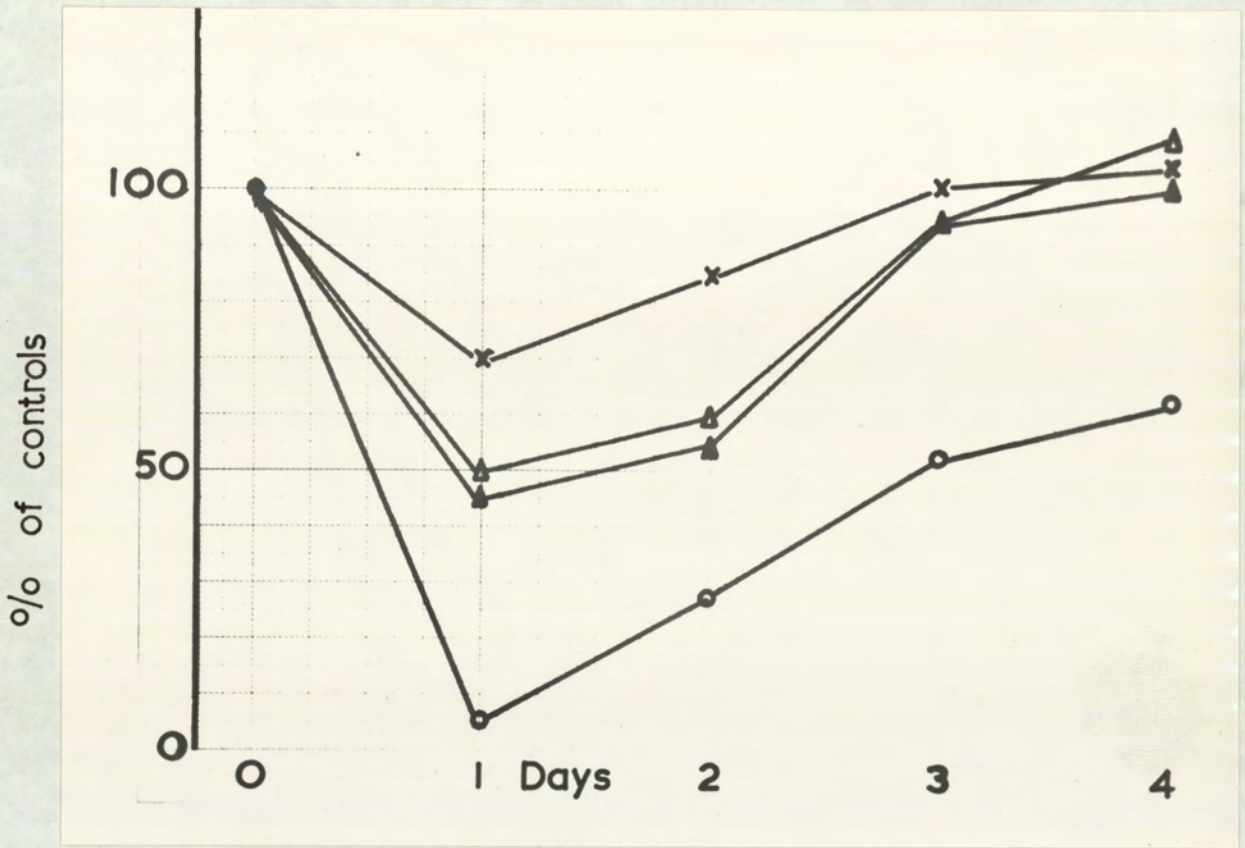

Fig.24 Conscious cat blood pressure. Effect of a single dose of reserpine (100μg/kg intravenously) on the pressor responses to angiotensin, 25ng/kg (open triangles), angiotensin, 200ng/kg (closed triangles), noradrenaline, 200ng/kg (crosses) and tyramine, 50μg/kg (open circles). All points plotted are the mean of three consecutive responses expressed as a percentage of the control responses.

Results taken from three cats.

medulla were responsible for the second component. After reserpine (50-100µg/kg) the response to angiotensin (200ng/kg) was reduced in height by about 50% and was changed into a prolonged monophasic response.

Effect of tetrabenazine.

Tetrabenazine (1-25mg/kg intraperitoneally) caused no change in the resting blood pressure or resting heart rate. At doses in excess of 5mg/kg it caused some central depression but this was not as marked as that caused by reserpine at a dose level of 250µg/kg. Tetrabenazine caused no relaxation of the nictitating membranes, diarrhoea or change of body weight.

At dose of less than 5mg/kg tetrabenazine caused no reduction in the pressor responses to any of the four pressor agents. At a dose of 5mg/kg there was some reduction in the responses to tyramine but the other responses were unaffected. The effect of tetrabenazine (25mg/kg) is illustrated in Fig.25. At this dose level tetrabenazine caused some reduction in the responses to angiotensin, tyramine, noradrenaline and McN-A-343.

Effect of syrosingopine in conscious cats.

Syrosingopine (500µg/kg intraperitoneally) caused a reduction in both systolic and diastolic blood pressures. This was maximal about 6 hours after the administration of syrosingopine at which time the reduction was 15-20 mm Hg. This substance also caused a very slight bradycardia but had no effect on behaviour or body weight. In about half

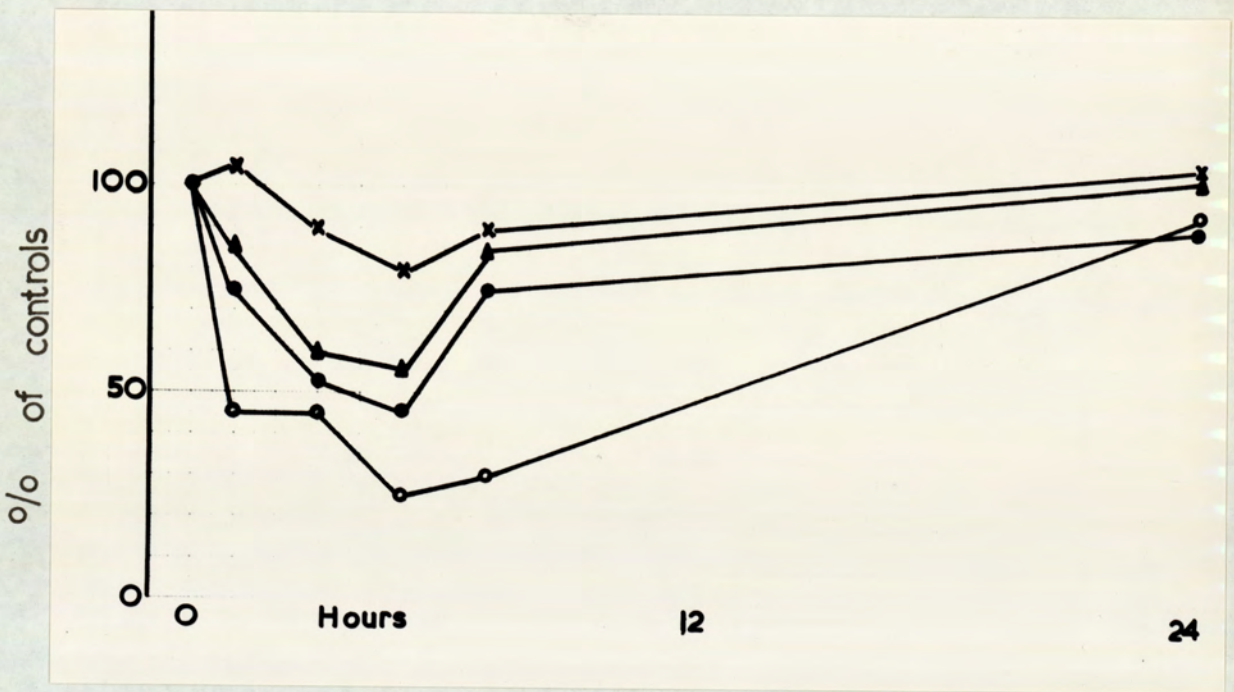

Fig.25 Conscious cat blood pressure. Effect of tetrabenazine (25mg/kg intraperitoneally) on the pressor responses to angiotensin, 50ng/kg (triangles), noradrenaline, 200ng/kg (crosses), McN-A-343, 15μg/kg (closed circles) and tyramine, 50μg/kg (open circles). All points plotted are the mean of three consecutive responses expressed as a percentage of the control responses.

Results taken from three cats.

of the experiments there was some diarrhoea after syrosingopine (500μg/kg) and in all experiments there was a marked relaxation of the nictitating membranes within 30 minutes of administration of the drug. Fig.26 shows the effect of syrosingopine (500μg/kg) on the responses to angiotensin, tyramine, McN-A-343 and noradrenaline. The responses to noradrenaline were unaltered by this treatment. The responses to angiotensin and McN-A-343 were reduced immediately after the administration of syrosingopine and although the responses to McN-A-343 were most affected, the maximal reduction of responses to both substances occurred after about 6 hours after which they showed a parallel recovery. Syrosingopine caused an initial enhancement of the responses to tyramine after which there was a marked reduction which was maximal after about 8 hours.

Effect of reserpine in conscious cats pretreated with pempidine.

The exact location of noradrenaline within the neuronal storage sites is not clearly understood. Sedvall (1964) and Sedvall & Thorsen (1965) showed that noradrenaline within the neuronal stores could be divided into two portions by its response to reserpine. The larger portion was actively depleted by reserpine whereas the smaller portion was only depleted by nerve impulses and the failure of released noradrenaline to rebind into the storage sites after treatment with reserpine. These workers showed that

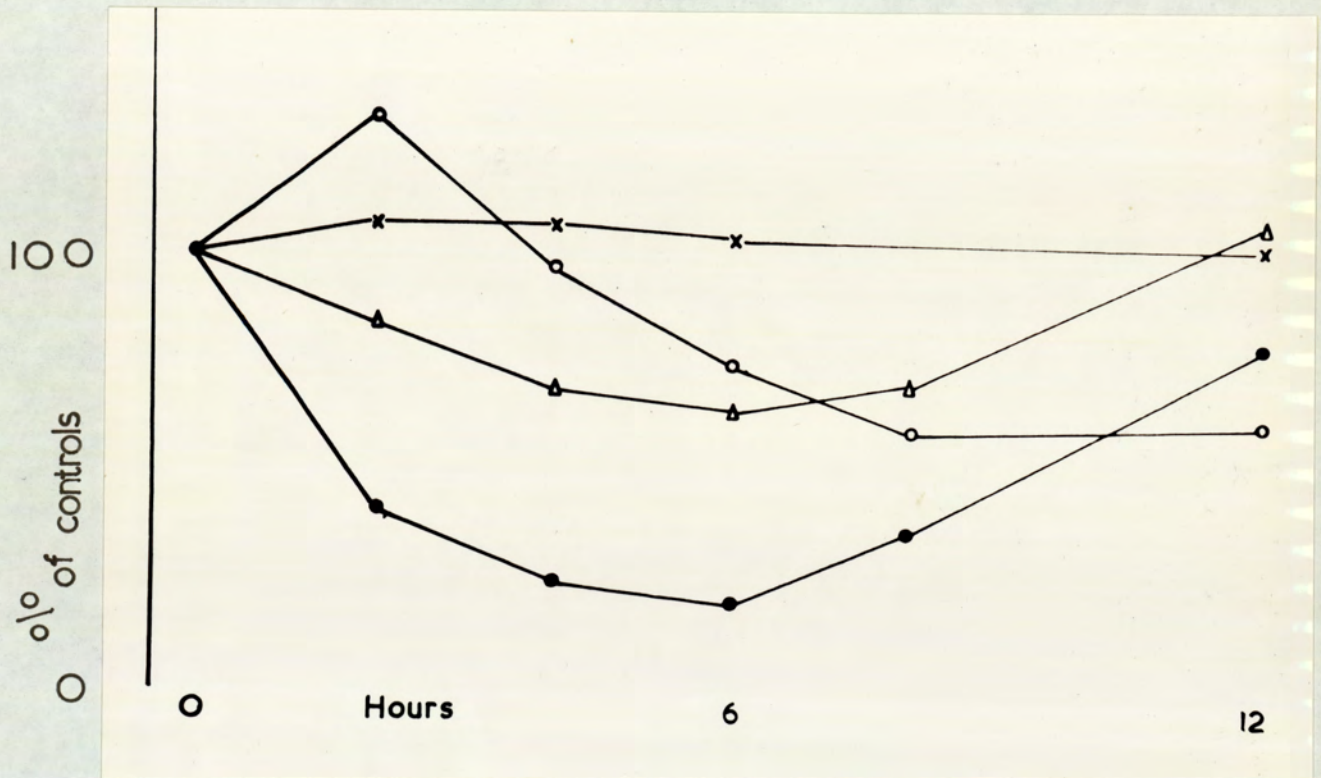

Fig.26 Conscious cat blood pressure. Effect of syrosingopine (500μg/kg intraperitoneally) on the pressor responses to angiotensin, 50ng/kg (triangles), noradrenaline, 200ng/kg (crosses), McN-A-343, 15μg/kg (closed circles) and tyramine, 50μg/kg (open circles). All points plotted are the mean of three consecutive responses expressed as a percentage of the control responses.

Results taken from three cats.

lack of activity along the post-ganglionnic sympathetic nerves prevented the depletion of the smaller pool of noradrenaline. The size of the responses after nerve stimulation was determined by the noradrenaline content of the smaller reserpine resistant pool and was apparently independent of the larger pool.

In order to determine the storage pool of the noradrenaline involved in the angiotensin pressor response in conscious cats animals were pretreated with pempidine (5mg/kg intravenously every 8 hours for 32 hours) prior to the administration of reserpine (50µg/kg). This treatment was intended to protect the noradrenaline pool from which nerve impulses release transmitter without protecting the larger pool. The index of complete ganglion blockade was taken as the complete relaxation of the nictitating membranes and the abolition of reflex bradycardia after injection of pressor drugs.

Pretreatment with pempidine caused an increase in the pressor responses to all four substances. Fig.27 shows the effect of a single dose of reserpine (50µg/kg) on the pressor responses to angiotensin, noradrenaline, tyramine and McN-A-343 in pempidine treated cats. The responses to tyramine and noradrenaline were reduced to a similar extent as in non-ganglion blocked cats. The responses to both angiotensin and McN-A-343 were reduced more rapidly than in non-ganglion blocked cats and this was followed by a more rapid recovery such that 24 hours after the administration of reserpine the responses to both

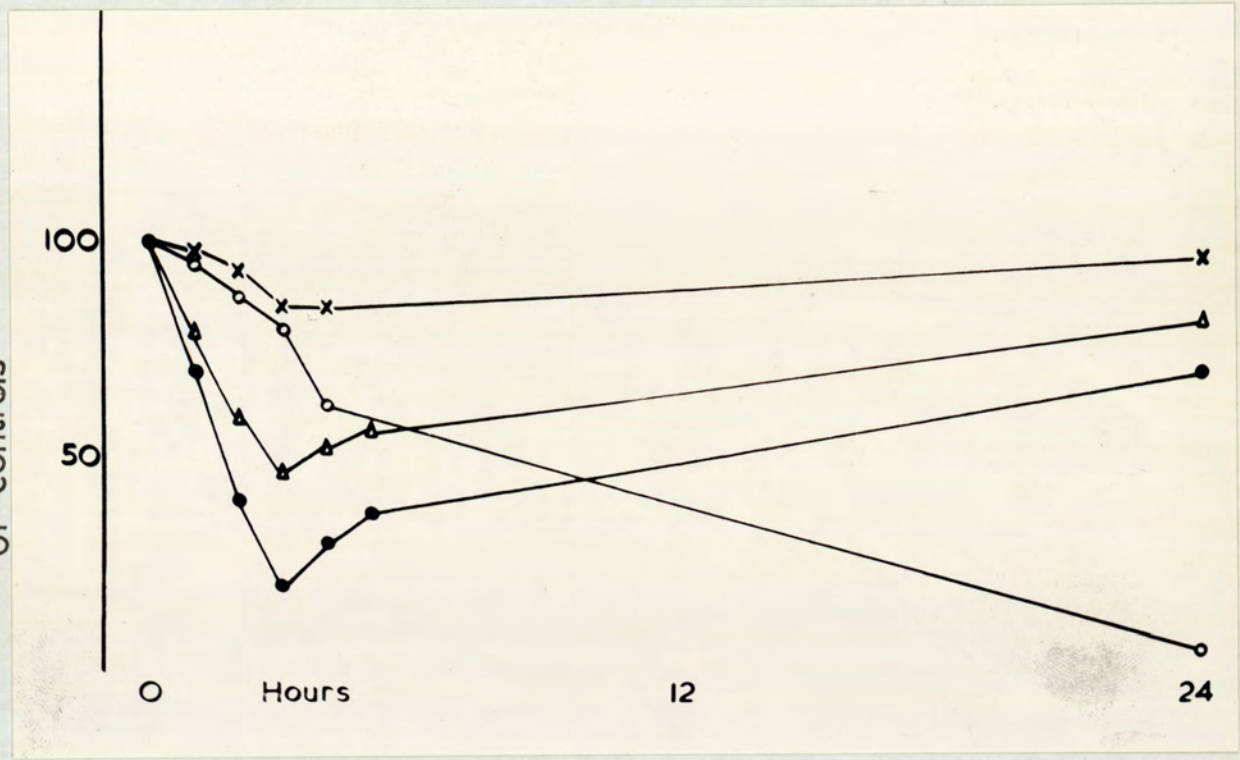

Fig.27 Conscious cat blood pressure. Effect of reserpine (50μg/kg intravenously) on the pressor responses to angiotensin, 50ng/kg (triangles), noradrenaline, 200ng/kg (crosses), McN-A-343, 15μg/kg (closed circles) and to tyramine, 50μg/kg (open circles) in cats pretreated with pempidine (5mg/kg intravenously) every 8 hours for 32 hours prior to the administration of reserpine. All points plotted are the mean of three consecutive responses expressed as a percentage of the control responses after pempidine.

Results taken from three cats.

pressor substances had returned to almost control level.

Discussion.

The experiments described in this chapter indicate that reserpine and syrosingopine cause a reduction in the pressor responses to angiotensin in conscious cats. The reduction in the angiotensin responses occurred after small doses of reserpine and when maximal accounted for about 50% of the angiotensin responses. Although small doses of reserpine reduced the angiotensin responses by about 50% there was no further reduction of the responses when the dose of reserpine was increased. The failure of tetrabenazine to cause a reduction of the angiotensin responses similar to that caused by reserpine or syrosingopine suggests that the action of reserpine in reducing pressor responsiveness to angiotensin is peripheral rather than central.

The mechanism of action of reserpine appears to be due to depletion of neuronal noradrenaline (see next chapter in which the experiments which suggest this mechanism are described).

There are a number of possible peripheral sites of the noradrenaline involved in the angiotensin pressor response. Angiotensin is known to stimulate the release of catecholamines from the adrenal medulla of most species (see for example Feldberg & Lewis, 1964;1965). It seems probable that in the present experiments release

of medullary amines occurred only after the administration of large doses of angiotensin when the pressor response was biphasic and the second component appeared to be due to release of medullary amines. The reduction of the responses to angiotensin was, however, similar over a wide dose range. The reduction of angiotensin responses by reserpine would therefore appear to be due to depletion of noradrenaline which was not restricted to the adrenal medulla.

The most likely sites of storage of the noradrenaline are the sympathetic nerve endings. Sedvall (1964) and Sedvall & Thorsen (1965) have shown, in anaesthetised cats, that neuronal noradrenaline is present in two functionnal states which can be distinguished by the use of large doses of reserpine. One pool would seem to contain the noradrenaline released by nerve impulses and the other that released by indirect sympathomimetics. The present experiments were designed to determine which of these two pools is involved in the acute pressor response to angiotensin. Sedvall (1964) and Sedvall & Thorsen (1965) found that reduction or abolition of activity along the post-ganglionnic sympathetic nerves slowed the depletion of the pool from which nerve impulses release noradrenaline. Unlike the findings of these workers in the present experiments reducing activity along the post-ganglionnic sympathetic nerves by pretreatment with pempidine accelerated both the onset of block of responses to McN-A-343 and their subsequent recovery. The mechanism of the nerve blocking action of reserpine in ganglion blocked cats was

presumably due to depletion of noradrenaline. In conscious cats this depletion was apparently accelerated by pretreatment with pempidine as was the subsequent recovery. Pretreatment with pempidine did not alter the effect of reserpine on the pressor responses to tyramine. The responses to angiotensin were reduced more rapidly after reserpine in ganglion blocked cats than in non-ganglion blocked animals. The reduction of angiotensin responses was almost identical along the time axis (see Fig.27) as the reduction of responses to McN-A-343. Responses to both substances also showed similar recovery along the time axis. These results would suggest that the noradrenaline in the pool from which it is released by nerve impulses is also involved in the acute pressor responses to angiotensin. This idea is supported by the similar recovery of responses to both angiotensin and McN-A-343 after reserpine in non-ganglion blocked cats and by the experiments with syrosingopine. Syrosingopine is known to deplete neuronal noradrenaline from peripheral sites (Brodie, 1960). This depletion is maximal 4-8 hours after administration of the drug. The responses to tyramine were initially enhanced by syrosingopine but were markedly reduced about 8 hours after syrosingopine when depletion was presumably maximal. Suprisingly, however, the responses to McN-A-343 and to angiotensin were reduced almost immediately after the administration of syrosingopine and the nictitating membranes were also relaxed within 30 minutes of the administration of the drug. The mechanism of this rapid

block of sympathetically mediated responses is not clear but is probably due to depletion of neuronal noradrenaline stores although responses to tyramine were enhanced immediately after syrosingopine and there was some delay before they were reduced. It is possible that syrosingopine might deplete the pool from which nerve impulses release noradrenaline more rapidly than it depletes the larger pool with which tyramine interacts.

The time course of the reduction of the responses to McN-A-343 and angiotensin and their subsequent recovery was identical after reserpine alone or in combination with pempidine and after syrosingopine and could be clearly distinguished from the time course of the reduction and recovery of responses to tyramine. The pressor responses to McN-A-343 are due to stimulation of sympathetic ganglia (Roszkowski, 1961) and are therefore mediated by the release of noradrenaline from the pool from which nerve impulses cause transmitter release. The similarity of the changes in the responses to McN-A-343 and the changes in the responses to angiotensin, caused by these depletion procedures, therefore suggests that the acute angiotensin pressor response in conscious cats is partly determined by the noradrenaline content of the pool, within the neuronal store, from which nerve impulses release noradrenaline and is independent of the larger pool from noradrenaline is released by indirect sympathomimetic amines.

Chapter 3.

Reversal of the anti-angiotensin action of reserpine by restoration of neuronal noradrenaline stores.

In the previous chapter it was shown that reserpine caused a reduction of the acute angiotensin pressor responses in conscious cats The experiments described in this chapter were carried out in order to determine whether the anti-angiotensin action of reserpine was mediated by depletion of neuronal noradrenaline.

Results.

In the following experiments attempts to reverse the effects of reserpine were carried out in cats which had received reserpine (50µg/kg/day intravenously) for the seven days immediately before the experiments began.

Effect of infusions of noradrenaline.

Noradrenaline was infused at a rate of 2µg/kg/minute for 1-2 hours. At this dose level noradrenaline caused a small well sustained increase in the blood pressure and slight bradycardia. Pressor sensitivity to angiotensin, tyramine, noradrenaline and McN-A-343 was tested 30 minutes after completion of the infusion. These infusions were without effect on the pressor responses to angiotensin and McN-A-343 but caused a slight increase in the responses to tyramine and a small decrease in the responses to noradrenaline.

Effect of infusions of dopa

Dopa was infused at a rate of 20μg/kg/minute for 1-2 hours and pressor sensitivity to angiotensin, noradrenaline, tyramine and McN-A-343 tested 30 minutes after completion of the infusion. These infusions were without effect on the pressor responses to any of the four pressor agents tested.

Effect of infusions of α-methyl dopa

α-methyl dopa was infused at 20μg/kg/minute for 2 hours and pressor sensitivity tested 30 minutes after completion of the infusions. In all experiments α-methyl dopa caused a marked recovery of the pressor responses to angiotensin, tyramine and McN-A-343 although the extent of the recovery varied from experiment to experiment. The reversal of the effect of reserpine was usually most marked for the responses to tyramine although complete reversal of the responses to both angiotensin and McN-A-343 occurred in one experiment out of three and the recovery was almost complete in the remaining two experiments. One of these experiments in which α-methyl dopa reversed the anti-angiotensin action of reserpine is illustrated in Fig.28. Thus, the reduction of the pressor responses to angiotensin, tyramine and McN-A-343 after daily treatment with reserpine (50μg/kg) could be reversed by infusions of α-methyl dopa.

Effect of tranylcypromine in reserpine treated cats.

Treatment with the monoamine oxidase inhibiting drug

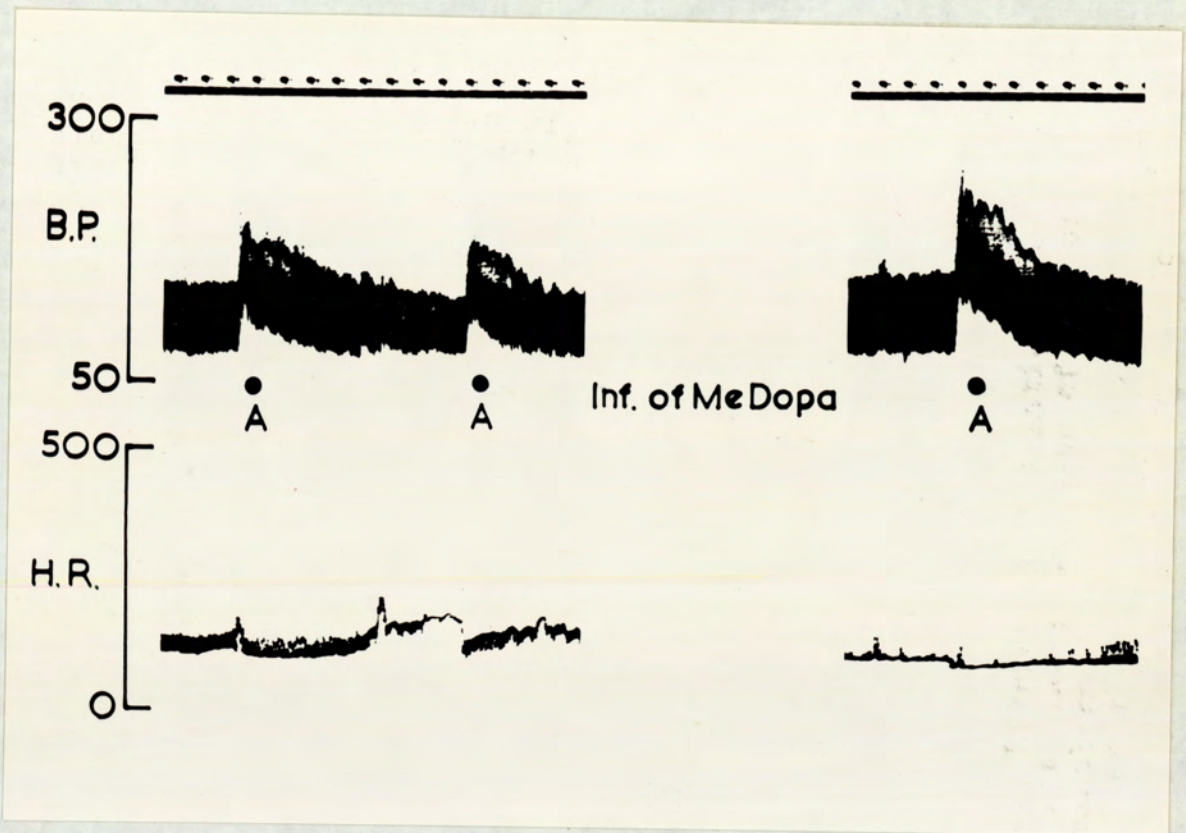

Fig.28 Conscious cat blood pressure. Reversal of the anti-angiotensin action of reserpine by intravenous infusion of ∝-methyl dopa. In the left hand panel responses to angiotensin (50ng/kg) A are shown after administration of reserpine. The right hand panel shows the response to the same dose of angiotensin 30 minutes after a slow intravenous infusion of ∝-methyl dopa. Time scale in minutes.

tranylcypromine (5mg/kg intravenously) caused some recovery of the pressor responses to angiotensin, tyramine and McN-A-343 16-20 hours after treatment. Fig.29 illustrates the recovery of the responses to angiotensin, tyramine and McN-A-343 16 hours after tranylcypromine in a cat pretreated with reserpine (50µg/kg/day for 7 days).

Effect of infusions of noradrenaline in reserpinised cats treated with tranylcypromine.

Prior to the inhibition of monoamine oxidase infusions of noradrenaline caused only a slight increase in the responses to tyramine after reserpine and did not increase the reduced responses to angiotensin and McN-A-343. Infusion of noradrenaline (2µg/kg/minute for two hours) in cats treated with tranylcypromine (5mg/kg), 16 hours before beginning the infusion, caused a marked reversal of the anti-tyramine action of reserpine and also a less well marked recovery of the pressor responses to angiotensin and McN-A-343.

Effect of infusions of dopa in reserpinised cats treated with tranylcypromine

Infusions of dopa (20 µg/kg/minute for two hours) in cats treated with tranylcypromine (5mg/kg), 16 hours before beginning the infusion, caused marked reversal of the anti-tyramine effect of reserpine and also some recovery of the pressor responses to both angiotensin and McN-A-343.

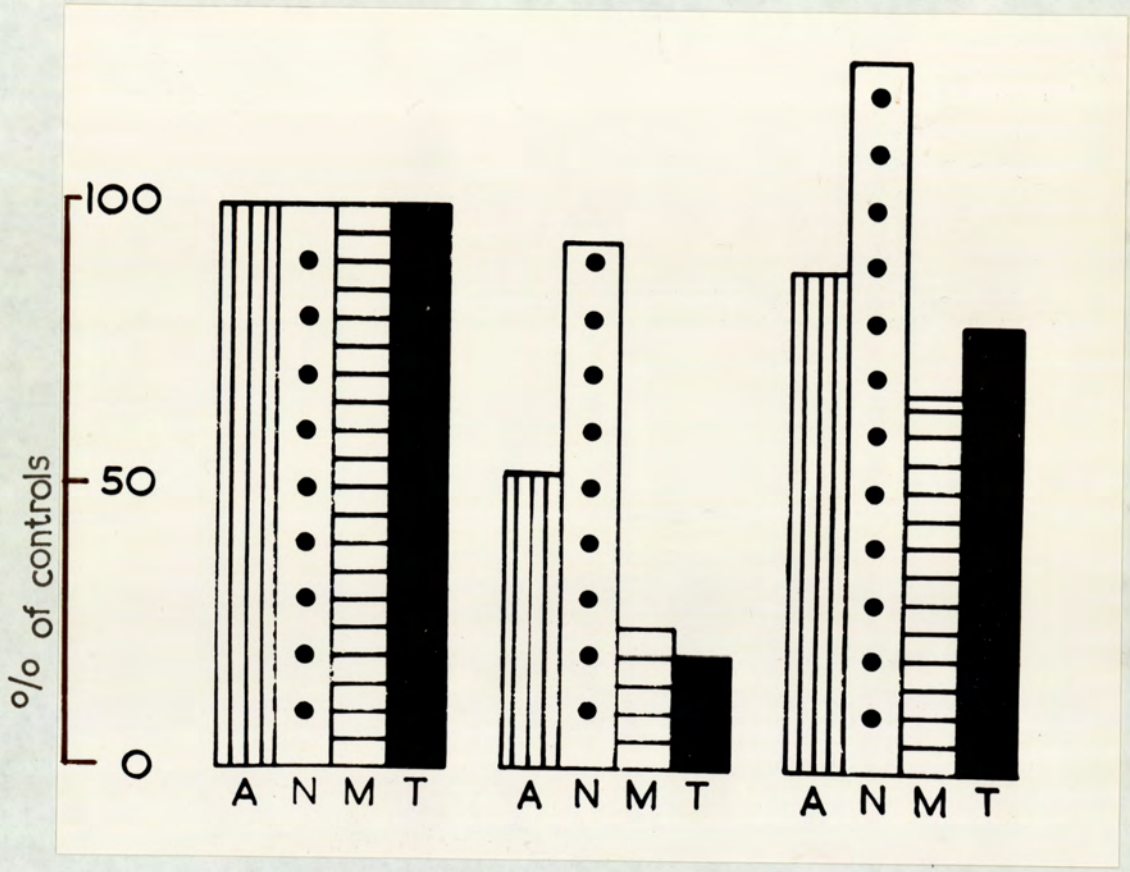

Fig.29 Conscious cat blood pressure. The left hand histogram shows the responses to angiotensin (50ng/kg) A, noradrenaline (200ng/kg) N, McN-A-343 (15μg/kg) M and tyramine (50μg/kg) T prior to drug treatment. These control responses are expressed as 100%. The centre histogram shows the responses to these pressor agents after reserpine (50μg/kg/day intravenously for 7 days). The responses are expressed as a percentage of the control responses. The right hand histogram shows the responses, expressed as a percentage of their controls, 16 hours after tranylcypromine (5mg/kg intravenously).

Results taken from three cats.

Discussion.

Reserpine causes a reduction in the pressor responses to tyramine, angiotensin and McN-A-343 in conscious cats. It would seem possible that this action of reserpine is mediated by the depletion of neuronal noradrenaline. The actions of reserpine which are due to depletion of neuronal noradrenaline can usually be reversed by replenishment of neuronal noradrenaline stores (see for example Burn & Rand, 1958;1960). In order to examine whether the anti-angiotensin action of reserpine was due to depletion of neuronal noradrenaline the effect of replenishing the neuronal noradrenaline stores on the pressor responses to angiotensin was examined.

Infusions of noradrenaline (Burn & Rand, 1958) or one of its precursors (Burn & Rand, 1960) have been reported to reverse the anti-tyramine action of reserpine by replenishment of neuronal noradrenaline responses. In the experiments described in this chapter, however, infusions of both noradrenaline and of its precursor dopa caused only a small increase in the responses to tyramine in reserpinised cats. The responses to angiotensin and to McN-A-343 were not changed after these infusions. After inhibition of monoamine oxidase, however, infusions of noradrenaline or of dopa did cause some recovery of the responses to tyramine, angiotensin and McN-A-343 in reserpine treated cats. Pretreatment with the monoamine oxidase inhibiting drug tranylcypromine alone caused some recovery of the responses to angiotensin, tyramine and McN-A-343 but after tranylcypromine treatment infusions of both

noradrenaline and dopa caused further recovery of the responses to angiotensin, tyramine and McN-A-343. It would therefore appear that inhibition of monoamine oxidase is necessary for the reversal of the effects of reserpine in conscious cats and that replenishment of the neuronal stores is only possible if the noradrenaline infused or synthesised is protected from metabolism by monoamine oxidase before becoming bound into neuronal storage sites. Clarke & Leach (1968) also found that in pithed rats reversal of the anti-tyramine actions of reserpine could only be achieved by infusion of noradrenaline or of its precursors after inhibition of monoamine oxidase. Reversal of the effects of reserpine was also achieved by infusion of α-methyl dopa. This substance is immune to metabolism by monoamine oxidase and can presumably therefore replenish the neuronal noradrenaline stores in normal animals.

The experiments described in this chapter indicate that in conscious cats replenishing the neuronal noradrenaline stores after their depletion by reserpine can only be achieved after inhibition of monoamine oxidase. When the stores are replenished, however, the anti-angiotensin effect of reserpine is reversed. Thus it would appear that the anti-angiotensin action of reserpine is due to depletion of neuronal noradrenaline.

Chapter 4.

Effect of increasing neuronal noradrenaline levels on the pressor responses to angiotensin, noradrenaline, tyramine and McN-A-343.

In the previous chapters it was shown that the acute pressor responses to intravenous injections of angiotensin in conscious cats were reduced by depletion of peripheral neuronal noradrenaline stores with reserpine and restored when the stores were replenished. Since reduction of the neuronal noradrenaline content reduced the pressor action of angiotensin it seems likely that increasing neuronal noradrenaline levels would potentiate the pressor action of angiotensin. Such an effect would add further evidence to the hypothesis that the acute pressor effect of angiotensin is partly mediated by the release of neuronal noradrenaline.

Effect of intravenous infusions of noradrenaline.

Intravenous infusions of noradrenaline (2μg/kg/minute) caused a small, variable increase in the resting blood pressure and slight bradycardia. The responses to angiotensin, tyramine, noradrenaline and McN-A-343 were retested 30 minutes after completing a 2 hour infusion of noradrenaline. There was no change in the responses to either angiotensin or McN-A-343. The responses to tyramine were, however, slightly increased whilst those to noradrenaline were slightly reduced.

Effect of pretreatment with a monoamine oxidase inhibitor.

Nialamide (20mg/kg intravenously) caused no change in resting blood pressure although there was a marked reduction in the resting heart rate. The bradycardia was maximal about 48 hours after administration of nialamide at which time the rate was slowed by up to 50 beats per minute. Treatment with nialamide caused the cats to become more friendly and playful and they were thus more restless during experiments.

Pressor sensitivity to angiotensin, noradrenaline, tyramine and McN-A-343 was retested 48 hours after nialamide administration. Nialamide caused a massive increase in the pressor potency of tyramine such that the dose of tyramine required to cause a pressor response similar to that caused by a 50 µg/kg dose initially was only 6µg/kg following nialamide. The responses to both angiotensin and McN-A-343 were increased by about 60% and those to noradrenaline by about 30% after nialamide. Fig.30 is a histogram showing the potentiation of the responses to angiotensin, noradrenaline, tyramine and McN-A-343 after nialamide. The potentiation of the responses to angiotensin, noradrenaline and McN-A-343 persisted for about 14 days and that of tyramine for about 21 days.

Effect of infusions of dopa in nialamide treated cats.

Infusions of noradrenaline had little or no effect on the pressor responses to angiotensin and McN-A-343 and caused

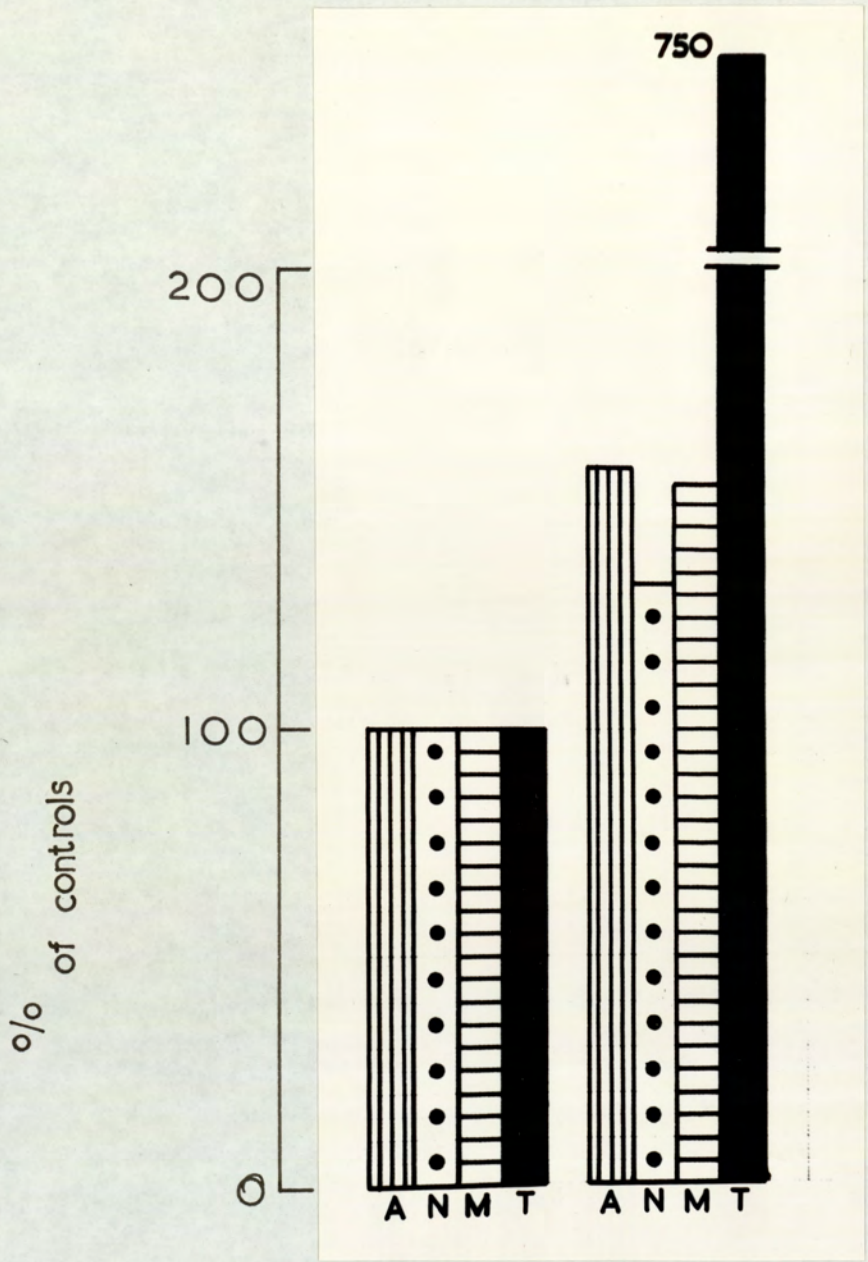

Fig.30 Conscious cat blood pressure. Effect of nialamide (20mg/kg intravenously) on the pressor responses to angiotensin (50ng/kg) A, noradrenaline (200ng/kg) N, McN-A-343 (15µg/kg) M and to tyramine (50µg/kg) T. The left hand histogram shows the control responses expressed as 100% and the right hand histogram the responses 48 hours after nialamide administration expressed as a percentage of their controls.

Results taken from three cats

only a small increase in the responses to tyramine. This may have been due to the failure of noradrenaline infusions to increase neuronal noradrenaline levels. In the experiments described in the previous chapter it was shown that the effects of reserpine could only be reversed by α -methyl dopa or by noradrenaline or by dopa after inhibition of monoamine oxidase. The failure of infused noradrenaline to enhance the pressor responses to tyramine might be due to the rapid metabolism of infused noradrenaline by monoamine oxidase.

In one cat dopa (2mg/kg) was infused intravenously over a period of 30 minutes. This had little immediate effect on the resting blood pressure but about 10 minutes after the completion of the infusion there was a slowly developing pressor response which persisted for about 15-20 minutes after which the blood pressure returned to normal. When maximal the systolic pressure rose to about 450 mm Hg and the diastolic to 150 mm Hg. Suprisingly, the cat showed no signs of distress during this massive pressor response but then died shortly after the blood pressure had returned to normal. Prior to the administration of dopa the cat had appeared to be in good health and it seems likely that death was caused by the combination of nialamide and dopa.

In two other cats dopa (200µg/kg) was infused intravenously over a period of 1 hour. This treatment had no effect on the resting blood pressure and did not alter the pressor actions of angiotensin, tyramine or McN-A-343.

Discussion.

Although depletion of noradrenaline by reserpine or syrosingopine reduced the pressor potency of angiotensin in conscious cats infusions of noradrenaline and dopa, designed to increase neuronal noradrenaline levels, had little or no effect on the pressor responses to angiotensin. These infusions also had little effect on the responses to tyramine As tyramine is known to exert its effects by release of neuronal noradrenaline the failure of these infusions to enhance the responses to tyramine suggests that they did not increase the neuronal noradrenaline content. The most likely explanation of this is that the infused noradrenaline was rapidly metabolised by monoamine oxidase before it had the opportunity to bind into neuronal stores. This explanation is supported by the finding described in the previous chapter that noradrenaline or dopa infusions were ineffective in reversing the actions of reserpine prior to inhibition of monoamine oxidase.

The monoamine oxidase inhibiting drug, nialamide, caused an increase in the responses to all four pressor substances. The actions of monoamine oxidase inhibiting drugs on the neuronal noradrenaline stores in complex and has been recently reviewed by Iversen (1967). There is considerable evidence that monoamine oxidase inhibition leads to increased noradrenaline content within neuronal stores. Treatment with nialamide caused a marked increase in the responses to angiotensin, tyramine, noradrenaline and McN-A-343. The enhancement wasmost pronounced for tyramine but this was probably due, at least in part,

to the reduced metabolism of tyramine after monoamine oxidase inhibition. The responses to noradrenaline were presumably enhanced by the failure of monoamine oxidase to make its usual contribution to the metabolism of noradrenaline The simplest interpretation of the potentiation of the responses to angiotensin and McN-A-343 following nialamide is that the neuronal noradrenaline levels were increased thus resulting in an increased sympathetic component of the acute pressor responses to these substances. The experiments which might have added further evidence to the hypothesis that nialamide enhanced the responses to angiotensin by increasing neuronal noradrenaline levels proved technically difficult. Dopa, after monoamine oxidase inhibition, would be expected to increase neuronal noradrenaline levels and might therefore be expected to further potentiate the responses to angiotensin, tyramine and McN-A-343. A dose of 2mg of dopa proved fatal to the only cat thus tested. At a lower dose (200μg/kg) dopa was not toxic but failed to increase the responses to tyramine suggesting that this dose was insufficient to increase the neuronal noradrenaline levels. It was not suprising therefore that this smaller dose of dopa did not further enhance the acute pressor responses to angiotensin.

The results reported in this chapter support the hypothesis that the acute pressor responses to angiotensin in conscious cats are mediated partly by release of neuronal noradrenaline.

Chapter 5.

An analysis of the role of noradrenaline in the acute pressor responses to angiotensin in conscious cats.

In the previous three chapters it was shown that the acute pressor response to angiotensin in the conscious cat was determined, in part, by the content of the neuronal noradrenaline stores. Thus, depletion of the stores leads to a reduction in the responses to angiotensin whilst loading of the stores enhanced the responses.

Although the results obtained using reserpine, tetrabenazine and syrosingopine suggested that noradrenaline at the peripheral sympathetic nerve endings was involved in the acute angiotensin response the role of the noradrenaline was not established. This chapter describes experiments carried out in an attempt to determine the nature of the action of noradrenaline in acute angiotensin pressor responses.

Results.

Effect of adrenergic neuron blockade by bethanidine.

Bethanidine (3mg/kg intravenously) caused a lowering of the resting blood pressure, resting heart rate and complete relaxation of the nictitating membranes. These effects were maximal 3-6 hours after administration of bethanidine. When maximal the fall in resting heart rate was about 50 beats per minute and both systolic and diastolic blood pressures were reduced by 15-20 mm Hg.

The effect of this dose of bethanidine on the pressor responses to angiotensin, tyramine, noradrenaline and McN-A-343 is shown in Fig.31. This dose of bethanidine caused a marked reduction of the responses to McN-A-343 which persisted for about 24 hours and a transient reduction of the responses to tyramine. The responses to noradrenaline were considerably enhanced but the responses to angiotensin were unchanged.

Effect of autonomic ganglion blockade by pempidine

Pempidine (5mg/kg intravenously every 8 hours for 32 hours) caused a marked fall in both systolic and diastolic blood pressures. The mean resting blood pressure after pempidine was lowered by as much as 40 mm Hg and was much more constant than in normal animals. The heart rate also became very steady after pempidine although there was little or no change in the rate. Pempidine also caused complete relaxation of the nictitating membranes.

The effect of pempidine (5mg/kg) on the responses to pressor agents is illustrated by Fig.32. There was marked potentiation of all pressor responses although this was most pronounced for the responses to tyramine and McN-A-343. Both these substances caused a marked tachycardia of 50-80 beats per minute after ganglion blockade. The responses to noradrenaline were also enhanced and there was some tachycardia but not as great as that caused by either tyramine or McN-A-343. The pressor responses to angiotensin were potentiated by pempidine treatment

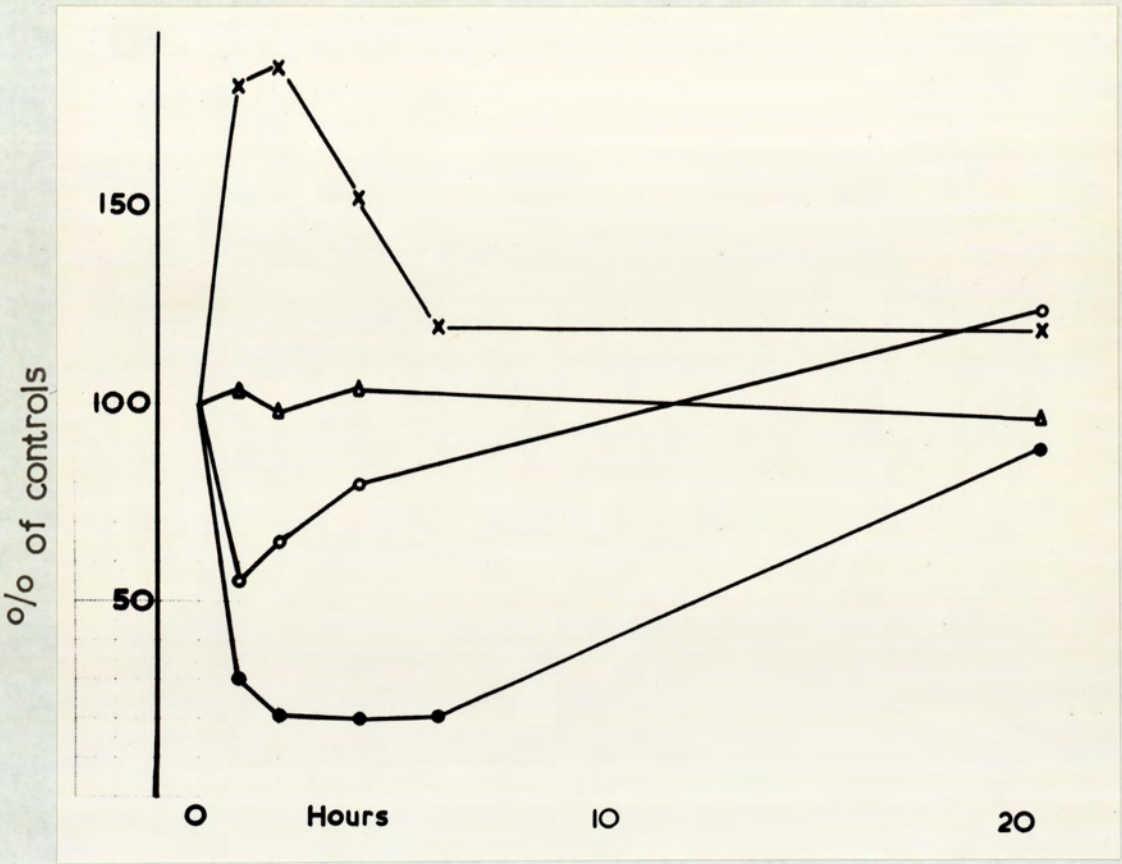

Fig. 31 Conscious cat blood pressure. Effect of bethanidine (3mg/kg intravenously) on the pressor response to angiotensin, 50ng/kg (triangles), noradrenaline, 200ng/kg (crosses), McN-A-343, 15μg/kg (closed circles) and tyramine, 50μg/kg (open circles). All points plotted are the mean of three consecutive responses expressed as a mean of the control responses.

Results taken from three cats.

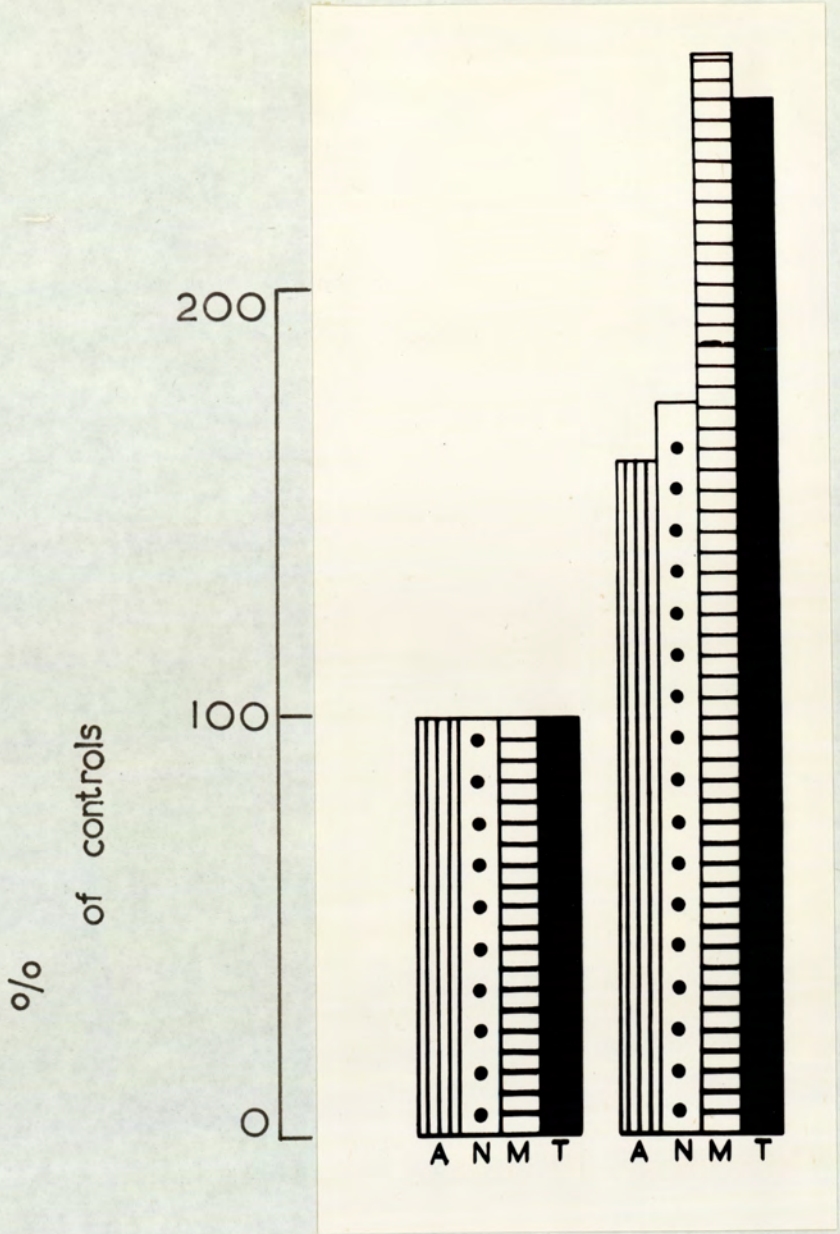

Fig. 32 Conscious cat blood pressure. Effect of pempidine (5mg/kg intravenously every 8 hours for 32 hours) on the pressor responses to angiotensin, 50ng/kg at A, noradrenaline, 200ng/kg at N, McN-A-343, 15μg/kg at M and tyramine, 50μg/kg at T. The left hand histogram shows the responses prior to pempidine expressed as 100% and the right hand histogram the responses after pempidine expressed as a % of their controls. The results are the mean of the findings in three cats.

but angiotensin had no effect on the heart rate.

Effect of blockade of adrenergic α- receptors by phentolamine

Phentolamine (3.5mg/kg intravenously) caused a small lowering of the resting blood pressure and an immediate tachycardia: the heart rate reaching as much as 300 beats per minute. The tachycardia did not persist and the heart rate returned to normal after about 10-15 minutes. Phentolamine also caused complete relaxation of the nictitating membranes.

Fig.33 shows the effect of phentolamine on the responses to angiotensin, tyramine, noradrenaline and McN-A-343. The responses to noradrenaline and tyramine were abolished by phentolamine and there was a marked reduction of the responses to McN-A-343. Phentolamine did not, however, cause any reduction of the responses to angiotensin. Recovery of the blocked responses to the other pressor substances was complete after about 10 hours.

Effect of blockade of adrenergic β-receptors by propranolol

Propranolol (1mg/kg intravenously) caused a fall of about 20 beats per minute in the resting heart rate but was without effect on the resting blood pressure and on the nictitating membranes.

The effect of propranolol on the responses to angiotensin, tyramine noradrenaline and McN-A-343 is shown in Fig.34. The responses to angiotensin, noradrenaline

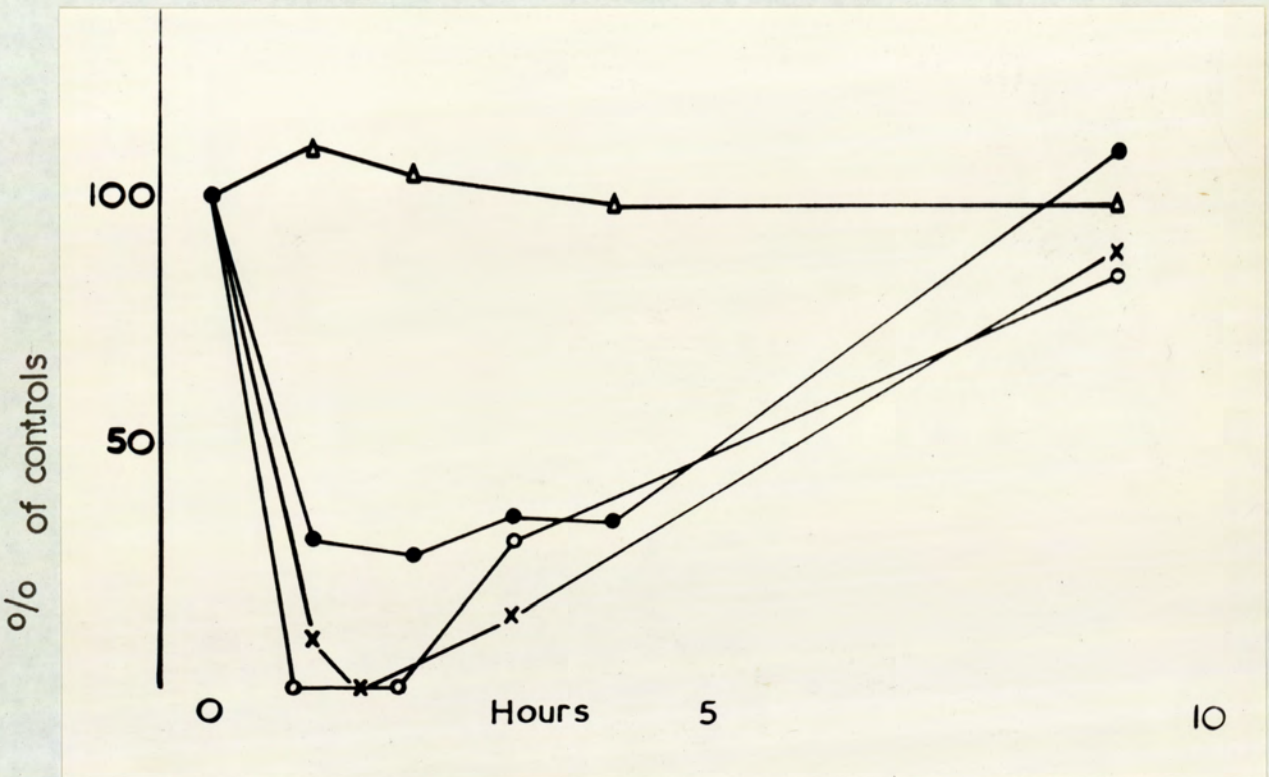

Fig. 33 Conscious cat blood pressure. Effect of phentolamine (3.5 mg/kg intravenously) on the pressor responses to angiotensin, 50ng/kg (triangles), noradrenaline, 200ng/kg (crosses), McN-A-343, 15μg/kg (closed circles) and tyramine, 50μg/kg (open circles). All points plotted are the mean of three consecutive responses expressed as a percentage of their controls.

Results taken from three cats

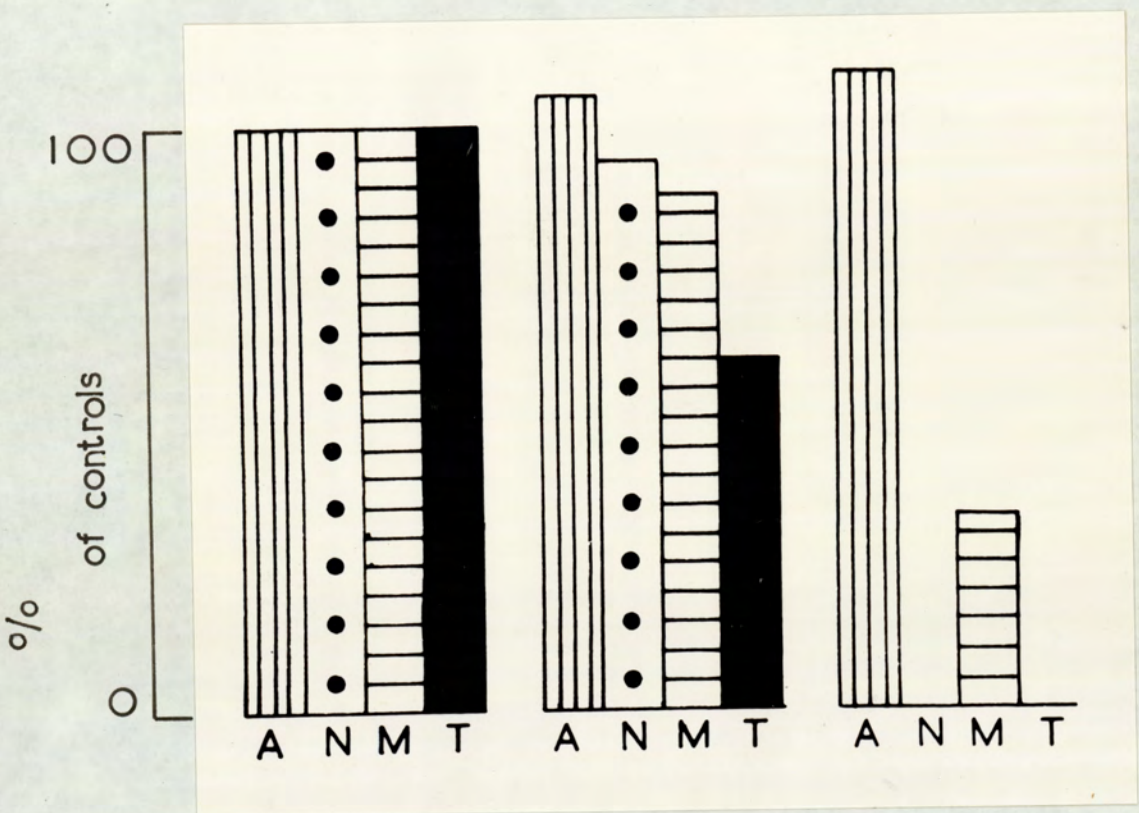

Fig. 34 Conscious cat blood pressure. Histogram showing the effects of α- and β-adrenergic receptor blockade on the pressor responses to angiotensin, 50ng/kg (A), noradrenaline, 200ng/kg (N), McN-A-343, 15μg/kg (M) and tyramine, 50μg/kg (T). The left hand histogram shows the responses prior to drug administration expressed as 100%. In the centre histogram the responses are shown, expressed as a percentage of their controls, after propranolol (1mg/kg intravenously) and in the right hand panel after both propranolol (1mg/kg intravenously) and phentolamine (3.5mg/kg intravenously).

Results taken from three cats

and McN-A-343 were almost unaltered but the responses to tyramine were reduced by 40% after propranolol.

Effect of simultaneous blockade of adrenergic α- and β - receptors by phentolamine and propranolol

Phentolamine (3.5mg/kg) was given to cats after first establishing the effects of propranolol on pressor substances. Fig.34 shows the effect of combined phentolamine and propranolol treatment on the responses to angiotensin, tyramine, noradrenaline, and McN-A-343. This treatment abolished the responses to noradrenaline and tyramine and markedly reduced the responses to McN-A-343. The responses to angiotensin were unaffected.

Effect of inhibition of neuronal noradrenaline uptake by desipramine

Desipramine (2.5mg/kg intravenously) had no effect on resting blood pressure, heart rate, behaviour or on the nictitating membranes.

The effect of desipramine on the pressor responses to angiotensin, noradrenaline, tyramine and McN-A-343 is shown in Fig.35. Desipramine abolished the responses to tyramine and greatly enhanced the responses to noradrenaline. The responses to angiotensin were slightly enhanced and those to McN-A-343 were slightly reduced. All the responses, except those to tyramine, had recovered after 12 hours.

Desipramine (2.5mg/kg) in cats pretreated with reserpine (100μg/kg intravenously 24 hours before desipramine) caused an enhancement of the responses to noradrenaline

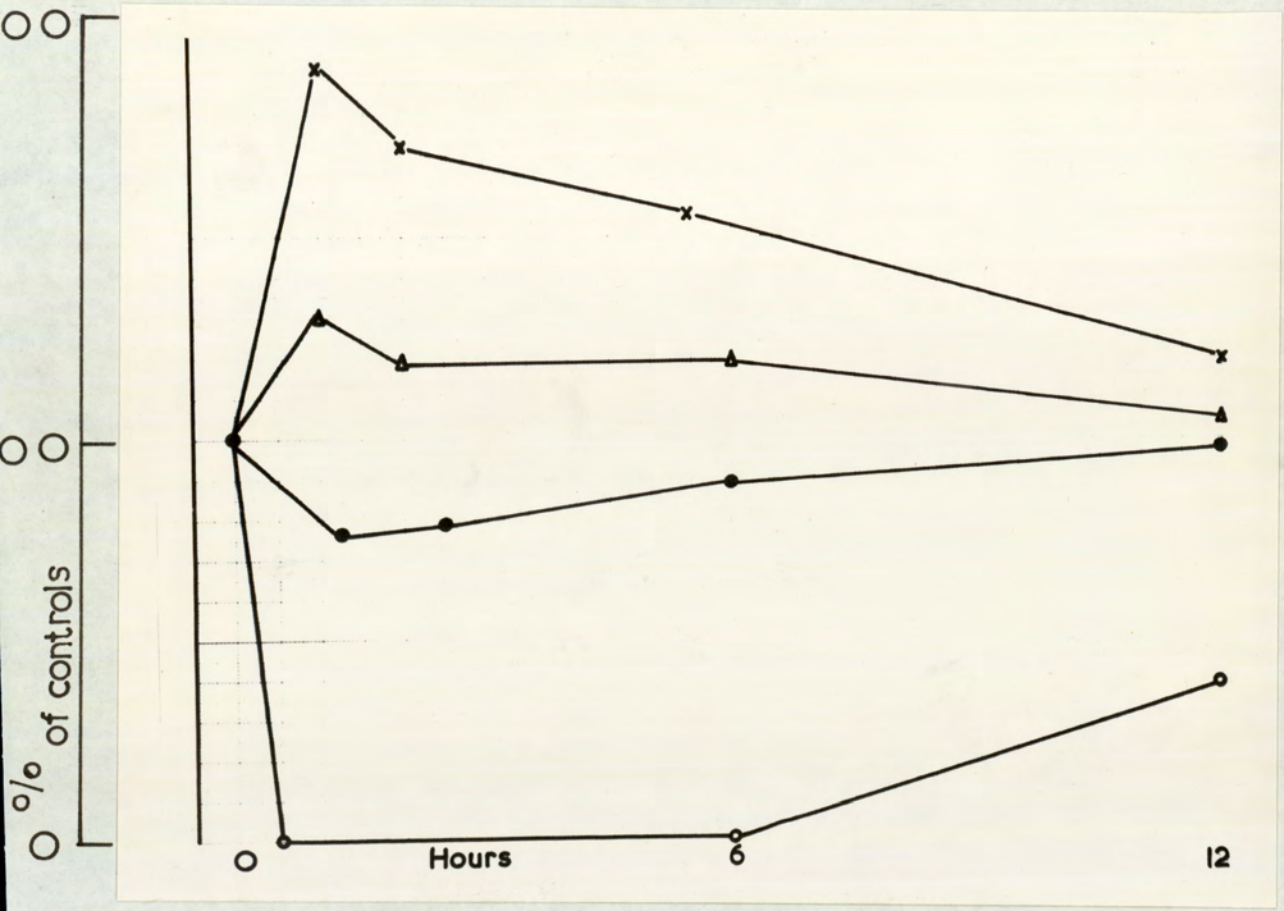

Fig.35 Conscious cat blood pressure. Effect of desipramine (2.5mg/kg intravenously) on the pressor responses to angiotensin, 50ng/kg (triangles), noradrenaline, 200ng/kg (crosses), McN-A-343, 15μg/kg (closed circles) and tyramine, 50μg/kg (open circles). All points plotted are the mean of three consecutive responses expressed as a percentage of the control responses.

similar to or slightly less than the enhancement in non-reserpinised cats. After reserpine, however, desipramine caused no increase in the pressor responses to angiotensin.

Discussion.

The experiments described in this chapter were carried out to establish the role of neuronal noradrenaline in the acute angiotensin pressor response in conscious cats.

There are a number of ways in which noradrenaline at the sympathetic nerve endings could contribute to the acute angiotensin pressor responses.

Angiotensin has been widely reported to cause a systemic pressor response by an action within the central nervous system (see for example Bickerton & Buckley, 1961). This action is probably mediated by the peripheral sympathetic nervous system. Angiotensin has also been reported to stimulate sympathetic ganglia and facilitate ganglionnic transmission (Haefely et al, 1965). Either of these actions of angiotensin might be expected to elicit a systemic pressor responses mediated by release of noradrenaline from the sympathetic nerve endings. The responses to angiotensin were not, however, reduced by bethanidine or by pempidine or by blockade of adrenergic receptor sites suggesting that the sympathetic component of the angiotensin response was independent of central stimulation or of stimulation of sympathetic ganglia.

Angiotensin stimulates the release of catecholamines from the adrenal medulla of many species (see review by

Vane, 1969). In Chapter 2 of this section it was suggested that the adrenal medulla only contributed to the pressor response after large doses of angiotensin. The results obtained in this chapter also indicate that there is no adrenal medullary component in the response to angiotensin at a dose level of 50ng/kg. Thus, a mixture of phentolamine and propranolol did not reduce the pressor responses to angiotensin and in pempidine treated cats angiotensin, unlike noradrenaline, did not cause tachycardia. Release of catecholamines from the adrenal medulla following the administration of angiotensin might be expected to cause some tachycardia.

The significance of the results obtained with desipramine is not clear. The simplest interpretation of the increased responses to angiotensin after desipramine is that angiotensin causes the release of neuronal noradrenaline . After release the noradrenaline is normally metabolised by being rebound into the neuronal stores. Desipramine prevents this rebinding and consequently enhances the pressor response to angiotensin. The failure of desipramine to potentiate angiotensin in reserpine treated animals supports this idea.

The role of noradrenaline released by angiotensin is not clear. The failure of phentolamine and propranolol, in combination, to reduce the responses to angiotensin indicates that noradrenaline released by angiotensin does not contribute to the angiotensin pressor responses by an action on α- or β-adrenergic receptors. Thus, it appears

that angiotensin releases noradrenaline from neuronal storage sites but that the noradrenaline contributes to the angiotensin pressor response by an action independent of α- and β-adrenergic receptors. This suggests the possibility of receptors other than those designated as α- or β- on which noradrenaline might act and by which it contributes to the angiotensin pressor responses. The nature of such hypothetical receptor sites is undetermined.

A further possible mechanism by which noradrenaline could contribute to the pressor action of angiotensin without acting on α- or β-adrenergic receptor sites is to influence the metabolism of angiotensin. Depletion of noradrenaline reduces the responses to angiotensin whilst increasing neuronal noradrenaline levels increases the responses to noradrenaline. Angiotensin is known to be metabolised by angiotensinases. It is possible that noradrenaline slows the metabolism of angiotensin and thus high neuronal noradrenaline levels reduce the normal rate of angiotensin metabolism and consequently increase the responses to angiotensin. Conversely depletion of noradrenaline increases the rate of metabolism of angiotensin and consequently reduces its pressor action. No studies of the influence of noradrenaline on angiotensin metabolism have been made.

The results described in this chapter fail to establish the role of noradrenaline in the acute pressor response to angiotensin. The results described eliminate a number of possible roles and although two possible mechanisms have been suggested they are very hypothetical.

SECTION 4. THE EFFECT OF SOME BIVALENT METAL CHELATING COMPOUNDS ON THE PRESSOR RESPONSES TO ANGIOTENSIN AND NORADRENALINE IN PITHED RATS AND ANAESTHETISED CATS.

Chapter 1.

The anti-angiotensin action of sodium diethyl-dithiocarbamate (DDC).

Schwyzer (1963) found that angiotensin formed amorphous precipitates with Cu^{++} and Zn^{++} and postulated that this precipitate might be the active pressor form of angiotensin. This hypothesis was supported by the finding of Gascon & Walaszek (1966) that osajin, an isoflavone derivative which also forms complexes with Cu^{++} and Zn^{++} specifically antagonised the contractile action of angiotensin on guinea-pig isolated ileum. Osajin, however, was subsequently shown to possess no *in vivo* anti-angiotensin activity (Walaszek, personal communication).

Since sodium diethyl-dithiocarbamate (DDC) is known to chelate bivalent metal ions (see review by Goldstein, 1966) this substance has been examined as a potential antagonist of the pressor action of angiotensin in pithed rats and anaesthetised cats.

Results.

Effect of DDC on pressor responses to angiotensin and noradrenaline in pithed rats.

DDC (5-25 mg/kg intravenously) caused a small pressor response and initially enhanced the pressor responses to

angiotensin (2-50ng) and to noradrenaline (5-50ng). The enhancement was usually maximal after about 1 hour and although the extent of the enhancement varied from experiment to experiment the responses to angiotensin and noradrenaline were usually increased by up to 50% of their control height and duration. Fig.36 illustrates the enhancement of the responses to angiotensin and noradrenaline in the pithed rat after DDC. The enhancement was followed by a slow decline in the responses to both angiotensin and noradrenaline but the responses to angiotensin declined more rapidly than did those to noradrenaline such that 2-3 hours after DDC the angiotensin responses were often abolished whilst those to noradrenaline were either slightly enhanced, unaffected or reduced by up to 50% of their control size. Fig.37 illustrates an experiment in which the responses to angiotensin were abolished after DDC whilst the responses to noradrenaline were enhanced. The changes in the responses to both angiotensin and noradrenaline after DDC were similar at all doses of these pressor agents tested. Thus there was an initial increase in the responses to both angiotensin and noradrenaline followed by a decline in the responses at all doses.

DDC (5-25 mg/kg intravenously) affected the pressor responses to electrical stimulation of the spinal sympathetic outflow in the same way as it did the responses to injected noradrenaline thus causing an initial enhancement followed by a small decline.

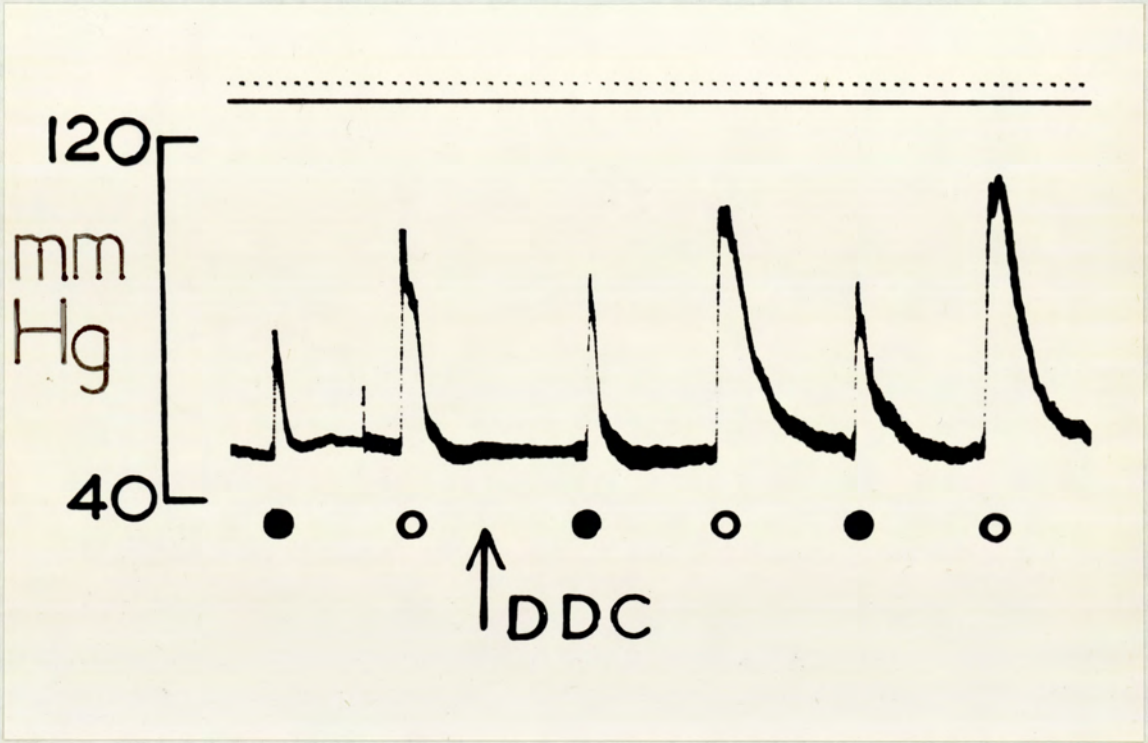

Fig.36 Pithed rat blood pressure preparation. Enhancement of the pressor responses to noradrenaline, 20ng (closed circles), and to angiotensin, 10ng (open circles) after DDC, 10mg/kg intravenously. Time scale in minutes.

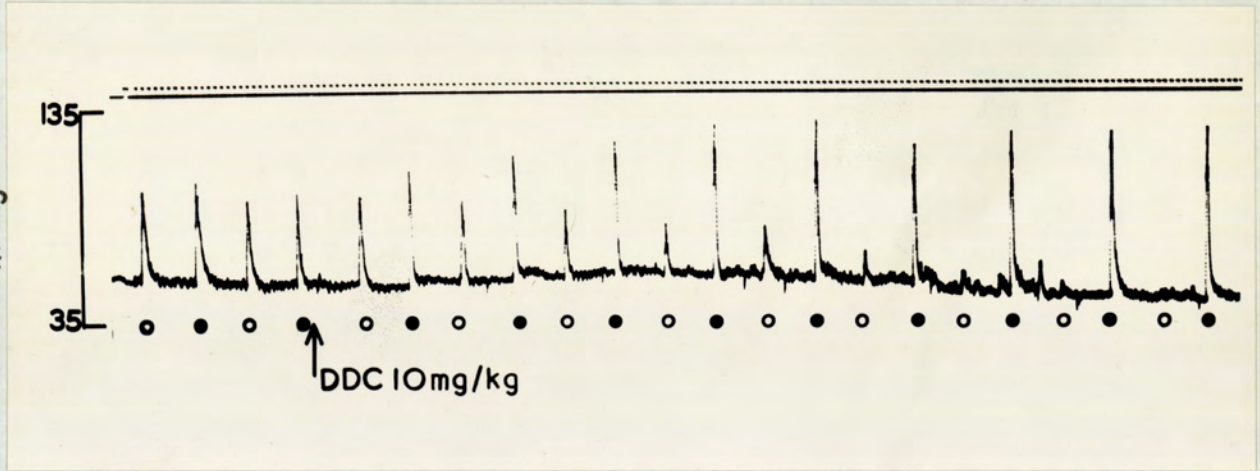

Fig. 37 Pithed rat blood pressure preparation. Effect of DDC, 10mg/kg intravenously, on the pressor responses to angiotensin, 5ng (open circles), and to noradrenaline, 20ng (closed circles). The responses to angiotensin were abolished whereas the responses to noradrenaline were enhanced. Time scale in minutes.

At doses in excess of 25mg/kg, DDC caused an initial enhancement of the responses to angiotensin, noradrenaline and stimulation of the spinal sympathetic outflow. This enhancement of the responses was then followed by a decline which was more rapid than the decline following smaller doses of DDC, this being especially true for the angiotensin responses.

Pretreatment of rats with reserpine (5mg/kg intraperitoneally 18 hours before pithing) had little or no effect on the pressor responses to angiotensin (2-50ng) or noradrenaline (5-50ng) prior to DDC. In these rats however, the initial enhancement and subsequent decline of the responses to angiotensin was accelerated in onset whereas the effect of DDC on the responses to noradrenaline was unaltered by reserpine.

Removal of the adrenal glands 30 minutes before pithing did not alter the responses to angiotensin or noradrenaline although the resting blood pressure was usually lower in these rats than those with intact adrenal glands. In adrenalectomised rats the resting blood pressure fell slowly after the administration of DDC (5-25mg/kg) and the responses to angiotensin and noradrenaline declined as the blood pressure fell. Reduction of the dose of DDC to 1mg/kg slowed the fall of the blood pressure after DDC but the pressor responses to angiotensin and noradrenaline nevertheless declined slowly as the blood pressure fell.

Effect of DDC on pressor responses to angiotensin and noradrenaline in anaesthetised cats.

In anaesthetised cats DDC (10-50mg/kg intravenously) enhanced the pressor responses to angiotensin (50-100ng/kg) and noradrenaline (200ng/kg). The enhancement was maximal 1-2 hours after DDC. The extent of the enhancement varied from experiment to experiment but was usually about 50% in both height and duration. The responses to both pressor agents subsequently declined slowly until they returned to control size but did not undergo further reduction.

Adrenalectomy was carried out in anaesthetised cats by one of three methods. In all cases the blood supply to and from the adrenal glands was dissected free of surrounding tissues. In some experiments ligatures were then tied tightly around these vessels and the adrenal glands were removed. In other experiments after tying ligatures around the vessels to and from the adrenal glands the glands were left in situ. In the third group of experiments the vessels were not ligatured but the adrenal glands were isolated from the circulation by the application of artery forceps to the vessels when required.

Adrenalectomy caused no reduction in the pressor responses to angiotensin at doses of 50ng/kg or less but did slightly reduce the pressor response to larger doses as shown in Fig.38A. After adrenalectomy the resting

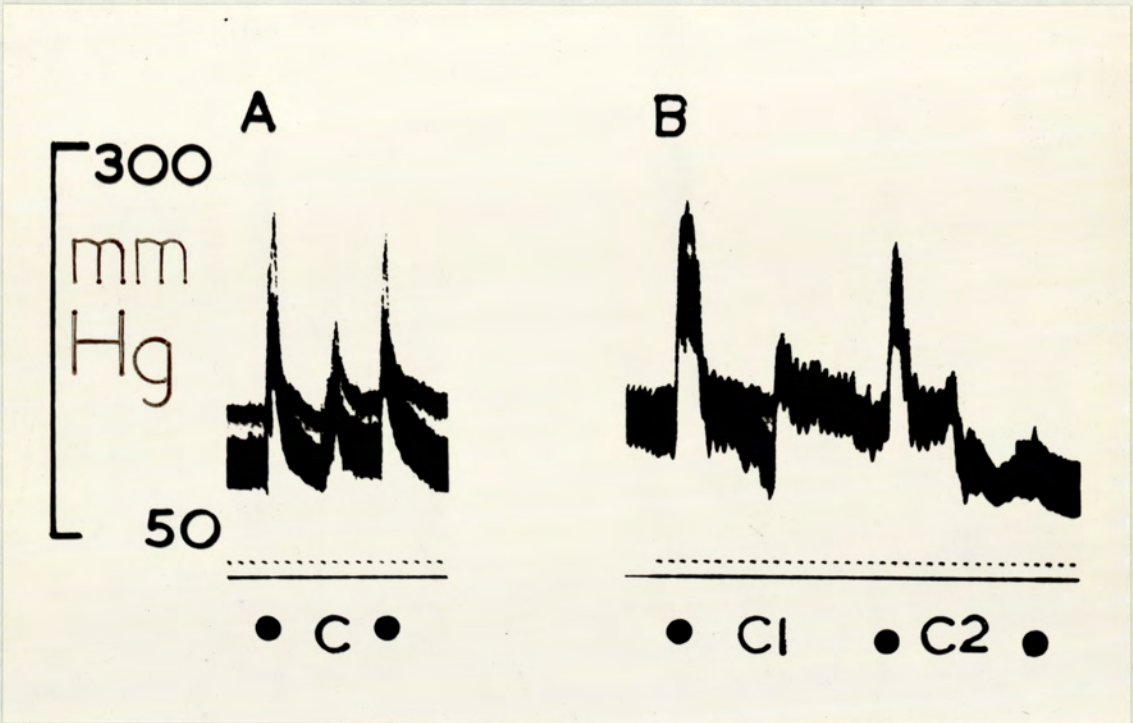

Fig.38 Anaesthetised cat blood pressure preparation. The left hand panel A shows the effect of isolation of both adrenal glands from the circulation on the pressor response to angiotensin, 100ng/kg at the closed circles, prior to the administration of DDC.
B shows the reduction in the pressor responses to angiotensin after adrenalectomy in a cat treated with DDC, 20mg/kg intravenously, 2 hours before adrenalectomy. At C1 the blood vessels to and from one adrenal gland were ligatured and at C2 the other adrenal gland was isolated from the circulation by a similar ligature. Time scale in minutes.

blood pressure usually declined very slowly over the course of 1-4 hours and the responses to angiotensin and noradrenaline showed a similar decline. In 2 experiments out of 9, however, there was no decline in the resting blood pressure after adrenalectomy. In these two experiments DDC (20mg/kg intravenously) caused an initial enhancement of the responses to angiotensin and noradrenaline. This enhancement was then followed by a decline in the responses to both substances. The decline in the responses to angiotensin was more rapid than the decline of the noradrenaline responses and in both experiments the responses to angiotensin were reduced to less than 50% of their controls when the noradrenaline responses were similar to the controls. This is illustrated by Fig.39.

In the experiments where the adrenal blood supply was clamped but the adrenal glands left in situ the responses to angiotensin were unaltered or slightly reduced by clamping prior to DDC. Similar clamping of the adrenal glands 3-4 hours after the administration of DDC reduced the responses to angiotensin by up to 80% of the control responses. Fig.38B illustrates an experiment in which clamping of the blood supply to each of the adrenal glands caused a marked reduction in the response to angiotensin.

Pretreatment with reserpine (0.25mg/kg/day intraperitoneally for 3 days or 0.8 mg/kg intraperitoneally 18 hours prior to the experiment) reduced the pressor

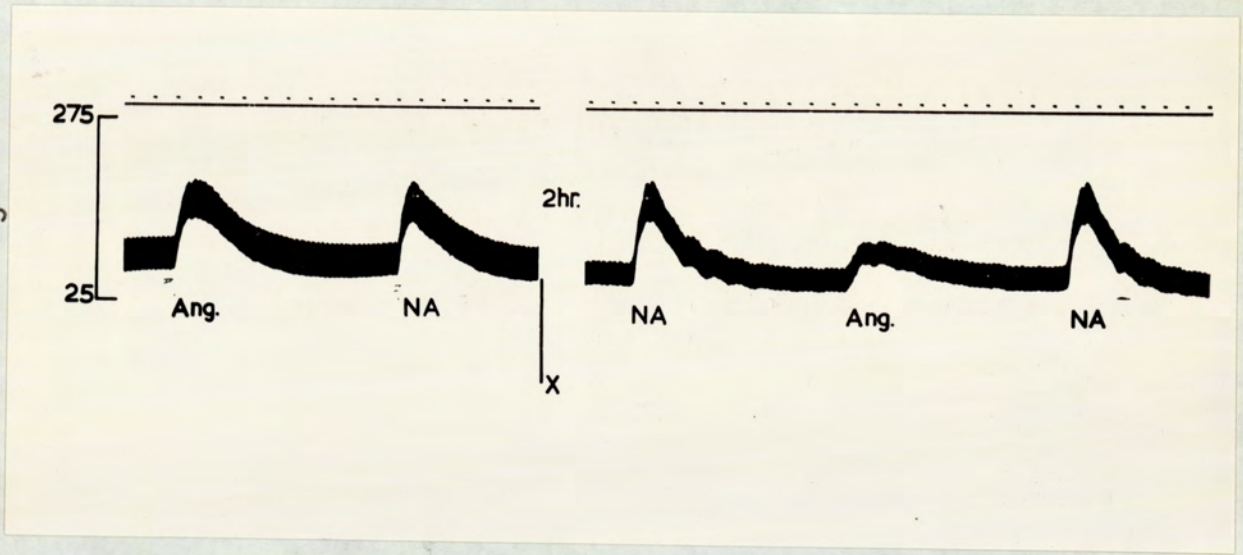

Fig.39 Anaesthetised cat blood pressure preparation. The anti-angiotensin action of DDC in an adrenalectomised, anaesthetised cat. Responses to angiotensin, 50ng/kg, at Ang and to noradrenaline, 200ng/kg, at NA are shown after adrenalectomy but prior to administration of DDC. Two hours after the administration of DDC, 20mg/kg intravenously, the responses to angiotensin were reduced but those to noradrenaline were unaltered. Time scale in minutes.

responses to angiotensin (50-100ng/kg) but did not alter the responses to noradrenaline (200ng/kg). For these experiments a dose of 200ng/kg of angiotensin was used. The results obtained in these experiments were essentially similar to those experiments in non-treated cats.

A mixture of phentolamine (3.5mg/kg) and propranolol (2.5mg/kg) administered intravenously abolished the pressor actions of injected adrenaline and noradrenaline and caused a small increase in the responses to angiotensin. In these cats DDC (25mg/kg intravenously) caused an initial enhancement of the responses to angiotensin and noradrenaline. The responses to both pressor agents then returned to their control size.

Discussion.

Schwyzer (1963) suggested that angiotensin might complex with Cu^{++} or Zn^{++} *in vivo* to produce its active pressor form; it might therefore be expected that the pressor action of angiotensin would be prevented by the lack of these ions for complex formation. DDC is known to chelate bivalent metal ions *in vivo*. This property of DDC has been employed in pharmacological studies as chelation of Cu^{++} inhibits the enzyme dopamine-β-hydroxylase (see review by Goldstein, 1966).

The immediate effect of DDC on the pressor responses to angiotensin and noradrenaline, in both pithed rats and anaesthetised cats, was to enhance these responses. The

extent of the enhancement varied from experiment to experiment but was usually of the order of a 50% increase in both height and duration of the responses. Enhancement of responses to noradrenaline after Cu^{++} chelation has been frequently reported (see review by Chaberek & Martell, 1959) and was confirmed in the present experiments. The mechanism of the enhancement of the responses to angiotensin is not clear. In rats pretreated with reserpine the responses to angiotensin show little enhancement after DDC suggesting that the enhancement of the angiotensin responses by DDC involves noradrenaline. In cats, however, reserpine did not interfere with the enhancement of angiotensin responses after DDC.

In anaesthetised cats with intact adrenal glands DDC caused no reduction in the responses to angiotensin but in adrenalectomised cats and pithed rats there was evidence that DDC antagonised the pressor action of angiotensin at a time when the pressor responses to noradrenaline were unaffected. In the majority of cats in which adrenalectomy was carried out prior to the administration of DDC the resting blood pressure declined slowly after DDC and the pressor responses to both angiotensin and noradrenaline showed a parallel decline. In a minority of experiments the resting blood pressure remained normal after adrenalectomy and in these experiments DDC specifically antagonised the pressor action of angiotensin. Acute adrenalectomy prior to the administration of DDC was either

without effect or caused a small reduction of pressor responses to angiotensin. After DDC, however, acute adrenalectomy caused a very marked reduction in the responses to angiotensin. It would thus appear that DDC had a dual action on angiotensin pressor responses in anaesthetised cats. On the one hand DDC reduced the responses to the "direct" effects of angiotensin but also greatly enhanced the adrenal medullary component of the responses. In cats with intact adrenal glands these two opposing actions of DDC appeared to cancel one another but after adrenalectomy only antagonism of the "direct" action of angiotensin was seen. Alternative means of removing the adrenal component of the angiotensin responses were tried since adrenalectomy proved too drastic for prolonged survival of the majority of cats after DDC. Pretreatment with reserpine to deplete adrenal catecholamines, however, did not reveal the anti-angiotensin action of DDC and neither did the use of adrenergic receptor blocking drugs sufficient to abolish the cardiovascular actions of adrenaline and noradrenaline.

The mechanism of the anti-angiotensin action of DDC in pithed rats and adrenalectomised, anaesthetised cats is not certain. Chelation of Cu^{++} and/or Zn^{++} would seem the most likely explanation of this action. Chelation of Cu^{++} by DDC is known to inhibit the enzyme dopamine-β-hydroxylase and thus interfere with the synthesis of noradrenaline. It would seem unlikely that inhibition of this enzyme contributes to the anti-angiotensin action of DDC.

Inhibition of dopamine-β-hydroxylase usually requires the administration of larger doses of DDC (Goldstein, 1966) and at a time when the responses to angiotensin were abolished in the pithed rat the responses to electrical stimulation of the spinal sympathetic outflow (Gillespie & Muir, 1967) were unaltered or only slightly reduced suggesting no interference with neuronal noradrenaline stores.

An alternative method by which chelation of Cu^{++} and/or Zn^{++} ions could antagonise the pressor action of angiotensin has been suggested by Schwyzer (1963). He postulated that angiotensin might complex with these ions to produce its active pressor form. Chelation of these ions by DDC would lead to their becoming inaccessible for complex formation with angiotensin and consequently production of the active form of angiotensin would be prevented.

The next chapter describes the results obtained from experiments in which other bivalent metal ion chelating compounds were examined for anti-angiotensin action in order to examine the hypothesis that antagonism of angiotensin pressor activity follows chelation of Cu^{++} and/or Zn^{++}.

Chapter 2.

The effect of penicillamine, colchicine and ascorbic acid on pressor responses to angiotensin and noradrenaline in pithed rats.

In the previous chapter DDC was shown to antagonise the pressor action of angiotensin in the pithed rat and in adrenalectomised cats. This action of DDC was thought to be mediated by chelation of Cu^{++} and/or Zn^{++}. This chapter describes some experiments in which penicillamine, colchicine and ascorbic acid, three compounds which chelate bivalent metal ions *in vivo*, were also tested as potential antagonists of angiotensin.

Results.

1. Penicillamine.

Penicillamine (1-100mg/kg intravenously) caused an increase in the pressor responses to both angiotensin (5-20ng) and to noradrenaline (10-50ng). This enhancement was almost immediate in onset and varied in extent from experiment to experiment but was usually of the order of an increase of 50-100% in height and a smaller increase in duration. The enhancement of these responses usually persisted for about 2 hours and the responses then returned to their control size. Increasing the dose of penicillamine to 1 g/kg caused a widening of the pulse pressure, which was due entirely to an increase in the systolic pressure, and a slight reduction in the pressor responses to both angiotensin and noradrenaline.

2. Ascorbic acid.

Ascorbic acid (1-500mg/kg intravenously) caused an increase in the pulse pressure by increasing the systolic blood pressure and caused a marked enhancement of the responses to both angiotensin and noradrenaline. This enhancement was almost immediate in onset and when maximal after a dose of 500mg/kg was of the order of a 500% increase in both height and duration. The enhancement of the responses to both angiotensin and noradrenaline persisted for the duration of all experiments.

3. Colchicine.

Doses of colchicine of less than 100µg/kg administered intravenously were without effect on the responses to angiotensin and noradrenaline. In larger doses colchicine reduced the responses to angiotensin and to noradrenaline. At a dose of 1mg/kg the reduction was small and only transient but increasing the dose of colchicine increased both the extent and duration of the reduction of the responses to both pressor agents and doses in excess of 25mg/kg abolished the responses to angiotensin and noradrenaline and these did not subsequently recover.

Discussion.

In the previous chapter DDC was reported to specifically antagonise the pressor action of angiotensin in pithed rats. It seemed possible that this action of DDC might be mediated by its ability to chelate bivalent metal ions

(Goldstein, 1966). This hypothesis was based on the suggestion of Schwyzer (1963) that angiotensin complexed with Cu^{++} and/or Zn^{++} *in vivo* to take its active form. Chelation of these ions would prevent such a complex and thus prevent production of the active pressor form of angiotensin. In this chapter three drugs known to chelate bivalent metal ions (Chaberek & Martell, 1959) have been examined as potential antagonists of angiotensin. None of these drugs showed specific anti-angiotensin activity although colchicine did reduce the responses to both angiotensin and noradrenaline. The action of colchicine would appear to be independent of chelation of bivalent metal ions since it caused no increase in the responses to noradrenaline as occurred in the experiments with the other chelating agents and which has been well documented for chelating agents (see review by Chaberek & Martell, 1959).

As neither penicillamine nor ascorbic acid showed any anti-angiotensin action at any dose but caused a large enhancement of the pressor responses to both angiotensin and noradrenaline it would appear that the anti-angiotensin activity of DDC was independent of chelation of bivalent metal ions. Chelation of these ions is known to enhance the responses to noradrenaline and the large enhancement of these responses in the experiments with penicillamine and ascorbic acid suggests that these substances were acting as chelating agents. The enhancement of the responses to noradrenaline after DDC also suggests that chelation of bivalent metal ions followed the administration of DDC

but the subsequent decline of the responses to both pressor agents, especially the responses to angiotensin, suggests that DDC might exert a dual action. It may be that DDC initially exerted the usual action of a chelating agent which is to enhance the responses to both angiotensin and noradrenaline but in addition DDC antagonised the pressor action of angiotensin. Pretreatment with reserpine reduced the enhancement of the angiotensin responses and accelerated the onset of the anti-angiotensin in pithed rats. In cats the anti-angiotensin action of DDC was only revealed after adrenalectomy. The contribution of the adrenal medulla to the angiotensin response in cats was small, prior to the administration of DDC but was subsequently increased. This increase was probably due, in part at least, to potentiation of the responses to catecholamines from the adrenal medulla after chelation of bivalent metal ions.

The anti-angiotensin action of DDC would thus appear to be independent of its ability to chelate bivalent metal ions which in fact obscures its action as an atangonist of angiotensin in cats. The mode of action of DDC as an angiotensin antagonist remains to be determined but it would seem possible that if this action of DDC could be separated from its chelating action the development of a specific antagonist might be possible.

GENERAL DISCUSSION.

GENERAL DISCUSSION.

The action of angiotensin at sympathetic nerve endings has been widely studied in recent years. The first parts of this Thesis are concerned with further studies into the nature of this interaction.

Angiotensin would appear to interact with the sympathetic nerve endings in several ways. Thus there is considerable evidence that angiotensin specifically increases the responses of tissues to noradrenaline released from peripheral sympathetic nerves in both whole animals (McCubbin & Page, 1963a,b) and in isolated tissues (Benelli et al,1964; Su, 1965: Thoenen et al, 1965). Second, in some vascular beds the vasoconstrictor action of angiotensin is dependent on an intact sympathetic innervation and is greatly reduced by procedures abolishing sympathetic tone. This has been demonstrated in isolated vascular muscle/sympathetic nerve preparations (Zimmerman,1962; Laverty, 1963), in whole animals (McGiff & Fasy,1964:1965) and in human studies (Johnsson et al, 1965; Scroop & Whelan,1966;1968; Henning & Johnsson,1967). Finally, intravenous infusions of noradrenaline or substances which release neuronal noradrenaline e.g. indirect sympathomimetics or ganglion stimulants enhance the pressor responses to intravenous injections of angiotensin (Haas & Goldblatt,1959; Schmitt & Schmitt,1967a,b: Pals & Fulton,1968).

In experiments using the rabbit isolated ear artery preparation it was shown that the vasoconstrictor action of angiotensin is weak in the absence of sympathetic tone

but is greatly enhanced if administered during sympathetic stimulation. The action of angiotensin was similarly enhanced during infusion of indirect sympathomimetic amines but not during infusion of noradrenaline. Drugs which prevent the release of noradrenaline from sympathetic nerve endings or drugs which block the adrenergic α-receptors abolish the enhancement of the vasoconstrictor action of angiotensin during sympathetic stimulation. The simplest interpretation of these results is that angiotensin normally releases noradrenaline from sympathetic nerve endings and owes part of its vasoconstrictor action to this release. The release of noradrenaline by other procedures (for example sympathetic nerve stimulation) may facilitate or be facilitated by angiotensin. This hypothesis is supported by a number of observations. First, tyramine, a substance known to produce its vasoconstrictor action through release of endogenous noradrenaline, is also potentiated during sympathetic stimulation. Second, this hypothesis accounts for the fact that exogenous noradrenaline does not enhance the angiotensin vasoconstriction. Third, in some preparations the response to angiotensin during sympathetic stimulation consisted of two separate peaks; presumably the first was due to angiotensin itself and the second to noradrenaline released by angiotensin. The enhanced single response seen in other experiments are likely to have been due to the superimposition of the two peaks. Finally, phentolamine, which acts by blocking α-receptors, prevented the sympathetic enhancement of angiotensin presumably by preventing the vasoconstrictor action of the noradrenaline released by angiotensin.

Facilitation of the release of noradrenaline would also explain the widely reported enhancement of the responses of tissues to sympathetic stimulation caused by angiotensin (see Introduction for references). Angiotensin is usually reported to specifically enhance responses mediated by endogenous noradrenaline although there are reports of potentiation of the responses to exogenous noradrenaline by angiotensin (Panisset & Bourdois,1967: 1968a,b.c,; Schmitt & Schmitt,1967a,b). Enhancement of the responses to endogenous noradrenaline by angiotensin was observed only occasionally and then only transiently in experiments using the rabbit isolated ear artery preparation. As angiotensin also simultaneously reduced the responses to injected noradrenaline it seems likely that angiotensin has a non-specific desensitising action on this tissue.

Angiotensin selectively potentiated the pressor responses to endogenous noradrenaline in the pithed rat preparation. The action of angiotensin appeared to occur mainly at the sympathetic nerve endings. The potentiation of the responses to endogenous noradrenaline persisted in the majority of experiments for some hours after the completion of the angiotensin infusion. The failure of angiotensin to cause a simultaneous enhancement of the pressor responses to exogenous noradrenaline adds further evidence to the hypothesis that release of noradrenaline by other procedures may be facilitated by angiotensin. If the action of angiotensin was mediated by inhibition of the metabolism of noradrenaline e.g. by

inhibition of uptake into neuronal storage sites as first suggested by Palaic & Khairallah (1967a,b) interference with the metabolism of injected noradrenaline would also be expected. An alternative hypothesis to explain the action of angiotensin at sympathetic nerve endings has been proposed by Pals & Fulton (1968). These workers postulated that there might be synergism between angiotensin and noradrenaline possibly at the adrenergic α-receptors. This hypothesis also fails to adequately explain the differential action of angiotensin on endogenous and exogenous noradrenaline although it does explain their observation, in the pithed rat, that the pressor responses to angiotensin are enhanced during intravenous infusion of noradrenaline. The potentiation of the responses to angiotensin by infusions of noradrenaline has been reported by Schmitt & Schmitt (1967a,b) and by Pals & Fulton (1968) and these results lead to the hypothesis of Pals & Fulton (1968). The potentiation of responses to angiotensin during infusions of noradrenaline has been confirmed in the present experiments. The potentiation developed gradually with time and was associated with a parallel increase of the pressor responses to tyramine, a substance known to exert its pressor action by release of neuronal noradrenaline. The potentiation of the angiotensin responses during noradrenaline infusions was prevented by phentolamine but unaltered by pretreatment with desipramine. This would suggest that the enhancement of the angiotensin responses is mediated via adrenergic α-receptors. Pals & Fulton (1968)

have suggested that synergism occurs between angiotensin and noradrenaline at the α-adrenergic receptors. This hypothesis of Pals & Fulton provides an attractive explanation of the enhancement of the pressor responses to angiotensin during infusions of noradrenaline in the pithed rat. Unfortunately this hypothesis does not explain the majority of reported interactions between angiotensin and endogenous noradrenaline. Angiotensin has been frequently reported to interact with endogenous noradrenaline specifically in both whole animals (McCubbin & Page, 1963a,b) and in isolated tissues (Zimmerman,1962; Laverty,1963: Benelli et al,1964; Su,1965: Thoenen et al, 1965). In none of these reports was angiotensin found to interact with exogenous noradrenaline. If the interactions between angiotensin and noradrenaline were due simply to synergism at the adrenergic α-receptors it is unlikely that angiotensin would potentiate only endogenous noradrenaline in the majority of preparations.

The results of experiments in the rabbit isolated ear artery preparation and the earlier experiments in pithed rats showed an interaction between angiotensin and noradrenaline confined to endogenous noradrenaline. These results were explained by the hypothesis that angiotensin releases noradrenaline or facilitates its release by other procedures. The potentiation of pressor responses to angiotensin during infusions of noradrenaline in the pithed rat does not appear to support this hypothesis.

The responses to angiotensin and tyramine showed a similar enhancement during infusions of noradrenaline and both declined when the infusion was discontinued. This would suggest that angiotensin might release neuronal noradrenaline in a manner similar to the release caused by tyramine but the failure of desipramine to prevent the enhancement of responses to angiotensin during infusion of noradrenaline indicates that there is a difference between the mechanism of the enhancement of angiotensin and tyramine. The enhanced responses to tyramine probably followed an increase in the neuronal noradrenaline content associated with infusion of noradrenaline but as the responses to angiotensin were enhanced during infusion of noradrenaline in desipramine treated rat it is unlikely that an increased neuronal noradrenaline content explains the enhancement of responses to angiotensin.

Although it seems unlikely that the enhancement of angiotensin responses during noradrenaline infusions is due to increased release of neuronal noradrenaline the most probable explanation of the specific interaction between angiotensin and endogenous noradrenaline reported by many groups of workers (see Introduction for references) and in this Thesis is that angiotensin acts, in part, by increasing release of noradrenaline by other procedures. Angiotensin is known to exert a part of its action on the aortic strip preparation of a number of species by the release of noradrenaline from stores within the muscle wall (see for example Distler et al, 1965a,b). Angiotensin also releases

labelled noradrenaline from rabbit isolated atria (Beaulnes et al,1966) and stimulates the release of catecholamines from the adrenal medulla of many species (see Introduction for references).

Measurement of facilitation of the release of noradrenaline by angiotensin has proved difficult and has provided conflicting evidence. Sakuri & Hashimoto (1965) found that in rabbits infusions of angiotensin, unlike infusions of tyramine, caused no increase in the urinary excretion of catecholamines or their derivatives. Simultaneous infusion of both angiotensin and tyramine, however, led to a far greater excretion of catecholamines than did the infusion of tyramine alone. Zimmerman & Whitmore (1967) and Zimmerman & Giesslen (1968) found that the increased responses of the dog isolated hind-limb preparation and of the dog renal artery preparation in vivo respectively to sympathetic nerve stimulation caused by angiotensin were associated with an increased release of noradrenaline at the sympathetic nerve endings. In contrast to these observations Thoenen et al (1965) and Hertting & Suko (1966) found that the enhanced responses of the cat spleen to splenic nerve stimulation after angiotensin were not due to an increased release of noradrenaline at the splenic nerve endings.

The role of the sympathetic nervous system in the acute pressor response to injections of angiotensin has been studied in conscious cats Previous studies using drugs which impair peripheral sympathetic function have

suggested that the acute pressor action of angiotensin is independent of the sympathetic nervous system in anaesthetised animals and in a limited number of studies using conscious human subjects (see Introduction for references).

The experiments in conscious cats have shown clearly that depletion of peripheral neuronal noradrenaline reduces the acute pressor response to intravenous angiotensin. Pretreatment with both reserpine and syrosingopine reduced the pressor potency of angiotensin by up to 50% but tetrabenazine, which depletes only central amines (Brodie,1960) did not reduce the pressor potency of angiotensin. Restoration of the peripheral neuronal noradrenaline stores led to a recovery of the angiotensin responses and in normal cats increasing the neuronal noradrenaline content by treatment with a monoamine oxidase inhibiting drug increased the pressor potency of angiotensin.

The maximal reduction of the responses to angiotensin following the depletion of peripheral noradrenaline was about 50% suggesting that the remaining 50% of the angiotensin response was independent of noradrenaline.

Sedvall (1964) and Sedvall & Thorsen (1965) have shown that in anaesthetised cats large doses of reserpine actively deplete the majority of the noradrenaline at the sympathetic nerve endings but that the noradrenaline released in response to nerve impulses is not actively depleted by reserpine and only disappears as a result of its displacement by nerve impulses and the blockade of

re-uptake by reserpine. In the experiments described in this Thesis cats were pretreated with pempidine to abolish activity along the post-ganglionnic sympathetic nerves. This treatment was expected to protect the noradrenaline released by nerve impulses whilst permitting the depletion of the majority of the noradrenaline. Surprisingly, it was found that in conscious cats treated with pempidine the block of the responses to nerve stimulation by reserpine was accelerated. This acceleration was presumably due to pempidine. The depletion of the majority of the noradrenaline by reserpine, assessed by the pressor responses to tyramine, was not altered by pretreatment with pempidine. In these experiments the responses to angiotensin showed a similar reduction and subsequent recovery, in terms of time, as did the responses to nerve stimulation (McN-A-343). This suggests that the noradrenaline content of the pool from which nerve impulses release noradrenaline determines the pressor potency of angiotensin in conscious cats. In addition to the results in cats treated with reserpine after ganglion blockade there is other evidence implicating the pool from which nerve impulses release noradrenaline, rather than the larger pool, with the pressor action of angiotensin. In normal cats the recovery of the responses to angiotensin after reserpine treatment closely resembled the recovery of the responses to McN-A-343 and preceded the recovery of the responses to tyramine by some days. In experiments in which syrosingopine was used to deplete peripheral noradrenaline the responses to McN-A-343 were more rapidly

reduced than were the responses to tyramine. In these experiments the responses to angiotensin showed a similar reduction and recovery, in terms of time, as did the responses to McN-A-343 and were clearly distinguished from the reduction and recovery of the tyramine responses. Thus the bulk of evidence implicates the noradrenaline pool from which nerve impulses release the sympathetic transmitter in the acute pressor response to angiotensin in conscious cats. When this pool is depleted the pressor potency of angiotensin is reduced.

The role of noradrenaline in the acute angiotensin pressor response remains obscure. Drugs which block the adrenergic α- and β-receptors, adrenergic neuron blocking agents, autonomic ganglion blocking drugs and drugs preventing the uptake into neuronal noradrenaline stores had little effect on the responses to angiotensin and in no case was the angiotensin response reduced after treatment with one of these drugs. Without being able to determine the role of noradrenaline in the acute pressor response following the administration of angiotensin these results appear to eliminate a number of possible roles.

There is considerable evidence that angiotensin can cause a pressor response by an action within the central nervous system. This central pressor action of angiotensin has been variously reported to be mediated via the peripheral sympathetic nervous system (Bickerton & Buckley,1961) and by inhibition of vagal tone (Scroop & Lowe,1968). As

neither autonomic ganglion blocking drugs nor adrenergic neuron blocking drugs reduce the pressor action of angiotensin it is unlikely that a central action of angiotensin contributes to its pressor action following intravenous injection in conscious cats.

The failure of bethanidine to reduce the angiotensin pressor responses also excludes the possibility that angiotensin owes a part of its pressor action to stimulation of sympathetic ganglia or its ability to facilitate ganglionic transmission (Haefely et al,1965).

Angiotensin is also known to stimulate the release of adrenal medullary catecholamines (see back for references). In the present experiments with conscious cats there was evidence of an adrenal medullary component in the pressor response only after large doses of angiotensin and smaller doses did not appear to cause release of medullary amines. It seems unlikely therefore that reserpine reduced the responses to angiotensin by reduction or depletion of adrenal medullary catecholamines.

The results of experiments in the rabbit isolated artery prepartion were explained by the hypothesis that angiotensin normally releases noradrenaline and/or facilitates the release of noradrenaline by procedures such as nerve stimulation. This hypothesis explained the results of these experiments and was supported by the experimental results of many workers. Thus, the vasoconstrictor action of angiotensin in the anaesthetised dog renal artery (McGiff & Fasy,1964; 1965), in the vasculature of the human hand <u>in vivo</u> (Johnsson et al,1965: Scroop & Whelan,1966;1968) and on a

variety of isolated vascular beds (Zimmerman,1962: Laverty, 1963: Day & Owen,1968a,b) is dependent on an intact sympathetic innervation and abolition of sympathetic tone reduces the action of angiotensin. This was confirmed in the isolated rabbit ear artery experiments. The pressor action of angiotensin in whole animals is not reduced by drugs such as pempidine which reduce or abolish vascular sympathetic tone or by procedures such as pithing. This has been widely reported (see Introduction for references) and in this Thesis the marked pressor action of angiotensin in pithed rats has been confirmed. In addition pempidine failed to reduce the pressor action of angiotensin in conscious cats. It is possible that the responses to angiotensin were reduced by these procedures which reduce or abolish vascular sympathetic tone but that the simultaneous abolition of cardiovascular reflexes opposed the effect of reducing vascular sympathetic tone such that the pressor action of angiotensin was unaltered. Thus it would appear that the poor vasoconstrictor action of angiotensin in many isolated vascular beds is caused by the lack of sympathetic tone. In addition there are reports that in whole animals (McGiff & Fasy,1964:1965) and in human subjects (Johnsson et al,1965: Scroop & Whelan, 1966:1968) when a single vascular bed is examined the vasoconstrictor action of angiotensin is dependent on the sympathetic tone of that vascular bed and procedures reducing this tone reduce the constrictor potency of angiotensin in that vascular bed. Nevertheless procedures which abolish

sympathetic tone do not reduce the systemmic pressor potency in whole animals. The explanation of these two groups of apparently conflicting results may become clear when further studies on the interaction between angiotensin and the sympathetic nervous system have been made.

The final part of this Thesis is concerned with the examination of a number of bivalent metal ion chelating compounds for specific anti-angiotensin activity. The significance of the findings has been discussed in the section describing the experimental results. Of the substances tested only DDC showed anti-angiotensin activity. In pithed rats DDC specifically antagonised the pressor action of angiotensin. In anaesthetised cats the action of DDC appeared more complex than its action in pithed rats. In these cats the anti-angiotensin action of DDC could be revealed only after adrenalectomy. The simplest explanation of this is that DDC exerts a dual action on the pressor response to angiotensin in anaesthetised cats. Thus it may be that one action of DDC is to oppose the "direct" effect of angiotensin but in normal animals this is opposed by a second action of DDC which is to potentiate the release of catecholamines from the adrenal medulla by angiotensin. Only after adrenalectomy therefore did DDC convincingly antagonise the pressor action of DDC in anaesthetised cats.

The anti-angiotensin action of DDC appears to be independent of bivalent metal ion chelation (which in fact may mask the anti-angiotensin action) since other

bivalent metal ion chelating compounds did not antagonise the pressor effect of angiotensin.

It is suggested that separation of the anti-angiotensin action of DDC from its metal chelating action might permit the development of a specific angiotensin antagonist. This, however, is unlikely until the mechanism of the anti-angiotensin action of DDC is more clearly understood.

ACKNOWLEDGEMENTS.

I would like to thank Dr. M. D. Day for his constant help, encouragement and criticism during the past three years, and thank Dr. P. S. J. Spencer and Dr. J. M. Harris for allowing me to work in their departments.

The work described in the first part of this Thesis was undertaken at Brighton College of Technology during the tenure of a grant from Brighton Education Committee and the later parts were undertaken at the University of Aston in Birmingham supported by a grant awarded to Dr. Day by the Medical Research Council.

REFERENCES.

Aars,H. (1968a). Early resetting of aortic baroreceptor activity in hypertension. J.Physiol.,Lond.,197, 21-22P.

Aars,H. (1968b). Aortic baroreceptor activity in normal and hypertensive rabbits. Acta physiol. scand.,72, 298-309.

Aars,H. & Akre,S. (1968). Effect of angiotensin on sympathetic nerve activity. Acta physiol. scand.,74, 134-141.

Aiken,J.W. & Reit,E. (1968). Stimulation of the cat stellate ganglion by angiotensin. J.Pharmac.exp.Ther., 159, 107-114.

Ajzen,H. & Woods,J.W. (1967). Blood pressure responses to angiotensin II following aldosterone and adrenalectomy. Acta physiol.latinoam.,17, 131-136.

Alexander,N. & De Cuir,M. (1967). Sinoaortic baroreflex system and early rise in renal hypertensive rabbits. Am.J.Physiol.,213, 701-705.

Axelrod,J., Whitby,L.G. & Hertting,G. (1961). Effect of psychotropic drugs on uptake of H^3-norepinephrine by tissues. Science N.Y.,133, 383-384.

Barajas,L. (1964). The innervation of the juxtaglomerular apparatus. An electron microscopic study of the innervation of the glomerular arterioles. Lab.Invest.,13, 916-930.

Baum,T. (1963). Vascular reactivity of reserpine-pretreated dogs. J.Pharmac.exp.Ther.,141, 30-35.

Baum,T. (1969). Sympathetic responses in cardiac tissue in experimental hypertension. J.Pharmac.exp.Ther.,165, 176-180.

Baum,T. & Shropshire,A.T. (1967). Vasoconstriction induced by sympathetic stimulation during development of hypertension. Am.J.Physiol.,212, 1020-1024.

Beaulnes,A., Brodeur,J. & Gariepy,G. (1964). Sur le mecanisme de l'action cardiotrope de l'angiotensine. L'Union Medicale, 93, 205.

Beaulnes,A., Nantel,A. & Panisset,J-C. (1966). Angiotensin-norepinephrine interactions in cardiac muscle. Fedn Proc. Fedn Am.Socs exp.Biol.,25, 288.

Benelli,G., Della Bella,D. & Gandini,A. (1964). Angiotensin and peripheral sympathetic nerve activity. Br.J.Pharmac.,22, 211-219.

Benetato,Gr., Haulica,I., Uluita,M., Bubuiana,E., Mocodean,J., Stefanescu,P. & Suhaciu,G. (1964). The central nervous action of angiotensin on aldosterone secretion and electrolyte balance. Int.J.Neuropharmac.,3, 565-570.

Bianchi,A., De Schaepdryver,A.F., De Vleeschhouwer,G.R. & Preziosi,P. (1959). Mecanisme de l'action hypertensive de l'hypertensine synthetique. Archs int.Pharmacodyn. Ther.,119, 497-501.

Bickerton,R.K. & Buckley,J.P. (1961). Evidence for a central mechanism in angiotensin induced hypertension. Proc.Soc.exp.Biol.Med.,106, 834-836.

Bohr,D.F. & Uchida,E. (1967). Individualities of vascular smooth muscles in response to angiotensin. Circulation Res.,20-21, Suppl.11.,135-145.

Boura,A.L.A. & Green,A.F. (1964) in Evaluation of Drug Activities, Volume 1. Academic Press. London.

Bourdois,P. & Panisset,J-C. (1968). Mesenteric nerve stimulation by intracarotid administration of angiotensin. Rev.Can.Biol.,27, 167-169.

Braun-Menendez,E., Fasciolo,J.C., Leloir,L.F. & Munoz,J.M. (1940). The substance causing renal hypertension. J.Physiol., Lond.,98, 283-298.

Brodie,B.B. (1960). Selective release of norepinephrine and serotonin by reserpine-like compounds. Diseases of the nervous system. Suppl. to Volume 21.

Brodie,B.B., Costa,E., Croppetti,A. & Matsumoto,C. (1968). Interaction between desipramine,tyramine and amphetamine at adrenergic neurons. Br.J.Pharmac.,34, 648-658.

Brody,M.J. (1964). Cardiovascular responses following immunological sympathectomy. Circulation Res.,15, 161-167.

Brown,J.J., Chapius,G. & Robertson,J.I.S. (1963). The effect of long-continued intravenous infusions of angiotensin in the rabbit. Lancet,i, 1356-1357.

Bunag,R.D., Page,I.H. & McCubbin,J.W. (1966a). Neural stimulation of release of renin. Circulation Res.,19, 851-858.

Bunag,R.D., Page,I.H. & McCubbin,J.W. (1966b). Influence of dietary sodium on stimuli causing renin release. Am.J.Physiol.,211, 1383-1386.

Burn,J.H. & Rand,M.J. (1958). The action of sympathomimetic amines in animals treated with reserpine. J.Physiol., Lon 144, 314-336.

Burn,J.H. & Rand,M.J. (1960). The relation of circulating noradrenaline to the effect of sympathetic stimulation. J.Physiol.,150, 295-305.

Cessi,C. & Bano,A. (1964a). Effetto pressorio dell'angiotensina nel coniglio reserpinizzato. Chir.Patol.sper.,12, 28-34.

Cessi,C. & Bano,A. (1964b). Influenza degli adrenolitici beta sull'azione pressoria dell'angiotensina. Chir.Patol. sper.,12, 7-12.

Cession,G. (1964a). Stimulation par l'angiotensine du systeme adrenergique chez l'homme. Bull.Acad.r.Med.Belg., 4, 615-628.

Cession,G. (1964b) Action antagoniste de la cinnarizine vis-a-vis de l'angiotensine in vivo. C.r.Seanc.Soc.Biol., 158, 1754-1756.

Cession,G & Cession-Fossion,A. (1963). Action excito-surrenalienne de l'angiotensin chez le rat. C.r Seanc.Soc. Biol.,157, 1830-1833.

Chaberek,S & Martell,A.E. (1959) in Organic Sequestering Agents. John Wiley & Sons Inc New York.

Clarke,D E. & Leach,G D.H. (1968). The influence of bretylium on the interactions of infused sympathomimetic amines and tyramine in the reserpine-treated pithed rat. Br.J.Pharmac.,32, 392-401.

Conway,J. (1955). The behaviour of the blood pressure in normal and hypertensive rabbits in response to l-noradrenaline and ganglion block by hexa- or penta-methonium. J.Physiol., Lond.,127, 69-80.

Conway,J. (1958). Vascular reactivity in experimental hypertension measured after hexamethonium. Circulation, 17, 807-810.

Cranston,W.I., Lavery,H., Lowe,R.D & Rosendorff,C. (1968). The central pressor effect of angiotensin in the rabbit. J.Physiol.,Lond.,198, 30-31P

Daniels,A E. & Buckley,J.P. (1968). Relation of the centrally evoked pressor effect of angiotensin II to the calcium ion. Europ.J Pharmac.,4, 152-164.

Day,M D., McCubbin,J.W. & Page,I.H. (1965). Limited hypertensive effects of infusion of angiotensin. Am.J.Physiol., 209, 264-268.

Day,M.D. & Owen,D A.A. (1968a). The interaction between angiotensin and sympathetically induced vasoconstriction in the isolated perfused central ear artery of the rabbit. Arch.Pharmak.exp.Path.,259, 164-165.

Day,M.D. & Owen,D.A.A. (1968b). The interaction between angiotensin and sympathetic vasoconstriction in the isolated artery of the rabbit ear. Br.J.Pharmac.,34, 499-507.

Day,M.D. & Owen,D.A.A. (1969). Enhancement by angiotensin of pressor responses to endogenous noradrenaline in pithed rat. Br.J.Pharmac.,35, 355P-356P.

Day,M.D., Owen,D.A.A. & Warren,P.R. (1968). An adrenergic neuron blocking action in isolated tissues. J.Pharm.Pharmac., 20, Suppl. 130S-134S.

de la Lande,I.S. & Rand,M.J. (1965). A simple isolated nerve-blood vessel preparation. Aust.J.exp.Biol.med.Sci., 43, 639-656.

de la Lande,I.S., Cannell,V.A. & Waterson,J.G. (1966). The interaction of serotonin and noradrenaline on the perfused artery. Br.J.Pharmac.,28, 255-272.

de la Lande,I.S., Paton,W.D.M. & Waud,B. (1968). Output of sympathetic transmitter in the isolated rabbit ear. Aust.J.exp.Biol.med.Sci.,46, 727-738.

DeMuylder,C.G. (1952) cited by Page & McCubbin (1968) in Renal Hypertension. Year Book Medical Publishers. Chicago.

Deodhar,S.D., Haas,E. & Goldblatt,H. (1964). Production of antirenin to homologous renin and its effect on experimental renal hypertension. J.exp.Med.,119, 425-432.

DeQuattro,V. & Sjoerdsma,A. (1968). Catecholamine turnover in normotensive and hypertensive man: effects of anti-adrenaline drugs. J.clin.Invest.,47, 2359-2373.

Devaux,C., Alexandre,J-M., Menard,J., Meyer,P. & Milliez,P (1968). Mise en evidence d'un inhibiteur naturel d'origine renale du systeme renine-angiotensine chez l'homme. C.r.Seanc.Soc.Biol.,266, 2029.

Dexter,L. & Haynes,F.W. (1942). Search for renin and determination of hypertensin precursor in plasma of normal and hypertensive patients. J.clin.Invest.,21, 627.

Dickinson,C.J. & Lawrence,J.R. (1963). A slowly developing pressor response to small concentrations of angiotensin. Lancet,i, 1354-1356.

Dickinson,C.J. & Yu,R. (1967a). The progressive pressor response to angiotensin in the rabbit. J.Physiol.,190, 91-99.

Dickinson,C.J. & Yu,R. (1967b). Mechanisms involved in the progressive pressor response to very small amounts of angiotensin in concious rabbits.Circulation Res.,20-21, Suppl.II, 157-165.

Dickinson,C.J., De Swiet,M. & De Schaepdryver,A.F. (1968). Adrenal medullary contribution to the progressive pressor response to angiotensin in the rabbit. Arch int.Pharmacodyn. Ther.,176, 304-306.

Distler,A., Liebau,H. & Wolff,H.P. (1965a). Action of angiotensin on sympathetic nerve endings in isolated blood vessels. Nature,Lond.,207, 764-765.

Distler,A., Liebau,H. & Wolff,H.P (1965b). Untersuchungen uber der mechanismus des pressorischen effektes von angiotensin. Verh.dt.Ges.inn.Med.,71, 437-441.

Dock,W. (1940). Vasoconstriction in renal hypertension abolished by pithing. Am.J.Physiol.,130, 1-8.

Dorr,L.D. & Brody,M.J. (1966). Preliminary observations on the role of the sympathetic nervous system in development and maintenance of experimental renal hypertension. Proc.Soc.exp.Biol.Med.,123, 155-158.

Doyle,A.E. & Black,H. (1955). Reactivity to pressor agents in hypertension. Circulation,12, 974-980.

Doyle,A.E. & Smirk,F.H. (1955). The neurogenic component in hypertension. Circulation,8, 543-552.

Dunlop,D. & Shanks,R.G. (1969). Inhibition of the carotid sinus reflex by the chronic administration of propranolol. Br.J.Pharmac.,36, 132-143.

Ellis,D.E. & Reit,E. (1969). Inhibition by lidoflazine of the contractile response of the rat isolated colon to angiotensin. Br.J.Pharmac.,35, 132-140.

Farmer,J.B. & Campbell,I.K. (1967). Ca and Mg ions: influence on the response of an isolated artery to sympathetic nerve stimulation, noradrenaline and tyramine. Br.J.Pharmac., 29, 319-328.

Farr,W. & Grupp,G. (1966). Sympathetically mediated effects of angiotensin. Fedn Proc. Fedn Socs.exp Biol.,25, 288.

Farr,W. & Grupp,G. (1967). Sympathetically mediated effects of angiotensin on the dog heart in situ. J.Pharmac.exp.Ther.,156, 528-537.

Feldberg,W. & Lewis,G.P. (1964). The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. J.Physiol.,Lond.,171, 98-108.

Feldberg, W. & Lewis,G.P. (1965). Further studies on the effects of peptides on the suprarenal medulla of cats. J.Physiol.,Lond.,178, 239-251.

Freeman,N.E. & Page,I.H. (1937). Hypertension produced by constriction of the renal artery in sympathectomized dogs. Am.Heart J.,14, 404-414.

Gascon,A.L. & Walaszek,E.J. (1966). Inhibition of valyl^5 angiotensinamide II by osajin. J.Pharm.Pharmac.,18, 478.

Gillespie,J.S. & Muir,T.C. (1967). A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. Br.J.Pharmac.,30, 78-87.

Glenn,F., Child,C.G. & Page,I.H. (1938). The effect of destruction of the spinal cord on hypertension artificially produced in the dog. Am.J.Physiol.,122, 506-510.

Glenn,J.F. & Anderson,E.E. (1967). Reversible renovascular hypertension. A Rev.Med.,18, 219-228.

Godfraind,T. (1968) in Pharmacology of hormonal polypeptides and proteins. Plenum Press New York.

Godfraind,T., Kaba,A. & Polster,P (1966). Specific antagonism to the direct and indirect action of angiotensin on the guinea-pig ileum. Br.J.Pharmac.,28, 93-104.

Gokhale,S.D., Gulati,O.D., Kelkar,L.V. & Kelkar,V.V. (1966). Effect of some drugs on human umbilical artery in vitro. Br.J.Pharmac.,27, 332-346.

Goldblatt,H., Lynch,J., Hanzal,R.F. & Summerville,W.W. (1934). Studies on experimental hypertension. I. J.exp.Med., 59, 347-379.

Goldstein,M. (1966). Inhibition of norepinephrine biosynthesis at the dopamine-β-hydroxylation stage. Pharmac. Rev.,18, 77-82.

Gordon,D.B. & Nogueira,A. (1962). Increased vascular reactivity in experimental hypertension. Circulation Res.,10, 269-273.

Gordon,D.B. & Stephenson,M.P. (1967). Increased vascular response to angiotensin during ganglion blockade. Am.J. Physiol.,212, 1033-1036.

Graham,J.D.P & Al Katib,H. (1967). Adrenolytic and sympatholytic properties of 2-halogenoalkylamines in the vas deferens of the guinea-pig. Br.J.Pharmac.,31, 42-55.

Graham,M.H., Aboud,F.M. & Eckstein,J W. (1965). Effect of cocaine on cardiovascular responses in intact dogs. J.Pharmac.exp Ther.,150, 46-52.

Green,A.F. & Boura,A.L A. (1964) in Evaulation of Drug Activities, Volume 1. Academic Press. London.

Grimson,K.S. (1941). The sympathetic nervous system in neurogenic and renal hypertension. Archs Surg.,Lond.,43, 284-305.

Gross,F. (1966) in Antihypertensive Therapy. Springer-Verlag. Berlin.

Gross,F., Bock,K.D. & Turrian,H. (1961). Untersuchungen uber die Blutdruckwirkung von angiotensin. Hev.physiol. pharmac.Acta.,19, 42-57.

Haas,E. & Goldblatt,H. (1959). Effects of an antihypertensive drug, pentolinium. Am.J.Physiol.,196, 763-768.

Haefely,W., Hurlimann,A. & Thoenen,H. (1965). Effects of bradykinin and angiotensin on ganglionnic transmission. Biochem.Pharmac.,14, 1393.

Hall,G.H., Gomersall,J.C.R. & Heneage,E. (1967). A simple device for recording blood pressure or for intravenous injection of drugs in the concious unrestrained cat. Physiol. Behav.,3, 205-206.

Halliday,R.D. & Buckley,J.P (1962). Central hypertensive effects of angiotensin II. Int.J.Neuropharmac.,1, 43-47.

Hedwall,P. (1968). Effect of rabbit antibodies against angiotensin II on the pressor response to angiotensin II and renal hypertension in the rat. Br.J.Pharmac., 34, 623-629.

Henning,M. (1969). Noradrenaline turnover in renal hypertensive rats. J.Pharm.Pharmac.,21, 61-63.

Henning,M. & Johnsson,G. (1967). Interference of phenoxybenzamine and guanethidine with the vasoconstrictor responses of noradrenaline and angiotensin in the hand. Acta pharmac.tox.,25, 373-384.

Hertting,G. & Suko,J. (1966). Influence of angiotensin, vasopressin or changes in flow rate on vasoconstriction, changes in volume and H^3noradrenaline release following postganglionnic sympathetic nerve stimulation in the isolated cat spleen. Br.J.Pharmac.,26, 686-696.

Helmer,O.M. (1958). Studies on renin antibodies. Circulation.17, 648-652.

Heymans,C., Bouckaert,J.J., Elaut,L. Bayless,F. & Samaan,A. (1937). Hypertension arterielle chronique par ischemie renale chez le chien totalement sympathectomise. C.r.Seanc.Soc.Biol.,126, 434-436.

Hines,E.A. (1940). The significance of vascular hypertension as measured by the cold-pressor response. Am.Heart J.,19, 408-416.

Houssay,B.A. & Braun-Menendex,E. (1942). The role of renin in experimental hypertension. Br.med.J.,2, 179-181.

Hughes,I.E. (1968a). Chronotropic and pressor responses to angiotensin in rats anaesthetised with urethane. Europ.J.Pharmac.,3, 189-195.

Hughes,I.E. (1968b). An investigation of the effects of angiotensin on the release of neurohumeral transmitters at motor, adrenergic and cholinergic nerve terminals. J.Pharm.Pharmac.,20, 116-124.

Ikeda,M., Fujii,J., Murata,K., Terasawa,F., Osawa,T., Hosoda,S., Kurihara,S., Kimata,S. & Okinaka,S. (1963) cited by Page & McCubbin (1968) in Renal Hypertension. Year Book Medical Publishers. Chicago.

Iversen,L.L. (1967) in The uptake and storage of noradrenaline in sympathetic nerves. Cambridge University Press.

James,T.N. (1965). Absence of direct chronotropic action of angiotensin infused into the sinus node artery. Am.J.Physiol.,209, 571-576.

Johnson,C.A. & Wakerlin,G.E. (1940). Antiserum for renin Proc.Soc.exp.Biol.Med.,44, 277-281.

Johnsson,G., Henning,M. & Ablad,B. (1965). Studies on the mechanism of the vasoconstrictor effect of angiotensin II in man. Life Sciences,4, 1549-1554.

Judson,W.E & Helmer,O.M. (1965). Diagnostic and prognostic values of renin activity in renal venous plasma in renovascular hypertension. Hypertension,13, 79-89.

Khan,J.R., Skeggs,L.T., Shumway,N.P & Wisenbaugh,P.E. (1952). The assay of hypertensin from the arterial blood of normotensive and hypertensive human beings. J.exp.Med., 95, 523-529.

Kaneko,Y., McCubbin,J.W. & Page,I.H. (1960). Mechanism by which serotonin, norepinephrine and reserpine cause central vasomotor inhibition. Circulation Res.,8, 1228-1234.

Kaneko,Y., McCubbin,J.W. & Page,I.H. (1961). Ability of vasoconstrictor drugs to cause adrenal medullary discharge after sensitization by ganglion stimulating agents. Circulation Res.,9, 1247-1254.

Kaneko,Y., Takeda,T., Nakajima,K. & Ueda,H. (1966). Effect of angiotensin on the pressor response to tyramine in normotensive subjects and hypertensive patients. Circulation Res.,19, 673-680.

Kaumann,A.J., Zuberbuhler & Taquini,A.C. (1964). Influence of desmethylimipramine on the pressor responses to angiotensin. Archs int. Pharmacodyn.Ther.,149, 69-77.

Kennedy,A C., Luke,R.G., Briggs,J.D. & Barr- Stirling,W. (1965). Detection of renovascular hypertension. Lancet,ii, 963-968.

Kezdi,P. (1962). Mechanism of the carotid sinus in experimental hypertension. Circulation Res.,11, 145-152.

Kezdi,P. & Wennemark,J.R. (1964). Baroreceptor and sympathetic activity in experimental renal hypertension in the dog. Circulation,17, 785-790.

Khairallah,P A. & Page,I.H. (1961). Mechanism of action of angiotensin and bradykinin on smooth muscle in situ. Am.J.Physiol.,200, 51-54.

Krasney,J.A., Paudler,F.T., Smith,D.C., Davis,L.D. & Youmans,W.B. (1965). Mechanism of cardioaccelerator action of angiotensin. Am.J.Physiol.,209, 539-544.

Krasney,J.A., Paudler,F.T , Hogan,P.M., Lowe,R.F & Youmans,Y.B. (1966). Peripheral adrenergic basis for cardioaccelerator action of angiotensin. Am.J.Physiol., 211, 1447-1450.

Krasney,J.A., Thompson,J.L. & Lowe,R.F. (1967). Cardiac effects of angiotensin injections into perfused right coronary artery. Am.J.Physiol.,213, 134-138.

Krieger,E.M. & Marseillan,R.F (1966). Neural control in experimental renal hypertension. The role of baroreceptors and splanchnic fibres. Acta physiol.latinoam.,16, 343-352.

Laragh,J.H., Cannon,P.J. & Ames,R.P. (1964). Interaction between aldosterone secretion, sodium and potassium balance and angiotensin activity in man: Studies in hypertension and cirohosis. Can.med.Ass.J.,90, 248-256.

Laurence,D.R. & Nagle,R.E. (1963). The effects of bretylium and guanethidine on the pressor responses to noradrenaline and angiotensin. Br.J.Pharmac.,21, 403-413.

Laverty,R. (1962). Relation in rat hind-limb vessels between nervous vasomotor tone and the response to vasoconstrictor drugs. Br.J.Pharmac.,18, 451-464.

Laverty,R. (1963). A nervously mediated action of angiotensin in anaesthetised rats. J.Pharm.Pharmac.,15, 63-68.

Levy,B. & Ahlquist,R.P. (1962). A study of sympathetic ganglionic stimulants. J.Pharmac.exp Ther.,137, 219-228.

Lewis,G.P & Reit,E. (1965). The action of angiotensin and bradykinin on the superior cervical ganglion of the cat. J.Physiol.,Lond.,179, 538-553.

Lewis,G.P & Reit,E. (1966). Further studies on the actions of peptides on the superior cervical ganglion and suprarenal medulla. Br.J.Pharmac.,26, 444-460.

Lewis,B.V. (1968). The responses of isolated sheep and human umbilical arteries to oxygen and drugs. J.Obstet. Gynaec.Br.Commonw.,75, 87-91.

Liebau,H., Distler,A & Wolff,H.P. (1965). The noradrenaline releasing effect of angiotensin II in isolated blood vessels. Acta endocr.,Copenh. Suppl.,100 No.138.

Liebau,H., Distler,A. & Wolff,H.P. (1966). Untersuchengen zur indirekten sympathomimetischen wirkung von angiotensin an isolierten blutgefaben. Klin.Wschr.,44, 322-326.

Louis,W.J. & Doyle,A.E. (1966). The pressor response to noradrenaline and tyramine during angiotensin tachyphylaxis in the dog. Clin.Sci.,31, 247-254.

Luke,R.G., Kennedy,A C., Briggs,J.D., Struthers,N.W., Watt,J.K., Short,D.W. & Barr Stirling,W. (1968). Results of surgery in hypertension due to renal artery stenosis. Br.med.J.,2, 76-80.

McCubbin,J.W., Green,J.H. & Page,I.H. (1956). Baroreceptor function in chronic renal hypertension. Circulation Res., 4, 205-210.

McCubbin,J.W. (1958). Carotid sinus participation in experimental renal hypertension. Circulation,17, 791-797.

McCubbin,J.W. & Page,I.H. (1963a). Neurogenic component of chronic renal hypertension. Science N.Y.,139, 210-215.

McCubbin,J.W. & Page,I.H. (1963b). Renal pressor system and neurogenic control of arterial pressure. Circulation Res.,12, 553-559.

McCubbin,J.W., de Moura,R.S., Page,I.H. & Olmsted,F. (1965). Arterial hypertension elicited by subpressor amounts of angiotensin. Science N.Y.,149, 1394-1395.

McGiff,J.C. & Fasy,T.M. (1964). Blockade of the renal vascular activity of angiotensin. Circulation,30, III-124.

McGiff,J.C. & Fasy,T.M. (1965). The relationship of the renal vascular activity of angiotensin II to the autonomic nervous system. J.clin.Invest.,44, 1911-1923.

McGregor,D.D. (1965). The effect of sympathetic nerve stimulation on the vasoconstrictor responses in perfused mesenteric blood vessels of the rat. J.Physiol.,Lond.,177, 21-30.

McGregor,D.D. & Smirk,F.H. (1968). Vascular responses in mesenteric arteries from genetic and renal hypertensive rats. Am.J.Physiol.,214, 1429-1433.

Machova,J. & Boska,D. (1967). A study of the action of angiotensin on the superior cervical ganglion in comparison with other ganglion stimulating agents. Europ.J.Pharmac., 1, 233-239.

Maebashi,M. Miura,Y. & Yoshinaga,K. (1968). Renin inhibitor in plasma of uraemic patients. Lancet,i, 1408-1409.

Marchetti,G.V., Merlo,L. & Noseda,V. (1965). Effects of angiotensin on coronary circulation. Arzneimittel-Forsch., 15, 1244-1247.

Miele,E. (1966). On the mechanism of the potentiating of the angiotensin pressor effect by guanethidine and desmethylimipramine. Med.Pharmac.exp.,15, 35-44.

Nashold,B.S., Mannarino,E. & Wunderlich,M. (1962). Pressor depressor blood pressure responses in the cat after intraventricular injections of drugs. Nature,Lond.,193, 1297-1298.

Niijima,A. & Winter,D.L. (1968). Baroreceptors in the adrenal glands. Science N.Y.,159, 434-435.

Nishith,S.D., Davis,L.D. & Youmans,W.B. (1962). Cardioaccelerator action of angiotensin. Am.J.Physiol., 202, 237-240.

Nishith,S.D., Ganguly,A.K., Ramanathan,R.R. & Sreepathi Rao, S.K. (1966). Cardiac accelerator mechanisms of angiotensin. Ind.J.Med.Res.,54, 923-931.

Oken,D.E. & Biber,T.U.L. (1968). Biologically effective immunization against angiotensin. Am.J.Physiol.,214, 791-795.

Ostrovsky,D., Sen,S., Smeby,R.R. & Bumpus,F.M. (1967). Chemical assay of phospholipid renin pre-inhibitor in canine and human blood. Circulation Res.,21, 497-507.

Page,I.H. (1940). The vasoconstrictor action of plasma from hypertensive patients and dogs. J.exp.Med.,72, 301-310.

Page,I.H. & Heuer,G.J. (1937a). Treatment of essential and malignant hypertension by section of anterior nerve roots. Archs intern.Med.,59, 245-298.

Page,I.H. & Heuer,G.J. (1937b). The effect of splanchnic nerve resection on patients suffering from hypertension. Am.J.med.Sci.,193, 820-841.

Page,I.H., Kaneko,Y. & McCubbin,J.W. (1966). Cardiovascular reactivity in acute and chronic renal hypertensive dogs. Circulation Res.,18, 379-387.

Page,I.H. & Olmsted,F. (1961). Hemodynamic effects of angiotensin, norepinephrine and bradykinin continuously measured in unanaesthetised dogs. Am.J.Physiol.,201, 92-97.

Page,I.H. & McCubbin,J.W. (1968) in Renal Hypertension. Year Book Medical Publishers. Chicago.

Palaic,D. & Khairallah,P.A. (1967a). Inhibition of noradrenaline uptake by angiotensin. J.Pharm.Pharmac., 19, 396-397.

Palaic,D. & Khairallah,P.A (1967b). Effect of angiotensin on uptake and release of norepinephrine by brain. Biochem. Pharmac.,16, 2291-2298.

Pals,D.T. (1968). Effect of bretylium tosylate on angiotensin pressor responses in the pithed rat. Archs int.Pharmacodyn.Ther.,173, 417-425.

Pals,D.T. & Fulton,R.W. (1968). Interrelationship between angiotensin and vascular adrenergic receptors. Am.J.Physiol., 214, 506-512.

Pals,D.T. & Masucci,F.D. (1968). Effect of cocaine, desipramine and angiotensin on uptake in tissues of pithed rats. Nature,Lond. , 217, 772-773.

Pals,D.T., Fulton,R.W. & Masucci,F.D. (1968). Angiotensin, cocaine and desipramine: Comparison of effects on blood pressure responses to norepinephrine, tyramine and phenylephrine in the pithed rat. J.Pharmac.exp.Ther., 162, 85-91.

Panisset,J-C. (1967). Effect of angiotensin on the release of acetylcholine from preganglionnic and postganglionnic nerve endings. Can.J.Physiol.Pharmac.,45, 313-317.

Panisset,J-C., Biron,P. & Beaulnes,A. (1966). Effects of angiotensin on the superior cervical ganglion of the cat. Experientia,22, 394-395.

Panisset,J-C. & Bourdois,P (1967). Effect of angiotensin on sympathetic nerve endings: Inhibition of uptake of norepinephrine Fedn Proc. Fedn Am.Socs exp.Biol.,26, 466.

Panisset,J-C. & Bourdois,P (1968a). Action de l'angiotensine sur le systeme nerveux sympathique. L'Union Medicale,97, 1220-1225.

Panisset,J-C. & Bourdois,P. (1968b). Action de l'angiotensine sur le systeme nerveux sympathique. Path.Biol.,Paris,16, 505-509.

Panisset,J-C. & Bourdois,P. (1968c). Effect of angiotensin on the response to noradrenaline and sympathetic nerve stimulation, and on ^{3}H-noradrenaline uptake in cat mesenteric blood vessels. Can.J.Physiol.Pharmac.,46, 125-131.

Peach,M.J., Cline,W.H. & Watts,D.T (1966). Release of adrenal catecholamines by angiotensin II. Circulation Res.,19, 571-575.

Peart,W.S. (1959). Hypertension and the kidney. II- Experimental basis of renal hypertension. Br.med.J.,2, 1421-1429.

Pickens,P.T., Dunstan,H.P., Bumpus,F.M. & Page,I.H. (1965). Measurement of plasma renin activity in hypertension. Hypertension,13, 90-96.

Piper,P.J. & Vane,J.R. (1967). The assay of catecholamines released into the circulation of the guinea-pig by angiotensin. J.Physiol.,Lond.,188, 20-21P.

Poisner,A.M. & Douglas,W.W. (1965). The requirement for Ca in adrenomedullary secretion by histamine, serotonin, angiotensin and bradykinin. Fedn Proc. Fedn Am.Socs exp Biol.,24, 488.

Robertson,A.A., Hodge,J.V., Laverty,R. & Smirk,F.H. (1968). Tissue catecholamine levels in normotensive, genetically hypertensive and renal hypertensive rats. Aust.J.exp.Biol. med.Sci.,46, 689-696.

Robertson,P.A & Rubin,D. (1962). Stimulation of intestinal nervous elements by angiotensin. Br.J.Pharmac.,19, 5-12.

Robinson,R.L. (1965). Stimulation of the adrenal catecholamine output by angiotensin and bradykinin in vitro. Fedn Proc. Fedn Am.Socs exp.Biol.,24, 488.

Robinson,R.L. (1967). Stimulation of the catecholamine output of the isolated, perfused adrenal gland of the dog by angiotensin and bradykinin. J.Pharmac.exp.Ther.,156, 252-258.

Rosas,R., Montague,D., Gross,M. & Bohr,D.F. (1965). Cardiac action of vasoactive peptides in the rat. Circulation Res.,16, 150-161.

Ross,G. (1967). Effects of angiotensin on the heart and coronary circulation of the dog before and after β-adrenergic blockade. Cardiovascular Res.,1, 255-262.

Ross,G. & White,F.N. (1966). Role of catecholamine release in cardiovascular response to angiotensin. Am.J.Physiol.,211, 1419-1423.

Roszkowski,A.P. (1961). An unusual type of sympathetic ganglion stimulant. J.Pharmac.exp Ther.,132, 156-170.

Sakuri,T. & Hashimoto,Y. (1965). The vasoconstrictor action of angiotensin in relation to catecholamine. Jap.J.Pharmac.,15, 223-233.

Schaper,W.K.A., Jageneau,A.H.M., Xhonneux,R., Van Nueten,J. & Janssen,P A.J. (1963). Cinnarizine (R516), a specific angiotensin blocking coronary vasodilator. Life Sciences, 12, 963-974.

Schlittler, E. (1967) in Medicinal Chemistry, Volume 7. Academic Press. London.

Schmitt,H. & Schmitt,H. (1967a). Modifications by catecholamines of pressor responses to angiotensin in pithed rats. Rev.Can. Biol.,26, 265-267.

Schmitt,H. & Schmitt,H. (1967b). Interrelations entre catecholamines et angiotensine. C.r.Seanc.Soc.Biol.,161, 753-756.

Schmitt,H. & Schmitt,H. (1968a). Modifications des effets hypertenseurs de l'angiotensine par les agents adrenergiques et antiadrenergiques. Archs int.Pharmacodyn.Ther.,171, 31-46.

Schmitt,H. & Schmitt,H. (1968b). Increased activity in sympathetic nerves induced by angiotensin. Rev.Can.Biol., 27, 255-257.

Schumann,H.J. & Guther,W. (1967). Untersuchungen zum wirkungmechanismus von angiotensin am isolierten aortenpraparatund am blutdruck von ratten und meerschweinchen. Arch.Pharmak.exp.Path.,256, 169-182.

Schwyzer,R. (1963). Chemical structure and biological activity in the field of polypeptide hormones. Pure appl. Chem.,6, 265-295.

Scroop,G.C. & Lowe,R.D. (1968). Central pressor effect of angiotensin mediated by the pararsympathetic nervous system. Nature,Lond.,220, 1331-1332.

Scroop,G.C. & Whelan,R.F (1966). A central vasomotor action of angiotensin in man. Clin.Sci.,30, 79-90.

Scroop,G.C. & Whelan,R.F. (1968). The effects of alpha-adrenergic receptor blockade and sympathetic denervation on the pressor action of angiotensin in man. Aust.J.exp Biol. med.Sci.,46, 563-572.

Sedvall,G. (1964). Short term effects of reserpine on noradrenaline levels in skeletal muscle. Acta physiol. scand.,62, 101-108.

Sedvall,G. & Thorsen,J. (1965). Adrenergic transmission at vasoconstrictor nerve terminals partially depleted of noradrenaline. Acta physiol.scand.,64, 251-258.

Sen,S., Smeby,R.R. & Bumpus,F.M. (1967). Isolation of a phospholipid renin inhibitor from kidney. Biochemistry, N.Y.,6, 1572-1581.

Sen,S., Smeby,R.R. & Bumpus,F.M. (1969). Plasma renin activity in hypertensive rats after treatment with renin preinhibitor. Am.J.Physiol.,216, 499-503.

Severs,W.B., Daniels,A.E., Smookler,H.M., Kinnard,W.J. & Buckley,J.P. Interrelationship between angiotensin II and the sympathetic nervous system. J.Pharmac.exp Ther., 153, 530-537.

Severs,W.B., Daniels,A.E. & Buckley,J.P. (1967). On the central hypertensive effect of angiotensin II. Int.J. Neuropharmac.,6, 199-205.

Shipley,R.E. & Tilden,J.H (1947). A pithed rat preparation suitable for assaying pressor substances. Proc.Soc.exp.Biol.Med.,64, 453-455.

Sigg,E.B., Soffer,L. & Gyermek,L. (1963). Influence of imipramine and related psychoactive agents on the effect of 5-hydroxytryptamine and catecholamines on the cat nictitating membrane. J.Pharmac.exp.Ther.,142, 13-20.

Smookler,H.M. & Buckley,J.P. (1965). Centrally mediated cardiovascular effects of angiotensin II and norepinephrine administered into the lateral ventricle of anaesthetised cats. Fedn Proc. Fedn Am.Socs exp.Biol.,24, 489.

Smookler,H.M., Severs,W.B., Kinnard,W.J. & Buckley,J.P. (1966). Centrally mediated cardiovascular effects of angiotensin II. J.Pharmac.exp.Ther.,153, 485-494.

Somlyo,A.V., Woo,C-Y. & Somlyo,A.P. (1965). Responses of nerve-free vessels to vasoactive amines and polypeptides. Am.J.Physiol.,208, 748-753.

Staszewska-Barczak,J. & Konopka-Rogatko,K. (1967). Pressor activity and release of catecholamines by angiotensin and some of its analogues. Bull.Acad.pol.Sci.Cl.II Ser.Sci. biol.,15, 503-510.

Staszewska-Barczak,J. & Vane,J.R. (1967). The release of catecholamines from the adrenal medulla by peptides. Br.J.Pharmac.,30, 655-667.

Su,C. (1965). Angiotensin: receptor site and effect on sympathetic transmission in the isolated pulmonary artery. Fedn Proc. Fedn Am.Socs exp.Biol.,24, 489.

Suzuki,A. & Matsumoto,H. (1966). The mechanism of contracting action of angiotensin on the isolated aortic strip of rabbit. Kobe J.med.Sci.,12, 167-178.

Taquini,A.C. (1963). Neurogenic control of peripheral resistance in renal hypertension. Circulation Res.,12, 562-567.

Taquini,A.C & Fasciolo,J.C. (1946). Renin in essential hypertension. Am.Heart J.,32, 357-363.

Taquini,A.C., Blaquier,P.C. & Bohr,D.F. (1961). Neurogenic factors and angiotensin in etilogy of hypertension. Am.J.Physiol.,201, 1173-1175.

Thoenen,H., Hurlimann,A. & Haefely,W. (1965). The effect of angiotensin on the responses to post-ganglionnic sympathetic stimulation of the cat spleen; lack of facilitation of norepinephrine liberation. Med.Pharmac.exp.,13, 379-387.

Thuransky,K. (1966). Continuous blood pressure measurement in non-anaesthetised animals. Acta Physiol.Acad.Sci.Hung., 29, 33-40.

Tigerstedt,R. & Bergman,P.G. (1898). Niere und Kreislauf. Skand.Arch.Physiol.,8, 223-271.

Trendelenburg,U. (1966). Observations on the ganglion stimulating action of angiotensin and bradykinin. J.Pharmac. exp.Ther.,154, 418-425.

Turker,R.K. & Kayaalp,S.O. (1967a). Inhibitory effect of angiotensin on the intestinal motility of the cat and its relation to sympathetic nervous system. Archs int. Physiol.Biochem.,75, 735-744.

Turker,R.K. & Kayaalp,S.O. (1967b). Effect of lidoflazine on norepinephrine, angiotensin and serotonin responses in isolated smooth muscle preparations. Experientia,23, 647.

Turker,R.K. & Karahuseyinoglu,E. (1968). Interaction of cocaine with angiotensin and tyramine on the isolated rabbit aortic strip. Experientia,24, 921-922.

Vander,A.J. (1965). Effects of catecholamines and the renal nerves on renin secretion in anaesthetised dogs. Am.J.Physiol.,209, 659-662.

Vander,A.J. (1967). Control of renin release.Physiol. Rev.,47, 359-382.

Vane,J.R. (1969). The release and fate of vasoactive hormones in the circulation. Br.J.Pharmac.,35, 209-242.

Van-Nueten,J.M., Dresse,A. & Dony,J. (1964). Action antagoniste de la cinnarizine vis-a-vis de l'angiotensine *in vitro*. C.r.Seanc.Soc.Biol.,158, 1750-1754.

Verney,E.B. & Vogt,M. (1938). An experimental investigation into hypertension of renal origin, with some observations on convulsive "uraemia". Q.Jl.exp.Physiol.,28, 253-303.

Volicer,L., Scheer,E., Hilse,H. & Visweswaram,D. (1968). The turnover of norepinephrine during experimental hypertension in rats. Life Sciences,7, 525-532.

Vogt,M. (1965). Release of medullary amines from isolated perfused adrenal gland of the dog. Br.J.Pharmac.,24, 561-565.

Wagermark,J., Ungerstedt,U. & Ljungquist,A. (1968). Sympathetic innervation of the juxtaglomerular cells of the kidney. Circulation Res.,22, 149-153.

Wakerlin,G.E. (1958). Antibodies to renin as proof of the pathogenesis of sustained renal hypertension. Circulation,17, 653-657.

Warter,J., Schwartz,J., Bloch,R., Velly,J., Imbs,J-L. & Desaulles,E. (1967). Renine et hypertension arterielle. Path.Biol.,Paris,15, 619-624.

Wathen,R.L., Kingsbury,W.S., Stouder,D.A., Schneider,E.G. & Rostorfer,H.H. (1965). Effects of infusions of catecholamines and angiotensin II on renin release in anaesthetised dogs. Am.J.Physiol.,209, 1012-1024.

White,F.N. & Ross,G. (1966). Adrenal-dependent responses to angiotensin in the cat. Am.J.Physiol.,210, 1118-1121.

Yu,R. & Dickinson,C.J. (1965). Neurogenic effects of angiotensin. Lancet,ii, 1276-1277.

Zimmerman,B.G. (1962). Effect of acute sympathectomy on responses to angiotensin and norepinephrine. Circulation Res.,11, 780-787.

Zimmerman,B.G. (1964). Influence of several autonomic blocking agents on segmental vascular reactivity. J.Pharmac. exp.Ther.,146, 200-208.

Zimmerman,B.G. (1967). Evaluation of peripheral and central components of action of angiotensin on the sympathetic nervous system. J.Pharmac.exp.Ther.,158, 1-10.

Zimmerman,B.G. & Gomez,J. (1965). Increased responses to sympathetic stimulation in the cutaneous vasculature in presence of angiotensin. Int.J.Neuropharmac.,4, 185-193.

Zimmerman,B.G. & Whitmore,L. (1967). Effect of angiotensin and phenoxybenzamine on release of norepinephrine in vessels during sympathetic nerve stimulation. Int.J.Neuropharmac., 6, 27-38.

Zimmerman,B.G. & Gisslen,J. (1968). Pattern of renal vasoconstriction and transmitter release during sympathetic stimulation in presence of angiotensin and cocaine. J.Pharmac.exp Ther.,163, 320-329.

Zussman,W.V. (1967). Renal catecholamine stores following experimental hypertension in the rat. Angiology,18, 741-751.