THE MECHANISMS INVOLVED IN THE RESPONSES ELICITED BY INTRACEREBROVENTRICULAR ADMINISTRATION OF RENIN AND ANGIOTENSIN IN THE CONSCIOUS CAT

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ABSTRACT

Angiotensin II is an octapeptide formed by the action of the enzyme renin on a glycoprotein substrate. It has potent pharmacological effects on the central nervous system causing water drinking and vasopressor responses. Recently enzyme systems capable of the synthesis and destruction of angiotensin have been isolated from brain tissue.

The cat does not readily drink water and drinking had not been elicited by central administration of drugs. This thesis describes the dipsogenic effects of renin and angiotensin in the cat. Intracerebroventricular (icv) administration of renin, angiotensin I and II each induced drinking in the water-replete cat and in addition angiotensin I and II were effective dipsogens when given intravenously (iv).

Peptide inhibitors which specifically block the synthesis or biological action of angiotensin II were used to analyse these dipsogenic effects. It was established that drinking caused by icv renin and angiotensin I was mediated by angiotensin II. Drinking elicited by iv angiotensin I or II was shown to be due to the action of angiotensin II within the brain. The results also indicated that systemic angiotensin I may be converted to angiotensin II in the brain.

The effects of angiotensin II may be mediated by release of central neurotransmitters. This hypothesis was investigated using autonomic blocking drugs. The evidence indicated that central β-adrenoceptor or dopamine receptor mechanisms were involved.

Drinking behaviour in the cat was also elicited by administration of hypertonic saline, polyethylene glycol or β-adrenoceptor agonists. A preliminary study of the contribution of renin and angiotensin to

these responses was made using a peptide inhibitor.

In the final chapter the centrally mediated vasopressor effects of angiotensin II was studied in the conscious cat and rabbit. The results obtained in the rabbit were interesting since they suggested that the response was mediated by release of a humoral agent from the brain.

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INTRODUCTION

THE RENIN-ANGIOTENSIN SYSTEM

The term 'renin' was introduced by Tigerstedt & Bergman (1898) to describe a vasopressor principle contained in a saline extract of rabbit kidney. Many years later renin was found to be a proteolytic enzyme which released a vasoactive polypeptide from a substrate in the plasma. (Page & Helmer, 1940; Braun-Menendez, Fasciolo, Leloir & Munoz, 1940.) This polypeptide was subsequently named angiotensin (Braun-Menendez & Page, 1958). Two forms of angiotensin could be separated by countercurrent distribution, a decapeptide and an octapeptide. These were named angiotensin I and II respectively (Skeggs, Marsh, Kahn & Shumway, 1954). The amino acid sequence of the decapeptide isolated from horse plasma substrate was elucidated and was:-

$$Asp^{1} - Arg^{2} - Val^{3} - Tyr^{4} - Ile^{5} - His^{6} - Pro^{7} - Phe^{8} - His^{9} - Leu^{10}$$

(Lentz, Skeggs, Woods, Kahn & Shumway, 1956). The same sequence was also found in angiotensin I from human and porcine sources (Gross, 1971). However, angiotensin I obtained from ox plasma substrate had valine in place of isoleucine at position 5 (Elliott & Peart, 1956). Further studies showed that the octapeptide was formed from the decapeptide by enzymic removal of the $His^{9} - Leu^{10}$ dipeptide. The enzyme responsible for this conversion was found in plasma (Skeggs, Kahn & Shumway, 1956). The structures of the angiotensin polypeptides were subsequently confirmed by synthesis (Rittel, Iselin, Kappeler, Rinkier & Schwyzer, 1957; Schwarz, Bumpus & Page, 1957; Schwyzer, Rinkier, Iselin, Rittel, Kappeler & Zuber, 1958.).

The formation and destruction of angiotensin II

Renin, a protein with a molecular weight between 40,000 and 50,000, has been detected in the kidneys of most vertebrates (Peart, 1965). In the mammalian kidney, the enzyme occurs in granules within the juxtaglomerular cells (Cook, 1971). Renin is released from these cells into the circulation, a process controlled by three major factors. These are intrarenal receptors, sympathetic nerve activity and humoral agents (Davis, 1973). The two intrarenal mechanisms are baroreceptors in the afferent glomerular arteriole and the macula densa of the juxtaglomerular apparatus. The first responds to changes in intraluminal pressure and the second is sensitive to variations in sodium ion concentration. It is thought that both receptors contribute to normal release of renin but that the baroreceptor mechanism is dominant in pathological states, such as haemorrhage (Davis, 1973). Renal sympathetic nerve activity can modulate renin release by indirect and direct effects. The afferent arterioles are innervated by this system and changes in arteriolar constriction affect the renal baroreceptor. The degree of constriction also affects glomerular filtration rate which influences the sodium load delivered to the macula densa. The juxtaglomerular cells are also innervated by the sympathetic system and increased activity causes renin release mediated by a β-adrenoceptor mechanism. (Loeffler, Stockigt & Ganong, 1972.) These β-adrenoceptors can also be stimulated by catecholamines released from the adrenal medulla (Assaykeen, Goldfien & Ganong, 1970). Conversely two other humoral agents, angiotensin II and vasopressin, inhibit renin release (Bunag, Page, & McCubbin, 1967). These latter agents may act physiologically

as a negative feedback control (Shade, Davis, Johnson, Gottshall & Spielman, 1973).

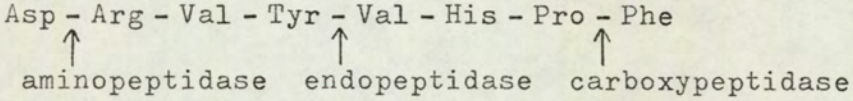
Although the kidney remains the main source of renin, renin-like enzymes have been isolated from extrarenal tissues (Gross, 1971). The main sources of these enzymes were blood vessels, adrenal gland, salivary glands, uterus and brain. These enzymes have slightly different physical properties from renal renin and in some tissues the enzyme level did not decline after the animal was nephrectomised. This suggested that the enzymes were synthesised in the respective tissues. These tissue enzymes have been named isorenins (Boucher, Ganten, Granger & Genest, 1972).

Renin substrate is composed of several heterogeneous glycoproteins, which have the same amino acid content but different carbohydrate moieties (Skeggs, Lentz, Hochstrasser & Kahn, 1963). These amino acids form a tetradecapeptide which contains the sequence of angiotensin I. The peptide can be released from glycoprotein by trypsin and has also been synthesised (Skeggs, Kahn, Lentz & Shumway, 1957, 1958). This synthetic substrate has been used to elucidate the site of action of renin. The enzyme split the substrate at a Leu - Leu bond to yield angiotensin I and a tetrapeptide.

In experiments using rabbit isolated aorta, angiotensin I was found to be almost inactive but it elicited a response after being incubated with plasma (Helmer, 1957). Skeggs et al (1956) had described a chloride-dependent enzyme in plasma which converted angiotensin I to II; this suggested that the octapeptide was the biologically active form. For several years it was thought that this plasma enzyme was the most important factor in angiotensin I conversion

but Ng & Vane (1968) presented evidence against this view. They found that the rate of conversion in dog blood was relatively slow but that rapid conversion occurred in the pulmonary vascular bed. Their results suggested that tissue enzymes in the lung were more important than plasma enzymes. Angiotensin I converting enzymes have since been isolated from many tissues (Cushman & Cheung, 1971). In vivo conversion of angiotensin I in the systemic circulation is negligible (Vane, 1969) thus the role, if any, of these extrapulmonary tissue enzymes is not clear.

In early experiments it was noted that angiotensin was destroyed by blood and by tissue extracts (Page & Helmer, 1940). This inactivation involved enzymes which were named angiotensinases. Three types of enzyme have been identified, aminopeptidases, endopeptidases and carboxypeptidases. Their sites of action are indicated in the following diagram:-



The plasma contains all three types (Khairallah & Page, 1967) but they may not have a significant effect on the in vivo catabolism of angiotensin. The half-life of angiotensin II in dog blood was 100 - 200 sec. but when the peptide was injected intravenously 50 - 70% of the dose was removed in one 15 sec. circulation. (Hodge, Ng & Vane, 1967.) The cerebral, hepatic, renal and femoral vascular beds all removed angiotensin II from the circulation but none was removed in the pulmonary vasculature. These results suggested that catabolism occurred at tissue sites but it is not known if

intracellular or membrane-bound enzymes are responsible. Angiotensin II can also be eliminated from the circulation by protein binding or renal excretion.

The pharmacology of renin and angiotensin

It is generally accepted that most of the biological effects of the renin-angiotensin system are mediated by angiotensin II. There is evidence that the decapeptide has weak agonist actions in some tissues, such as the adrenal medulla (Peach, 1974) but the effects are qualitatively the same as the octapeptide. Early pharmacological studies were performed using small quantities of angiotensin isolated from natural sources. After synthetic preparations became available, extensive investigations of the properties of angiotensin II were possible. In most studies the peptide used was Hypertensin-CIBA, an analogue of Val^5 angiotensin II in which the β-carboxyl of aspartic $acid^1$ was replaced by a neutral amide. This analogue has similar pharmacological activity and potency to both naturally occurring forms of angiotensin II in all preparations in which they have been compared (Gross, 1971.). Angiotensin II has a wide spectrum of activity on the cardiovascular system, the nervous system and mechanisms regulating salt and water homeostasis. Some of the important peripheral actions will be briefly discussed below.

Cardiovascular effects

Angiotensin II is the most potent naturally occurring pressor substance, being 10 - 20 times more potent by weight than noradrenaline. Single intravenous injections elicited a dose related pressor response in the dog, cat, rabbit and rat (Gross & Turrian, 1960). The response to small doses given at regular

intervals was repeatable and constant but larger doses caused tachyphylaxis. Vasoconstriction occurred in the precapillary resistance vessels (Folkow, Johansson & Mellander, 1960) mainly in the splanchnic, renal and skin vasculature (Forsyth, Hoffbrand & Melmon, 1971). The vasoconstriction was due to a direct effect on blood vessels since it was not reduced by pithing, adrenalectomy or autonomic blocking drugs (Finch & Leach, 1969; Day & Owen, 1970 a.b.).

In contrast to its in vivo activity, angiotensin II has usually been found to be less potent than noradrenaline on isolated vascular smooth muscle (Bohr, 1974). Arteries from different species and different vascular beds varied considerably in their sensitivity to the peptide. Bohr & Uchida (1967) classified arteries according to their response to angiotensin II. They found three types, those giving sustained contractions, those exhibiting transient contraction and non-responders. Isolated veins also gave non-uniform responses to angiotensin II.

Myotropic effects of angiotensin II could also be demonstrated in intestinal and uterine smooth muscle both in vitro and in situ (Gross & Turrian, 1960).

Neurogenic effects

Angiotensin II interacts with the autonomic nervous system at several peripheral sites; the adrenal medulla, ganglia and adrenergic neurone terminals.

Felberg & Lewis (1964) demonstrated that angiotensin II injected into the coeliac artery of an eviscerated cat caused contraction of the nictitating membrane. The response was abolished by

adrenalectomy suggesting that it was mediated by release of adrenal catecholamines (Feldberg & Lewis, 1964; 1965). Angiotensin II was shown to cause direct release of amines from the canine isolated adrenal gland (Robinson, 1967) and this has been confirmed in other species (Peach, 1974).

Two effects on autonomic ganglia have been reported, a direct stimulant action and a modulating effect on impulse transmission. Close arterial injection of angiotensin II into the superior cervical ganglion of an anaesthetised cat caused contraction of the nictitating membrane. The response was abolished by ganglionectomy but not by intravenous hexamethonium (Lewis & Reit, 1965). In the same type of experiment Panisset, Biron & Beaulnes (1966) demonstrated that low doses of angiotensin II (10^{-11}g) inhibited ganglionic transmission but higher doeses facilitated it.

Zimmerman & Gomez (1965) reported that angiotensin II increased vasoconstriction in the cutaneous vasculature of the dog elicited by sympathetic nerve stimulation. This suggested an action of the peptide on adrenergic neurones. This may be either facilitation of transmitter release (see Zimmerman, Gomer & Liao, 1972) or inhibition of noradrenaline reuptake (Khairallah, 1972). A further action of angiotensin on adrenergic neurones has recently been described, the peptide can stimulate catecholamine biosynthesis at the tyrosine hydroxylase stage (Roth, 1972).

Effects on salt and water balance

Angiotensin II exerts a peripheral influence on salt and water balance by acting on the kidney and the adrenal cortex.

Intravenous infusions of the peptide can cause increased or

decreased excretion of salt and water, depending on the dose administered. Generally, small doses elicit antidiuresis and antinatriuresis and large doses have the opposite effect (Bock, Brown, Lever, Robertson & Masson, 1968). The effect on renal function can be due to vasoconstriction in the glomerular or medullary beds or a direct action on tubule cells. Thurau (1974) reviewed the evidence for a tubular mechanism and concluded that the limited evidence supported a distal tubule site of action. In rat kidney cortex slices, low doses of angiotensin II (10^{-12} g ml^{-1}) caused an increased sodium extrusion which was consistent with an antinatriuretic action. (Munday, Parsons & Poat, 1971.)

The relationship between the kidney and aldosterone release was established by ablation studies; nephrectomy reduced aldosterone secretion after haemorrhage (Davis, Carpenter, Ayers, Holman & Bahn, 1961). Simultaneously, other workers reported that in man infusions of angiotensin II caused increased plasma levels and urinary excretion of aldosterone (Genest, Nowaczynski, Koiw, Sandor & Biron, 1960; Laragh, Angers, Kelly & Leiberman, 1960). Subsequently, release of aldosterone by angiotensin II has been shown in other vertebrates and the effect can be demonstrated in adrenal cortex slices. The peptide stimulates aldosterone biosynthesis by increasing conversion of cholesterol to pregnenolone. (Davis, 1974.) It has recently been reported that the heptapeptide, des-Asp^1 angiotensin II, was more potent than the octapeptide in causing aldosterone release. (Peach, 1972.) The renin-angiotensin system seems to be an important regulator of aldosterone release in homeostasis and disease; it functions as a negative feedback

mechanism.

Structure-activity relationships

In addition to the parent peptide many structural analogues of angiotensin II have now been prepared by solid phase synthesis. These have been used to determine the relative importance of each amino acid side chain to the agonist activity of angiotensin II (Khosla, Smeby & Bumpus, 1974). The essential features for myotropic and vasopressor activity were:- a) six amino acids from the C terminus b) a phenolic hydroxyl side chain at position 4 c) an imidazole ring at 6 d) proline at 7 and e) an aromatic amino acid with a free carboxyl group at 8.

EFFECTS OF ANGIOTENSIN II ON THE CENTRAL NERVOUS SYSTEM

In 1961, it was generally accepted that "angiotensin has a strong peripheral vasoconstrictor action and no action on the central nervous system" (Page & Bumpus, 1961). This view was challenged by results obtained using a cross-circulation preparation in anaesthetised dogs. (Bickerton & Buckley, 1961). In this preparation the cerebral circulation of a "recipient" dog was perfused, via the carotid arteries, with blood from a "donor" dog. The vertebral arteries and venous sinuses of the recipient were occluded to prevent leakage between the cerebral and systemic circulations. Thus the only communication mechanism between the head and the trunk of the recipient was the nervous system. Angiotensin II injected into the cerebral circulation of the recipient elicited a systemic pressor response which was abolished by peripheral α-adrenoceptor blockade (Bickerton & Buckley, 1961). Other vasoconstrictor substances did not cause a similar response and the response to angiotensin II

occurred even after denervation of the carotid bodies (Buckley, Bickerton, Halliday & Kato, 1964). This suggested that the effect was due to a direct action on central neurones and was not a secondary phenomenon associated with cerebral vasoconstriction.

In the last ten years the effects elicited by administration of angiotensin II into the vertebral arteries, the cerebrospinal fluid and the brain tissue have been widely studied. The most important centrally mediated actions of angiotensin II involve the regulation of salt and water balance and the control of the cardiovascular system. These actions will be discussed below.

Penetration of angiotensin into the brain

The access to the brain of blood-borne solutes is limited by a complex system of passive physical structures and active transport mechanisms. In the systemic circulation solutes leave capillaries through the small gaps between the epithelial cells but in the brain blood vessels these gaps are closed by "tight junctions". These junctions form a "blood-brain barrier" across which only lipid soluble substances can easily diffuse. There are however certain regions where the "barrier" is absent, these include the neurohypophysis, the area postrema and the subfornical organ (Wilson & Brodie, 1961). These areas provide a gateway for polar compounds to enter the brain. A second route of entry into the brain is via the cerebrospinal fluid (CSF). There is no barrier between the blood and the epithelium of the choroid plexus, the organ which secretes CSF. Substances may be transported across the epithelium from the blood to the CSF, the latter fluid being in communication with the extracellular fluid of the brain.

In studies in anaesthetised dogs, it was demonstrated that half of an administered dose of angiotensin II ($2\,\mu g\,ml^{-1}$) was removed during a single circulation through the head. (Hodge et al, 1967.) The fate of the peptide was not determined, it may have entered the brain or been metabolised to biologically inactive products. In a subsequent study, angiotensin II could not be detected in the dog cisternal CSF after a one hour intravenous infusion of the peptide ($200ngKg^{-1}min^{-1}$) (Ganten, Marquez-Julio, Granger, Hayduk, Karsunky, Boucher & Genest, 1971). This did not necessarily indicate that the peptide did not enter the CSF since it may have been metabolised before reaching the sampling site. Mouw, Bonjour, Malvin & Vander (1971) found that 60% of an administered dose of angiotensin II was metabolised during perfusion through the dog cerebral ventricular system.

Richardson & Beaulnes (1971) used a histochemical method to detect angiotensin II. The peptide was coupled to horseradish peroxidase and injected intravenously into mice ($10\mu gKg^{-1}$ angiotensin II). After 5 minutes the staining-reaction product could be detected in vesicles within choroid plexus cells, however Brightman (1967) obtained similar results after injecting only horseradish peroxidase. Autoradiographic studies with C^{14}-angiotensin II in the mouse demonstrated that 5 minutes after intravenous injection of the peptide ($70\mu gKg^{-1}$), the label was located in the choroid plexus, third ventricle, cerebral aqueduct and surrounding brain tissue (Volicer & Loew, 1971). These studies are difficult to interpret since the very large doses used would have caused large sustained pressor effects.

The earliest study of the distribution of angiotensin II was performed in normal and nephrectomised rats by Bumpus, Smeby, Page & Khairallah (1964). They found that immediately after a 20 minute intravenous infusion of tritiated angiotensin II ($23\mu gKg^{-1}min^{-1}$) the radioactivity level of brain tissue was lower than that of plasma. However, 30 minutes later the radioactivity level of the brain had increased and was greater than that of the plasma. The labelled material isolated from the brain had a different electrophoretic mobility to that of angiotensin II, thus it may have been a metabolite. In the later studies in mice, mentioned above, the radioactive label was accumulated in the brain after intravenous injection of C^{14} angiotensin II (Volicer & Loew, 1971; Richardson & Beaulnes, 1971). The most useful study which has been reported was that performed by Osborne, Pooters, Angeles d'Auriac, Epstein, Worcel & Meyer (1970) using small doses of high specific activity C^{14} angiotensin II (25 - 100ng). After intravenous injection in anaesthetised rats, the tissue : plasma radioactivity ratio for several brain regions was measured at 2, 5 and 30 minutes. Their results showed that the ratio was less than unity at all times for all the regions including the preoptic area and floor of the fourth ventricle. The only region which concentrated the label was the pituitary gland which lies outside the blood brain barrier.

The limited data compiled in the above experiments do not conclusively establish that small blood-borne doses of angiotensin II penetrate the CNS. However since it has been demonstrated that infusion of low doses of angiotension II via the vertebral arteries can elicit centrally mediated responses (see

Severs & Daniels-Severs, 1973), small amounts of the peptide may enter the brain. It is likely that areas without a blood brain barrier are involved since the pituitary, area postrema and subfornical organ have all been implicated in the central effect of angiotensin II.

The brain renin-angiotensin system

Recent studies have established that a separate renin-angiotensin system exists in the central nervous system. This suggests that the centrally mediated effects may have a physiological role.

A renin-like enzyme has been isolated from rat, dog and human brain tissue (Ganten, Minnich, Granger, Hayduk, Brecht, Barbeau, Boucher & Genest, 1971; Fischer-Ferraro, Nahmod, Goldstein & Finkielman, 1971; Wan, Ravenell, Avery & Stephen , 1972). In dog (Ganten, Marquez-Julio et al, 1971) and human (Wan et al, 1972) brain, isorenin activity was found in the frontal cortex, caudate nucleus, thalamus, hypothalamus, midbrain, pons and medulla. The enzyme concentrations in each region were similar. Subcellular fractionation on sucrose gradients revealed that most of the renin activity in brain and kidney homogenates was located in the lysosomal fraction. In addition, brain isorenin was found in significant quantities in the synaptosome fraction (Minnich, Ganten, Barbeau & Genest, 1972). This latter observation may indicate that isorenin was associated with nerve terminals.

Brain isorenin acted on homologous, heterologous and synthetic substrates and was thus different from "pseudorenin" (Skeggs, Lentz, Kahn, Dorer & Levine, 1969) a plasma enzyme which acts only on

synthetic material. Isorenin also differed in several respects from renal renin having a lower pH optimum, a different electrophoretic mobility and being less sensitive to hog antirenin. When equal amounts of brain isorenin and renal renin (measured by their activity with homologous dog substrate) were incubated with heterologous or synthetic substrate, the isorenin had greater activity (Ganten, Marquez-Julio et al, 1971). Despite these different characteristics, it was possible that the brain enzyme could have been derived from plasma renin. In the dog, no renin could be detected in CSF after plasma renin levels had been raised by sodium restriction or infusion of exogenous renin (Ganten, Marquez-Julio et al, 1971). This suggested that plasma renin did not enter the brain and that isorenin was endogenous. Further evidence that isorenin synthesis occurred in the brain was provided by results of experiments in nephrectomised animals. Twelve days after nephrectomy renin levels in the dog brain were not significantly different from those in control animals although no renin could be detected in the blood plasma (Ganten, Marquez-Julio et al, 1971).

Brain isorenin concentration in the dog was also affected by age, administration of steroids or by electrolyte restriction. In puppies, brain isorenin activity increased during the first year after birth whereas the plasma level declined in that period. Aldosterone (2mg day^{-1}, 8 days) and progesterone (25mg day^{-1}, 28 days) had opposite actions on brain enzyme levels, the former causing a decrease and the latter an increase. In contrast plasma renin levels fell after both steroids. Potassium restriction for 18 days exerted a differential effect on isorenin levels. The concentration in the

thalamus increased, that in the medulla decreased but there was no change in the cortex (Ganten, Minnich et al, 1971). These results may indicate that central renin formation is regulated by changes in electrolyte balance initiated by steroids.

Angiotensin-like material was also isolated from the brain tissue of the nephrectomised dog and rat (Fischer-Ferraro et al, 1971). The material was unequally distributed, the highest concentration being in the hypothalamus. The brain-stem, cortex and basal nuclei also contained angiotensin but no activity was found in the cerebellum. Countercurrent distribution revealed two peaks corresponding to angiotensin I and II; the decapeptide was the more abundant. This result was confirmed by Ganten, Marquez-Julio et al (1971), since the vasopressor response elicited by angiotensin extracted from the brain was prevented by anti-angiotensin I but not anti-angiotensin II. Ganten's group also isolated a protein from the dog brain which acted as a renin substrate. The pathophysiological significance of the system has recently been emphasised by the isolation of an angiotensin-like substance from the CSF of normal and hypertensive humans (Finkielman, Fischer-Ferraro, Diaz, Goldstein & Nahmod, 1972).

Converting enzyme with similar properties to those of enzymes in peripheral tissues has been identified in rat brain (Yang & Neff, 1972). The enzyme was found in the microsomal fraction. It was not uniformly distributed, the striatum, cerebellum and pituitary containing high concentrations and the hypothalamus and cortex low concentrations. In the pituitary the activity was mainly in the posterior lobe (Yang & Neff, 1973). The differential distribution of

brain converting enzyme might act as a regulator to allow local formation of angiotensin II at a receptor site.

There are also brain enzymes which can inactivate polypeptides, one found in dog and rat brain degrades angiotensin but not bradykinin or vasopressin (Goldstein, Diaz,Finkielman, Nahmod & Fischer-Ferraro, 1972). The main substrate was angiotensin II but the decapeptide was also inactivated.

Centrally mediated actions of angiotensin on salt and water balance

The volume and composition of body fluid are maintained by complex regulatory mechanisms for the intake and excretion of water and electrolytes. It is necessary to discuss these mechanisms before an adequate account can be given of the central actions of angiotensin. Since this thesis is primarily concerned with the dipsogenic actions mediated by the renin-angiotensin system, the control of drinking mechanisms will be emphasised.

a) The double depletion hypothesis

The body fluid is distributed in two compartments, intracellular fluid (ICF) and extracellular fluid (ECF). It has been proposed that primary drinking behaviour, in which there is a relative or absolute lack of body water (Fitzsimons, 1972), is initiated by depletion of either fluid compartment. This has been named "the double depletion hypothesis" (Epstein, 1973). The hypothesis states that depletion of either compartment triggers mechanisms which have inputs to an integrating centre in the brain. These inputs can be afferents from peripheral and central nervous receptors or humoral agents. Integration leads to a specific motivational state to which the animal responds by seeking and ingesting water. The mechanisms

which regulate renal conservation of water are also activated by these inputs. Some of the evidence on which this hypothesis was based will be given below.

b) ICF depletion

Gilman (1937) found that dogs drank twice as much water after intravenous injection of hypertonic saline than after hypertonic urea. He concluded that drinking was caused by ICF depletion since sodium ions did not effectively penetrate cell membranes. This view has been confirmed in other studies using solutes which remain in the ECF, (see Blass, 1973). Although cellular dehydration occurs in all tissues, most evidence indicates that the receptors which detect the deficit are in the brain. The location of these receptors was first demonstrated by Andersson (1953) who elicited drinking in the goat by injecting hypertonic saline into the hypothalamus. Later studies in the rat (Blass & Epstein, 1971) and the rabbit (Peck & Novin, 1971) indicated that the lateral preoptic area (LPO) was the sensitive region. Bilateral lesions of the LPO in both species reduced drinking elicited by systemic injection of hypertonic saline but not ad libitum drinking or that caused by ECF depletion. These groups also demonstrated that microinjections of hypertonic sodium chloride or sucrose solution into the LPO elicited only drinking behaviour. The concept that these receptors are sensitive to osmotic pressure has recently been questioned by Andersson (1971). He believes that the receptors are sensitive to CSF sodium ion concentration; this idea will be discussed later. There is also recent evidence suggesting that peripheral osmoreceptors in the hepatic portal vein may contribute to the control of water intake (Kozlowski

& Drzewiecki, 1973).

c) ECF depletion

Sodium depletion in the dog induced drinking despite the fact that water moved from the ECF into the cells causing cellular overhydration (Holmes & Cizek, 1951). This suggested that ECF depletion was also a potent dipsogenic stimulus.

ECF comprises a vascular component, plasma and an extravascular component, interstitial fluid. Since there are many sensory nerve endings in the vasculature but few, if any, in the interstitial space, the receptors which detect ECF deficit are probably in the vascular system. Stretch "baroreceptors" within the walls of the capacitance vessels near the right atrium are known to control release of antidiuretic hormone (Share, 1969). Impulses from these receptors are transmitted in the vagus and activate ascending and descending pathways in the medulla oblongata. It seems likely that these or similar receptors are involved in initiating drinking.

Direct depletion of the vascular compartment caused drinking in the rat (Fitzsimons, 1961) but was not a reliable stimulus in other species (Fitzsimons, 1972). A less drastic method of depleting the plasma volume was introduced by Fitzsimons (1961), this involved injecting hyperoncotic solutions into the interstitial space. This procedure withdraws fluid from the vascular system and sequesters it in the interstitium, thus inducing a relative depletion of ECF. Further support for the view that receptors in the vasculature were implicated was gained from experiments involving ligation of the vena cava (Fitzsimons, 1964). When the rat inferior vena cava was tied just above the kidney, venous return was reduced by 30%. This

apparent plasma deficit initiated drinking behaviour.

There is evidence that in addition to direct activation of central integrating mechanisms, a humoral factor is involved in the response to certain types of ECF depletion. It seems likely that the descending pathways from the medulla initiate reflex release of renin which acts as a dipsogenic stimulus by liberating angiotensin. This evidence will be fully discussed below.

d) Central nervous mechanisms

Central neurones which affect drinking behaviour have been identified by making lesions or by stimulating at specific brain loci. The importance of the hypothalamic region was demonstrated in early experiments. Electrical stimulation of the perifornical hypothalamus in the goat induced copious drinking (Andersson & McCann, 1955). Their observations were subsequently confirmed in the rat, dog and pigeon (see Stevenson, 1967). Further evidence was obtained by making lesions in the lateral hypothalamic area in the rat; this caused either adipsia or aphagia (Montemurro & Stevenson, 1957). Detailed analysis of this "lateral hypothalamic syndrome" showed that adipsic rats did not drink in response to ICF or ECF depletion (Epstein, 1971). The hypothalamic region together with the hippocampus, septum, amygdala and parts of the neocortex and midbrain form the "limbic system". The components of this system are connected by major neural pathways including the fornix, mammillothalamic tract, stria terminalis and median forebrain bundle. Many of these structures in the limbic system are also involved in the central nervous drinking mechanisms. Fisher & Coury (1962) demonstrated that application of carbachol to limbic structures in the rat

elicited drinking. Rats with septal lesions exhibit hyperdipsia and show exaggerated drinking in response to hypovolaemic but not hyperosmotic stimuli (Blass & Hanson, 1970). This suggested that the septum exerts an inhibitory action on drinking induced by extracellular stimuli. The amygdala, however, appeared to have a facilitating action on water intake since its ablation reduced drinking but stimulation increased water intake (Grossman & Grossman, 1963). Mogenson (1973) recently summarised his experiments on the effect of amygdalar or septal stimulation on drinking elicited by hypothalamic stimulation. He found that stimulation in the dorsomedial septum facilitated, but stimulation in the ventrolateral septum inhibited drinking induced by hypothalamic stimulation. Electrical stimulation of the amygdala inhibted hypothalamically induced drinking in most experiments.

A hypothesis consistent with this data is that an integration "centre" for dipsogenic stimuli exists in the lateral hypothalamus. Information from peripheral and central receptors and from other areas of the limbic system is first processed then transmitted to midbrain effector neurones. An alternative explanation for the effects of hypothalamic lesions and stimulation is that these simply interfere with fibre tracts passing through the region. The fact that afferent fibres from peripheral receptors, which pass through the area would be interrupted must also be considered.

e) Central neurohumoral transmitters

Since 1960 there have been numerous attempts to identify the central neurotransmitters which mediate drinking behaviour. Most studies have been performed using the rat and the evidence has not

been duplicated in other species.

Grossman (1960) reported that carbachol applied to the rat hypothalamus caused drinking whereas noradrenaline caused eating. Drinking was also increased after central administration of eserine (Stein & Seifter, 1962) suggesting that endogenous acetylcholine participated in drinking behaviour. Muscarinic receptor stimulation was involved since muscarine, but not nicotine, elicited a similar dipsogenic response (Stein & Seifter, 1962). This view was supported by evidence that central pretreatment with atropine reduced carbachol induced drinking. The cholinoceptive region extended beyond the hypothalamus to include limbic structures which formed the Papez circuit (Fisher & Coury, 1962).

In some experiments central injection of noradrenaline did elicit drinking in the rat (Myers, 1964a). Later, Lehr, Mallow & Krukowski (1967) found that systemic injection of isoprenaline induced drinking and considered that the amine might act centrally. Leibowitz (1971) extended this idea showing that intrahypothalamic injections of the β-adrenoceptor agonist elicited drinking. Central administration of propranolol reduced the response but similarly administered phentolamine was not an effective antagonist. An alternative view held by many other investigators is that isoprenaline acts by releasing a renal dipsogen. The evidence for this hypothesis has been clearly summarised by Fisher (1973). He found that the central threshold dose of isoprenaline was higher than the systemic one and that the central action was reduced after nephrectomy. Fisher suggests that isoprenaline acts on the kidney either directly by "leaking" from the brain or indirectly by central

stimulation of efferent neurones to the renal nerves. His ideas are supported by experiments involving section of the spinal cord and the use of labelled isoprenaline.

A third neurotransmitter which may be involved in drinking is dopamine. Ungerstedt (1971) reported that destruction of central catecholaminergic neurones in the rat using 6-hydroxydopamine caused adipsia and aphagia. Setler (1973) reported that intraventricular injection of the amine in the rat elicited drinking without increased feeding. The rats also suffered weakness and ataxia. A role for dopamine was also suggested by the report that intrahypothalamic injection of apomorphine which is known to stimulate dopamine receptors was dipsogenic in some rats (Fisher, 1973).

Setler (1973) developed a hypothesis that central cholingergic and dopaminergic mechanisms were respectively involved in the response to cellular and extracellular stimuli. She found that centrally administered atropine only reduced drinking induced by carbachol or hypertonic saline. Conversely, haloperidol given centrally only reduced drinking caused by hypovolaemic stimuli. Fisher (1973) performed similar experiments but found that both central cholinergic and dopaminergic mechanisms were involved in all types of dipsogenic stimuli. He did however find that the cholinergic component was greater in cellular dehydration and the dopaminergic component greater in extracellular dehydration.

The role of various central neurotransmitters in drinking mechanisms in other species is virtually unknown. Central administration of carbachol caused autonomic or emotional responses but not drinking in the cat (Myers, 1964b), monkey (Sharpe & Myers,

1969) and gerbil (Block, Vallier & Glickman, 1974). However, recently intrahypothalamic nicotine has been shown to elicit drinking in the monkey (Myers, Hall & Rudy, 1973). In the gerbil intracerebral injection of noradrenaline caused drinking which was not secondary to increased eating.

The role of renal factors in drinking

In 1954, a group of workers in Spain noticed that nephrectomised rats drank less water than either intact animals or those in which the ureters had been tied. They also observed that the drinking deficit of nephrectomised rats could be restored by injecting hog kidney extracts (Linazasoro, Jimenez-Diaz & Castro-Mendoza, 1954). They suggested that the kidney might be the source of a "thirst regulating hormone". Later, this result was confirmed by reports that injections of rat kidney cortex (Asscher & Anson, 1963) or crude hog renin (Nairn, Masson & Corcoran, 1956) elicited drinking.

During his investigations into the role of extracellular factors in drinking behaviour, Fitzsimons (1969) studied the effect of certain cardiovascular manipulations. He found that the drinking response following caval ligation or suprarenal aortic constriction was smaller in nephrectomised rats than in intact or uretal ligated ones. It was also demonstrated that constriction of the renal arteries induced drinking in the rat (Fitzsimons, 1969; Gutman, Benzakein & Chaimowitz, 1967). These results also implicated the kidney in drinking mechanisms.

Fitzsimons (1969) then extended the earlier observations, showing that the renal dipsogenic factor was present in the cortex

but not the medulla of the kidney. This result was consistent with the suggestion that the active principle was renin. In order to substantiate this view Fitzsimons (1969) compared the vasopressor and dipsogenic potencies of each fraction during the preparation of renin from hog kidney. He could not separate them at any stage. Further support was provided by results demonstrating that both vasopressor and dipsogenic activity of kidney extracts were suppressed by DOCA/saline pretreatment. Finally, an intravenous infusion of highly purified renin elicited drinking in nephrectomised and intact rats.

Further evidence which implicated renin in drinking behaviour came from experiments with hypotensive drugs. The β-adrenoceptor agonist, isoprenaline, (Lehr et al, 1967) and other hypotensive agents, hydralazine, phentolamine and phenoxybenzamine (Peskar, Leodolter & Hertting, 1970) increased water intake in the rat. The same drugs also caused increases in plasma renin concentration (Meyer, Peskar & Hertting, 1971) and both responses were prevented by nephrectomy (Meyer et al, 1971). It was suggested that renin release was mediated by an action on juxtaglomerular cell β-adrenoceptors. The β-agonists acted directly and the hypotensive agents indirectly, by reflex activation of the sympathetic-adrenal system. This suggestion was confirmed since systemic administration of propranolol reduced the effects of both types of drug but ganglion blockade only prevented the response to the hypotensive agents (Meyer, Rauscher, Peskar & Hertting, 1973).

Support for the dipsogenic role of renin has also been provided by clinical studies. Certain patients with renin-secreting

tumours have experienced "intense thirst" associated with elevated plasma renin levels; thirst was immediately abolished after bilateral nephrectomy (Brown, Curtis, Lever, Robertson, de Wardener & Wing, 1969).

Most of the biological actions of renin are mediated by angiotensin (Peart, 1965), thus its dipsogenic activity was investigated. Intravenous infusions of angiotensin II caused drinking in both the intact and nephrectomised rat (Fitzsimons & Simons, 1969). The doses necessary to elicit the response were very high and nephrectomised animals were more sensitive. Subsequently it has been demonstrated that drinking can be elicited using much lower doses of either angiotensin I or II (Hsiao & Epstein, 1973).

There were several possible mechanisms through which angiotensin could initiate drinking. Firstly, the pressor action could have altered the sensitivity of stretch baroreceptors in the vasculature to a pre-existing hypovolaemia. This mechanism seemed unlikely since equipressor infusions of vasopressin were not dipsogenic (Fitzsimons & Simons, 1969). A second possibility was that the polypeptide itself caused hypovolaemia by increasing capillary permeability. Haefli & Peters (1971) reported that iv injection of renin or angiotensin II into nephrectomised rats decreased plasma volume within 2 minutes. This result was not confirmed by other workers using intact or nephrectomised rats (Fitzsimons & Simons, 1969; Abdelaal, Mercer & Mogenson, 1974a). Since angiotensin II can stimulate aldosterone release, this mechanism might have been implicated, however the dipsogenic effect of the peptide was similar

in adrenalectomised and control rats (Fitzsimons & Simons, 1969).

Characteristics of the central dipsogenic response to angiotensin II

The hypothesis which has received most support was that angiotensin-induced drinking was due to a central nervous action. Booth (1968) first noted that injection of angiotensin II into the rostral hypothalamus of the rat elicited copious drinking. The central dipsogenic activity of angiotensin II in the rat was subsequently confirmed using intracerebral (Epstein, Fitzsimons & Rolls, 1970) and intracerebroventricular (Severs, Summy-Long, Taylor & Connor, 1970) injections. Unlike carbachol, angiotensin II has dipsogenic actions in several other species. Central dipsogenic responses have been demonstrated in the goat (Andersson & Westbye, 1970), the rhesus monkey (Setler, 1971; Myers et al, 1973; Sharpe & Swanson, 1974), the cat (Sturgeon, Brophy & Levitt, 1973; Cooling & Day, 1973), the gerbil (Block et al, 1974), the dog (Rolls & Ramsay, 1973), the dove, rabbit and guinea pig (Fitzsimons, 1971a). Another factor which suggested the central nervous system as the site of action was that in the rat the central threshold dose was at least a hundred times less than the systemic one (Epstein et al, 1970).

Extensive investigations of the central dipsogenic actions of angiotensin II have been made in the rat (Severs & Daniels-Severs, 1973). Drinking can be elicited with doses of 1 - 4000ng intracerebrally (Epstein et al, 1970; Severs et al, 1970). The response began from 10 seconds to 5 minutes after injection, it lasted for 5 to 10 minutes and the amount of water consumed varied from a few sips to 30ml (Epstein et al, 1970). In individual rats a

response could be elicited at 15 minute intervals and was stable over several months of testing (Epstein et al, 1970). In the rat angiotensin II was more specific than other agonists since it elicited only drinking behaviour, however in the monkey the peptide elicited dose-related yawning behaviour (Sharpe & Swanson, 1974). The dipsogenic stimulus was very potent since rats would awake from sleeping or struggle against manual restraint to drink. If water was not provided the animal would search the cage and lick spillage (Epstein et al, 1970). In experiments in which access to water was delayed, Rolls & Jones (1971) showed that drinking occurred up to 60 minutes after intracerebral injection.

Drinking elicited by intracerebral angiotensin II has similar characteristics to that induced by the most common natural stimulus, water deprivation. After both stimuli there was no hyperactivity, more isotonic saline than water was ingested (Rolls & Jones, 1971) and feeding was suppressed (McFarland & Rolls, 1972). Water deprived or angiotensin II treated rats would also drink water adulterated with quinine (Rolls, Jones & Fallows, 1972) or alcohol (Johnson & Anderson, 1973) and press levers to obtain water (Rolls et al, 1972; Graeff, Gentil, Peres & Covian, 1973). In the gerbil when lettuce was provided as the free-water source, angiotensin II ic or lettuce-deprivation both caused increased lettuce intake. This is good evidence that angiotensin has a physiological role in drinking behaviour (Block et al, 1974).

Fitzsimons (1971b) reported that intracerebral injection of hog renin, tetradecapeptide renin substrate and angiotensin I caused drinking in the rat. These agents probably act by local generation

of angiotensin II by interacting with the intrinsic brain renin-angiotensin system. He also examined the metabolic degradation products of angiotensin II. The heptapeptide, des-Phe^{8} angiotensin II, was inactive but the heptapeptide, des-Asn^{1} angiotensin II was half as active as the parent peptide. The 3 - 8 hexapeptide had about 10% of the activity of angiotensin II, however shorter chain fragments were very weak agonists. These results suggested that the structure-activity relationships for myotropic and vasopressor effects and those for the dipsogenic action were similar. A hexapeptide with phenylalanine at the C terminus were essential features. In a later report, Swanson, Marshall, Needleman & Sharpe (1973) confirmed that des-Phe^{8}-angiotensin II was inactive but found that if phenylalanine was replaced by either alanine, tyrosine or isoleucine, the dipsogenic action was restored. They suggested that the structural requirements for central dipsogenic activity in the rat were not as rigid as those for systemic actions. The central administration of another proteolytic enzyme, kallikrein, or other polypeptides, bradykinin, oxytocin or vasopressin, did not elicit drinking in the rat (Fitzsimons, 1971).

Location of the site of action of angiotensin II in the brain

Attempts to delineate the central site of action of angiotensin II have involved microinjection of the peptide into discrete brain loci in several species. In the rat, the septum, anterior thalamus, preoptic area and anterior hypothalamus were sensitive to large doses of angiotensin II. These doses occasionally elicited responses when injected into the lateral or ventromedial hypothalamus and the amygdala. The cerebellum, tegmentum, superior

colliculus, caudate nucleus and neocortex were insensitive (Epstein et al, 1970). When smaller doses were used the site of action was confined to the nucleus accumbens, preoptic area and anterior hypothalamus. A later study, in which smaller injection volumes were used, confirmed these results but the sensitive zone was located nearer to the midline (Swanson & Sharpe, 1973). Microinjection of angiotensin II into the septal, preoptic and anterior hypothalamic areas elicited drinking in the gerbil (Block et al, 1974). Myers et al (1973) demonstrated that a receptive area for angiotensin II in the monkey was situated caudally in the hypothalamus and mesencephalon. These workers did not study regions anterior to A.P. 15 but in a subsequent study (Sharpe & Swanson, 1974) another angiotensin-sensitive zone was located between A.P. 16 and A.P. 18. This region encompassed the septum, preoptic and anterior hypothalamus. Sites in the thalamus, midbrain reticular formation and hindbrain were not sensitive to angiotensin II. In the cat injection of angiotensin II into structures which form the limbic system elicited drinking (Sturgeon & Levitt, 1974). Sensitive regions were also found in the corpus callosum, substantia nigra and caudate nucleus but no drinking occurred after injections of angiotensin II into the cerebral cortex.

Although there was a wide distribution of angiotensin-sensitive areas, many were demonstrated in the limbic system or in sites connected with the hypothalamus. Thus it is likely that the outputs from these regions are integrated in the hypothalamic drinking "centre". At present only a few electrophysiological studies of the effect of angiotensin II on central neurones have been reported but the results

are consistent with this view. Application of the peptide to rat preoptic neurones caused either increases or decreases in their activity (Findlay, 1972). However, preoptic injections of angiotensin II did initiate an increase in the rate of discharge of hypothalamic neurones (Black, Mok, Cope & Mogenson, 1973). Similarly, intravenous or local injections of angiotensin II caused either increased or decreased hypothalamic neural activity (Wayner, Ono & Nolley, 1973).

The wide range of receptive sites could also be explained by diffusion or transport of the peptide from insensitive to sensitive regions. Johnson (1973) reported that in the rat the course of the injection cannula determined the sensitivity of intracerebral sites. When the cannula traversed the cerebral ventricles negative sites became positive, conversely if the cannula did not penetrate the ventricle positive sites were found to be negative. This suggested that the solution injected leaked into the ventricles along the track of the cannula to act on periventricular tissues. A possible site of action is the subfornical organ (SFO), a vascular ependymal structure which projects into the third ventricle. It has no blood-brain barrier and would thus be accessible to systemic angiotensin. The SFO in the rat has a low dipsogenic threshold to angiotensin II, the minimum dose being 0.1ng (Simpson & Routtenberg, 1973). Lesions in the SFO abolished drinking induced by injections of angiotensin into the preoptic area (Simpson & Routtenberg, 1973) or lateral ventricle (Phillips, Leavitt & Hoffman, 1974) and reduced the response to intravenous angiotensin II (Simpson, 1973). Electrophysiological studies produced conflicting results, in the

cat Felix & Akert (1974) found that microiontophoresis of the peptide increased SFO discharge but this was not confirmed in the rat (Phillips et al, 1974). A ventricular site of action is supported by the results in the rat (Severs et al, 1970) goat (Andersson & Westbye, 1970) monkey (Sharpe & Swanson, 1974) and in this study in the cat demonstrating that small icv doses of angiotensin II reliably elicit drinking.

Mechanism of the central dipsogenic action

The precise mechanism of the dipsogenic action of angiotensin II is unclear but it appears to have at least two components. The evidence cited above establishes that in animals in water-balance drinking can be elicited by injecting angiotensin II into the brain. This suggests that angiotensin has a direct central dipsogenic action which is independent of cellular or extracellular stimuli. The absence of interaction between two stimuli is evidence for functional independence of the mechanisms (Fitzsimons, 1972). Fitzsimons (1970) found that in the rat drinking elicited by intracerebral angiotensin II simply added to that induced by cellular or extracellular stimuli. His results were confirmed recently by Severs, Summy-Long & Daniels-Severs (1974).

However, Fitzsimons (1970) also found that subthreshold doses of angiotensin II combined with subthreshold cellular or extracellular stimuli to elicit drinking. This suggested that the peptide sensitised central integrating mechanisms to other dipsogenic stimuli. This view was also supported by Andersson's (1971) observations that combined icv infusion of angiotensin II in hypertonic saline caused a greater drinking response than the total

of their individual effects. This sensitising action may be important in the dipsogenic response to extracellular stimuli, since most of these stimuli release renin.

The action of angiotensin II on the central neurones controlling drinking behaviour may be direct or it may be mediated by release of central neurotransmitters. The release of these neurotransmitters by angiotensin II could be due to a post-synaptic action on specific receptors or to a presynaptic action at the nerve terminal. The role of neurotransmitters in the dipsogenic action of angiotensin II is considered in Chapter 3.

Effects of angiotensin II on release of antidiuretic hormone

The renal conservation of water is regulated by antidiuretic hormone (ADH) released from the neurohypophysis. This hormone acts on the distal tubules and collecting ducts of the kidney and increases their permeability to water. The mechanisms controlling the release of ADH and those initiating drinking behaviour are very similar. ADH release can be initiated by depletion of either the extracellular or intracellular fluid compartments and central cholinergic and adrenergic neurones are involved. An account of the mechanisms controlling the release of ADH has been given by Ginsburg (1968).

Severs et al (1970) first suggested that angiotensin II might cause the release of ADH. They found that the pressor response to icv angiotensin II in the rat was attenuated by lesions in the supraoptic nucleus or hypophysectomy. Further experiments demonstrated that icv administration of angiotensin II caused water retention for 90 minutes in prehydrated rats (Severs, Daniels-Severs, Summy-Long &

Radio, 1971). A similar antidiuretic response was observed in the goat after infusion of angiotensin II into the third ventricle (Andersson & Westbye, 1970). These indirect studies were extended by Bonjour & Malvin (1970), who reported that intravenous infusion of angiotensin II increased plasma ADH levels in the conscious dog. A greater increase in plasma ADH concentration occurred after infusion into the carotid arteries of the chloralosed dog, indicating a central site of action (Mouw et al, 1971). This hypothesis was confirmed since perfusion of an angiotensin II solution through the ventricular system of the anaesthetised dog was more effective than either intravenous or intracarotid infusions (Mouw et al, 1971). A second group was unable to repeat the results obtained by infusing angiotensin II intravenously or into the carotid arteries (Claybaugh, Share & Shimizu, 1972). However, this group found that intracarotid infusions of angiotensin II potentiated the release of ADH elicited by intravenous administration of hypertonic saline (Shimizu, Share & Claybaugh, 1973). Similarly, Olsson & Kolmodin (1974) demonstrated that the antidiuretic effect of intracarotid hypertonic saline in the goat was potentiated by icv infusion of angiotensin II. Thus angiotensin II may act directly or by reducing the threshold for other stimuli.

There are two possible sites of action for angiotensin II, the peptide could act on the supraoptic nucleus (SON) or directly on the neurohypophysis. Microiontophoretic application of angiotensin II into the cat SON (Nicoll & Barker, 1971) or to canine organ-cultured SON cells (Sukai, Marks, George & Koestner, 1974) caused an increase in their rates of discharge. However, other workers have shown that

angiotensin II can release ADH from rat isolated neurohypophyses (Gagnon, Cousineau & Boucher, 1973).

Central actions of angiotensin II on electrolyte balance

The central actions of angiotensin II on the intake and excretion of electrolytes were confusing since it caused increases in both parameters. In the rat several workers have found that angiotensin II injected intracerebrally (Fisher, 1973) or intraperitoneally (Wong & Whiteside, 1974) caused increased preference for sodium chloride solution rather than water. The saliuretic effects of icv administration of angiotensin II have been demonstrated in the rat (Severs et al, 1971) and the goat (Andersson & Westbye, 1970). The mechanisms involved in these responses have not been examined.

Centrally mediated cardiovascular actions of angiotensin II

The centrally mediated vasopressor effect of angiotensin II was first observed when the peptide was injected via the carotid artery into the cerebral circulation of an anaesthetised dog (Bickerton & Buckley, 1961). Since then extensive investigations of the response have been performed in several species. In most of these studies the peptide was administered either via the vertebral artery or by intracerebral injection into brain tissue or the CSF. The results obtained using these different routes of injection will be considered separately.

Vertebral artery administration

Dickinson & Lawrence (1963) demonstrated that in the conscious rabbit intravenous infusions of initially subpressor doses of angiotensin II produced a nervously mediated progressive pressor

response. They suggested that this might be caused by vasomotor centre ischaemia due to constriction of the hindbrain vasculature. This hypothesis was tested by comparing the pressor response elicited by infusions of angiotensin II into the vertebral artery with that elicited by a similar intravenous infusion. When small doses of angiotensin II were used the pressor response after vertebral artery administration was consistently greater than after intravenous administration (Dickinson & Yu, 1967). This result was confirmed in both conscious and anaesthetised rabbits (Ueda, 1968; Rosendorff, Lowe, Lavery & Cranston, 1970). Intravertebral artery infusions of low doses of angiotensin II which were non pressor when given intravenously, raised the blood pressure of anaesthetised mongrel (Ferrario, Dickinson & McCubbin, 1970) and greyhound (Lowe & Scroop, 1969) dogs and of conscious mongrels (Fukiyama, Page & McCubbin, 1971; Sweet, Kadowitz & Brody, 1971). Centrally mediated pressor responses have also been reported in anaesthetised cats (Keim & Sigg, 1971; Buckley, 1972) and conscious man (Ueda, 1968) after vertebral artery infusion of small doses of angiotensin II.

Site of action in the brainstem

Recent evidence does not support the view that angiotensin acts by causing vasoconstriction in the hindbrain blood vessels. It now appears that the peptide acts on specific neural structures in the brain-stem. The site of action was located by selectively ligating the hindbrain blood vessels or making lesions in the brain tissue of mongrel or greyhound dogs. Midbrain section did not affect the pressor response to vertebral artery infusions of angiotensin II indicating that hypothalamic and mesencephalic structures were

unimportant (Gildenberg, 1971; Joy, 1971). The ligation studies indicated that a response was only elicited if angiotensin had access to the caudal part of the medulla oblongata (Gildenberg, 1969; Joy & Lowe, 1970a).

The most suitable site in the medullary region was the area postrema, a neurovascular structure situated in the floor of the fourth ventricle. It is accessible to blood-borne angiotensin II since it is outside the blood brain barrier. Ueda (1968) showed that microinjection of several substances, including angiotensin II, into the area postrema of the cat caused a systemic vasopressor response. His group subsequently showed that increased unit activity occurred in the cat area postrema after angiotensin II was infused into the vertebral artery (Ueda, Katayama & Kato, 1972). Bilateral ablation or cooling of the area postrema in the dog abolished the vasopressor effect of intravertebral infusion of angiotensin II (Gildenberg, 1971; Joy & Lowe, 1970b) but not those elicited by acetylcholine or prostaglandin $F_{2\alpha}$ (Joy & Lowe, 1970b).

Ablation studies have also demonstrated a central component in the response to intravenous infusions of angiotensin II. In both the greyhound (Scroop, Katic, Joy & Lowe, 1971) and the rabbit (Joy & Lewis, 1971) bilateral ablation of the area postrema significantly reduced the pressor response to iv angiotensin but not that to iv noradrenaline.

Mechanism of action

The mechanism of action of angiotensin II in the brainstem has not yet been adequately explained. The area postrema overlies the nucleus vagi and has connections with the solitary tract nucleus.

This latter nucleus receives fibres from the glossopharangeal and carotid sinus nerves, thus angiotensin II might modify integration of impulses arriving at the vasomotor centre.

Iizuka & Peterson (1969) reported that the pressor response to hypothalamic, but not medullary, stimulation was increased during vertebral artery infusion of angiotensin II in the dog. Other workers were unable to confirm this effect in the cat (Suga, Manger & Reich, 1971; Keim & Sigg, 1971). Several groups have investigated the effect of intravertebral artery infusion of angiotensin II on the integration of baroreceptor afferent impulses. In the dog, reflex vasodilatation in the perfused hindlimb vasculature, elicited either by iv injection of noradrenaline or low carotid sinus pressure, was reduced by central infusion of the peptide (Sweet & Brody, 1970; Goldstein, Heitz & Brody, 1972). The depressor response and bradycardia induced by carotid sinus nerve stimulation was also inhibited during vertebral artery angiotensin II infusion (Fukiyama, 1972). A similar infusion potentiated reflex vasoconstriction initiated by low perfusion pressure in the carotid sinus (Goldstein et al, 1972) or the pressor response to carotid occlusion (Inoue, Smulyan & Eich, 1969). Central adrenergic neurones appeared to be involved in the central effects of angiotensin II since prior intravertebral infusion of clonidine reduced the response to the peptide (Katic, Lavery & Lowe, 1972). This inhibitory effect of clonidine could be reversed by adrenergic neurone blockers or phentolamine given centrally.

Efferent pathways

In most species the effects of intravertebral artery infusions

of angiotensin II were mediated by the sympathetic nervous system. Thus in the rabbit the pressor response was abolished by ganglion or α-adrenoceptor blockade (Ueda, 1968). However, in the dog the efferent pathways may be different in various breeds. The pressor response in the mongrel dog was entirely mediated by sympathetic mechanisms since it was abolished by cervical spinal cord section (Ferrario, Gildenberg & McCubbin, 1972) or guanethidine (Fukiyama, 1972) but not by atropine. In contrast the pressor response in the greyhound was not affected by procedures which disrupted sympathetic efferents but was markedly reduced by vagotomy or atropine (Scroop & Lowe, 1969). This suggested that withdrawal of parasympathetic tone caused an increased cardiac output leading to a rise in pressure. However, a "residual" pressor response which remained after vagotomy was abolished by bethanidine. The results obtained using vagotomy and atropine are difficult to interpret since both procedures cause large increases in heart rate . It may be that since greyhounds have tremendous cardiac reserve they respond to any stimulus by increasing cardiac output.

Direct evidence that vertebral artery infusion of angiotensin II caused increased sympathetic discharge was obtained in electrophysiological studies. Ueda (1968) reported an increase in superior cervical nerve activity in the rabbit and Fukiyama (1972) an increased splanchnic nerve discharge in the dog. Conflicting evidence was obtained in the cat. Keim & Sigg (1971) noted an increase in the preganglionic cervical sympathetic nerve prior to the pressor response. However, Suga et al (1971) did not detect any increase in splanchnic nerve discharge after either injection or

infusion of angiotensin II into the vertebral artery.

Intracerebral administration

Nashold, Mannario & Wunderlich (1962) first demonstrated the pressor response elicited by injection of angiotensin II into the lateral cerebral ventricle of the anaesthetised cat. A detailed analysis of this response was subsequently performed by the group led by Buckley (Buckley, 1972). In their studies this group perfused the ventricular system from the lateral ventricle to the cisterna magna using artificial cerebrospinal fluid. Angiotensin II injected into the perfusion solution elicited a dose related pressor response usually accompanied by tachycardia but occasionally by reflex bradycardia (Smookler, Severs, Kinnard & Buckley, 1966). In later studies, it was shown that icv administration of angiotensin II caused a pressor response in the anaesthetised dog (Severs, Daniels & Buckley, 1967; Gildenberg, Ferrario & McCubbin, 1973) and rabbit (Rosendorff et al, 1970) and the conscious rat (Severs et al, 1970) rabbit (Rosendorff et al, 1970) or goat (Andersson, Eriksson & Fernandez, 1971).

Central site of action

Experiments involving selective brain lesions and variation of the cannula position established that in the cat, angiotensin II acted in the midbrain. Cerveau isolé transection and midbrain lesions each reduced the vasopressor response, indicating that suprapontine mesencephalic structures were important (Severs, Daniels, Smookler, Kinnard & Buckley, 1966; Severs et al, 1967). The response was also attenuated by excluding the aqueduct and fourth ventricle from the perfusion system. Since direct

application of the peptide to the outflow from the aqueduct did not elicit an effect, a periaqueductal site of action was probable.

In 1967, Enoch & Kerr described a sympathetic vasopressor pathway which originated in the hypothalamus, synapsed in the subnucleus medialis then turned abruptly lateral and terminated in the nucleus mesencephalicus profundus. Deuben & Buckley (1970) presented evidence which suggested that angiotensin II acted on this tract. They found that the angiotensin-sensitive area lay between planes 4 - 5 anterior to the Horsley - Clarke zero. The subnucleus medialis lies at plane 4 anterior and lesions at that locus, but not at loci posterior to it, abolished the response to icv angiotensin II. Earlier experiments in the same preparation had demonstrated that intrahypothalamic injections of angiotensin II did not cause a rise in blood pressure (Severs et al, 1966). Similarly, the nucleus mesencephalicus profundus and deeper tegmental structures were also insensitive to angiotensin II (Deuben & Buckley, 1970). These results implied that the subnucleus medialis of the periaqueductal grey matter was the site of action.

Recent evidence obtained in experiments in the dog has established that there are two separate sites of action in the brain for angiotensin II. In these studies destruction of the area postrema abolished the pressor response to angiotensin II given via the vertebral artery but did not affect the response to icv angiotensin II. Conversely, midbrain lesions, which did not attenuate the hypertensive response to vertebral artery infusions of angiotensin II, abolished the effect of icv angiotensin II (Gildenberg et al, 1973).

The centrally mediated cardiovascular effects of angiotensin II

appear to involve adrenergic mechanisms, but the exact mode of action is obscure. In the cat depletion of brain catecholamine levels, by icv injection of either metaraminol or reserpine, reduced the pressor response. The response was also reduced by central injection of pargyline (Smookler, 1965) or infusion of noradrenaline (Smookler et al, 1966) which increased the level of catecholamines in the brain. However, central administration of the peptide did not have any significant effect on the concentration of noradrenaline in several regions of the brain (Daniels & Buckley, 1968). In the rat, the pressor response to icv angiotensin II also involved central adrenergic mechanisms since it was reduced by central injection of phentolamine or propranolol (Severs et al, 1971).

Efferent mechanisms

The peripheral sympathetic nervous system and adrenal medulla were the efferent pathways involved since the response was abolished after C_1 spinal cord section in the cat (Smookler et al, 1966) and rabbit (Rosendorff et al, 1970). This was confirmed in the cat by using autonomic blocking drugs. Systemic administration of either phenoxybenzamine, pronethalol or a compound which prevented release of adrenal catecholamines all reduced the pressor response (Severs et al, 1966). However, in the rat only systemic administration of phenoxybenzamine reduced the pressor response to icv angiotensin II. Systemic administration of phentolamine or β-adrenoceptor blockers did not affect the response and hexamethonium potentiated it (Severs et al, 1970, 1971). It has been suggested that the pressor response in the rat is mediated by release of vasopressin.

The basis of the project

The centrally mediated cardiovascular actions elicited by icv administration of angiotensin II in the anaesthetised cat were examined by Buckley's group (Buckley, 1972). The aim of this project was to examine the central actions of the peptide in the conscious cat thus avoiding any complications introduced by anaesthesia. The original intention was to study the cardiovascular effects but the potent dipsogenic effect of angiotensin II was also observed. At that time there were no other reports of drinking behaviour elicited by central administration of drugs in the cat. This observation was thus pursued and experiments to analyse the mechanism of the dipsogenic response of renin and angiotensin form the major part of this thesis.

METHODS

Cats

Mongrel cats of either sex weighing between 2 and 4Kg were used in this study. The animals were housed in separate cages and given one meal of tinned cat food (Whiskas, Petfoods Ltd.) each day. Water was available ad libitum.

Cannulation of the lateral cerebral ventricle

a) Modification of the Collison cannula

In 1953, Feldberg & Sherwood described a cannula which could be used to inject drugs into the cerebral ventricles of the cat. It consisted of a 12mm shaft connected to a butt bearing two screw threads. The lower thread allowed the device to be screwed into the skull and the upper thread accepted either a stilette or a screw-cap with a rubber diaphragm. It was named the "Collison" cannula after its designer.

In initial experiments in this study the Collison cannula was used, but several minor problems were encountered. In many cats the butt was too short so that it was often covered by skin to the level of the screw-cap. This made it difficult to hold the butt firmly whilst removing the stilette. Since the screw threads on the butt were in the same direction, the device was sometimes inadvertently loosened from the skull when removing the screw-cap. It was also noticed that the original Collison cannula had a large internal fluid volume which seemed unnecessary and could allow a proportion of a drug dose to remain within the cannula.

In order to overcome these defects the cannula was modified. Firstly, the length of the butt was increased so that the screw-thread for the cap was well clear of the skin. Secondly, the

directions of the screw threads were reversed with respect to each other. Thus any lateral pressure on the butt when removing the stilette tended to tighten the cannula into the skull rather than loosening it. In order to increase the anchorage, a flange was added at the base of the butt which could be embedded in acrylic cement during the cannulation. The "dead space" within the cannula was decreased by continuing the shaft tubing through the butt. Administration of drugs by injection through the rubber diaphragm was discontinued and a connector cap was made through which infusions could be given. The connector cap was constructed from 16 gauge hypodermic needle tubing, passed through, then soldered to a screw cap. A separate connector was used for each drug solution. A scale diagram of the cannula and its attachments is shown in Fig. 1. The modified cannula, like the original, was made from stainless steel and weighed twice as much (5.8g compared with 2.6g). This weight difference did not appear to disturb the cats. Fig. 2 illustrates the Collison and modified cannulae.

b) Cannulation procedure

The cannulation procedure has been photographed and various stages are illustrated in Fig. 3. The cat was anaesthetised using a mixture of oxygen (20%), nitrous oxide (80%) and halothane. The halothane concentration was 4% during induction and 1.5% was used for maintaining anaesthesia. When the cat was fully anaesthetised it was placed in a prone position with its head held firmly in the head-holder of a stereotaxic frame. The head was shaved and a parasaggital incision, 3cm long, was made slightly to the right of the midline. The periosteum was scraped from the skull and the

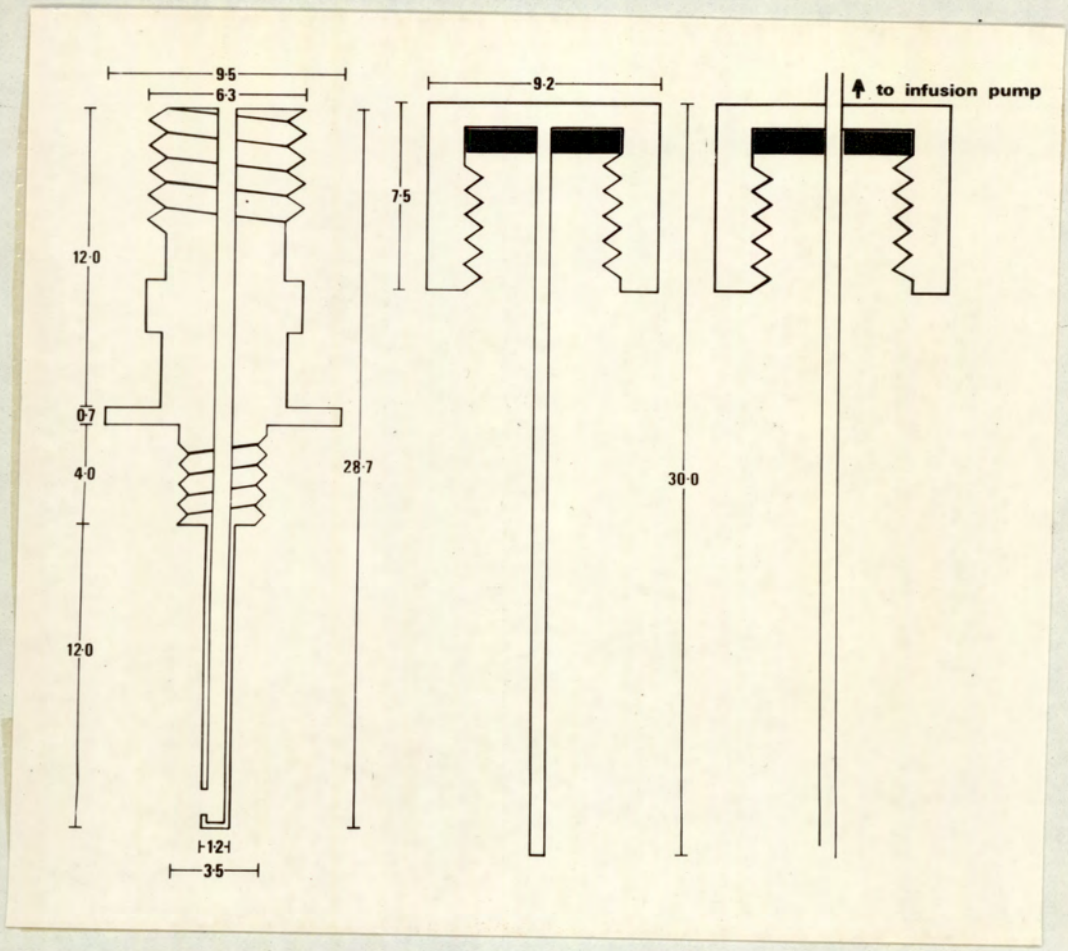


Fig. 1 A scale diagram showing cross-sections of the modified cannula, stilette and infusion cap used in experiments in the conscious cat. The measurements are in millimetres.

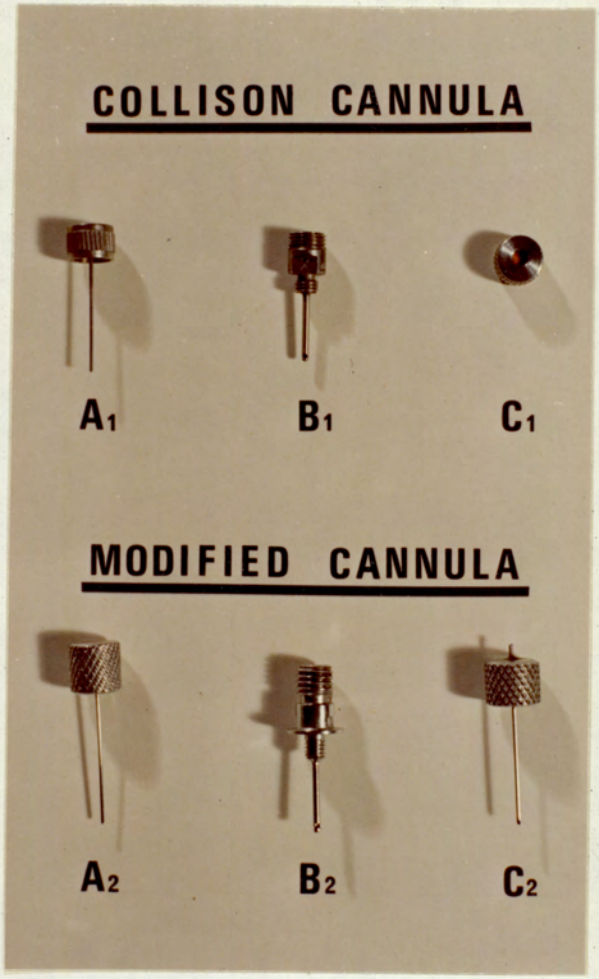
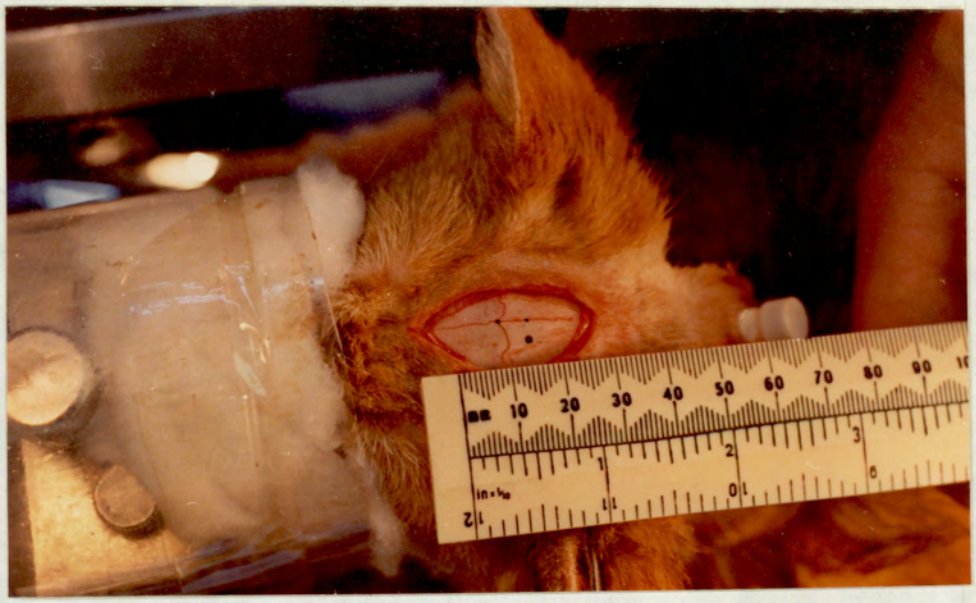


Fig. 2 A photograph showing both the Collison cannula and the modified device used in the present study.

Fig. 3 A series of photographs illustrating various stages in the procedure used to implant the intracerebroventricular cannula in the cat.

1. The incision and the position for the cannula
2. The hole drilled at the position indicated above
3. The guide tube in position
4. and 5. The cannula in position
6. The end of the procedure


mm 10 20 30 40 50 60 70 80 90
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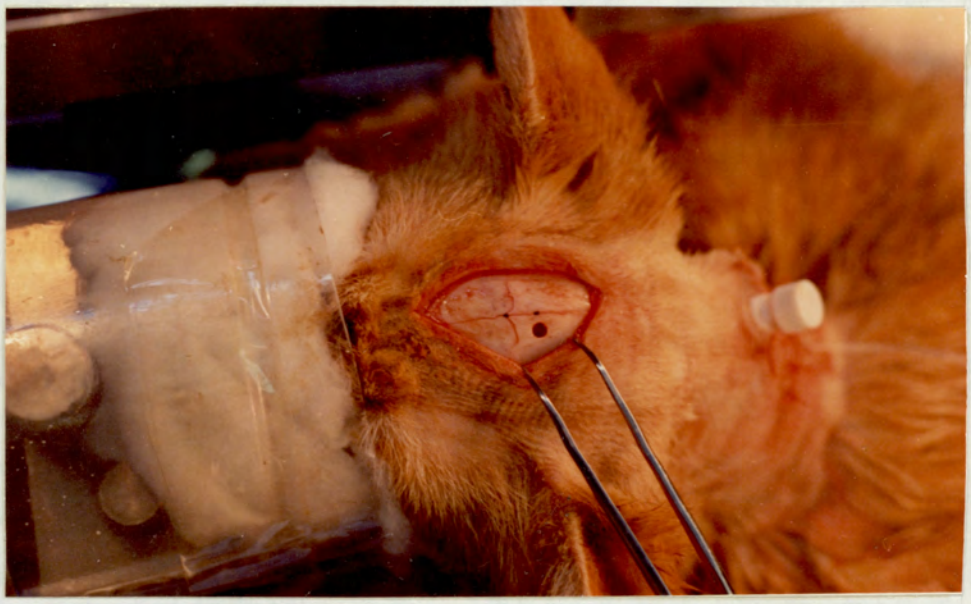
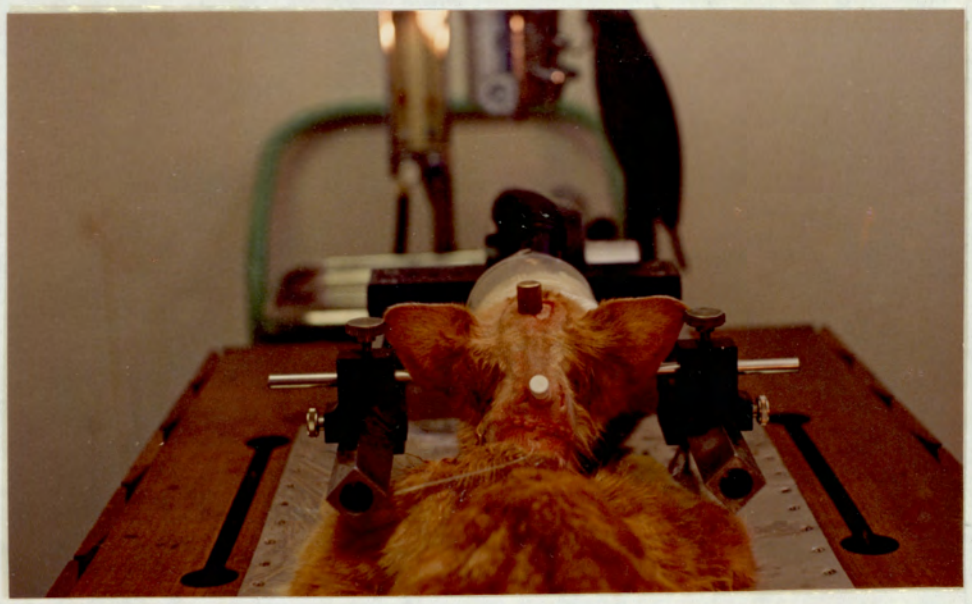
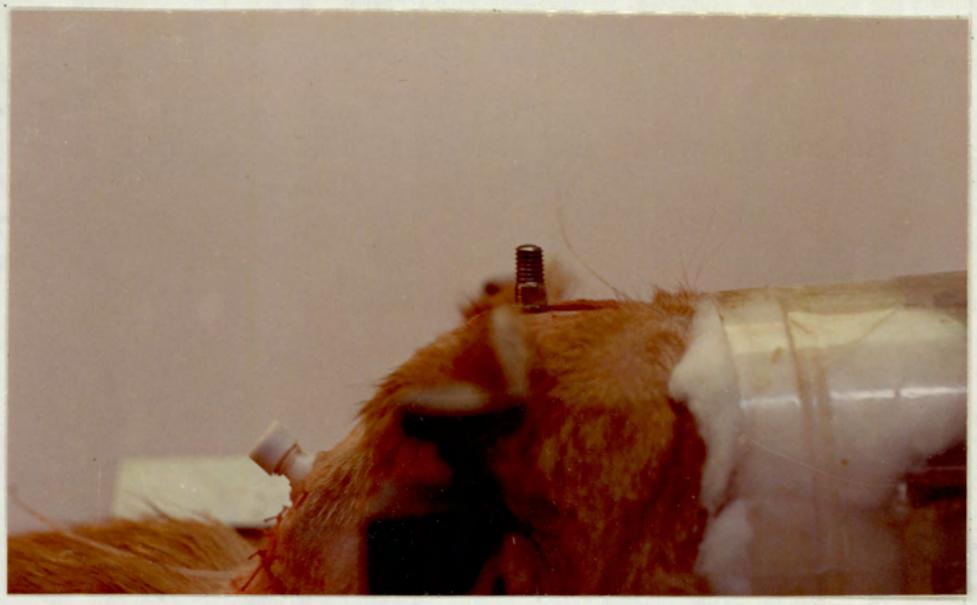
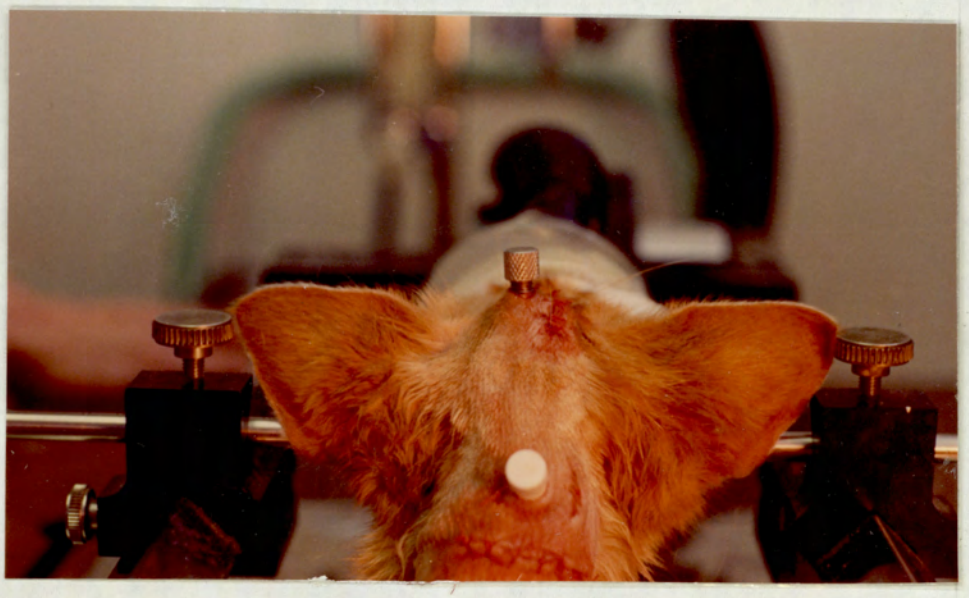








position for the cannula marked on the bone. The co-ordinates normally used were 6mm caudal to the coronal suture and 4mm lateral to the saggital suture. However, slight alterations were made when using either very small or very large cats. A hole was bored at the site marked using a dental drill, this hole was tapped, the meninges cut and a guide-tube, lacking a shaft, was inserted. A needle was passed through the guide-tube to create a track for the shaft of the cannula. The guide-tube was then removed and the cannula inserted, taking care to ensure that it remained vertical. The cannula was positioned so that the opening was directed medially towards the Foramen of Munro. After fixing the cannula with acrylic cement, the skin was drawn over the butt and a hole was made to allow the cannula to pass through. The initial incision was then sutured. The operation was performed under clean conditions to minimise the risk of infection. The instruments and the cannula were sterilised by steam before use and stored in a 2% aqueous solution of "Hibitane" (I.C.I. Ltd) during the operation. All surfaces were cleaned using disinfectant and steps were taken to avoid hairs entering the wound. At the end of the operation an intramuscular injection of a long acting penicillin combination ("Triplopen", Glaxo Ltd.) was given.

At the end of a series of experiments a solution of Pontamine Sky Blue dye was infused into the ventricles. The cat was killed about 30 minutes later by injecting an overdose of sodium pentobarbitone intravenously. The brain was removed, fixed in 4% formol saline then sectioned manually using a razor blade. The dye was usually detected in the lateral, third and fourth ventricles. A series of brain sections showing the rostral and caudal faces is

presented in Fig. 4.

Cannulation of blood vessels

In many cats cannulas were introduced to allow measurement of the arterial blood pressure and intravenous administration of drugs. These cannulas were implanted during the same operation, prior to the icv cannulation. The method used has been described in detail by Day & Owen (1970a). The right carotid artery was cannulated and the catheter inserted until the tip lay in the thoracic aorta. The blood pressure was monitored during cannulation to ensure that the tube did not enter the heart. The cannula was tied into the artery and passed beneath the skin to a Teflon valve secured at the back of the neck. The intravenous cannula was inserted into a branch of the right jugular vein, it also emerged at the back of the neck and was closed with a pin.

Arterial blood pressure was measured by means of a pressure transducer connected to an electronic recorder. The heart rate was obtained using a tachygraph triggered by the blood pressure pulse.

Procedure for experiments

After the cat had recovered from the operation it was allowed to become familiar with the surroundings used for the experiment. This was accomplished by placing the cat in the cage in the laboratory for about 6h each day for 4 days. During this acclimatisation period the cat was closely observed. Experiments were performed twice a week with at least 48h between the start of each.

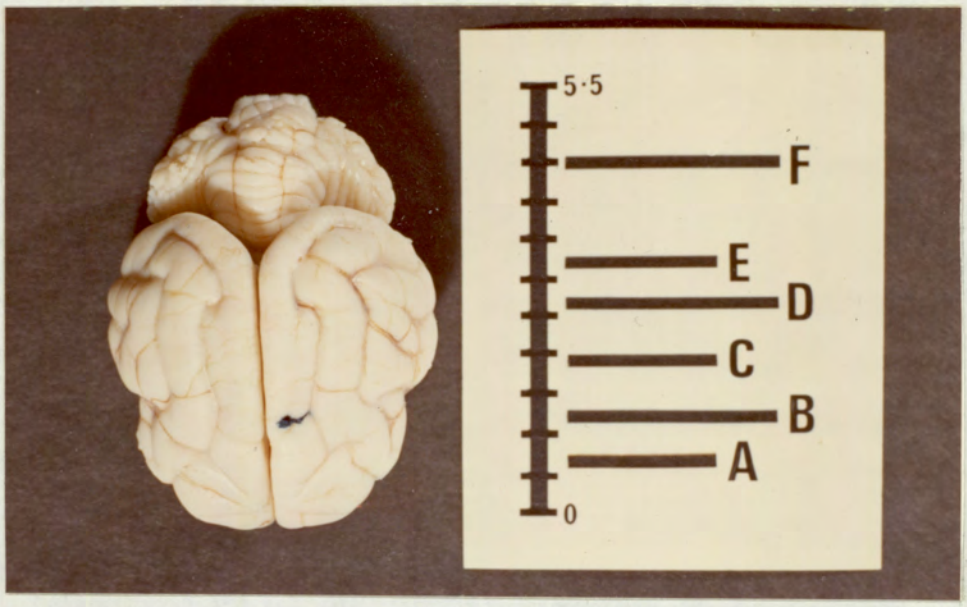
Drug solutions were administered into the lateral cerebral ventricle using a variable-speed constant infusion pump (S.R.I. Ltd.). The volume infused was 100μl at 25μl min^{-1}, which is approximately

Fig. 4 Photographs illustrating the distribution of Pontamine Sky Blue dye (100μl) infused into the lateral cerebral ventricle of the conscious cat.

Top. The whole brain. The letters A - F indicate the planes at which the brain was sectioned. Scale: 0 - 5.5cm, each division is 0.5cm.

Middle. The rostral faces of sections at planes A - F.

Bottom. The caudal faces of sections at planes A - F.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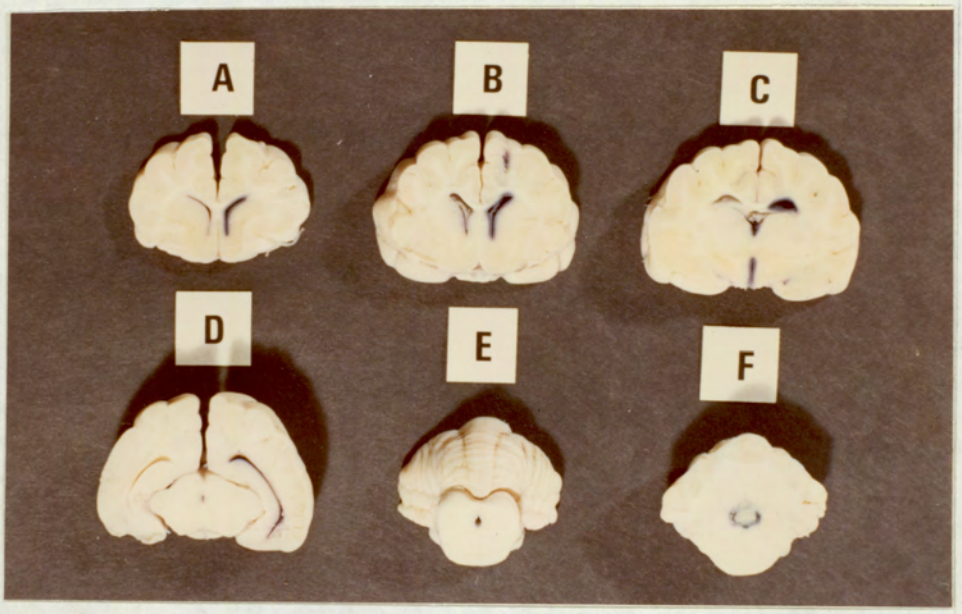
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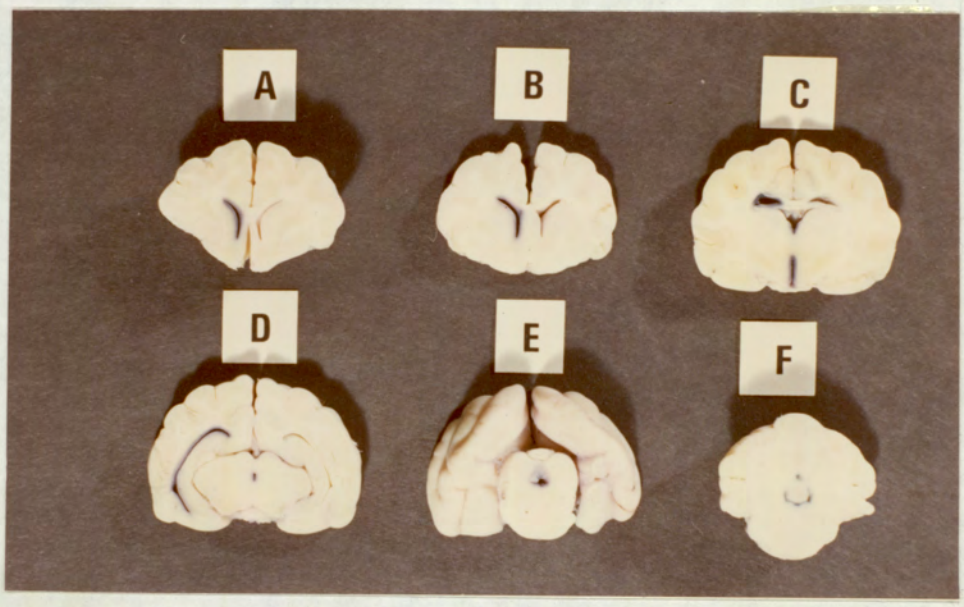
A
B
C
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F

the rate of formation of CSF in the cat (22.2 ± 6.8µl min^{-1}:Vates et al, 1964). Intravenous doses were given remotely either as single injections or continuous infusions.

The amount of water consumed was measured by weighing the bowl of water before and after the response. The loss by evaporation was corrected by measuring the weight change of a similar bowl of water placed outside the cage. In a recent report Sturgeon, Brophy & Levitt (1974) criticised the use of conventional water bowls and described a new drinking device for the cat. Their initial method consisted of measuring the volume of water by transferring fluid from the bowl to a measuring cylinder. They found this to be inaccurate since water adhered to the bowl and also the large cylinders used were only graduated in 5ml units. These difficulties did not arise in the present study when weight rather than volume was measured. The only difficulty which could arise was that removing the bowl from the cage might disturb the cat. This did not seem to be a problem in this study since after acclimatisation the cats usually dozed during experiments and were only disturbed by unusual stimuli. Sturgeon et al (1974) also found that their cats spilled water whilst drinking, this was not observed in these studies unless the bowl was full to the brim. Evaporation loss reported by Sturgeon et al (1974) was 0.75ml h^{-1} which was similar to that observed here (0.5ml h^{-1}).

Rabbits

New Zealand White rabbits weighing between 2-3Kg were used. The animals were individually housed and allowed free access to water and rabbit diet pellets.

Cannulation procedures

The cannulation procedures used in the rabbit were essentially the same as those employed in the cat. However, icv and intravascular cannulas were implanted at separate operations. The icv cannula was implanted at least 2 days before the experiment whilst blood vessel cannulation was performed 18h before the experiment . This procedure was adopted since the arterial cannula did not usually remain patent for more than 48h.

Cannulation of the lateral ventricle

The cannula used for icv administration of drugs was prepared from a 19G hypodermic needle. The shaft was cut to a length of 8mm and the hub filled with a quick-setting rubber solution. A wire stilette was inserted through the rubber seal to prevent debris entering the lumen during cannulation.

The rabbit was anaesthetised with oxygen, nitrous oxide and halothane mixture. The halothane concentration was 3% for induction and maintainance. The rabbit was placed in a prone position and the skull exposed through a 3cm parasaggital incision. The position for the cannula was marked, 2mm posterior and 2mm lateral to the bregma. Three small brass screws were then fixed to the skull at equidistant points 3mm from this mark. A hole just large enough for the shaft of the cannula to enter was then drilled at the position indicated. The cannula was inserted into the hole and bonded to the skull and the screws using acrylic cement. The skin was then sewn around the cannula. At the end of each experiment a dye was infused into the brain through the cannula and its distribution in the ventricles examined after removal of the brain.

Cannulation of blood vessels

This was performed in a similar manner to the procedure described for the cat. One minor difference was that the tip of the arterial cannula lay in the common carotid artery rather than the aorta.

Procedure for experiments

On the day of the experiment the rabbit was placed in the cage for 1h before the procedure started. Drugs were infused icv and iv using S.R.I. constant infusion pumps. The volume given centrally was 100µl over 4 minutes and that for systemic infusions 0.5ml in 4 minutes. The blood pressure was measured using a pressure transducer and the heart rate using a tachygraph triggered by the arterial pulse. These parameters were monitored using an electronic recorder.

Drugs Used

Angiotensin II amide (Val^5)	- "Hypertensin" CIBA
Angiotensin I (Ile^5)	- Gift Dr. M. Peach
Atropine methylnitrate	- BDH Ltd.
Atropine sulphate	- BDH Ltd.
Bethanidine sulphate	- Burroughs Wellcome & Co.
Carbamylcholine	- BDH Ltd.
Clonidine	- Boehringer
Dopamine hydrochloride	- Sigma Chemical Co.
Haloperidol	- Searle Ltd.
Halothane	- "Fluothane" ICI Ltd.
Hexamethonium bromide	- May & Baker Ltd.
6 Hydroxydopamine	- Sigma Chemical Co.
Isoetharine	- Gift Dr. R. Poyser
L-Isoprenaline sulphate	- BDH Ltd.
Leu^8angiotensin II (Ile^5)	- Dr. D. Regoli
Lignocaine hydrochloride	
Metaraminol bitartrate	- Merck, Sharpe & Dohme
L-Noradrenaline hydrochloride	- Sigma Chemical Co.
Pempidine tartrate	- May & Baker Ltd.
Pepstatin A	- Dr. H. Umezawa
Phenoxybenzamine hydrochloride	- SK & F Laboratories Ltd.
Phentolamine Methane Sulphonate	- CIBA
Pimozide	- Janssen
DL Pindolol	- Sandoz
Polyethylene glycol	- Liquid Macrogol 300 B.P.C.
Practolol	- ICI Ltd.

DL-Propranolol	- ICI Ltd.
D-Propranolol	- ICI Ltd.
L-Propranolol	- ICI Ltd.
Renin (Lyophylised hog)	- Nutritional Biochemical Corp.
Salbutamol	- Allen & Hanbury Ltd.
$Sar^{1}Ala^{8}$angiotensin II (Val^{5})	- Gift Norwich Pharmacal Co.
$Sar^{1}Ile^{8}$angiotensin II (Ile^{5})	- Gift Dr. F. Bumpus
$Sar^{1}Leu^{8}$angiotensin II (Ile^{5})	- Dr. D. Regoli
DL-Sotalol	- Mead Johnson
SQ 20881	- Gift E. Squibb Ltd.
Tolazoline Hydrochloride	- Boots Pure Drug Co. Ltd.

Drugs were dissolved in sterile 0.9% sodium chloride solution (Steriflex, Allen & Hanbury Ltd.).

Phenoxybenzamine was dissolved in distilled water.

Haloperidol and Pimozide were dissolved in 0.1M Tartaric acid and neutralised with 1N NaOH to pH 7.4.

The doses of drugs are expressed in terms of the base.

CHAPTER 1

Water drinking behaviour in the cat induced by the administration of renin or angiotensin and by other procedures

The cat has been widely used as an experimental animal but there have been few studies of the mechanisms controlling water drinking in this species. One reason for this lack of interest may be that normally a cat obtains most of its water from food, thus there is little spontaneous drinking. Carver & Waterhouse (1962) found that cats maintained on canned meat (70 - 80% moisture) obtained only 10% of their total water intake by drinking. This supported previous observations by Wolf (1958), who kept cats successfully without water for several months on diets of fresh salmon and beef. Another factor which has confused studies of drinking behaviour in the cat has been the choice of drinking fluid. Some investigators have used milk, not water, and their results are difficult to interpret since milk is highly palatable and can also be classed as food.

Early evidence that electrical stimulation of the brain initiated ingestive behaviour was obtained using the cat. Brügger (1943) found that hypothalamic stimulation elicited feeding and also caused the cat to drink milk. This work was extended by using intracerebroventricular (icv) injections of hypertonic sodium chloride solution as the stimulus. Miller (1961) used "moderately thirsty" cats and found that icv administration of hypertonic saline (2%) increased water drinking whilst icv administration of distilled water decreased it. These results were consistent with the view that drinking behaviour was regulated by central "osmoreceptors" but since the cats were "thirsty" no conclusions could be made about the

initiating stimulus. Glaser & Wolf (1963) reported that icv injection of hypertonic saline (2 - 3%) induced "heavy drinking of water" which occurred after the cat had suffered seizures. In these experiments the general central excitation could have induced drinking rather than specific stimulation of drinking mechanisms.

When the present studies were started there were no reports of drinking behaviour elicited in the cat by central administration of drugs. The possible central dipsogenic action of carbachol had been tested (Myers, 1964b; Macphail & Miller, 1968) but no drinking behaviour had been observed. Renin and angiotensin had been found to be potent central dipsogens in other species (Fitzsimons, 1972) thus an investigation of their activity in the cat was undertaken. The dipsogenic effects of central and systemic administration of components of the renin-angiotensin system are described in this chapter. Concurrently with this work a group in the U.S.A. independently investigated the central dipsogenic action of angiotensin II in the cat (Sturgeon et al, 1973). Their results will be compared with those obtained in this study. Spontaneous water drinking of cats in this laboratory was observed and attempts were made to induce drinking in the cat using some of the techniques previously employed in the rat.

Results

Observations of spontaneous water drinking behaviour in the cat

The six cats used in this study were housed in separate cages, fed with approximately 300g canned meat each day and allowed water ad libitum. The daily water intake of each cat from its water bowl was measured for a period of eight weeks. There were variations

ranging from 0 to 40g in the amount of water drunk by each cat per day but the mean daily intake was 6 ± 1g. Days on which no drinking occurred amounted to about 40% of the total observation period. The results from three animals are displayed in Fig. 1:1.

The dipsogenic effect of icv administration of angiotensin II

When angiotensin II was infused into the lateral cerebral ventricle of a cat previously allowed free access to water, it regularly induced water drinking. A dipsogenic effect was never obtained after an icv infusion of isotonic saline. Angiotensin II-induced drinking usually began 1 to 10 minutes after the start of the infusion and was normally completed within 30 minutes. In some cats drinking was continuous but other animals drank in short bursts, sometimes leaving the bowl between these. The stimulus was potent enough to make a sleeping cat awake to drink. In two cats in which drinking was voracious, the animals drank about 50g of water, vomited and then began to drink again. Thus the stimulus was powerful enough to overcome this distressing experience.

A typical experiment is illustrated in the series of photographs in Fig. 1:2. The first one shows the start of the infusion with the cat lying quietly on her bed. (The infusion pump, which was normally situated at the side of the cage, was placed on top so that it could be photographed.) In the second picture, taken 4'30" after the start of the infusion the cat began to move towards the water bowl. The next four photographs, taken after 4'40", 5'50", 8'55" and 12'10", show the cat in the act of drinking. Initially the cat stood above the bowl but then it appeared to show more interest in the water and finally crouched over the bowl. After 13'15", the cat

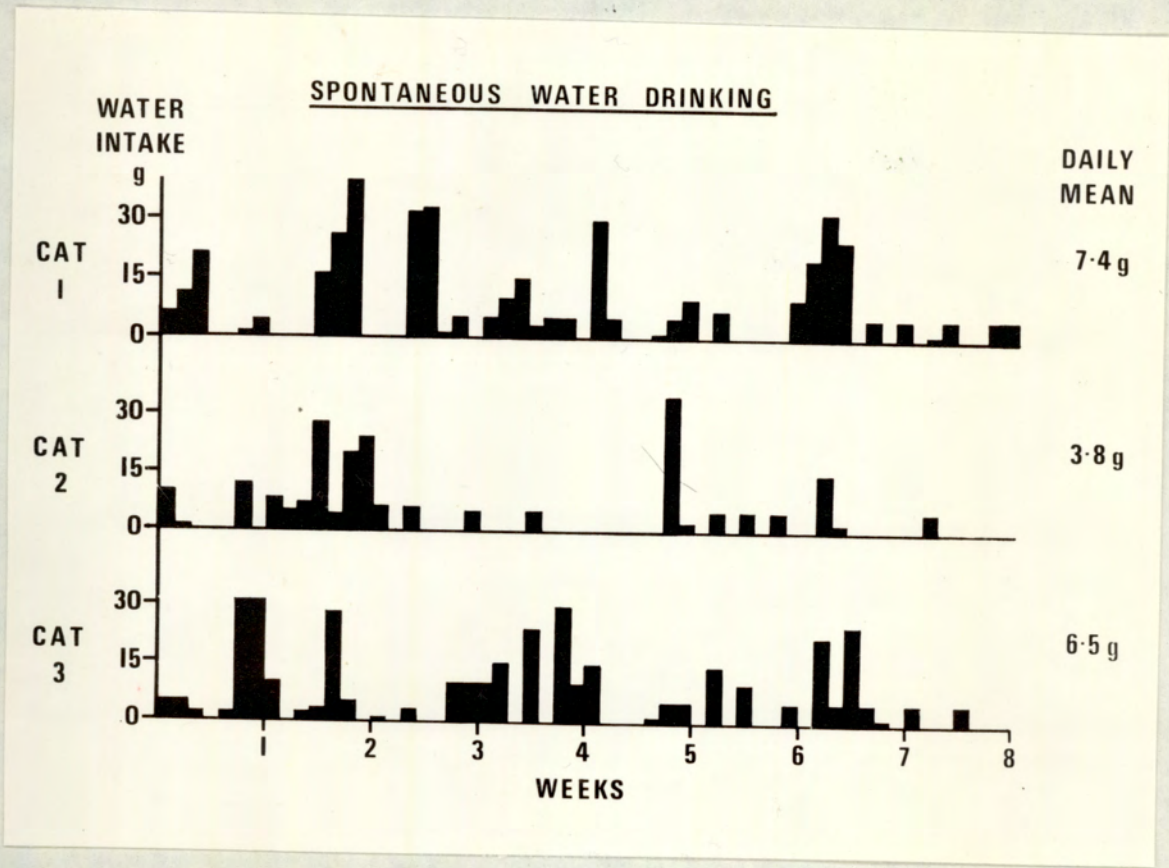
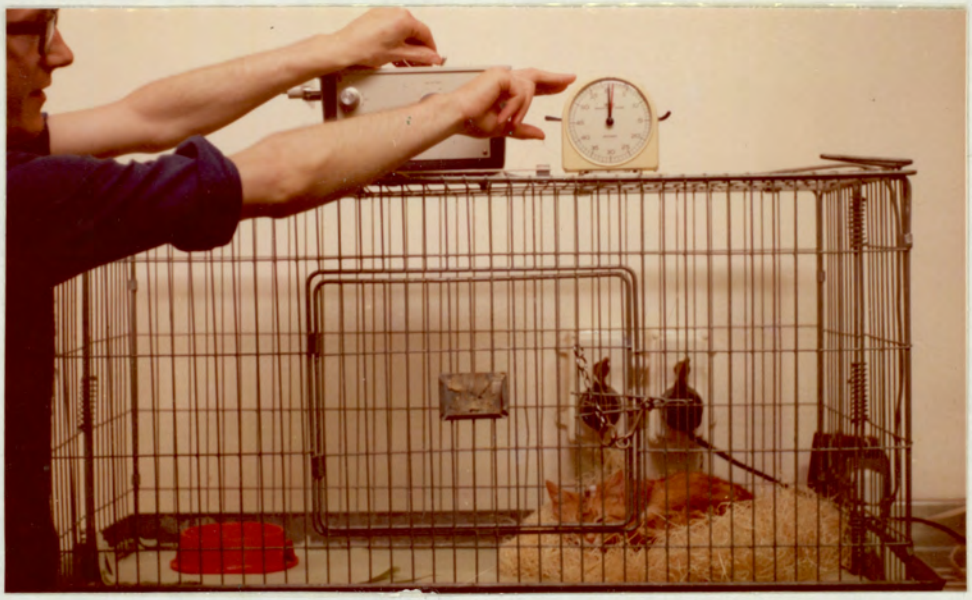
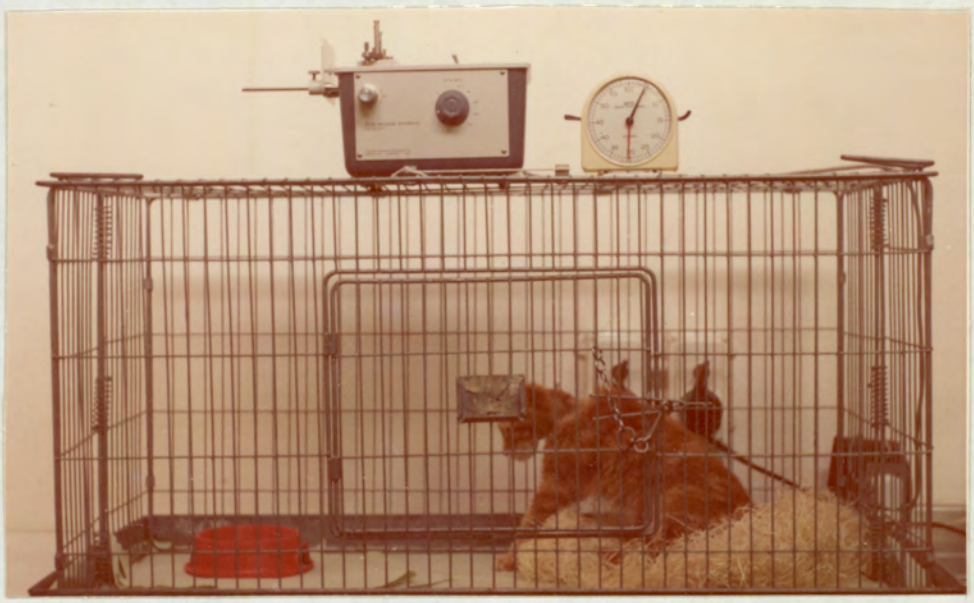
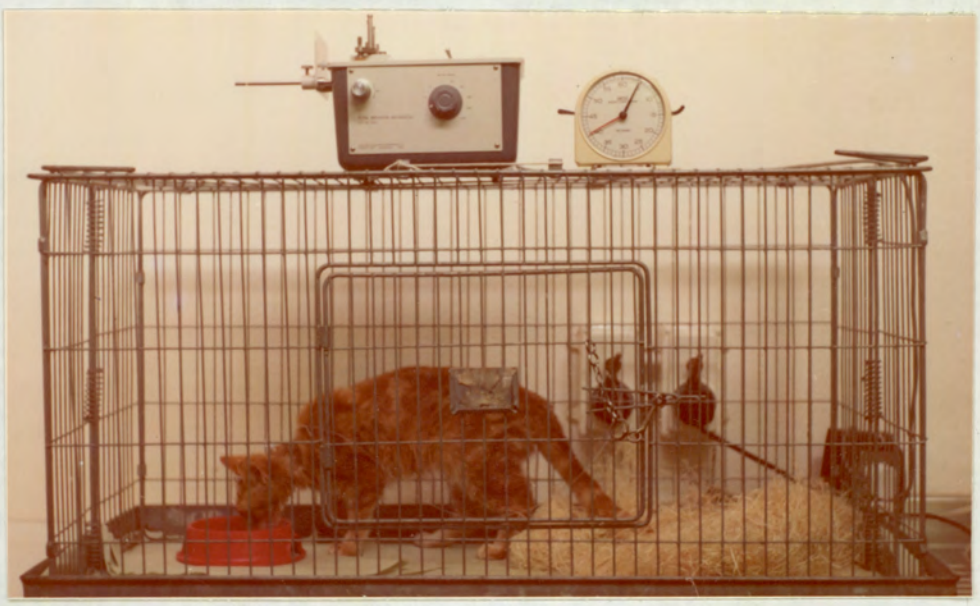


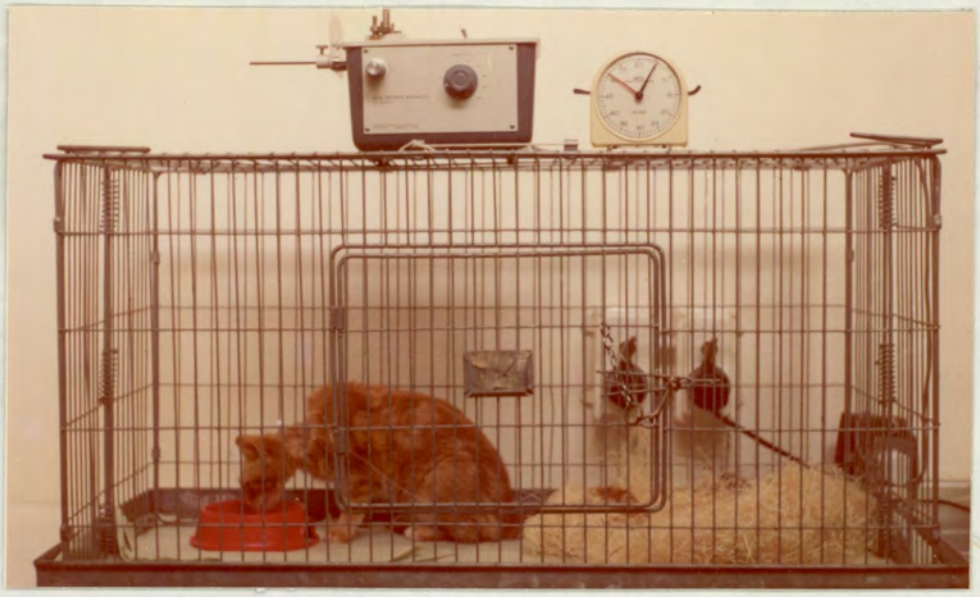
Fig. 1:1 Histograms representing the spontaneous daily water intake from the drinking bowl in each of 3 cats during an 8 week period.

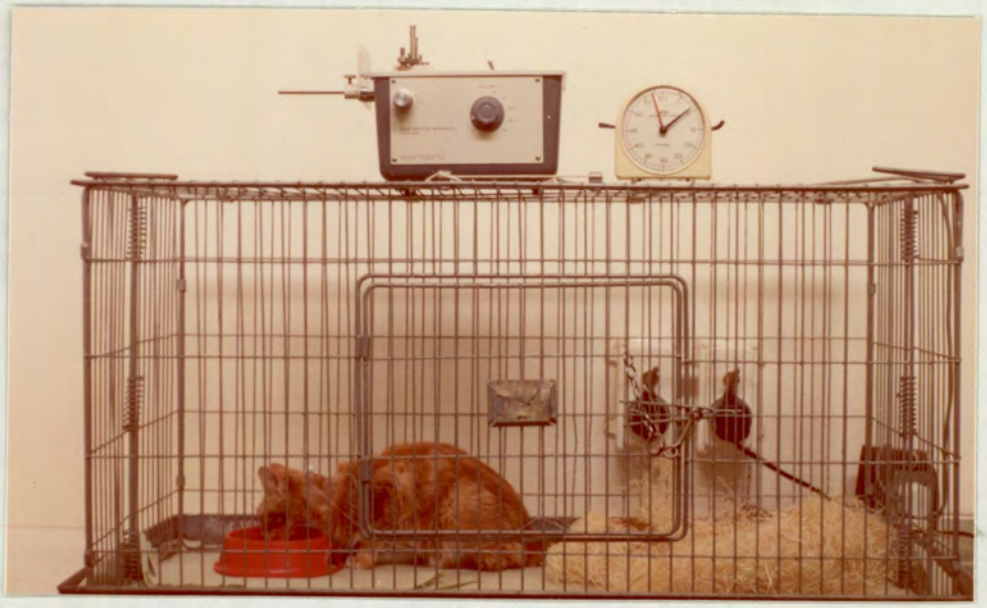
Fig. 1:2 A series of 9 photographs illustrating the drinking behaviour in the cat elicited by infusion of angiotensin II into the lateral cerebral ventricle. The clock shows the time after the start of the infusion at which each photograph was taken.

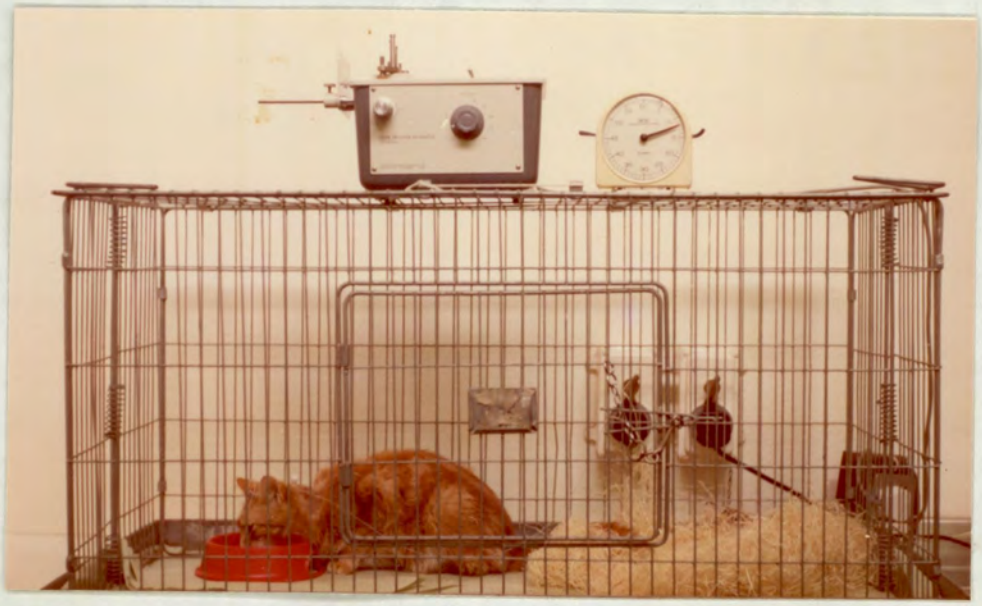


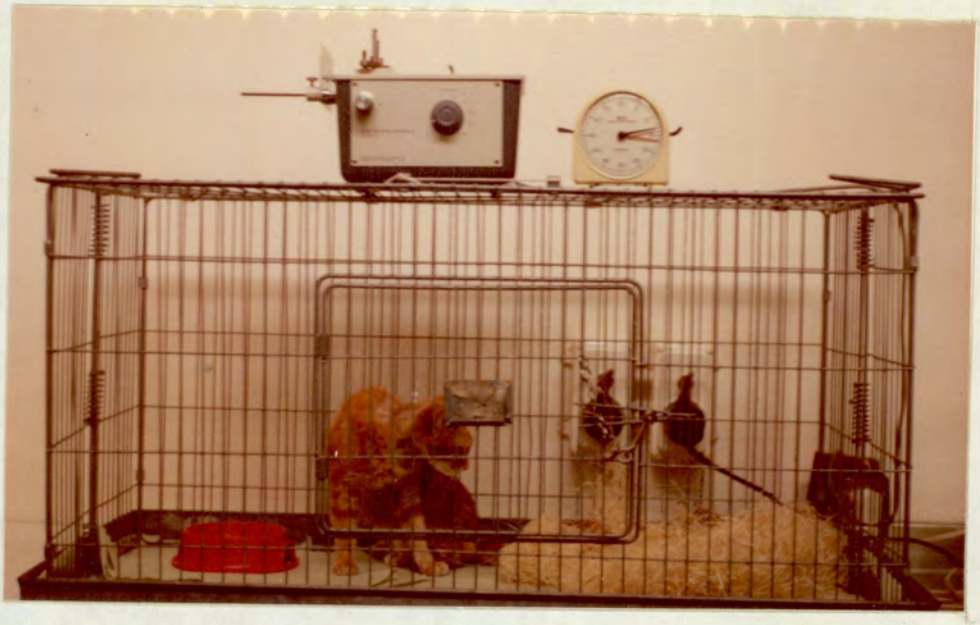


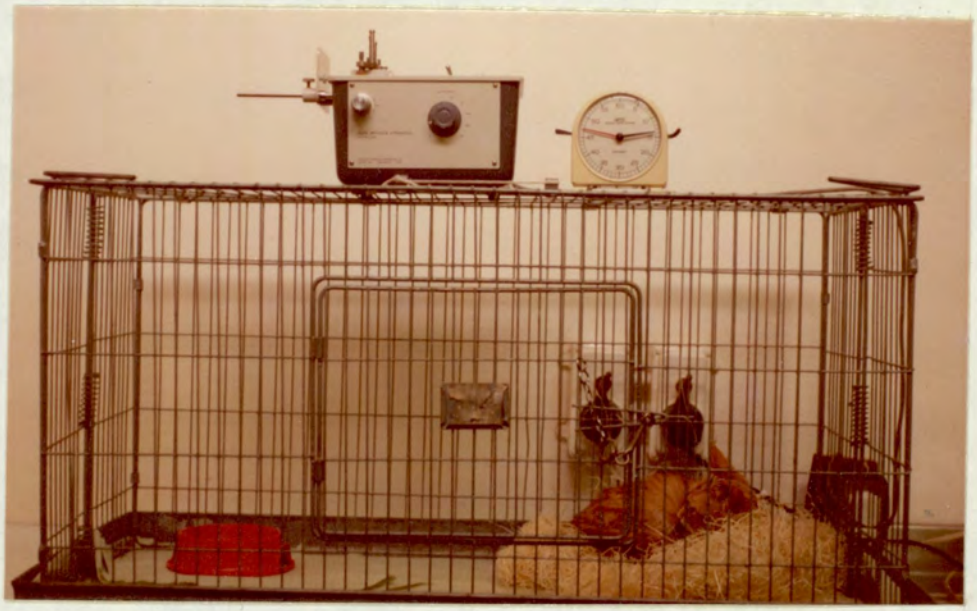


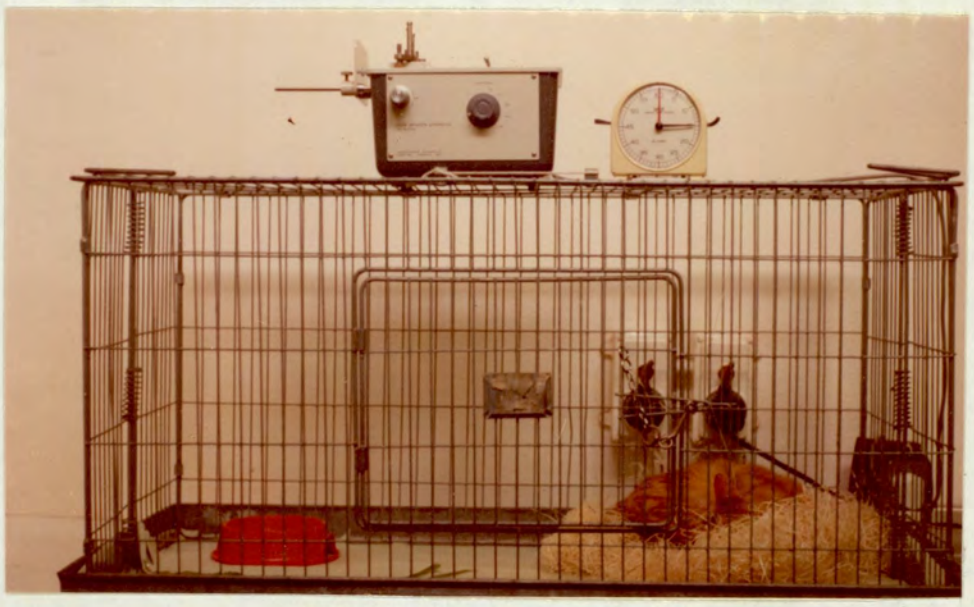












stopped drinking and moved back to her bed. The last two shots show the cat lying on her bed and finally sleeping. The dose of angiotensin II used was 1μg icv and the cat drank 87g water during the session.

Angiotensin II-induced drinking was obtained over a dose range 1ng to 4μg icv and the amounts of water consumed ranged from 5 - 180g in different experiments and with different doses. Drinking behaviour was dose related and a maximal response was usually elicited with doses of 100ng. This is illustrated in Fig. 1:3. The dipsogenic response could be elicited at hourly intervals for 4h but the amount of water consumed in response to the initial infusion was larger than that elicited by subsequent infusions. Fig. 1:4 shows the dipsogenic responses elicited by a supramaximal dose of angiotensin II (4μg) given icv every hour. The second, third and fourth responses are all significantly different to the first one. When the dose interval was extended to 2h, there was no significant difference between the dipsogenic responses to two icv infusions of angiotensin II. After a cat had been used on four or five separate occasions, the maximal dipsogenic response declined to about 50g and remained at this level for several months.

There are some reports that angiotensin II may facilitate intake of saline rather than water (see Fisher, 1973). In two cats, a comparison was made between the intake of water and 0.9% saline after icv infusion of angiotensin II (1μg). In four experiments the intake of saline solution was twice as large as the intake of water.

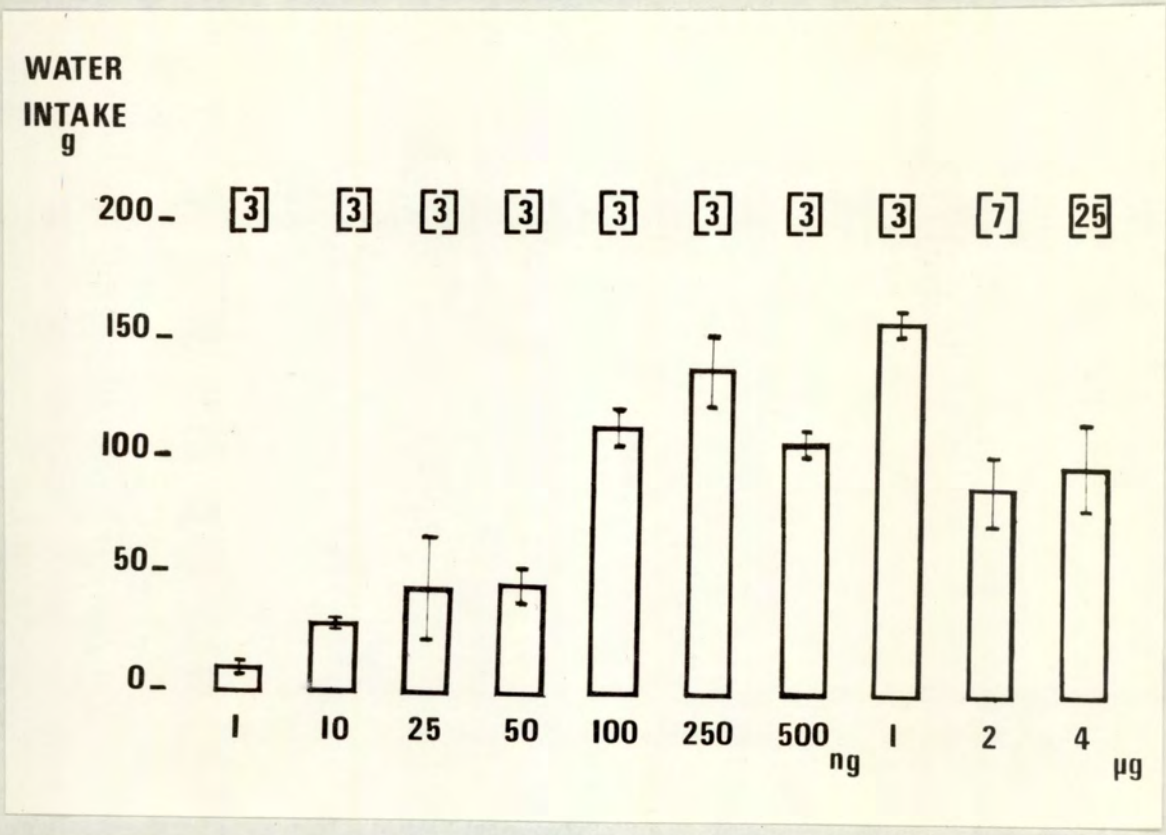


Fig. 1:3 Mean water drinking responses induced in cats by icv administration of angiotensin II. Figures in brackets refer to the number of cats tested at each dose-level; vertical bars are SE of means.

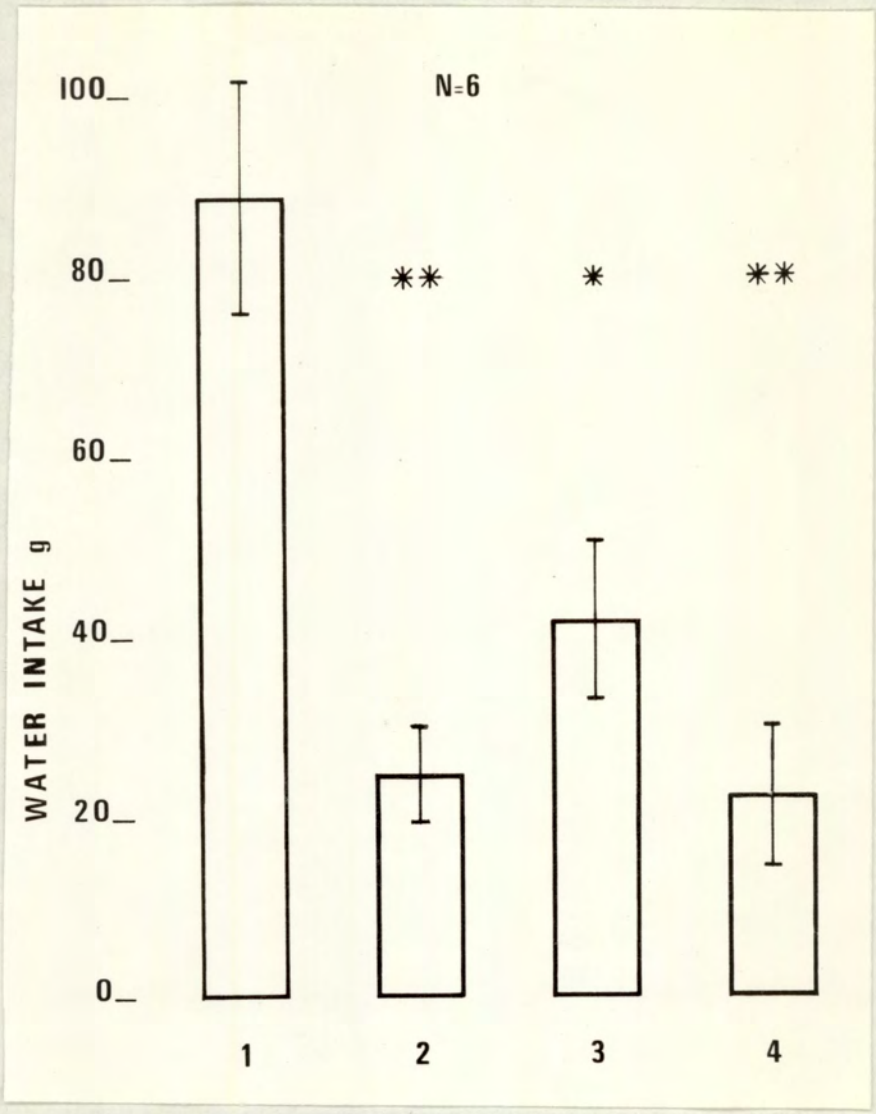


Fig. 1:4 Mean water drinking responses elicited in the cat by icv infusion of a supramximal dose of angiotensin II (4μg) at 1h intervals. Vertical bars indicate SE of means (** $p<0.01$, * $p<0.05$).

The interaction between icv angiotensin II and icv hypertonic solutions

In these experiments, using five newly cannulated cats, the dipsogenic response to angiotensin II icv dissolved in 0.9% saline was compared with that elicited by the same dose dissolved in hypertonic solutions of sodium chloride or sucrose. Experiments were performed on successive days and only one infusion was made in each session.

On the first day the dipsogenic effect of a submaximal control dose of angiotensin II icv (50ng) dissolved in 0.9% saline was recorded. The next day the effect of a similar volume of hypertonic saline (1.8%) was observed. The effect of a combined infusion of angiotensin II in hypertonic saline was tested on the next day. The results are illustrated in Fig. 1:5. Hypertonic saline icv elicited a brief dipsogenic response (8g) in only one cat. However, when a combined infusion was made the drinking response was twice as great as that elicited by icv angiotensin II in isotonic saline.

After an interval of two days the dipsogenic effect of an icv infusion of hypertonic sucrose solution and of angiotensin II dissolved in this solution were tested. Hypertonic sucrose did not elicit drinking and the effect of a combined infusion was not significantly different to that elicited by angiotensin II dissolved in 0.9% saline (Fig. 1:5). At the end of this series of experiments a supramaximal dose of angiotensin II was tested; this elicited a drinking response twice as large as the control.

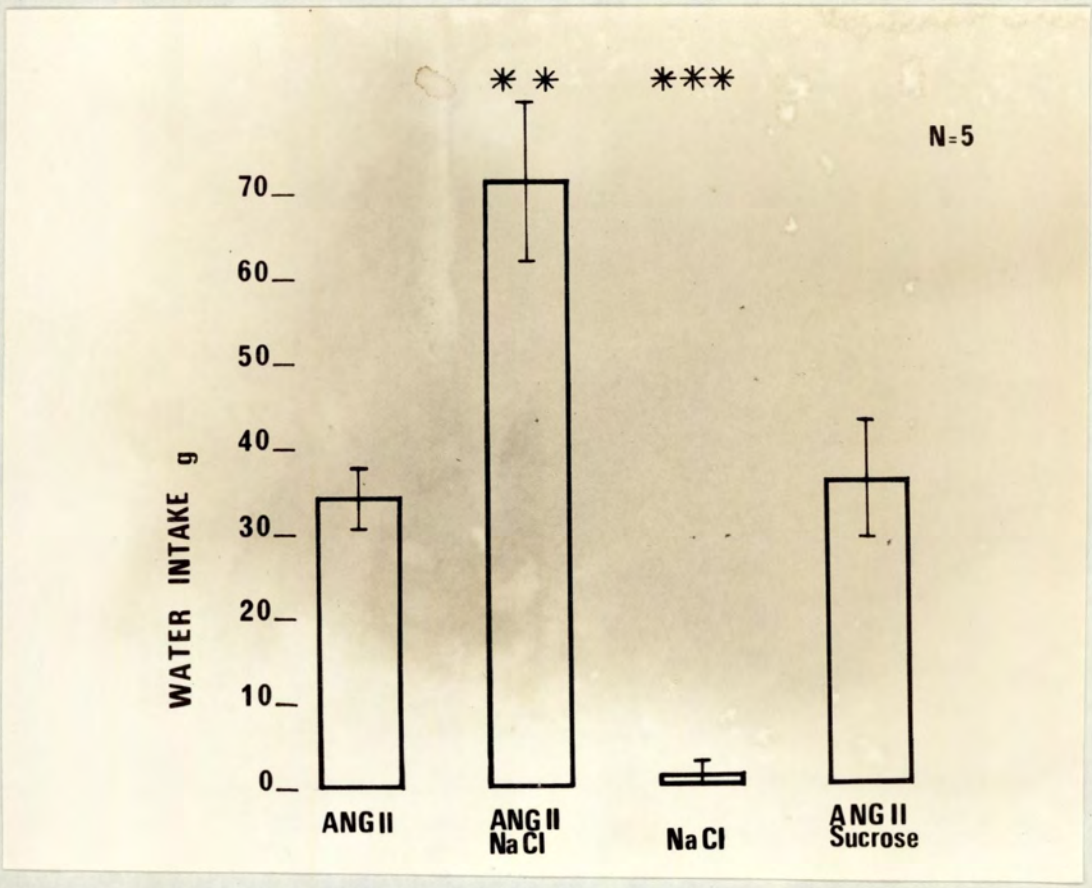


Fig. 1:5 Mean water drinking in the cat elicited by icv infusion of:- angiotensin II (50ng) in 0.9% NaCl solution, angiotensin II (50ng) in 1.8% NaCl solution, 1.8% NaCl solution and angiotensin II (50ng) in 21% sucrose solution. Vertical bars indicate SE of means (*** $p<0.001$, ** $p<0.01$).

The dipsogenic effect of icv angiotensin I and renin

In seven cats the effect of icv infusion of angiotensin I (50ng to 3μg) was investigated; it elicited a similar dipsogenic response to that induced by an equivalent dose of angiotensin II.

Hog renin (0.1 - 0.25 GbU) infused into the lateral ventricle initiated drinking in each of seven cats tested. The onset of the response was delayed by about 15 minutes and the duration prolonged, the response lasting for at least 1h. The enzyme could remain active for several hours in some animals. In one cat 0.25 GbU icv elicited drinking which lasted for 10h, the cat consuming 400g water.

The dipsogenic effect of intravenous administration of renin, angiotensin I or angiotensin II

Intravenous infusion of angiotensin II at doses of 10 to 100ngKg^{-1}min^{-1} for up to one hour caused sustained vasopressor responses in the five cats tested but did not elicit drinking. However, when the dose was increased to 200ngKg^{-1}min^{-1} drinking was elicited. At this dose the latent period was usually between 4 and 10 minutes and this period was proportionally shortened by doubling the dose to 400ngKg^{-1}min^{-1}. Thus the threshold dose was between 0.8 and 2μgKg^{-1}. There was no significant difference in the amounts of water consumed in response to the two dose levels. Drinking behaviour normally ceased after 5 - 10 minutes despite continued infusion of the peptide. The mean water intake in five cats each receiving a total intravenous dose of 4μgKg^{-1} was 36 ± 10g.

Angiotensin I (250ngKg^{-1}min^{-1}) also elicited drinking when infused intravenously in four cats. Infusion of renin (5 GbU in 10 minutes) in four cats which responded to iv angiotensin II,

caused a small response (9g) in only one animal.

It was noticed that drinking in response to intravenous angiotensin could only be elicited for about three weeks although the cats were still sensitive to icv angiotensin.

The dipsogenic effects of β-adrenoceptor agonists

Three β-adrenoceptor agonists, isoprenaline, salbutamol and isoetharine were injected subcutaneously in six cats. Each agonist elicited drinking behaviour. The onset was delayed, beginning between 30 and 60 minutes after injection and was completed within 2h. The results are presented in Table 1:1.

When infused icv, isoprenaline (50μg) induced drinking after a shorter latency (2 - 30 minutes) in ten of sixteen cats tested. The mean water intake was 37 ± 6g. In Fig. 1:6, a trace showing cardiovascular changes after such an infusion is displayed; the start and finish of drinking are also indicated.

In four cats which responded to icv isoprenaline (50μg), the dipsogenic response elicited was compared with that produced by a subcutaneous injection of the same dose administered on a different day. The mean dipsogenic response to icv infusion was 38 ± 10g and that to subcutaneous injection was 23 ± 9g. The difference between these means was not statistically significant.

The effect of icv infusion of hypertonic saline and of cholinomimetic or sympathomimetic drugs

In the experiments cited above icv infusion of 1.8% saline elicited drinking in only one of five cats. In two of the cats which did not respond, icv infusion of 5% and 10% saline was also ineffective. After these infusions the only behavioural response was miaowing. In

TABLE 1:1

Agonist	Dose ($\mu g\ Kg^{-1}$)	Water Intake ($g\ 2h^{-1}$)	N
Isoprenaline	50	26 ± 6	6
	25	22 ± 6	
Isoetharine	25	18 ± 5	6
Salbutamol	25	21 ± 5	6

The dipsogenic effect in the cat elicited by subcutaneous injection of β-adrenoceptor agonists.

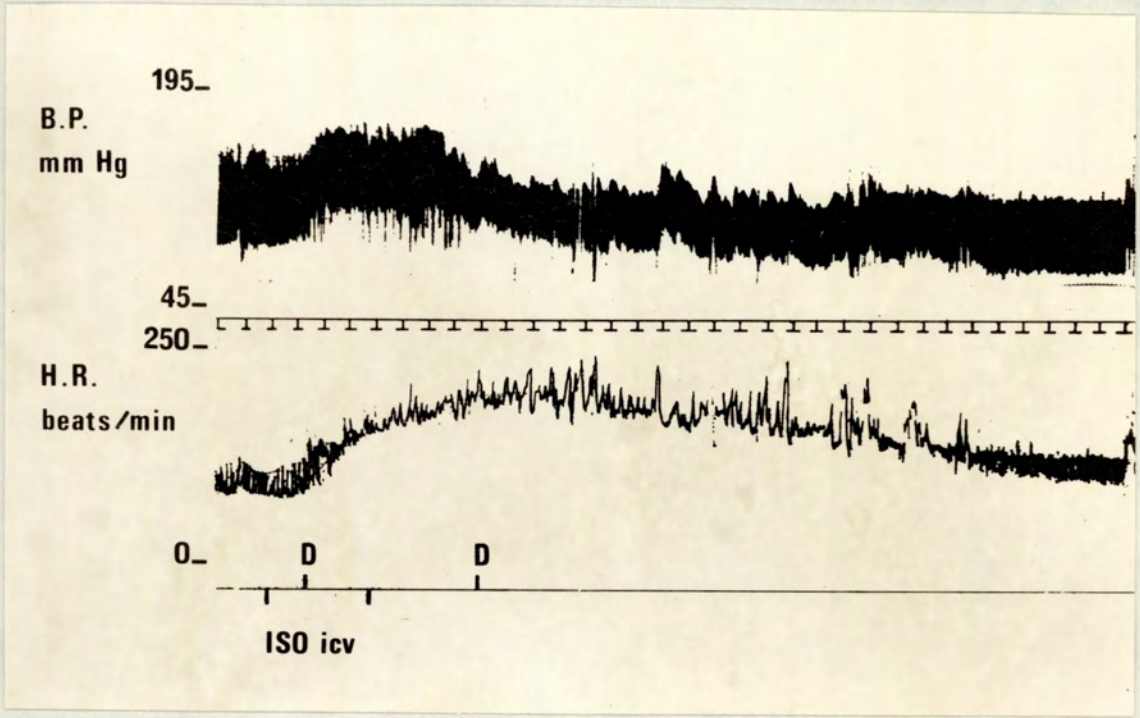


Fig. 1:6 Effect of a 4 minute icv infusion of isoprenaline (50µg) in the conscious cat. The trace illustrates changes in blood pressure and heart rate. Drinking behaviour occurred between the points marked "D".

four experiments carbachol (10 - 20μg) icv elicited a rage reaction, cats growled and became aggressive, but drinking was not observed. Intracerebroventricular infusions of noradrenaline (50μg) dopamine (50μg) or clonidine (1 - 20μg) did not elicit drinking in four experiments with each drug.

The effect of intravenous infusion of hypertonic sodium chloride solution

In a preliminary experiment, 10% sodium chloride solution was infused intravenously in one cat at 1ml min^{-1} for 50 minutes (i.e. 5g NaCl). This induced severe autonomic responses; increased heart rate and blood pressure, retching, defaecation, micturition, nystagmus and aggression. The cat began to drink 3h after the start of the infusion and continued intermittently during 24h, 240g water were consumed. This stimulus was very severe and smaller quantities of salt were infused in subsequent studies.

In two cats infusion of 10ml 5% saline in 20 minutes (i.e. 0.5g NaCl) elicited increased blood pressure and heart rate but not drinking. However, infusion of 20% saline at 0.25ml min^{-1} for 30 minutes (1.5g NaCl) elicited similar cardiovascular responses and drinking behaviour. Drinking began between 18 and 25 minutes after the start of the infusion and continued for 6h; mean water intake was 59g. The infusions initially caused the cats some discomfort and for this reason the effect was not examined in other animals.

The effect of subcutaneous injection of 50% W/V polyethylene glycol (PEG) contained in 0.9% sodium chloride solution

Polyethylene glycol solution (5ml Kg^{-1}) was injected subcutaneously in three cats. The drinking water was removed from

the cage after the injection and replaced 2h later. Drinking began in all cats within 30 minutes of access to water and was completed in two cats within 2h, mean water intake being 29g. In the third animal a prolonged response occurred and the cat drank 233g water in 24h. In control experiments, in which cats injected subcutaneously with isotonic saline ($5 ml Kg^{-1}$) were deprived of water for 2h, no drinking behaviour was observed. Examination of PEG treated cats 6h after the injection revealed that a pocket of fluid had accumulated beneath the skin, after 2 -3 days this fluid had disappeared.

The effect of arterial haemorrhage

In four cats amounts of blood ranging from 10 to $20 ml Kg^{-1}$ were removed through a catheter placed in the aorta at rates ranging from 2 - 20ml min^{-1}. The cats were observed for 6h and water intake recorded for 24h. There was no increase in water intake in any of these experiments.

The effect of ligation of the inferior vena cava

In three cats the inferior vena cava was ligated above the origin of the renal veins but below the hepatic veins. The 24h water intake was recorded for two days before and three days after the operation. Drinking behaviour did not begin for at least 6h after recovery from the anaesthetic, but the 24h intake was increased in each cat on the day of the operation. The water intake declined to the pre-ligation level within 2 days. The results obtained in each cat are presented in Fig. 1:7. The cats were not debilitated after the operation and survived for at least 2 weeks before being killed.

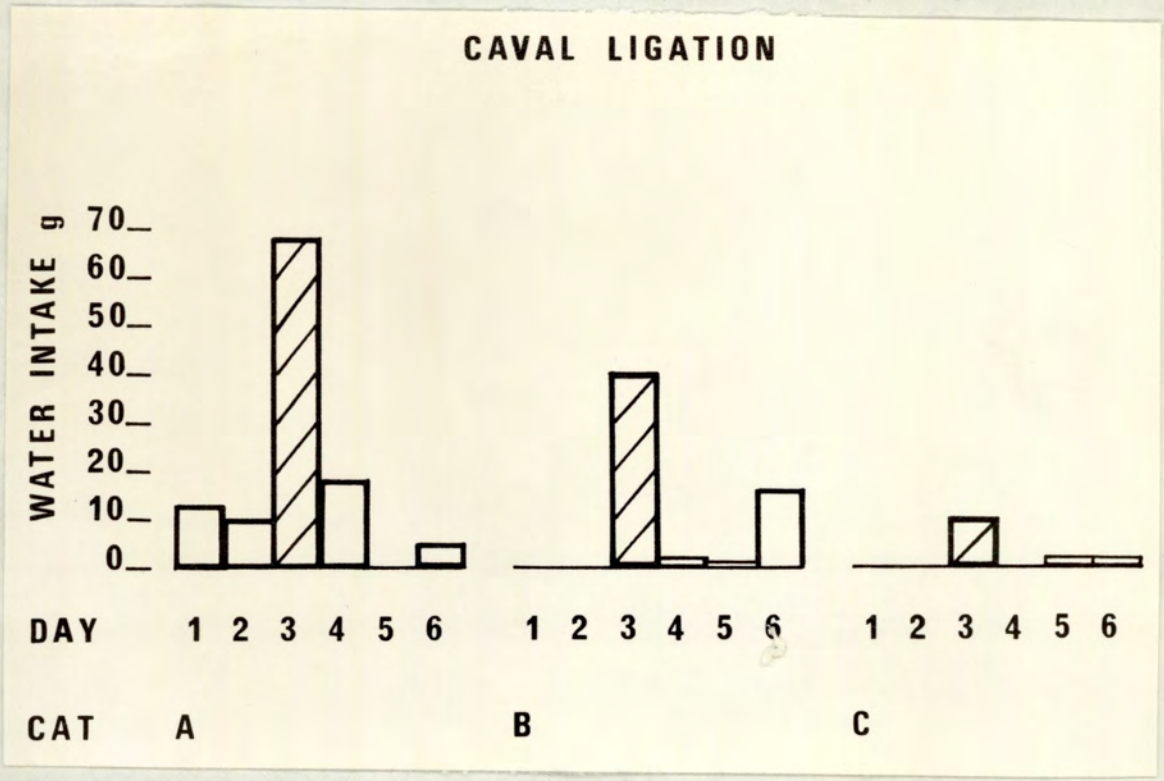


Fig. 1:7 The effect of ligation of the inferior vena cava, immediately above the renal veins, on the daily water intake from the drinking bowl in each of 3 cats. Values are represented by histograms, the hatched column shows the day of the operation.

Discussion

Spontaneous water drinking of cats in this laboratory was measured to provide baseline data and to determine if this was similar to that published by other workers. The results supported the report of Carver & Waterhouse (1962) that cats fed on canned meat did not drink much water. The range and daily mean water drinking were also similar to those reported by Scott & Jackson (1971). These workers found the range to be 0 - 70ml and mean drinking 13ml; the values in the present study were 0 - 40g and 6g respectively.

Central administration of renin, angiotensin I or angiotensin II caused drinking in water replete cats. These results confirm and extend the report that microinjection of angiotensin II into several areas of the cat brain elicits a dipsogenic response (Sturgeon et al, 1973). In the present study, the latent period, duration and magnitude of the dipsogenic response to central administration of the octapeptide were each similar to those reported by Sturgeon et al (1973). A minor difference between the results was that the maximum response in this investigation was elicited using a lower dose than that used in the experiments of Sturgeon's group. This may be due either to animal variation or because different sites of administration were employed. Sturgeon et al (1973) constructed their dose-response curve by combining the results from several positive drinking sites whereas in this work all responses were elicited by icv infusions. Another difference was the criterion adopted for a positive response. Sturgeon's group obtained drinking after central injection of a 5-ion solution used to dissolve

angiotensin II and only considered intakes in excess of 30ml as positive. In the present experiments no drinking was obtained after infusion of isotonic saline and all drinking responses were considered. Using this criterion drinking was induced with doses of 1ng angiotensin II icv. In a recent report (Sturgeon & Levitt, 1974) the neuroanatomical distribution of the angiotensin II-positive sites in the cat was discussed in detail. Analysis of this data shows that the largest dipsogenic response was reliably elicited by microinjections into the lateral ventricle. Recently, Johnson (1973) discovered that in the rat the sensitivity of central neurones to angiotensin II was dependent on the cannula traversing the cerebral ventricles. This suggested that the site of action might be close to the ventricular wall. Simpson & Routtenberg (1973) subsequently demonstrated that in the rat a periventricular structure, the subfornical organ, was extremely sensitive to the dipsogenic action of angiotensin II and was thus a likely target site. The results described here and those of Sturgeon & Levitt (1974) are consistent with a periventricular site of action in the cat.

The reduction in water-drinking in response to repeated central administration of angiotensin II at short intervals has also been observed in the rat (Epstein et al, 1970) and the monkey (Sharpe & Swanson, 1974). This phenomenon may be due to decreased receptor sensitivity, decomposition of the peptide or to an inhibitory effect of increased water loading. The first proposition seems unlikely since in the cat the mean response to the third infusion was significantly larger than that to the second one. The second suggestion is untenable since a solution could be kept at room

temperature overnight without apparent loss of activity. Thus the third proposal is the most likely explanation. This view is supported by the observation that when the dose interval was extended, allowing time for the water load to be eliminated, the response to a second infusion was not significantly reduced. The decline in the maximum response observed after several central infusions may be due to changes in sensitivity initiated by the mechanical disturbance of repeated infusions.

Central administration of angiotensin II in the rat can cause a larger intake of isotonic saline than water (Rolls & Jones, 1971; J. Buggy (1972) cited by Fisher, 1973). This may be due to a taste preference or because ingestion of water inhibits drinking mechanisms more efficiently than saline. A similar difference in fluid intake was observed in the two cats tested in this study. It could be advantageous if such a mechanism operated physiologically since saline is more effective than water in restoring the plasma deficit in hypovolaemia.

The central dipsogenic activity of renin and angiotensin I has been previously reported in the rat (Fitzsimons, 1971). The results in the cat confirm those observations in a different species. A renin substrate has been isolated from dog brain (Ganten, Marquez-Julio et al, 1971) and it seems likely that renin acts on a similar substrate in cat brain. There is also an angiotensin-converting enzyme in brain tissue which would facilitate the formation of angiotensin II (Yang & Neff, 1972). The role of these enzymes in the dipsogenic responses to renin and angiotensin I is discussed in Chapter 2.

Infusion of either angiotensin II or hypertonic saline into the third ventricle of the goat elicited drinking (Andersson & Westbye, 1970). When a combined infusion of angiotensin II in hypertonic saline was administered, the drinking response was greater than the sum of the individual responses. However, if angiotensin was dissolved in hypertonic sucrose or glucose solution the drinking response was reduced (Andersson, 1971). In the cat a similar positive central interaction between angiotensin II and sodium was observed. However, in two respects the results differed from those reported by Andersson's group. Firstly, hypertonic saline alone was not an effective central dipsogen and secondly angiotensin II dissolved in hypertonic sucrose was as effective as when it was dissolved in isotonic saline. Similar experiments in other species have produced conflicting results. Swanson, Sharpe & Griffin (1973) demonstrated that drinking elicited by a threshold central dose of angiotensin II in the rat was potentiated by using hypertonic saline as the injection vehicle. In contrast, Fitzsimons (1973) reported that changing the vehicle from isotonic saline to hypertonic solutions of sodium chloride, sucrose or dextrose did not affect the drinking behaviour elicited by a range of intracerebral doses of the peptide. Likewise, Sharpe & Swanson (1974) did not observe any differences in angiotensin II-induced drinking in the monkey when the peptide was dissolved in distilled water, isotonic saline or hypertonic saline.

Andersson (1971) proposed that the central receptors thought to be sensitive to osmotic pressure were sensitive to changes in CSF sodium ion concentration. He considered that angiotensin II acted

either by sensitizing these receptors to sodium or by altering membrane permeability to increase their exposure to sodium. The exact mechanism is obscure but there is evidence that the effect of angiotensin on hypothalamic neurone activity is potentiated if the peptide is administered in hypertonic saline (Wayner, Ono & Nolley, 1973). There is also considerable evidence that both central and systemic administration of angiotensin II sensitise drinking mechanisms to existing dipsogenic stimuli. Fitzsimons (1970) found that subthreshold central doses of angiotensin II in the rat act synergistically with subthreshold cellular or extracellular stimuli to cause drinking. Recently, a similar interaction has been reported in the goat between a subthreshold icv dose of angiotensin II and a subthreshold intracarotid infusion of hypertonic saline (Olsson & Kolmodin, 1974). Intravenous infusion of angiotensin II also reduced the threshold for drinking induced by iv administration of hypertonic saline in the rat (Hsiao & Epstein, 1973) and the dog (Kozlowski, Drzewiecki & Zurawski, 1972). In order to clarify this effect, a detailed investigation of the interaction of angiotensin II with various types of cellular and extracellular dipsogenic stimuli is needed.

Drinking behaviour in the cat was also elicited by intravenous infusion of angiotensin I or II. Fitzsimons & Simons (1969) first showed that intravenous infusion of angiotensin II in the rat caused drinking. These workers used very large doses but recently their results have been confirmed using lower doses of both angiotensin I and II (Hsiao & Epstein, 1973). The doses used in the cat were of the same order as those used by the latter group, however, they were

quite large when considered in terms of their systemic vasopressor activity. The drinking response to the systemic infusions was smaller and briefer than that produced by icv infusions. Drinking always ceased before the end of the infusion although the cat was capable of drinking more. This phenomenon is difficult to explain but a possible cause is the large sustained pressor response. This may have reflexly inhibited drinking or have disturbed the cat.

Renin has also proved an effective systemic dipsogen in the rat (Fitzsimons, 1969) but it was not very potent in these experiments in the cat. The animals used were all sensitive to intravenous angiotensin II and since renin elicited a prolonged pressor response it presumably released angiotensin II. It is however possible that the renin was bound to the blood vessel walls and released angiotensin locally at the vascular receptor sites; Thurston & Swales (1973) have suggested such a mechanism. An alternative explanation of the lack of dipsogenic effect is that the dose of renin employed was too low.

Drinking induced by systemic injection of isoprenaline was demonstrated in the rat by Lehr et al (1967); they suggested a central site of action. Leibowitz (1971) also favoured a central site of action since she found that intrahypothalamic injection of the amine caused drinking. The results obtained by other investigators do not support this view. The dipsogenic response to either central (Fisher, 1973) or systemic (Houpt & Epstein, 1971) injection of isoprenaline in the rat was abolished after nephrectomy. It was also noted that the peripheral threshold dose of isoprenaline was smaller than the central one (Fisher, 1973). These results supported the

hypothesis that drinking was mediated by release of renal renin. This view was confirmed by the demonstration that systemically administered isoprenaline caused both drinking and increased plasma renin levels, these effects being prevented by β-adrenoceptor blockade (Meyer et al, 1973).

Systemic administration of isoprenaline in the dog caused dose-related drinking behaviour (Fitzsimons & Szczepanska-Sadowska, 1974). These workers found that the response was not affected by nephrectomy but other workers obtained the opposite result (Rolls & Ramsay, 1973).

In the cat both icv and subcutaneous administration of isoprenaline caused drinking. The latency of onset was shorter and the water intake slightly, but not significantly, larger after central administration. In contrast to the rat, isoprenaline does not appear to 'leak' from the brain, since the cardiovascular effects of icv isoprenaline are reduced by peripheral ganglion blockade (Day & Roach, 1974). Thus these results suggest that there may be a central β-adrenoceptor mechanism which mediates drinking in the cat. Recently, Brophy & Levitt (1974) have reported that intracerebral injection of isoprenaline in the cat caused a larger drinking response than injection of other catecholamines. The mean water intake (26ml) was not however significant under their criterion. The sensitivity to systemic injection of isoprenaline in the cat differed from that in the dog and drinking was not increased by doubling the dose. Since systemic injection of two other β-receptor agonists elicited drinking responses of similar magnitude, this may indicate an "all or nothing" type of response. The mechanisms of the dipsogenic effect

of isoprenaline will be further discussed in Chapter 2.

In the dog (Gilman, 1937) and the rat (Fitzsimons, 1961a) intravenous infusion of hypertonic saline caused drinking and this was also demonstrated in the cat. It is generally accepted that drinking is initiated by stimulation of central receptors sensitive to osmotic pressure (or sodium concentration). Although previous workers have observed drinking in the cat after icv hypertonic saline, it was not observed in this study. This discrepancy is extremely puzzling, although it might be that the receptors are more accessible to blood-borne stimuli than to CSF borne stimuli.

Depletion of the extracellular fluid volume has been demonstrated as an important dipsogenic stimulus in the rat (Fitzsimons, 1972). 'Volume receptors' situated in the thoracic vasculature may initiate direct stimulation of central drinking mechanisms or cause reflex release of renin. Several methods have been used to cause absolute or relative extracellular fluid deficits; haemorrhage, sequestration of plasma in the extracellular spaces and ligation of the inferior vena cava.

The direct method, controlled haemorrhage, was not an effective dipsogenic stimulus in the cat. Fitzsimons (1961b) used this method successfully in the rat but Holmes & Montgomery (1951) removed up to 40% of the blood volume in the dog without eliciting drinking. The failure of this method can be due to either of two factors. After a small haemorrhage the plasma deficit is rapidly replaced by interstitial fluid, thus removing the stimulus. However, after a large haemorrhage an animal may suffer from shock and be so

debilitated that it cannot drink.

Injection of polyethylene glycol dissolved in saline into the extracellular space is a reliable dipsogenic stimulus in the rat (Fitzsimons, 1961). This result was confirmed in the cat.

A reduction in venous return can be effected by tying the inferior vena cava above the kidneys, this caused drinking in the rat (Fitzsimons, 1964). The same procedure increased 24h water intake in the cat but the latency of the onset of drinking was longer than in the rat.

It has been suggested that the renin-angiotensin system may mediate or facilitate drinking in response to hypovolaemia, since all three techniques cause renin release. Hall & Hodge (1971) measured plasma angiotensin levels in the anaesthetised cat after slow or rapid haemorrhage ($8.6 ml Kg^{-1}$ total) using a superfusion bioassay. They found that plasma angiotensin levels rose by $17.6 ng Kg^{-1} min^{-1}$ in both cases. This value is much lower than the intravenous dose of exogenous angiotensin II which elicited drinking in the present study. This may also explain the failure of haemorrhage to induce drinking in the cat. The role of angiotensin in drinking induced by caval ligation was not directly investigated but the cats which consumed most water after ligation were also the most sensitive to icv angiotensin II. Thus the renal dipsogen may be implicated in the response. The role of the renin-angiotensin system in the dipsogenic response to polyethylene glycol will be discussed in the next chapter.

CHAPTER 2

The effect of enzyme inhibitors and analogue antagonists of angiotensin II on drinking behaviour induced by renin, angiotensin and other dipsogenic stimuli

The investigation of the biological role of the renin-angiotensin system has been hindered by the lack of specific antagonists. An early approach to this problem was the use of immunological techniques to produce antisera against renin or angiotensin. Recently, great advances have been made in this area and several agents have been developed which either inhibit renin or angiotensin converting enzyme or which block angiotensin II receptors.

Under certain conditions the proteolytic activity of renin was similar to that of pepsin (Franze de Fernandez, Paladini & Delius, 1965). Thus when a pentapeptide pepsin inhibitor, pepstatin, was isolated from Streptomyces testaceus (Umezawa, Aoyagi, Morishima, Matsuzaki, Hamada & Takeuchi, 1970) it was logical to test it as a potential renin inhibitor. In vitro formation of angiotensin by incubation of hog renin with renin substrate was reduced by pepstatin (Gross, Lazar & Orth, 1972). The pentapeptide also reduced the vasopressor response elicited by intravenous injection of renin in the nephrectomised rat, suggesting that it inhibited angiotensin formation in vivo (Gross et al, 1972). This suggestion was recently confirmed when pepstatin was found to reduce renin-induced increases in plasma angiotensin concentration in the rat (Oster, Lazar & Hackenthal, 1974).

Inactivation of kinins and conversion of angiotensin I occur mainly in the pulmonary circulation (see Vane, 1969). Ng & Vane (1968) suggested that both processes involved the same enzymic

mechanism. A peptide fraction of Bothrops Jararaca venom had previously been found to inhibit kininases (Ferreira, 1966); the crude extract was also an effective converting enzyme inhibitor (Bakhle, 1968). The component peptides were separated, identified then synthesised (Ferreira, Bartelt & Greene, 1970; Ondetti, Williams, Subo, Plusec, Weaver & Kocy, 1971). The most potent of the synthetic inhibitors were a nonapeptide (SQ 20881) and a pentapeptide (SQ 20475). These peptides inhibit angiotensin I conversion in vitro (Bakhle, 1972) and reduce the vasopressor response to intravenous injection of angiotensin I (Greene, Camargo, Kreiger, Stewart and Ferreira, 1972).

The systematic investigation of the structure-activity relationships of angiotensin II and over two hundred analogues (see Khosla et al, 1974) led to the introduction of angiotensin II receptor antagonists. It was noticed that angiotensin II analogues substituted in position 8 with aliphatic amino acids were devoid of agonist activity but prevented the responses to the parent peptide (see Regoli, Rioux & Park, 1972). The inhibition of angiotensin II was competitive and the responses to other agonists were unaffected. Additional substitution of these analogues at position 1 with sarcosine (N-methyl glycine) increased their in vivo potency (Pals, Masucci, Denning, Sipos & Fessler, 1971; Türker, Hall, Yamamoto, Sweet & Bumpus, 1972; St Louis & Regoli, 1972). This enhanced activity could be due either to increased affinity for the receptor site or to protection from the action of aminopeptidases.

Some of the above inhibitors (Fig. 2:1) have been used to elucidate the mechanism and site of the dipsogenic action of renin

Fig. 2:1

The amino acid sequences of peptide antagonists of the renin-angiotensin system

Pepstatin A

isovaleryl LVal-LVal-4amino, 3hydroxy, 6methylheptanoyl -LAla-4 amino, 3hydroxy, 6methylheptanoic acid

SQ 20881

Pyr-LTry-LPro-LArg-LPro-LGlu-LIle-LPro-LPro

$Sar^{1}Ala^{8}$angiotensin II

Sar-LArg-LVal-LTyr-LVal-LHis-LPro-LAla

$Sar^{1}Ile^{8}$angiotensin II

Sar-LArg-LVal-LTyr-LIle-LHis-LPro-LIle

$Sar^{1}Leu^{8}$angiotensin II

Sar-LArg-LVal-LTyr-LIle-LHis-LPro-LLeu

Leu^{8}angiotensin II

LAsp-LArg-LVal-LTyr-LIle-LHis-LPro-LLeu

and angiotensin. The results obtained in the cat are described in this chapter. Experiments were also performed using a receptor blocker to determine the role of the renin-angiotensin system in different types of dipsogenic stimuli.

Results

The effect of icv pepstatin

Pepstatin (100μg) infused icv did not elicit a drinking response in four cats. However, the dipsogenic response to icv renin (0.1 GbU), given 15 minutes after central administration of pepstatin, was significantly reduced for at least 3h. The same icv dose of pepstatin did not affect drinking induced by icv angiotensin I (1.2μg) or angiotensin II (1μg). In all experiments control responses were obtained by giving icv infusions of 0.9% saline instead of pepstatin and at least 2 days were allowed between experiments. The results of these experiments are shown in Fig. 2:2.

The effect of icv SQ 20881

The angiotensin converting enzyme (ACE) inhibitor, SQ 20881, given icv (25μg) did not cause drinking but it significantly delayed the onset of the dipsogenic response to icv renin (0.1 GbU). In four cats, treated 15 minutes earlier with SQ 20881 icv, water intake 30 and 60 minutes after icv renin was greatly reduced but after 120 and 180 minutes, the mean cumulative response was not significantly different from the control mean. Drinking in response to icv angiotensin I (1.2μg) was also significantly reduced after icv SQ 20881 but that elicited by icv angiotensin II (1μg) was slightly increased. The results are shown in Fig. 2:3.

In three cats treated 15 minutes before with icv SQ 20881 (25μg),

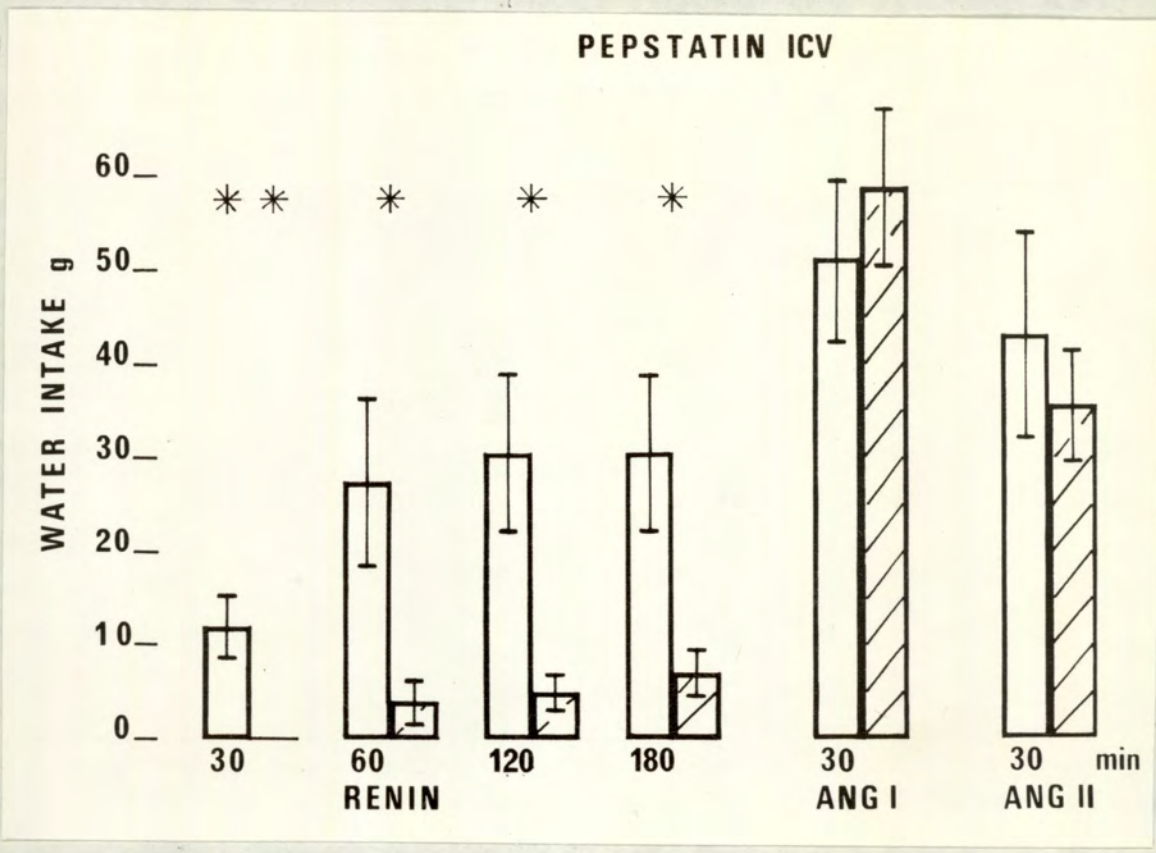


Fig. 2:2 Effect of icv pepstatin (100μg) on water drinking in 4 cats induced by icv renin (0.1 GbU), angiotensin I (1.2μg) and angiotensin II (1μg). Responses to renin at each time are cumulative intakes, responses to angiotensin I and II are intakes in the 30 minute period after administration of the dipsogen. Open columns represent control experiments and hatched columns represent experiments in which cats were pretreated with the inhibitor 15 minutes before the dipsogen. Vertical bars are SE of means (**$p < 0.01$; *$p < 0.05$).

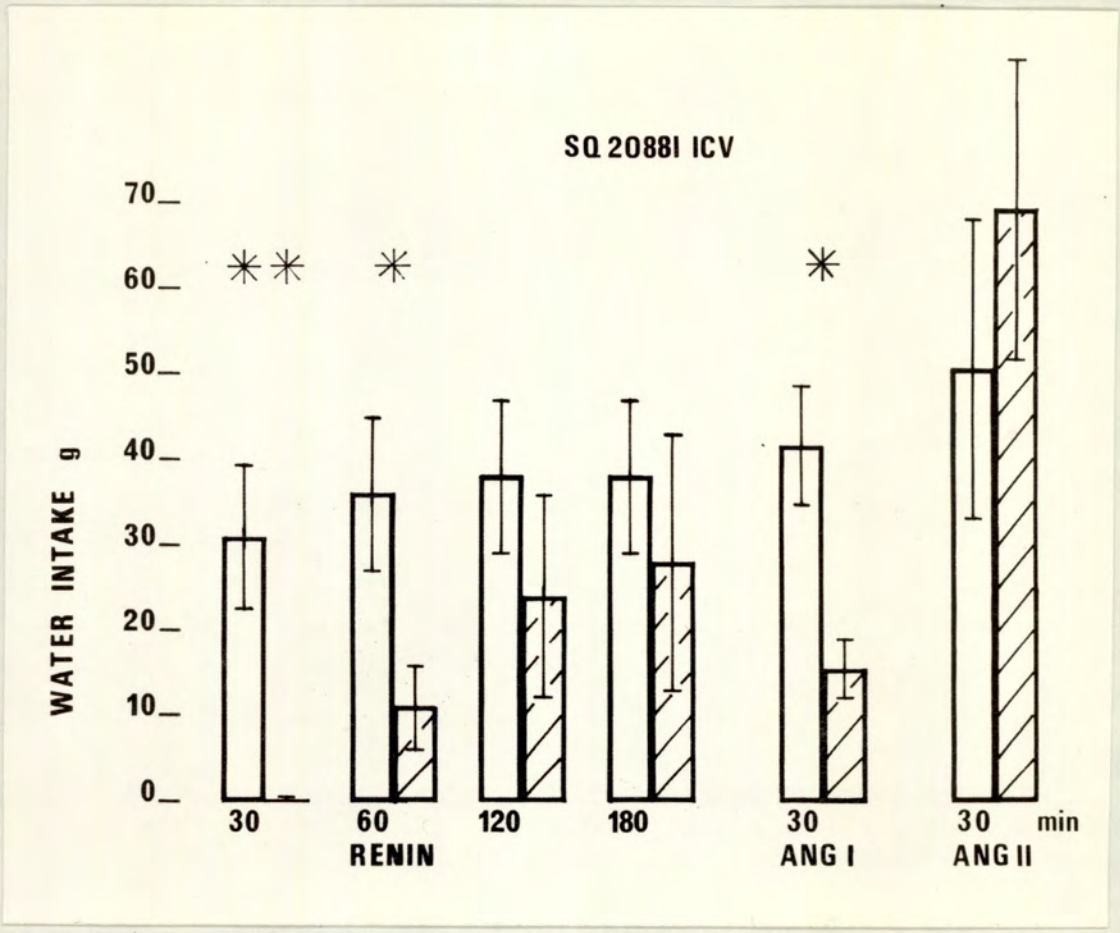


Fig. 2:3 Effect of icv SQ 20881 (25μg) on water drinking in 4 cats induced by icv renin (0.1 GbU), angiotensin I (1.2μg) and angiotensin II (1μg). Responses to renin at each time are cumulative intakes, responses to angiotensin I and II are intakes in the 30 minute period after administration of the dipsogen. Open columns represent control experiments and hatched columns experiments in which the cats were pretreated with the inhibitor 15 minutes before the dipsogen. Vertical bars are SE of means (**$p<0.01$; *$p<0.05$).

water consumption in response to an intravenous infusion of angiotensin I (500ng $Kg^{-1}min^{-1}$; 10 min.) was reduced from a mean in control experiments of 38 ± 11g to 11 ± 3g. Similar pretreatment did not reduce the dipsogenic response to iv infusion of angiotensin II (400ng $Kg^{-1}min^{-1}$; 10 min.).

In a single experiment in a conscious cat, illustrated in Fig. 2:4, intravenous injection of SQ 20881 (20μg Kg^{-1}) reduced the vasopressor response to iv injection of angiotensin I (30ng Kg^{-1}) without affecting that to angiotensin II (25ng Kg^{-1}). This demonstrates the systemic activity of this particular sample of inhibitor.

The effect of analogue antagonists of angiotensin II

a) Sarcosine^1 Alanine^8 angiotensin II (Sar^1Ala^8AII)

In five cats, infusions of angiotensin II (1μg) icv 30 and 150 minutes after an icv infusion of 100μl 0.9% saline elicited mean drinking responses of 46 ± 2g and 44 ± 2g respectively. Three days later the dipsogenic response to icv angiotensin II was recorded 30 and 150 minutes after an icv infusion of Sar^1Ala^8AII (5μg). The analogue itself had a weak dipsogenic effect in one cat (9g consumed) and the mean response to icv angiotensin II given 30 minutes later was significantly reduced to 7 ± 3g. However, 150 minutes after the analogue angiotensin-induced drinking had recovered to 38 ± 4g, which was not significantly different from the control. This is illustrated in Fig. 2:5.

In similar experiments the dipsogenic response to icv angiotensin I was also reversibly inhibited by central administration of Sar^1Ala^8AII (5μg). The mean drinking responses in the control experiments were 36 ± 7g and 29 ± 5g and those after pretreatment with

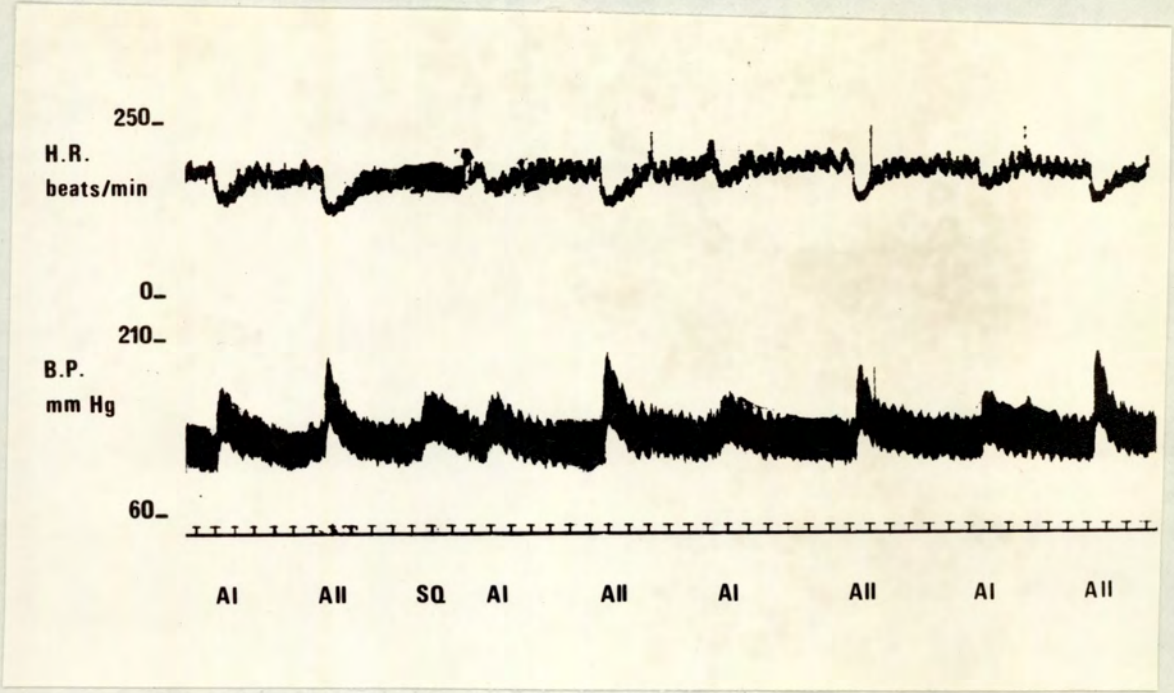


Fig. 2:4 A trace illustrating the effect of a single iv injection of SQ 20881, 20$\mu g\,Kg^{-1}$, (SQ) on the vasopressor responses elicited in the conscious cat by iv injection of angiotensin I, 30$ng\,Kg^{-1}$, (AI) or angiotensin II, 25$ng\,Kg^{-1}$, (AII).

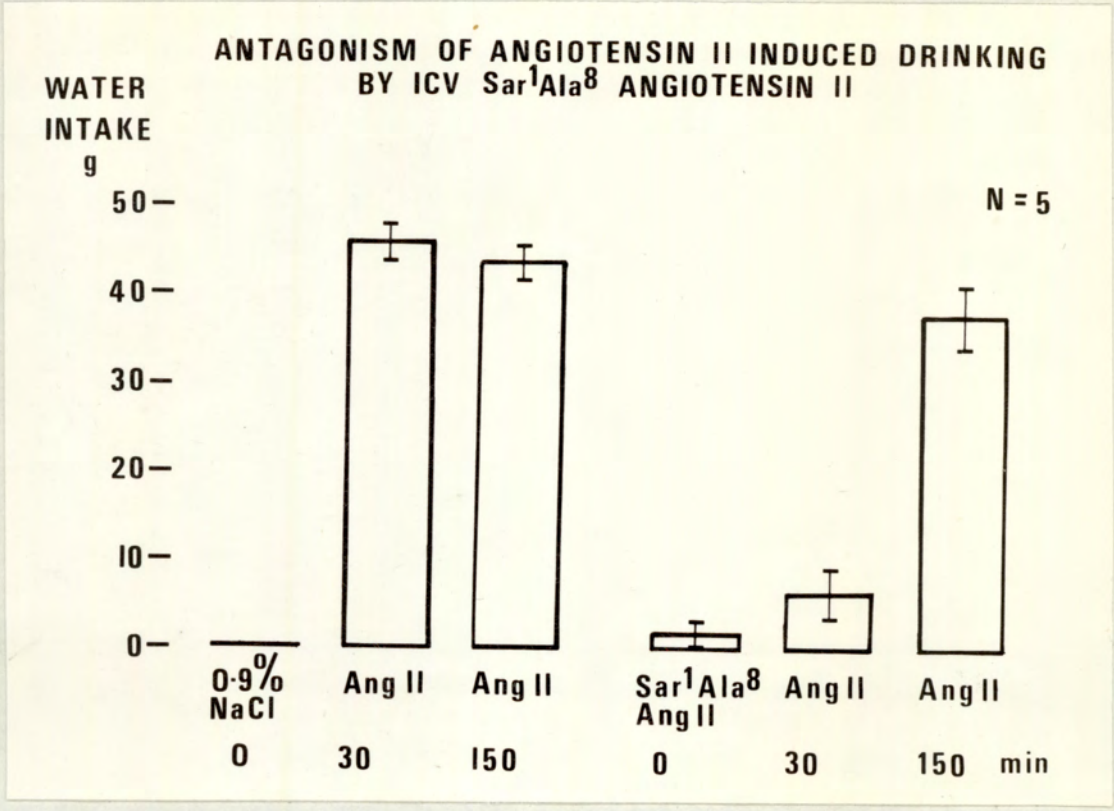


Fig. 2:5 Mean water drinking in 5 cats induced by icv angiotensin II (1μg). In control experiments angiotensin II was given 30 and 150 minutes after 0.9% NaCl solution icv (100μl). Three days later angiotensin II was given 30 and 150 minutes after Sar^1Ala^8angiotensin II icv (5μg). Vertical bars are SE of means.

the analogue were 9 ± 2g and 27 ± 5g at 30 and 150 minutes respectively.

When renin (0.1 GbU) was given icv 30 minutes after central infusion of Sar^1Ala^8AII, the onset of drinking was delayed and the magnitude of the response reduced. The effect of icv Sar^1Ala^8AII on drinking induced by icv renin, angiotensin I and II is summarised in Fig. 2:6.

Central administration of Sar^1Ala^8AII (5μg) inhibited drinking induced by intravenous infusion of either angiotensin II ($400ngKg^{-1}min^{-1}$; 10 min.) or angiotensin I ($500ngKg^{-1}min^{-1}$; 10 min.) administered 15 minutes later. The dipsogenic responses elicited by either infusion 195 minutes after the analogue were similar to those in control experiments. These results are expressed in Table 2:1.

Simultaneous intravenous infusion of Sar^1Ala^8AII ($2\mu gKg^{-1}min^{-1}$) and angiotensin II ($400ngKg^{-1}min^{-1}$; 10 min.) markedly reduced both the dipsogenic and pressor responses to angiotensin II. The analogue was infused for 10 minutes alone, then the combined infusion for 10 minutes and finally the analogue alone for a further 15 minutes. Infusion of angiotensin II alone 3h later elicited drinking and pressor responses similar to those obtained in control experiments. The mean drinking responses in control experiments were 28 ± 2g and 26 ± 2g and those in experiments with the analogue were 3 ± 1g and 34 ± 9g.

The dipsogenic effect of icv angiotensin II (0.1 - 1μg) was not affected by simultaneous intravenous infusion of Sar^1Ala^8AII ($2\mu gKg^{-1}min^{-1}$). Infusions of the analogue were commenced 10 minutes before angiotensin II and continued for 40 minutes.

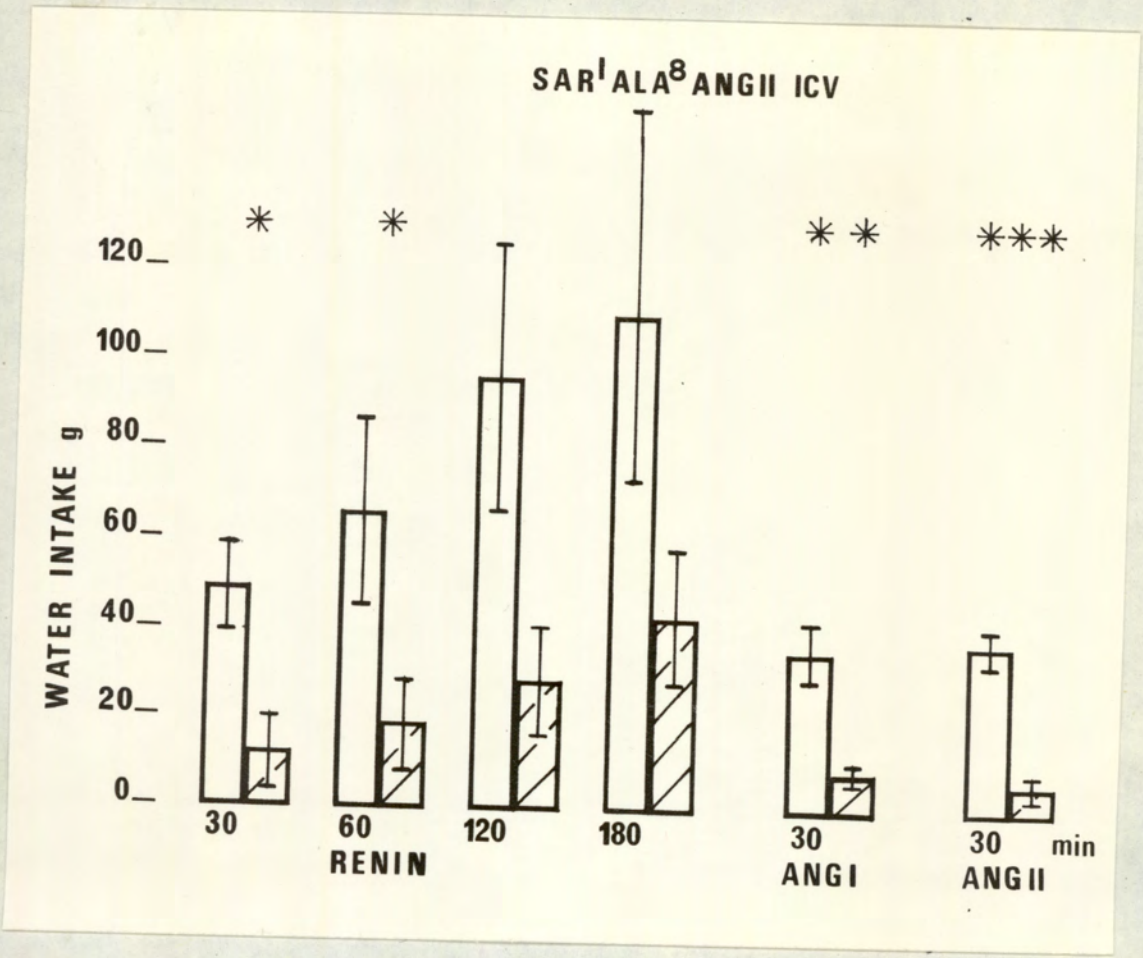


Fig. 2:6 Effect of icv Sar^{1}Ala^{8}angiotensin II (5µg) on water drinking in 4 cats induced by icv renin (0.1 GbU), angiotensin I (1.2µg) and angiotensin II (1µg). Responses to renin at each time are cumulative intakes, responses to angiotensin I and II are intakes in the 30 minute period after administration of the dipsogen. Open columns represent control experiments and hatched columns experiments in which the cats were pretreated with the analogue 30 minutes before the dipsogen. Vertical bars are SE of means (***$p<0.001$; **$p<0.01$; *$p<0.05$).

TABLE 2:1

Dipsogen	icv pretreatment	Drinking response at 15 & 195 min after pretreatment (g water ± SE)		N
		15 min	195 min	
iv angiotensin II	0.9% NaCl (100µl)	30 ± 11	25 ± 10	3
	Sar^1Ala^8AII (5µg)	1 ± 1**	30 ± 12	
iv angiotensin I	0.9% NaCl (100µl)	25 ± 1	26 ± 1	3
	Sar^1Ala^8AII (5µg)	1 ± 1**	16 ± 4	

Effect of icv pretreatment with Sar^1Ala^8 angiotensin II on the dipsogenic response to iv infusion of angiotensin I or II (**$p<0.01$).

Intravenous infusion of Sar^1Ala^8AII ($1\mu g Kg^{-1} min^{-1}$) selectively reduced the pressor response to iv injections of angiotensin II ($50ngKg^{-1}$) in the conscious cat but did not affect the pressor response to iv noradrenaline ($400ngKg^{-1}$). The results of one experiment are shown by the trace in Fig. 2:7. This confirms the systemic antagonist activity of the analogue.

b) Sarcosine^{1}Leucine^{8}angiotensin II (Sar^1Leu^8AII)

This analogue elicited a small dipsogenic response in two of four cats when given icv at a dose of $5\mu g$. The dipsogenic response to icv angiotensin II was reversibly inhibited after this analogue (Table 2:2).

c) Sarcosine^{1}Isoleucine^{8}angiotensin II (Sar^1Ile^8AII)

Drinking behaviour after icv administration of this analogue ($5\mu g$) was observed in only one of four cats. The dipsogenic response to icv angiotensin II was inhibited and this inhibition was more prolonged than that produced by the other analogues (Table 2:2).

d) Leucine^{8}angiotensin II (Leu^8AII)

This analogue was the only one used in this study which was not modified at position 1, it exhibited a greater agonist response than the others when infused icv ($5\mu g$). Drinking was observed in three of the four cats used. The analogue did however reversibly inhibit the dipsogenic action of icv angiotensin II (Table 2:2).

The vasopressor effects of intravenous injections of angiotensin II analogues

Since the analogues used exhibited weak dipsogenic activity, their agonist effects on the blood pressure of the conscious cat were examined. Each analogue was injected intravenously at a dose

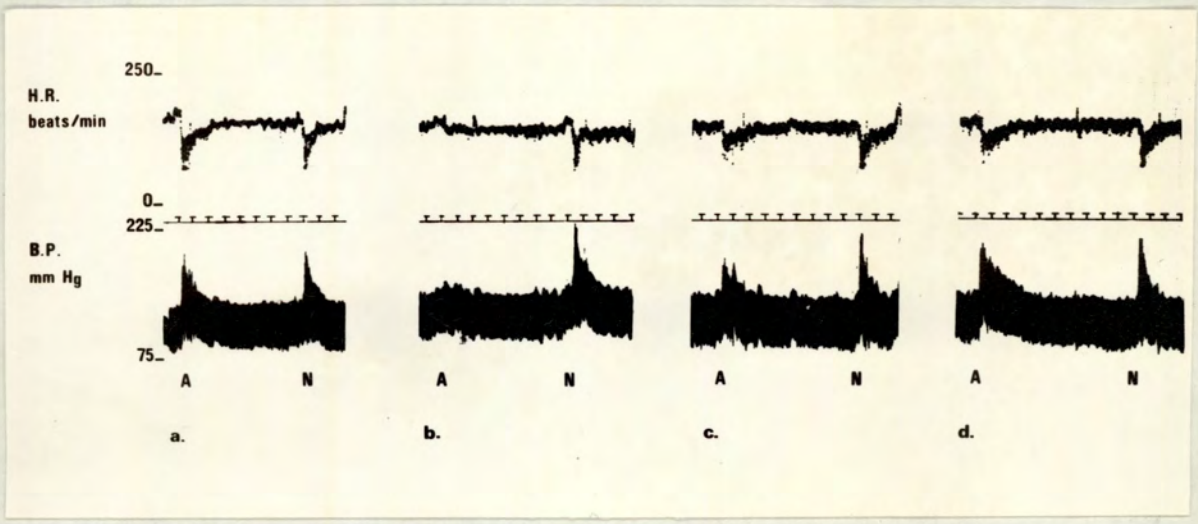


Fig. 2:7 Effect of 60 minutes iv infusion of $Sar^{1}Ala^{8}$angiotensin II, $1\mu g\,Kg^{-1}min^{-1}$, on the vasopressor responses elicited in the conscious cat by iv injection of angiotensin II, $50ng\,Kg^{-1}$, and noradrenaline, $400ng\,Kg^{-1}$

a) Before infusion of $Sar^{1}Ala^{8}$angiotensin II
b) 30 minutes after the start of the infusion
c) 10 minutes after the end of the infusion
d) 30 minutes after the end of the infusion

TABLE 2:2

icv pretreatment	Drinking response (g water ± SE)			N
	Pretreatment 0 min	AII (1μg) icv 30 min	AII (1μg) icv 150 min	
0.9% NaCl (100μl)	0	46 ± 2	44 ± 2	4
Sar^1Ala^8AII (5μg)	2 ± 2	7 ± 3**	38 ± 4	
0.9% NaCl (100μl)	0	38 ± 6	38 ± 8	4
Sar^1Leu^8AII (5μg)	5 ± 4	4 ± 2**	23 ± 4	
0.9% NaCl (100μl)	0	40 ± 8	40 ± 8	4
Sar^1Ile^8AII (5μg)	2 ± 2	0**	12 ± 2	
0.9% NaCl (100μl)	0	38 ± 8	37 ± 9	4
Leu^8AII (5μg)	15 ± 6	2 ± 2**	30 ± 7	

Effect of icv administration of angiotensin II-analogues on drinking elicited by icv angiotensin II. The drinking responses to saline, analogues and angiotensin II are 30 minute total intakes (**$p<0.01$).

of 5μg, 10 minutes after a standard dose of angiotensin II (50ng). The results of one experiment are illustrated in Fig. 2:8.

The effect of icv administration of Sar^1Ala^8AII on drinking induced by hypertonic saline, polyethylene glycol and isoprenaline

1) Drinking induced by iv infusion of 20% sodium chloride solution

In the two cats used, drinking was elicited by intravenous infusion of 7.5ml 20% saline in 30 minutes. The analogue (5μg) was infused icv 30 minutes before the start of the hypertonic saline infusion and a second dose of Sar^1Ala^8AII was given 2½h after the first to maintain the blockade. In control experiments 100μl 0.9% saline was infused icv instead of the antagonist analogue. In both cats the cumulative drinking response after central administration of Sar^1Ala^8AII was greater than that in control experiments. This result is depicted in the histograms in Fig. 2:9.

2) Drinking induced by subcutaneous injection of 50% w/v PEG in isotonic saline

In this experiment using three cats the analogue was administered icv at the same times as in the preceding experiment i.e. 30 minutes before the stimulus and a second dose 2½h later. The cats were injected with 5ml Kg^{-1} PEG solution and the drinking water removed from the cage for 2h. In analogue pretreatment experiments drinking during the first hour of access to water was slightly, but not significantly, reduced compared with control experiments. Cumulative drinking responses 2, 3 and 4h after access to the water were similar in pretreatment and control experiments. This result is illustrated in Fig. 2:10.

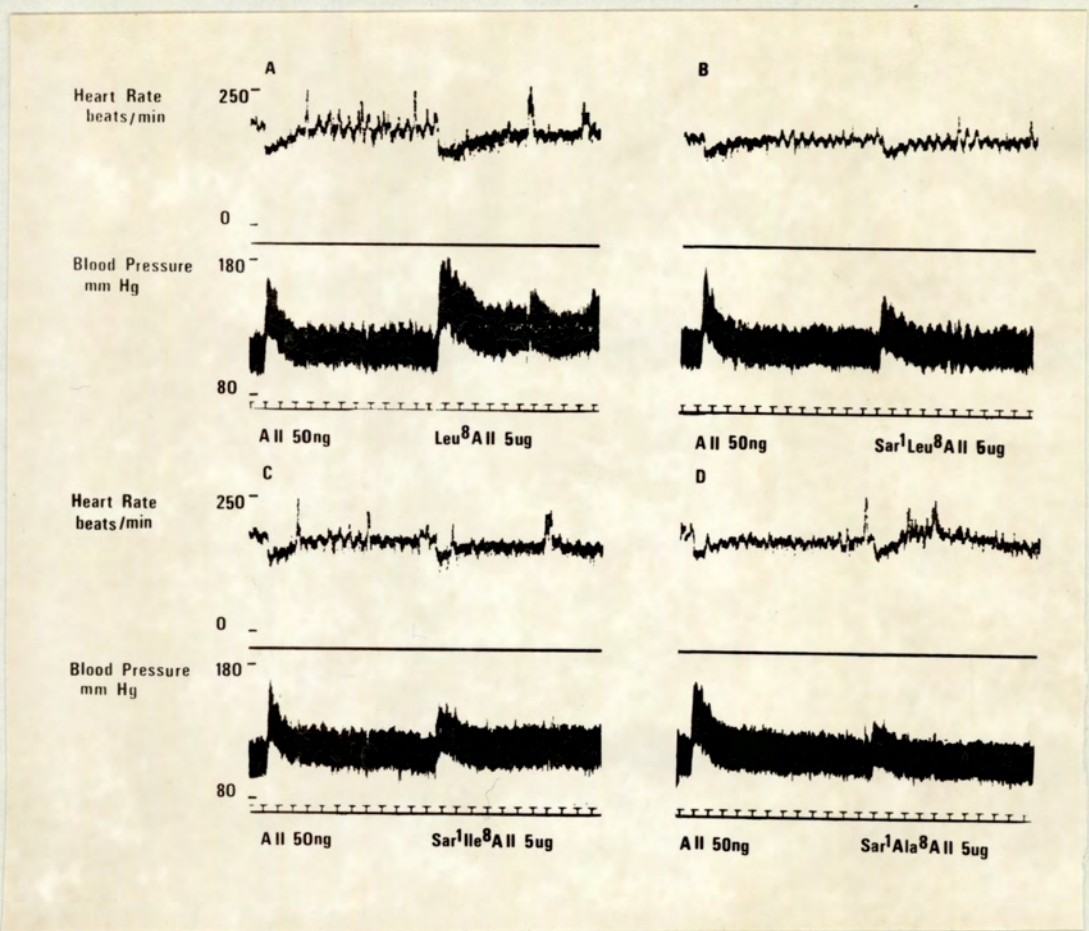


Fig. 2:8 The vasopressor response elicited in the conscious cat by single iv injections of 4 angiotensin II-analogues. The response elicited by iv injection of angiotensin II is shown for comparison.

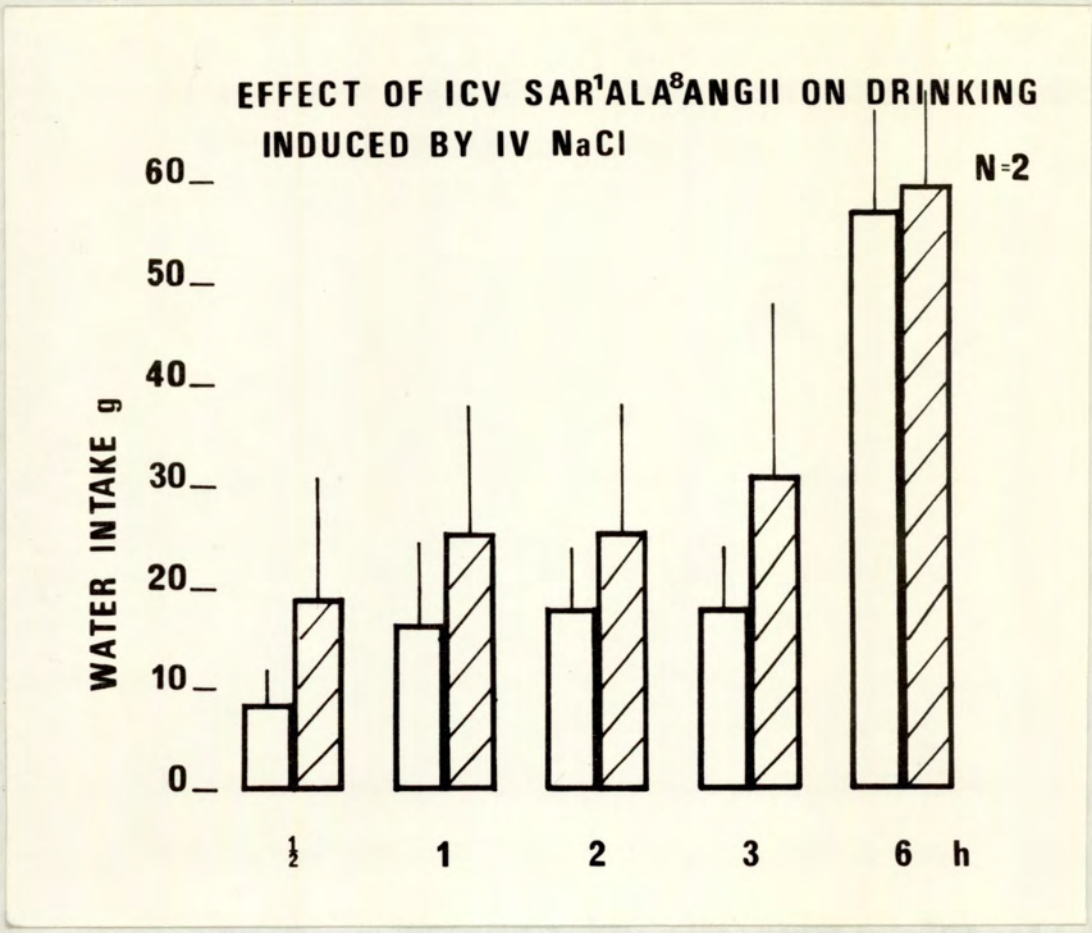


Fig. 2:9 The effect of icv administration of Sar^{1}Ala^{8} angiotensin II on drinking in the cat induced by iv infusion of 7.5ml 20% NaCl solution. The water intakes are cumulative values at each time. The analogue was given 30 minutes before infusion of hypertonic saline and a second dose was given $2\frac{1}{2}$h after the first.

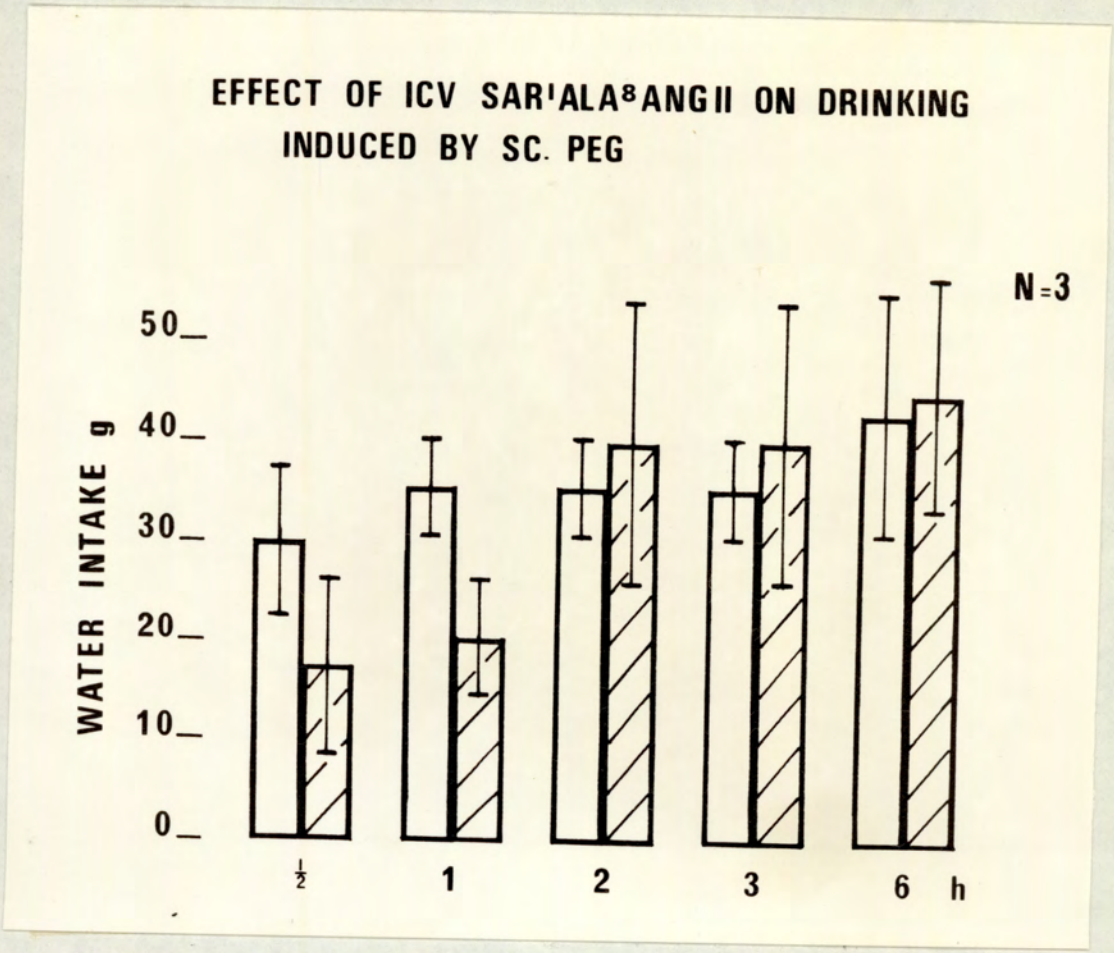


Fig. 2:10 The effect of icv administration of Sar^{1}Ala^{8} angiotensin II on drinking in the cat induced by subcutaneous injection of 5ml Kg^{-1} 50% w/v PEG in isotonic saline. Drinking water was withheld for 2h after PEG injection and the time-scale begins when water was replaced. Water intakes are cumulative values at each time. The analogue was given 30 minutes before PEG injection and a second dose 2$\frac{1}{2}$h after the first.

3) Isoprenaline-induced drinking

In two cats the effect of icv Sar^1Ala^8angiotensin II (5μg) given 30 minutes before an icv infusion of isoprenaline (50μg) was examined. Drinking in cats pretreated with the analogue was not significantly different from that in animals pretreated with isotonic saline, water intakes being 30g and 29g respectively.

In three cats the effect of similar pretreatment on drinking induced by subcutaneous injection of isoprenaline (50μg) was tested. In two cats pretreated with icv Sar^1Ala^8AII isoprenaline did not induce drinking but in the third cat there was no reduction in isoprenaline induced drinking. The mean water intakes in control and pretreated cats were 20 ± 4 and 7 ± 7 respectively.

Discussion

The dipsogenic effect of renin administered into the central nervous system may be mediated directly or may require the formation of angiotensin. In these experiments in the cat the drinking response to icv renin was markedly attenuated by prior central administration of the renin inhibitor pepstatin. This result supports the view that the dipsogenic effect of renin was mediated indirectly by releasing angiotensin from a brain renin substrate. The dose of the inhibitor used in this study was very large and it could be argued that the peptide exerted a non specific antidipsogenic effect. However, the drinking elicited by icv angiotensin I or II was not affected suggesting that the action of pepstatin was specific. The results agree with those obtained in the rat by Epstein, Fitzsimons & Johnson (1974).

Angiotensin I is a potent central dipsogen but it is not clear

whether it must first be converted to angiotensin II. In the cat central administration of an angiotensin converting enzyme inhibitor reduced the dipsogenic effect of both icv renin and angiotensin I but did not affect angiotensin II-induced drinking. These results support the hypothesis that the dipsogenic effects of the renin-angiotensin system are mainly mediated by the octapeptide. There was a small drinking response to angiotensin I after the inhibitor, which could be due to incomplete inhibition of brain converting enzyme or to a weak direct action of the decapeptide. Experiments in other laboratories using the rat have yielded inconsistent results. Severs, Summy-Long & Daniels-Severs (1973) reported that intraventricular administration of the nonapeptide inhibitor reduced the central dipsogenic effect of angiotensin I. Epstein et al (1974) extended these observations finding that intracerebral microinjection of the inhibitor reduced drinking induced by microinjection of renin, renin-substrate and angiotensin I. The opposite view was taken by Swanson, Marshall, Needleman & Sharpe (1973) who found no attenuation of angiotensin I-induced drinking when the decapeptide was injected intracerebrally 1 minute after a central injection of SQ 20881. However, their results could be attributed to the short dose interval between inhibitor and agonist. Their work was corroborated by Bryant & Falk (1973) who conducted a detailed analysis and found SQ 20881 to be ineffective whether it was injected intracerebrally or intraventricularly. Later, Lehr & Goldman (1974) repeated this work and they found that intracerebral administration of the inhibitor was ineffective but that icv administration selectively reduced angiotensin I-induced drinking. These discrepancies are difficult

to explain since the doses of inhibitor and agonist used were of the same order in all experiments. Another curious result was also obtained in the rat by Burckhardt, Peters-Haefli & Peters (1973) who found that intrahypothalamic injection of the inhibitor, SQ 20881, reduced the dipsogenic effect of a similar injection of renin but not of angiotensin I. In the rhesus monkey central administration of SQ 20881 also failed to inhibit angiotensin I-induced drinking.

In the experiments in the cat, the drinking response to icv angiotensin II was slightly increased after the central administration of the inhibitor. Similar increases in angiotensin II-induced drinking were also observed in the rat (Epstein et al, 1974; Bryant & Falk, 1973).

The view that renin-angiotensin induced drinking in the cat is principally mediated by angiotensin II received further support from experiments using competitive antagonists of the octapeptide. Central administration of Sar^1Ala^8AII reduced drinking induced by central infusion of renin, angiotensin I or II. Inhibition was reversible since after $2\frac{1}{2}$h the response to a second infusion of either angiotensin I or II was similar to that in control experiments. The reversible antagonism of angiotensin II was also seen using three other analogues of the octapeptide infused icv. Similar results were obtained in the rat using two sarcosine^1-substituted analogues (Epstein et al, 1974; Lehr & Goldman, 1974). When other analogues, Phe^4Tyr^8- and Ile^8-angiotensin II, were used in the rat no inhibition of angiotensin II-induced drinking was observed (Swanson et al, 1973). This failure may have been due to the short dose interval (1 minute) between antagonist and agonist. In a single experiment in the

rhesus monkey, Sharpe & Swanson (1974) also failed to block angiotensin-induced drinking with an intracerebral injection of Ile^8angiotensin II.

The analogues, Phe^4-Tyr^8-, Ala^8- and Ile^8-angiotensin II were reported to be potent dipsogens when they were injected into the brain of the rat (Swanson et al, 1973). It was proposed that central angiotensin receptors were dissimilar to those in the periphery. In the monkey, intracerebral injection of Ile^8-angiotensin II had only a weak dipsogenic effect but Phe^4Tyr^8-angiotensin II was a more potent dipsogen (Sharpe & Swanson, 1974). The different analogues used in the present study in the cat possessed weak central dipsogenic activity. They also exhibited weak vasopressor activity when injected systemically in the conscious cat, confirming a previous report in the rat (St Louis & Regoli, 1972). The dipsogenic and vasopressor potencies of each analogue were closely correlated suggesting that the compounds are weak partial agonists. Substitution with sarcosine at position 1 appeared to decrease the partial agonist activity since Sar^1Leu^8AII possessed less pressor and dipsogenic activity than Leu^8AII.

It has been assumed that the site of the dipsogenic action of angiotensin was in the central nervous system, since the central threshold dose was less than the systemic one. The present study in the cat has provided the first direct evidence to support this hypothesis. It was demonstrated that central administration of an angiotensin II receptor blocker reduced the dipsogenic response to intravenous infusion of angiotensin I or II.

An unexpected observation in this study was that inhibition of

brain converting enzyme reduced drinking elicited by intravenous infusion of the decapeptide but not that induced by the octapeptide. This implied that systemically administered angiotensin I entered the brain before being converted to angiotensin II. Although this would appear to be unlikely a similar phenomenon has been observed in the rat. Lehr, Goldman & Casner (1973) found that icv injection of SQ 20881 reduced drinking elicited by intraperitoneal administration of renin.

Intravenous infusion of Sar^1Ala^8 angiotensin II in the cat reduced drinking elicited by a simultaneous intravenous infusion of angiotensin II but not that elicited by icv angiotensin II. This indicates that the analogue penetrates the central nervous system but the amount entering was not sufficient to block the effects of locally applied high doses of the parent peptide. Vaughan, Gavras, Laragh & Koss (1973) first reported the inhibitory effect of systemic infusion of this analogue against drinking induced in the rat by injection of kidney extract. This result has recently been corroborated by Tang & Falk (1974) using systemic infusions of renin or angiotensin II as the dipsogenic stimulus.

The results of experiments with inhibitors summarised in this chapter suggested that these compounds might be useful in elucidating the role of the renin-angiotensin system in various dipsogenic stimuli. Preliminary experiments were performed in the cat using central administration of an angiotensin II antagonist. This procedure was adopted since it has been demonstrated that renin-angiotensin induced drinking is mediated by the central action of angiotensin II.

Drinking behaviour induced by systemic administration of hypertonic saline is not thought to involve the renin-angiotensin system. This opinion was confirmed since central administration of Sar^1Ala^8angiotensin II did not affect the cumulative dipsogenic response. Initially drinking increased, this may represent an interaction between hypertonic saline and the analogue similar to those between sodium and angiotensin II discussed in Chapter 1.

After central angiotensin II receptor blockade, the drinking induced by subcutaneous polyethylene glycol was initially reduced. However, the total water intake after 2h was similar to that in control experiments. This suggests that a small component of the response may be due to activation of the renin-angiotensin system. Drinking induced by PEG in the rat was reduced by systemic infusion of an angiotensin II antiserum (Abdelaal, Mercer & Mogenson, 1974). It has also been reported that there is a close correlation between plasma renin activity and water intake in the rat after this procedure (Leenen & Stricker, 1974). These results also suggested that the renin-angiotensin system was involved in the response, but other mechanisms are involved since polyethylene glycol-induced drinking can be elicited in nephrectomised rats (Fitzsimons, 1969).

Central administration of the analogue reduced drinking elicited by systemic injection of isoprenaline but did not affect that induced by icv isoprenaline. These results suggest that drinking is initiated by different mechanisms, the former by release of renin and the latter by central β-adrenoceptor stimulation. The use of inhibitors in the rat has not produced clear results. Intravenous infusion of angiotensin II-antiserum reduced drinking induced by

systemic isoprenaline (Abdelaal et al, 1974b) but this was not confirmed using systemic administration of $Sar^{1}Ala^{8}$angiotensin II (Tang & Falk, 1974).

CHAPTER 3

The effect of systemic and central administration of autonomic nervous system blockers on drinking behaviour induced by icv angiotensin II in the cat

The most likely candidates as neurohumoral transmitters in the central nervous system are acetylcholine, noradrenaline, dopamine and 5-hydroxytryptamine. These amines have been located in central neurones and are released by electrical stimulation of the brain. It has also been demonstrated that autonomic and behavioural responses can be initiated by central administration of these amines or drugs which modify their actions (see Vogt, 1969). There is evidence that three of these amines, acetylcholine, noradrenaline and dopamine, may be involved in central drinking mechanisms.

In 1960, Grossman elicited drinking behaviour in the rat by implanting crystals of carbachol or acetylcholine into the hypothalamus. This observation has been repeatedly confirmed in the rat but similar experiments in the cat (Myers, 1964b) monkey (Sharpe & Myers, 1969) and gerbil (Block et al, 1974) have been unsuccessful. Whilst the latter results limit the concept that central cholinergic neurones mediate drinking, the hypothesis cannot be totally rejected.

In the same study Grossman (1960) found that intrahypothalamic implants of noradrenaline depressed drinking but Myers (1964a) reported that in his experiments injection of the amine into the lateral hypothalamus stimulated drinking. Leibowitz (1971) suggested that these conflicting results could be due to noradrenaline acting on α- and β-adrenoceptors respectively. In her studies, isoprenaline applied to the hypothalamus elicited drinking which was prevented by

central pretreatment with propranolol. She also found that the reduction in drinking induced by central administration of noradrenaline was antagonised by central α-adrenoceptor blockade.

Recently, the central dipsogenic effect of catecholamines has been reinvestigated by Setler (1973). She found that intracerebral noradrenaline had a potent inhibitory action on drinking in water-deprived rats but that in water-replete animals it had a weak dipsogenic action. Dopamine injected into the cerebral ventricles was also an effective dipsogen. These results suggested that drinking might be mediated by stimulation of central dopamine receptors, on which noradrenaline exerted a weak action. This view was given qualified support by Fisher (1973), who reported that central administration of apomorphine, a dopamine receptor agonist, caused drinking in some rats.

There are several reports that central administration of angiotensin II may affect central cholinergic and adrenergic transmission. In the cat encephale isolé preparation, the output of acetylcholine from the cerebral cortex was enhanced by topical application or microinjection of nanogram doses of angiotensin II (Elie & Panisset, 1970). This suggested that the peptide facilitated the release of this transmitter.

In the rat, perfusion of a solution containing angiotensin II through the cerebral ventricles did not increase spontaneous release of noradrenaline (Palaic & Khairallah, 1968). In the same experiments the noradrenaline concentration in the outflowing solution increased after stimulation of the cut central end of the vagus. In earlier experiments, Palaic & Khairallah (1967) had

found that angiotensin II prevented the uptake of noradrenaline into the brain tissue. The authors proposed that the peptide acted by inhibiting neuronal reuptake of catecholamines.

The effect of the peptide on brain catecholamines in the rat was investigated by Minnich, Donaldson & Barbeau (1973). They found that icv injection of angiotensin II increased dopamine levels in the hypothalamus, striatum, brainstem and cerebellum and noradrenaline levels in the hippocampus and cerebellum. These results support the view that angiotensin II may modulate central catecholaminergic mechanisms. This effect may occur physiologically since it has been demonstrated that the distribution of endogenous angiotensin and noradrenaline in the brain are closely correlated (Fischer-Ferraro et al, 1971).

The above evidence is consistent with the hypothesis that the dipsogenic effect of angiotensin II could be mediated by either cholinergic or adrenergic transmitters. The results in this chapter describe experiments using various autonomic blocking drugs to examine the above hypothesis.

Results

Effect of intravenous administration of autonomic blocking drugs on drinking induced by icv angiotensin II

When a dose interval of 2h was allowed between two icv infusions of angiotensin II, the amounts of water consumed in response to each were not significantly different. The effects of intravenous administration of autonomic blocking drugs were investigated by comparing angiotensin II-induced drinking 1h before and 1h after administration of the blocker.

Drinking elicited by icv infusion of angiotensin II (1μg) was almost abolished after iv injection of hexamethonium (10mg Kg^{-1}) and significantly reduced after pempidine (5mg Kg^{-1}). The dipsogenic response was also significantly reduced following systemic administration of the muscarinic antagonist, methylatropine (1mg Kg^{-1}). These results are illustrated in Fig. 3:1.

In contrast, agents which affect peripheral adrenergic mechanisms did not affect angiotensin-induced drinking. After adrenergic neurone blockade with bethanidine (5mg Kg^{-1}), α-adrenoceptor blockade with phentolamine (5mg Kg^{-1}) and β-adrenoceptor blockade with sotalol (5mg Kg^{-1}), there was no significant reduction in drinking. A small reduction in angiotensin-induced drinking was observed after DL-propranolol (5mg Kg^{-1}) but this was not statistically significant. These results are shown in Fig. 3:2.

In all the above experiments the effectiveness of the blocking drugs was checked by comparing the cardiovascular responses to iv injection of an appropriate agonist before and after the antagonist.

Effect of icv administration of autonomic blocking drugs on drinking induced by icv angiotensin II

In these experiments the initial dipsogenic response induced by icv angiotensin II was compared with that elicited 1h after the central administration of the blocker. Preliminary studies indicated that when 100μl 0.9% saline was infused icv instead of the blocker there was no significant difference between the two angiotensin-induced responses. The doses of antagonists used in the study were similar to those used by other workers to inhibit drug-induced temperature (Feldberg & Saxena, 1971; Hall, 1972) or

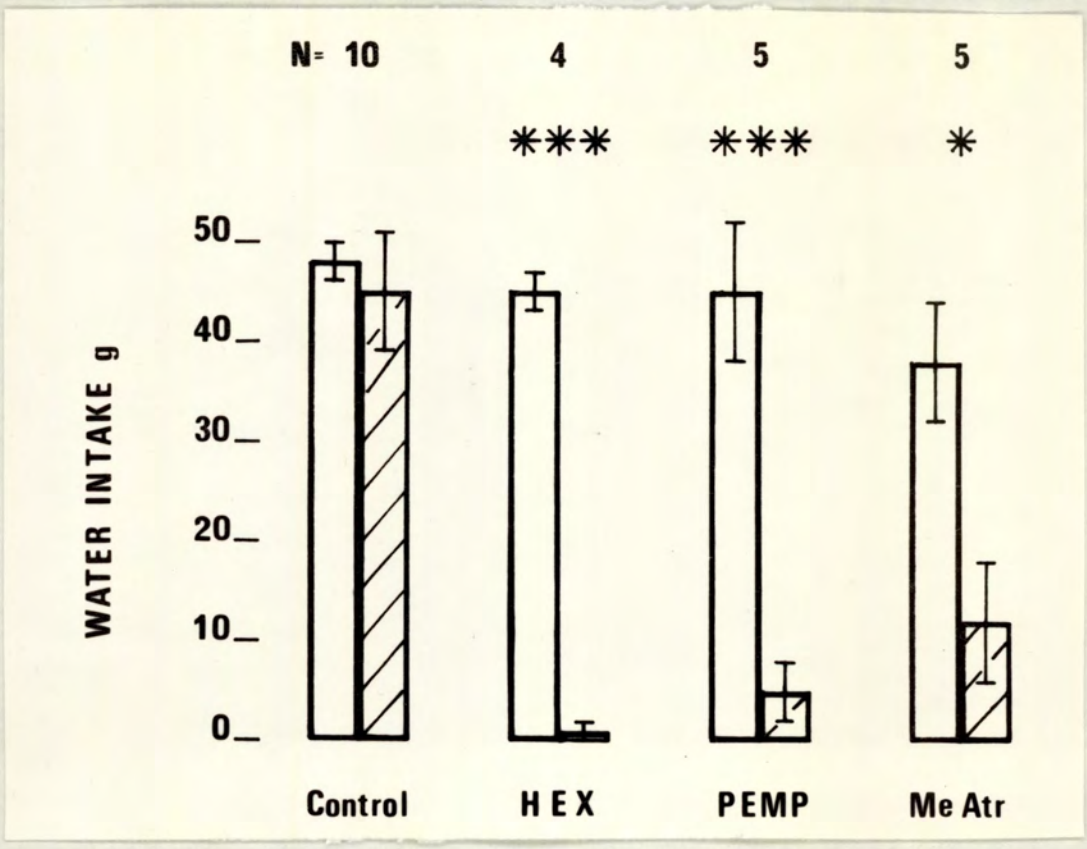


Fig. 3:1 The effect of iv hexamethonium ($10 mg\,Kg^{-1}$), iv pempidine ($5 mg\,Kg^{-1}$) and iv methyl atropine ($1 mg\,Kg^{-1}$) on drinking in the cat elicited by icv angiotensin II (1μg). Open columns are water intakes 1h before the blocker, hatched columns 1h after the blocker. Vertical bars are SE of means (***$p \leq 0.001$; *$p \leq 0.05$).

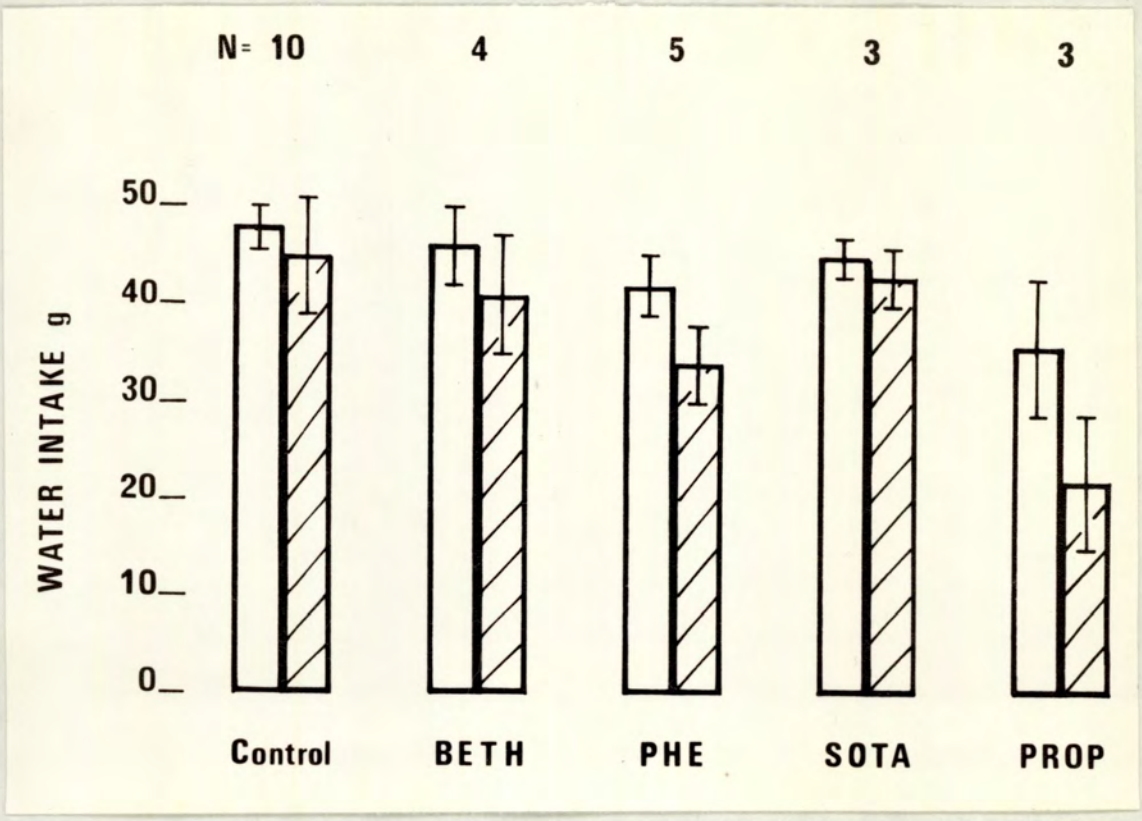


Fig. 3:2 The effect of iv bethanidine (5mg Kg^{-1}), iv phentolamine (5mg Kg^{-1}), iv sotalol (5mg Kg^{-1}) and iv propranolol (5mg Kg^{-1}) on drinking in the cat elicited by icv angiotensin II (1μg). Open columns are water intakes 1h before the blocker, hatched columns 1h after the blocker. Vertical bars indicate SE of means.

cardiovascular (Day & Roach, 1974) changes in the cat.

Atropine methonitrate and atropine sulphate icv

Central infusion of atropine methonitrate (200μg) caused a marked increase in heart rate and increased motor activity in the cat. In two animals minor convulsions lasting for 2 - 3 minutes occurred 20 minutes after the infusion ended. However, the dipsogenic effect of icv angiotensin II (1μg) was not significantly changed (Fig. 3:3). Central administration of atropine sulphate (200μg) was also ineffective as an antagonist of the dipsogenic response.

Pempidine and hexamethonium icv

In four experiments central administration of pempidine (200μg) did not significantly reduce angiotensin II-induced drinking. This is illustrated in Fig. 3:3. When hexamethonium (200μg) was used in two experiments a similar result was obtained.

Bethanidine icv

When bethanidine (400 or 600μg) was infused into the lateral ventricle, there was usually an increase in both blood pressure and heart rate. There was a dose related reduction of angiotensin-induced drinking in each of 5 cats. At the higher dose drinking induced by angiotensin II was abolished in 3 of these 5 animals (Fig. 3:3).

icv administration of α-adrenoceptor antagonists

Central administration of either tolazoline (600μg) or phenoxybenzamine (250μg) did not affect angiotensin-induced drinking. A third α-adrenoceptor blocker, phentolamine (125 or 250μg), reduced or abolished drinking elicited by angiotensin II. After the larger dose of phentolamine some of the cats seemed to be

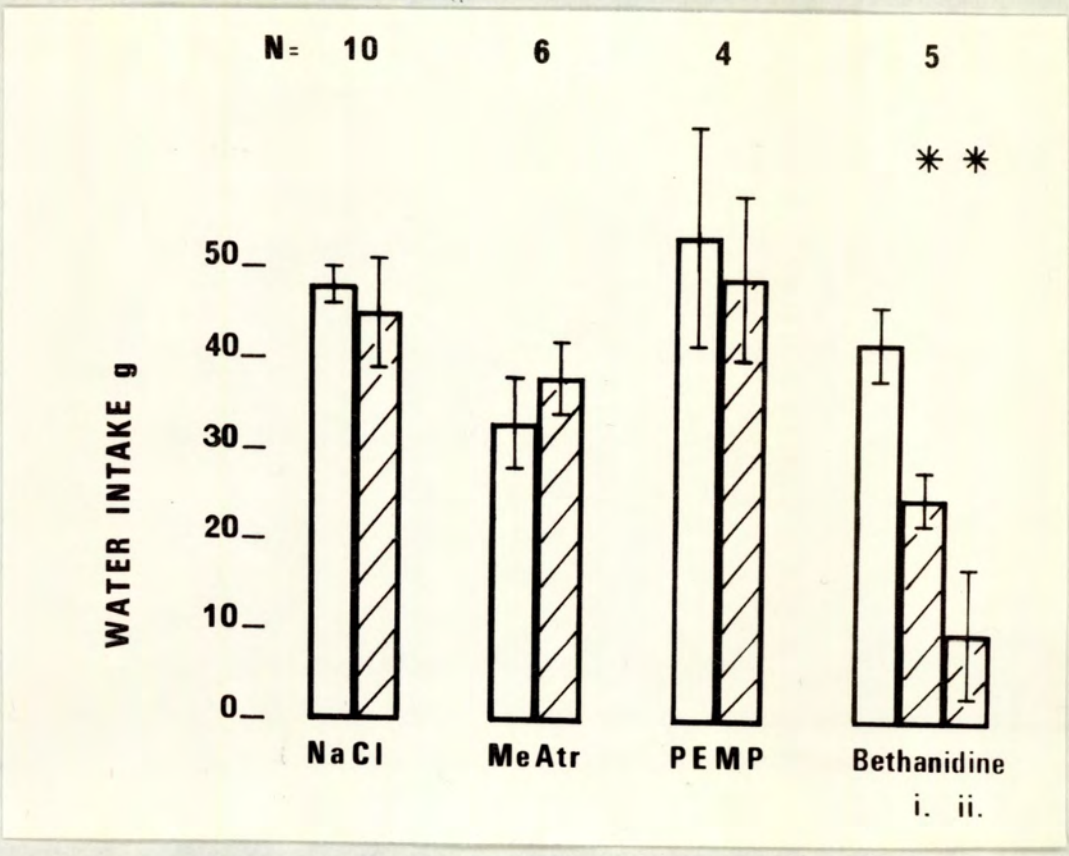


Fig. 3:3 The effect of icv methyl atropine (200μg), icv pempidine (200μg) and icv bethanidine (i) 400μg; ii) 600μg) on drinking in the cat elicited by icv angiotensin II (1μg). Open columns are water intakes 1h before the blocker, hatched columns 1h after the blocker. Vertical bars are SE of means (**$p<0.01$).

sedated and did not eat when returned to the home cage. The results of these experiments are shown in Fig. 3:4.

icv administration of β-adrenoceptor antagonists

In this study four β-adrenoceptor blockers were used, DL-propranolol (450μg), practolol (400μg), pindolol (500μg) and sotalol (750μg). After central administration of propranolol and pindolol, angiotensin-induced drinking was abolished and it was markedly reduced after infusion of the two other antagonists. These results are shown in Fig. 3:5.

It has been suggested that some of the actions of β-adrenoceptor blockers are dependent on their local anaesthetic actions, this effect was therefore examined. The L- and D-isomers of propranolol both exhibit membrane stabilising activity but only the former has β-adrenoceptor antagonist activity. In the cat icv administration of the L-isomer (250μg) significantly reduced angiotensin-induced drinking but similar administration of the D-isomer (250μg) resulted in only a small reduction of the response. When lignocaine (500μg) was infused icv it also caused a small non significant reduction in angiotensin-induced drinking. These results are illustrated in Fig. 3:6.

Effect of 'dopamine receptor' antagonists

In three cats icv infusion of haloperidol (200μg) markedly reduced drinking induced by icv angiotensin II. This drug was dissolved in tartaric acid, thus central administration of tartrate ions may have contributed to the effect.

In subsequent experiments haloperidol ($1 mg Kg^{-1}$) or pimozide ($1 mg Kg^{-1}$) were given intravenously since they easily penetrate the

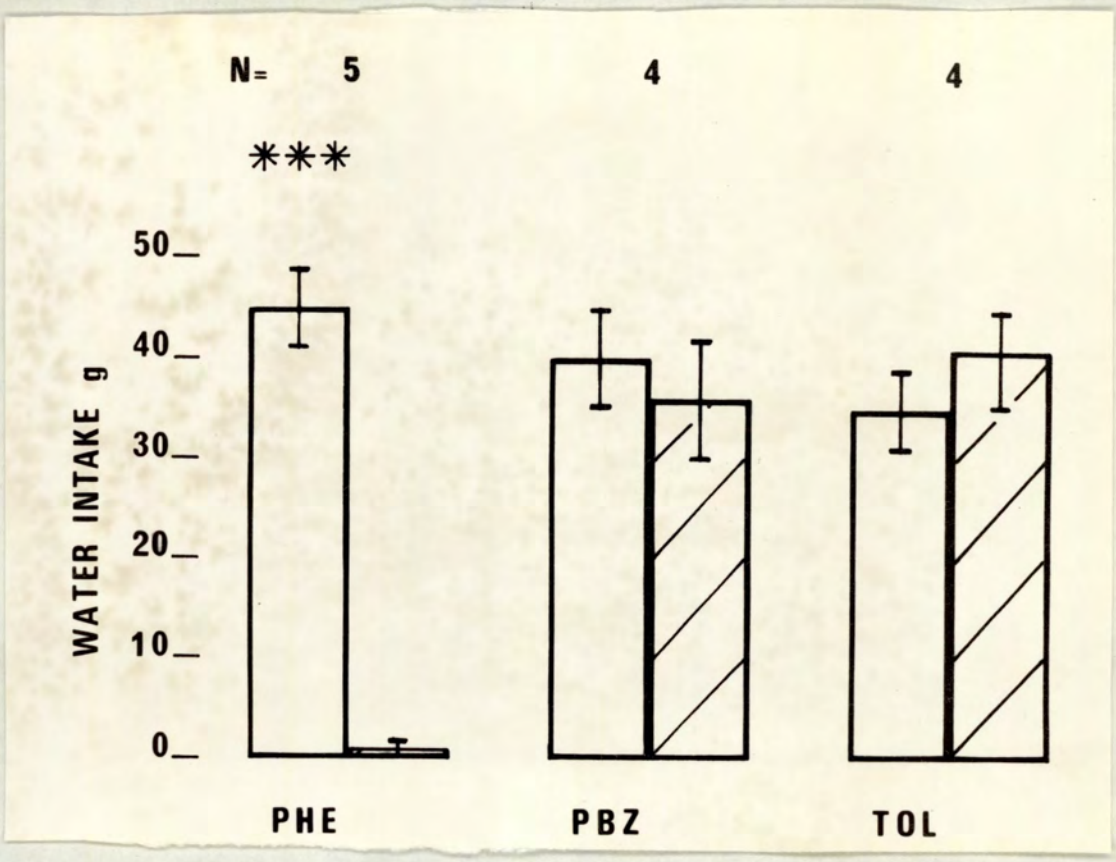


Fig. 3:4 The effect of icv phentolamine (250μg), icv phenoxybenzamine (250μg) and icv tolazoline (600μg) on drinking in the cat elicited by icv angiotensin II (1μg). Open columns are water intakes 1h before the blocker, hatched columns 1h after the blocker. Vertical bars indicate SE of means (***$p<0.001$).

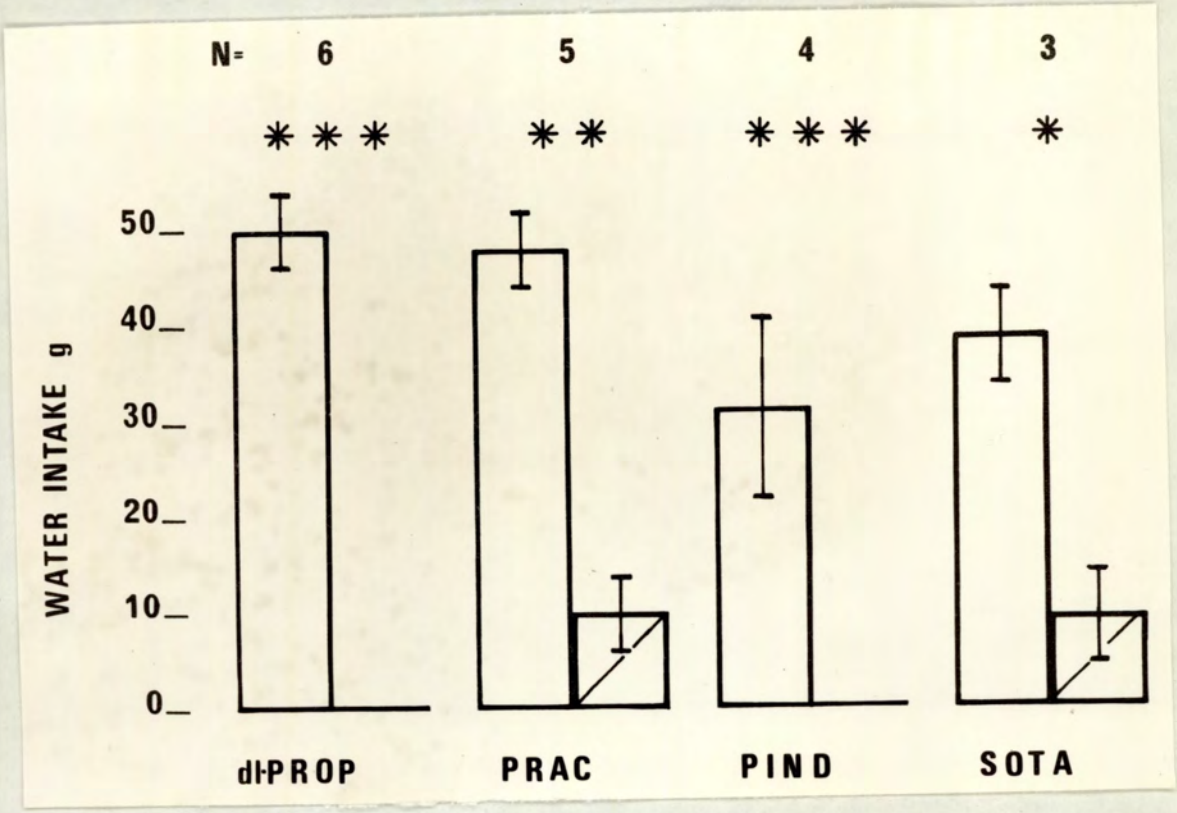


Fig. 3:5 The effect of icv DL-propranolol (450μg), icv practolol (400μg), icv pindolol (500μg) and icv sotalol (750μg) on drinking in the cat elicited by icv angiotensin II (1μg). Open columns are water intakes 1h before the blocker, hatched columns 1h after the blocker. Vertical bars are SE of means (***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$).

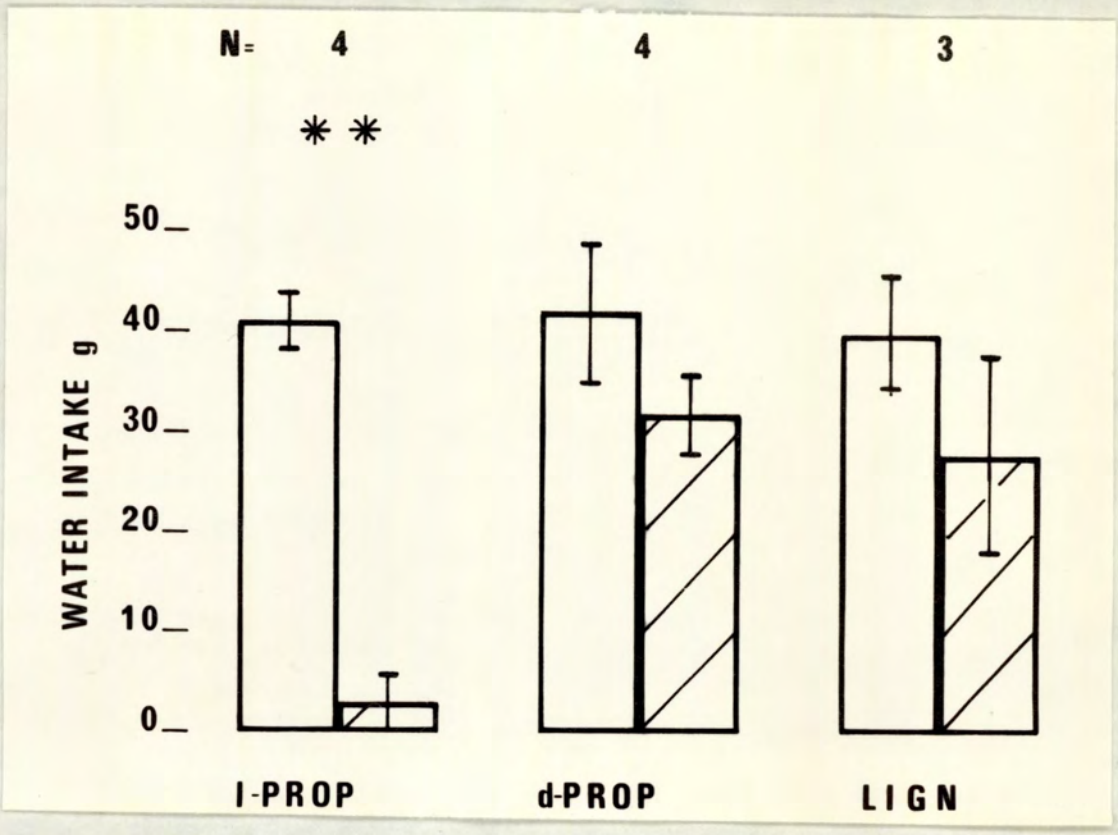


Fig. 3:6 The effect of icv L-propranolol (250μg), icv D-propranolol (250μg) and icv lignocaine (500μg) on drinking in the cat elicited by icv angiotensin II (1μg). Open columns are water intakes 1h before the blocker, hatched columns 1h after the blocker. Vertical bars are SE of means (**$p<0.01$).

central nervous system. In these experiments angiotensin II-induced drinking was also significantly reduced. The results of these experiments are summarised in Fig. 3:7. These neuroleptic drugs did not appear to affect the behaviour of the cats, the animals were alert, moved normally and ate their food.

Effect of intraventricular administration of metaraminol

Intraventricular administration of metaraminol has been reported to induce rapid depletion of central catecholamines in the cat (Smookler et al, 1966). In the present study, a control response to icv angiotensin II was elicited on the first day of the experiment. On the second day metaraminol (1mg) was infused icv, this elicited a large pressor response. Five hours later the dipsogenic effect of icv angiotensin II was tested. The central dipsogenic effect of angiotensin II was then investigated at 24h intervals for the next five days. In all three cats angiotensin II-induced drinking was abolished 5h after metaraminol. The inhibition was not permanent and the response gradually returned during the next four days. In one cat drinking returned to the control value but in the other two animals the maximum response was reduced to about 50% of the control.

Effect of icv 6-hydroxydopamine

The effect of 6-hydroxydopamine was investigated in two cats in which control dipsogenic responses to icv angiotensin II had been established. A total dose of 5mg 6-hydroxydopamine was administered by infusing 1mg per day icv on alternate days. The central dipsogenic effect of angiotensin II was retested 24h after the final dose of the amine. The response in both cats was only marginally reduced (10 - 20%) compared with the control. However, 1 week later the dipsogenic

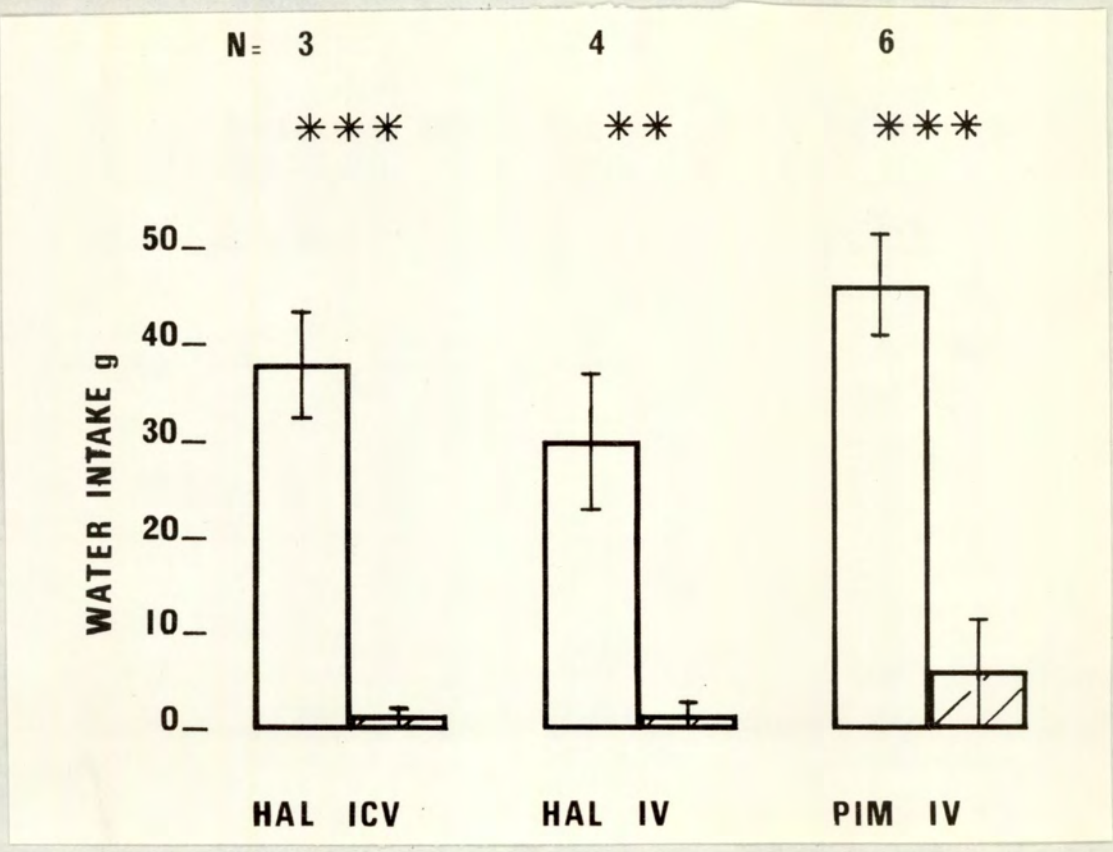


Fig. 3:7 The effect of icv haloperidol (200μg), iv haloperidol ($1mgKg^{-1}$) or iv pimozide ($1mgKg^{-1}$) on drinking in the cat elicited by icv angiotensin II (1μg). Open columns are water intakes 1h before the blocker, hatched columns 1h after the blocker. Vertical bars indicate SE of means (***$p \leq 0.001$; **$p \leq 0.01$).

response to icv angiotensin II in both cats was greatly reduced (70 - 80%).

Discussion

Pharmacological antagonists have been widely used in the rat to elucidate the role of cholinergic and catecholaminergic mechanisms in drinking induced by centrally administered angiotensin II. Most of these investigations have been concerned with the role of transmitters in the integrating mechanisms of the limbic system but in some, peripheral mechanisms have also been examined. The rat may not be a typical subject, being the only species in which carbachol is a reliable dipsogen, thus it is important to study this problem in other species. The present work has been performed in the cat and other groups have recently made preliminary studies in the rhesus monkey (Myers et al, 1973; Sharpe & Swanson, 1974).

After blockade of systemic nicotinic or muscarinic receptors, drinking induced by icv angiotensin II in the cat was markedly reduced. Peripheral receptors must have been involved since neither hexamethonium nor methylatropine, which are quaternary ammonium compounds, can penetrate the blood-brain barrier. In the rat, systemic administration of hexamethonium also inhibited angiotensin II-induced drinking (Severs et al, 1970) but subcutaneous injection of methylatropine was ineffective (Lehr & Goldman, 1973). This data implies that peripheral cholinergic mechanisms are involved in the efferent pathways which mediate the response. Although their nature is obscure, evidence from other sources suggests that these efferents also mediate drinking initiated by other stimuli. In the rat, De Wied (1966) found that intraperitoneal injection of hexamethonium reduced drinking elicited by subcutaneous injection of

hypertonic saline. Drinking induced by water-deprivation in the rat was also reduced by systemic injection of three quaternary ammonium muscarinic antagonists (Gerald & Maickel, 1969).

Peripheral adrenergic mechanisms did not appear to be involved in angiotensin-induced drinking in the cat. The only antagonist which reduced the response was propranolol. This may have been due to an action of propranolol in the CNS. Similar conclusions on the role of the peripheral sympathetic nervous system in angiotensin-induced drinking can be drawn from experiments in the rat (Severs et al, 1970, 1971; Lehr & Goldman, 1973).

Central cholinergic mechanisms did not appear to be involved in angiotensin-induced drinking in the cat, since the response was not attenuated after central administration of nicotinic or muscarinic antagonists. This result was not surprising since it is well established that central administration of cholinomimetic drugs do not elicit drinking in this species. In the rat administration of such antagonists has produced conflicting results. Fitzsimons & Setler (1971) reported that preoptic injections of atropine, which were sufficient to inhibit carbachol induced drinking, were ineffective against intracerebral angiotensin II. Their results have been confirmed by several other groups (see Severs & Daniels-Severs, 1973). In contrast, Severs et al (1970) found that following intraventricular injection of atropine or hexamethonium, the dipsogenic effect of icv angiotensin II was reduced. This result could be explained if central cholinoceptive sites outside the limbic system were involved in the response, as suggested by Lehr & Goldman (1973). The majority of the evidence available supports the view that

cholinergic mechanisms in the limbic system did not participate in the response. Setler (1973) has recently presented evidence that drinking following preoptic injections of carbachol and angiotensin II involve different mechanisms. She found that after a preoptic injection of noradrenaline the response to the cholinomimetic was reduced but that to the polypeptide was unaffected. In contrast to the results in the cat and the rat, preliminary studies in the monkey indicate that cholinergic neurones in the limbic system mediate the dipsogenic action of angiotensin II. Myers et al (1973) found that intrahypothalamic injections of nicotine or angiotensin II each elicited drinking and this response was prevented by central pretreatment with mecamylamine.

Fitzsimons & Setler (1971) proposed that catecholaminergic neurones in the limbic system were involved in the central dipsogenic action of angiotensin. In experiments in the rat, they demonstrated that a single intracerebral injection of 6-hydroxydopamine reduced drinking elicited by angiotensin II but not that elicited by carbachol. In the cat prolonged central administration of 6-hydroxydopamine, in doses reported to reduce brain catecholamines (Longo, 1973), did not immediately reduce angiotensin II-induced drinking. However, one week after the end of the treatment the response was reduced. This may have been due to a nonspecific action, such as the destruction of the ependyma and adjacent tissue (Longo, 1973). Although these experiments with 6-hydroxydopamine in the cat do not confirm the report of Fitzsimons & Setler (1971) their concept was supported by other experiments. Central administration of metaraminol, which rapidly depletes brain catecholamines (Smookler et al, 1966),

inhibited angiotensin II-induced drinking in the cat. The response was also reduced after central infusion of the adrenergic neurone blocker bethanidine.

The possibility that central catecholamines were involved in the angiotensin response was further investigated using α- and β-adrenoceptor antagonists. Central α-adrenoceptor blockade yielded inconsistent results, phentolamine markedly reduced angiotensin-induced drinking but neither phenoxybenzamine nor tolazoline affected it. Divergent results were also obtained in the rat by Lehr & Goldman (1973) but in their study intracerebral injection of tolazoline reduced drinking caused by angiotensin II whilst phenoxybenzamine and phentolamine were ineffective. Fitzsimons & Setler (1971) also found that intracerebral phentolamine was ineffective unless toxic doses were used. However, in other studies icv injection of either tolazoline or phentolamine reduced the dipsogenic action of similarly administered angiotensin II (Severs et al, 1971; Severs & Daniels-Severs, 1973). The variations in these results in both the cat and the rat cast doubt on the role of central α-adrenoceptor stimulation in the response to angiotensin II. This conclusion was supported by results demonstrating that neither clonidine nor noradrenaline were effective dipsogens in the cat (Chapter 1).

In contrast the results obtained using β-adrenoceptor blockers were consistent. Angiotensin II-induced drinking was significantly reduced after central administration of each β-antagonist. Propranolol and pindolol were the most potent, sotalol was least active whilst practolol had intermediate antagonist activity. A

similar order of potency has been demonstrated in blocking the peripheral and central cardiovascular actions of isoprenaline. In the rat intraventricular administration of several β-antagonists yielded inconsistent results and inhibitory effects were ascribed to local anaesthetic actions (Severs et al, 1971). Fitzsimons & Setler (1971) also considered that the effect of β-blockers was nonspecific since they found that D-propranolol was as effective as the L-form. This did not appear to be true in the cat since L-propranolol had a greater inhibitory effect than the D-isomer. This supports the hypothesis that the reduction in angiotensin-induced drinking was due to β-adrenoceptor block rather than to membrane stabilising effects. A similar conclusion can be drawn from the results showing that practolol and sotalol, which have no local anaesthetic action, were better inhibitors than lignocaine. The observation that central infusion of isoprenaline induced drinking in the cat is also consistent with the view that central β-adrenoceptors are involved in drinking mechanisms.

Recently, Setler (1973) summarised her work on the effects of depletion of brain catecholamines on angiotensin II-induced drinking in the rat. She found that the dipsogenic effect of angiotensin II was diminished when dopamine levels were reduced but noradrenaline levels were normal. Conversely when dopamine levels were normal but noradrenaline levels reduced there was no significant reduction in angiotensin-induced drinking. Neither procedure affected drinking elicited by carbachol. The implication that central dopaminergic neurones participated in the dipsogenic response was supported by her earlier report that central pretreatment with

haloperidol also inhibited it (Fitzsimons & Setler, 1971). This latter observation was confirmed in the cat. In addition it was demonstrated that pimozide, a specific dopamine antagonist also reduced angiotensin II-induced drinking. The effect of intracerebral haloperidol in the rat was not confirmed by Swanson et al (1973), however they used a very short dose interval (1 minute) between administration of the antagonist and angiotensin II. A similar negative result in the monkey was also obtained by the same group (Sharpe & Swanson, 1974).

CHAPTER 4

The cardiovascular actions elicited by intracerebroventricular administration of renin and angiotensin in the conscious cat and rabbit

The cardiovascular effects of icv administration of angiotensin II were thoroughly investigated using the chloralose-anaesthetised cat (Severs et al, 1966; Smookler et al, 1966). These workers established the dose-response relationship and examined the role of peripheral and central adrenergic mechanisms in the pressor response. Soloman (1972) extended their work by demonstrating that angiotensin I also caused a centrally mediated pressor response when it was injected into the lateral ventricle. He also studied the effect of central or systemic administration of converting enzyme inhibitors or angiotensin II analogues on these pressor responses.

Centrally mediated pressor responses to icv angiotensin II have also been reported in the conscious rat (Severs et al, 1970) rabbit (Rosendorff et al, 1970) and goat (Andersson et al, 1971).

In this chapter the cardiovascular effects of icv administration of renin and angiotensin in the conscious cat are discussed. The effect of central and systemic administration of autonomic blocking agents and central administration of angiotensin II analogue antagonists were also studied. Finally, an account of some experiments using conscious rabbits is presented.

Results

The effects of icv administration of angiotensin II in the conscious cat

In this study the dipsogenic and cardiovascular effects of icv administration of angiotensin II were studied simultaneously. This

procedure was unsatisfactory since the act of drinking caused increases in blood pressure and heart rate and it was difficult to separate the primary centrally mediated cardiovascular effects from those secondary to drinking. The results are therefore treated in a qualitative manner and no attempt was made to quantify them. In Fig. 4:1 & 2, the cardiovascular effects elicited by increasing doses of angiotensin II icv are illustrated. At low doses (1 - 50ng) there appears to be a slight pressor response (5 - 10mm Hg) upon which the effects due to drinking are superimposed. At higher doses (100ng - 4μg) there was usually an initial peak pressor response due to drinking, followed by a sustained response due to angiotensin II. In one of the traces in Fig. 4:2, water was withheld for 10 minutes after icv infusion of angiotensin II (1μg). The trace shows that the pressor response began after 1 minute and reached a plateau after 4 minutes, the pressure at that time being about 20mm Hg above the baseline. It was difficult to establish a dose-response relationship but the maximum response occurred at doses of 2 to 4μg. The effects on heart rate were variable, sometimes increases occurred before drinking began but usually the effects of drinking obscured the response. In many cats an elevated heart rate persisted after drinking ceased. High doses of angiotensin II often caused reflex bradycardia.

The effects of icv angiotensin I were similar to those elicited by the octapeptide.

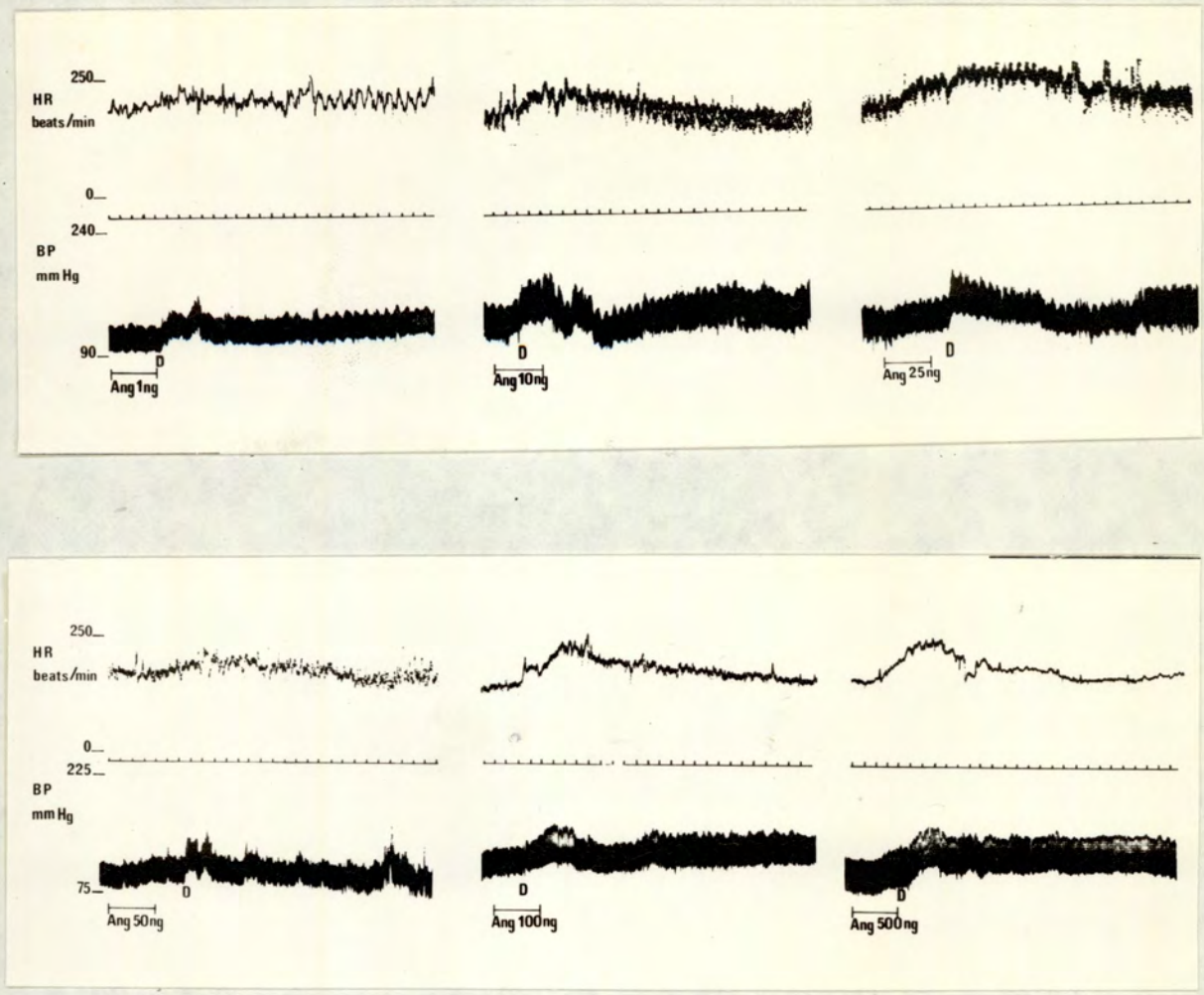


Fig. 4:1 Conscious cat. The cardiovascular responses elicited by icv infusion of increasing doses of angiotensin II. 'D' indicates drinking.

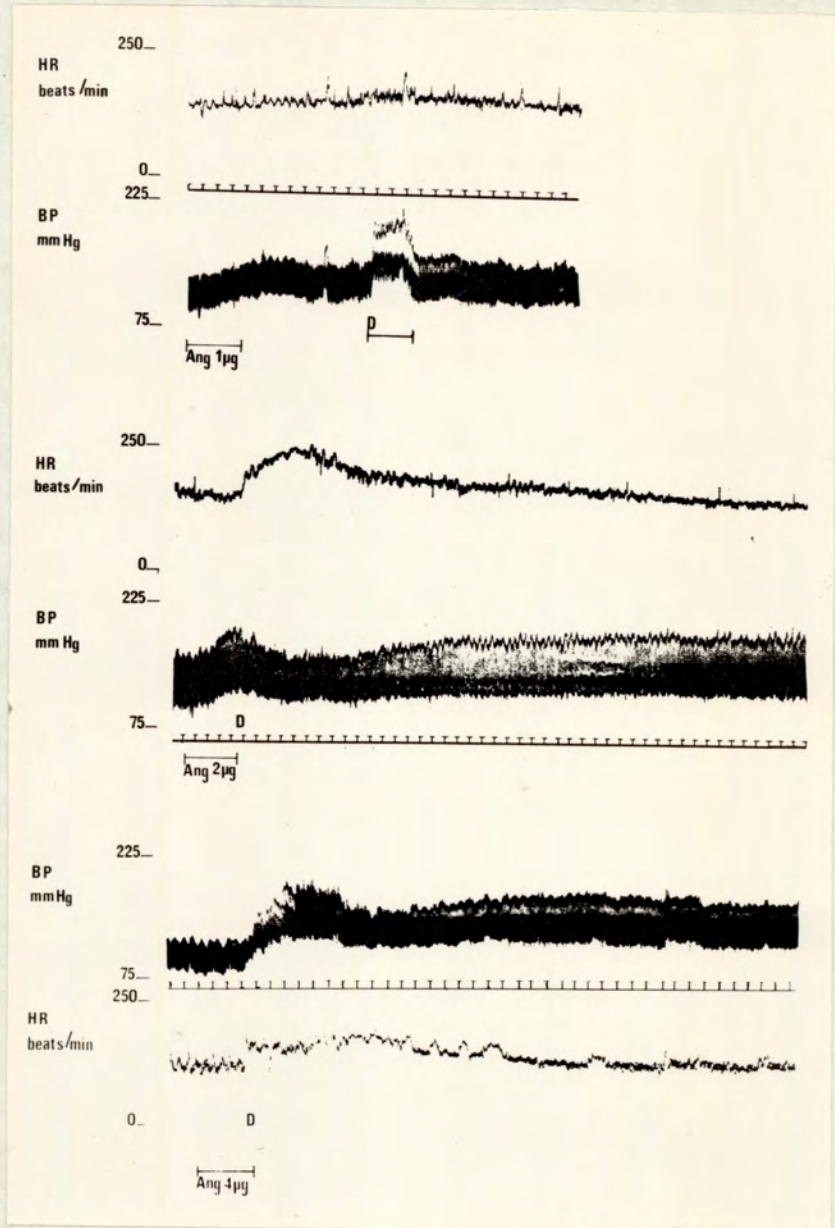


Fig. 4:2 Conscious cat. The cardiovascular responses elicited by icv angiotensin II.

Upper trace - 1µg
Middle trace - 2µg
Lower trace - 4µg.

'D' indicates drinking. In the upper trace water was withheld for 10 minutes after infusion of angiotensin.

The effect of intravenous administration of autonomic blocking agents on the cardiovascular responses elicited by icv angiotensin II

These results are also complicated since some antagonists reduce drinking and its associated cardiovascular phenomena. In most experiments the effects of icv angiotensin II (1μg) were examined 1h before and 1h after administration of the blocker. Systemic administration of pempidine ($5mg\,Kg^{-1}$) reduced the pressor response to icv angiotensin II in most experiments but in others the pressor response was unchanged (Fig. 4:3). The pressor response to icv angiotensin II was also reduced after systemic administration of bethanidine ($10mg\,Kg^{-1}$) or phentolamine ($5mg\,Kg^{-1}$) (Fig. 4:4). These two blockers did not affect drinking and its associated tachycardia. Intravenous administration of propranolol ($5mg\,Kg^{-1}$) reduced the pressor response, tachycardia and drinking to icv angiotensin II. However sotalol ($5mg\,Kg^{-1}$) only reduced the tachycardia (Fig. 4:5).

Peripheral autonomic blockade had variable effects on the responses elicited by large icv doses of angiotensin II (4μg). The pressor response was either slightly reduced, potentiated or unchanged. This may indicate that after administration of large doses of angiotensin II, some of the peptide overflowed into the systemic circulation.

The effect of icv administration of autonomic blocking agents on the cardiovascular response to icv angiotensin II

The pressor response to icv angiotensin II (1μg) was not markedly reduced 1h after icv infusion of bethanidine (400μg) (Fig. 4:6a). However, 1h after 600μg bethanidine icv (Fig. 4:6b) the centrally-mediated pressor response was markedly reduced.

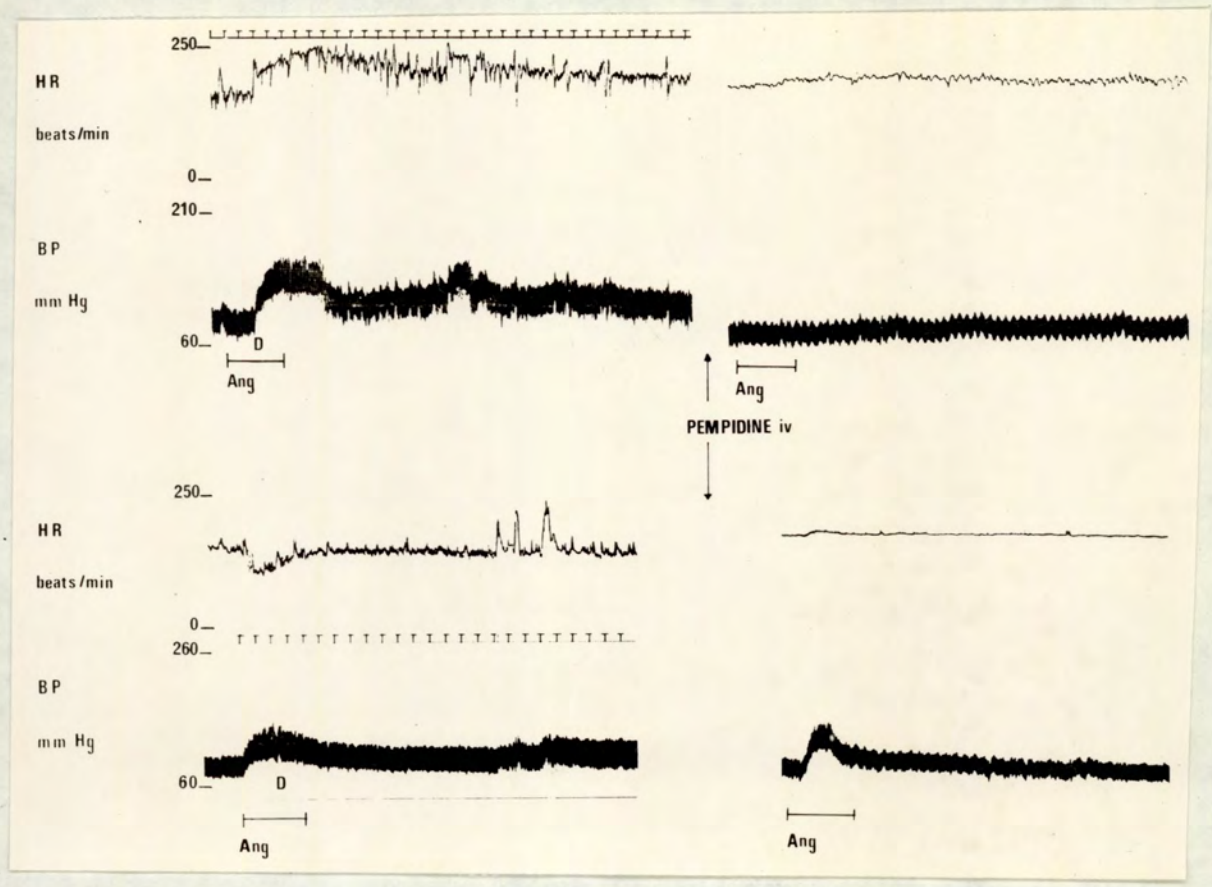


Fig. 4:3 Conscious cat. The effect of iv administration of pempidine (5mg kg^{-1}) on the cardiovascular responses elicited by icv angiotensin II (1μg). The traces illustrate the two types of effect observed in different cats. 'D' indicates drinking. In each case pempidine was given 1h before the second dose of angiotensin.

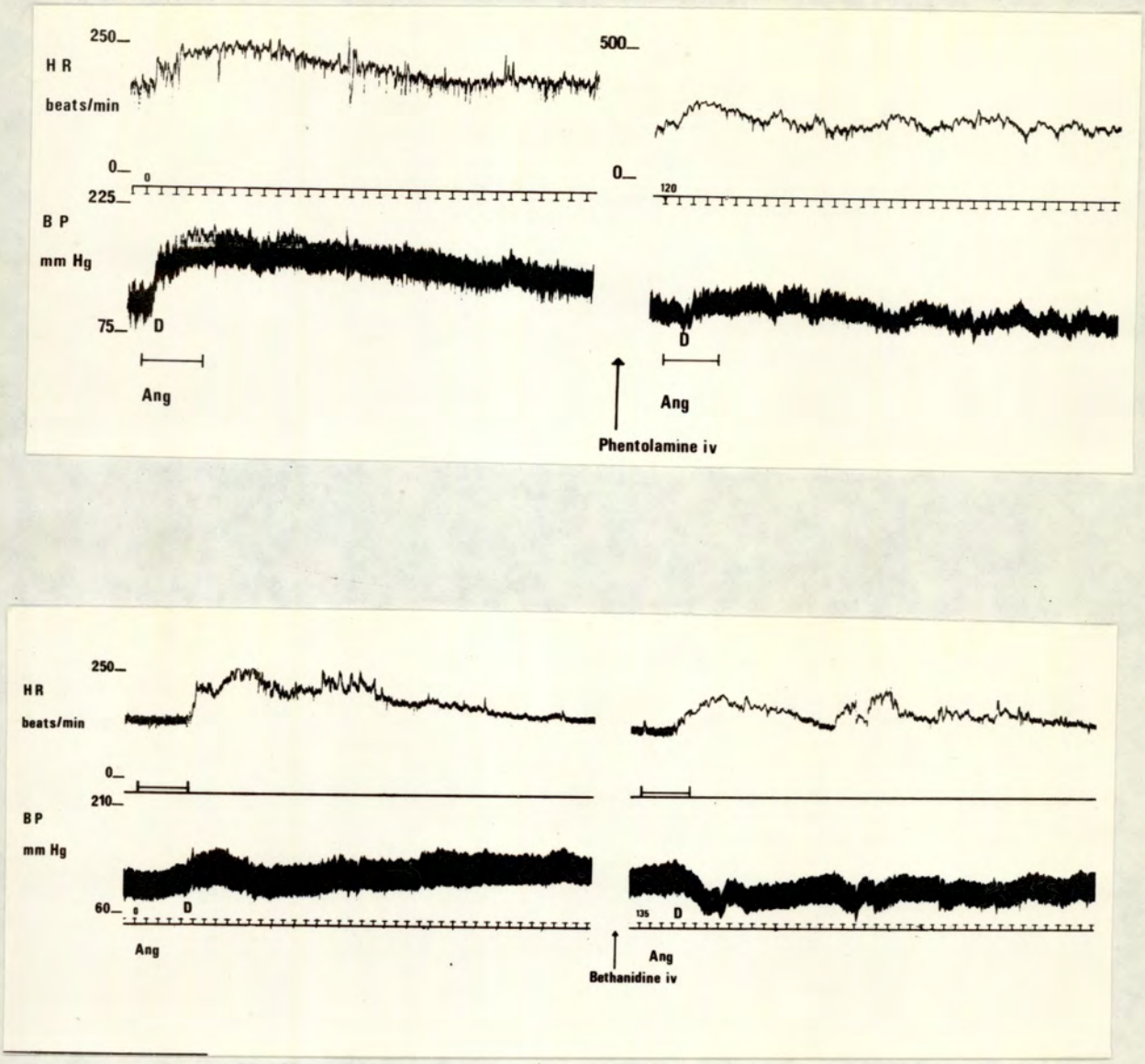


Fig. 4:4 Conscious cat. The effect of iv administration of phentolamine (5mg kg^{-1}) or bethanidine (10mg kg^{-1}) on the cardiovascular responses elicited by icv angiotensin II (1μg). 'D' indicates drinking. Each blocker was given 1h before the second dose of angiotensin.

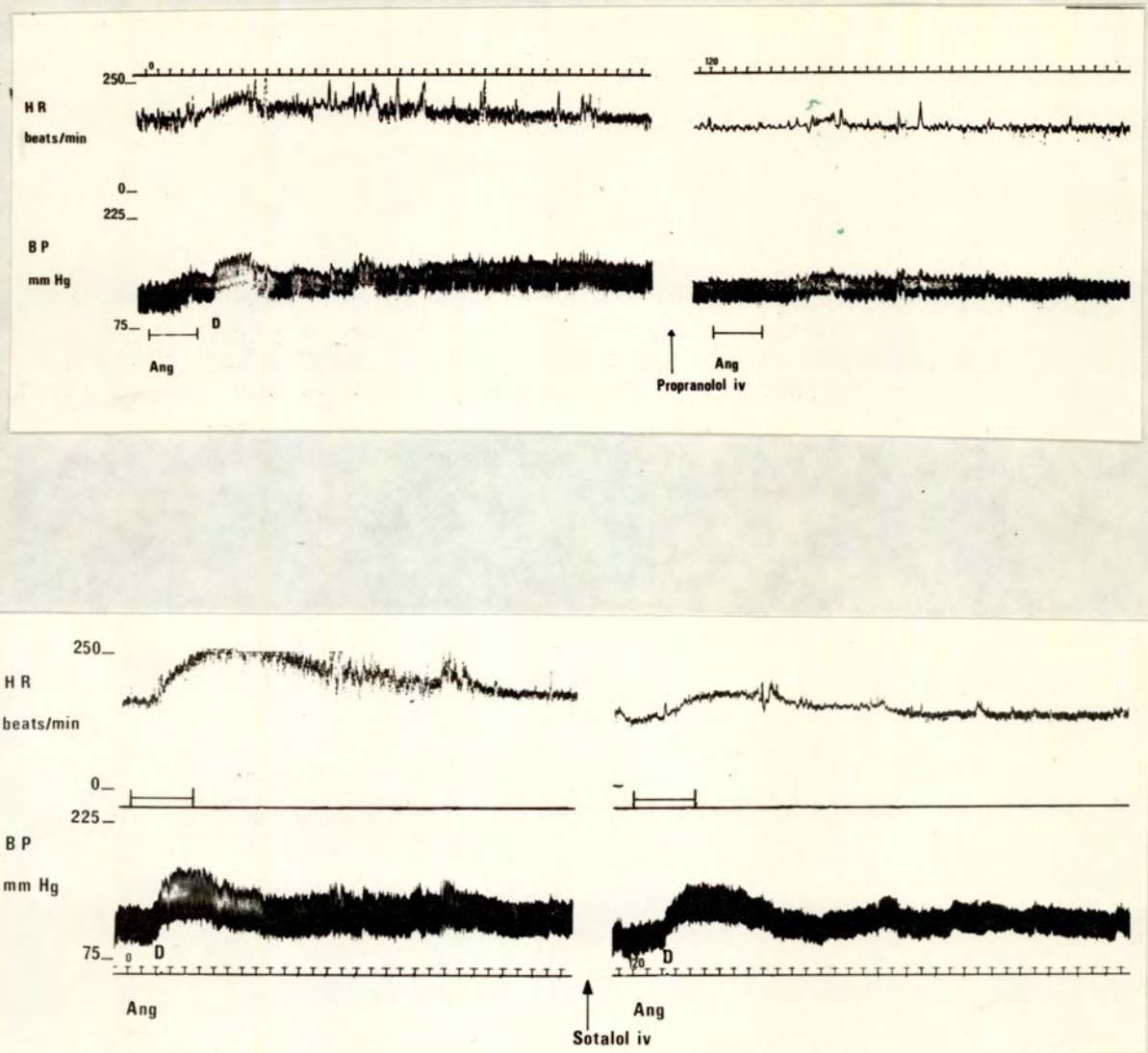
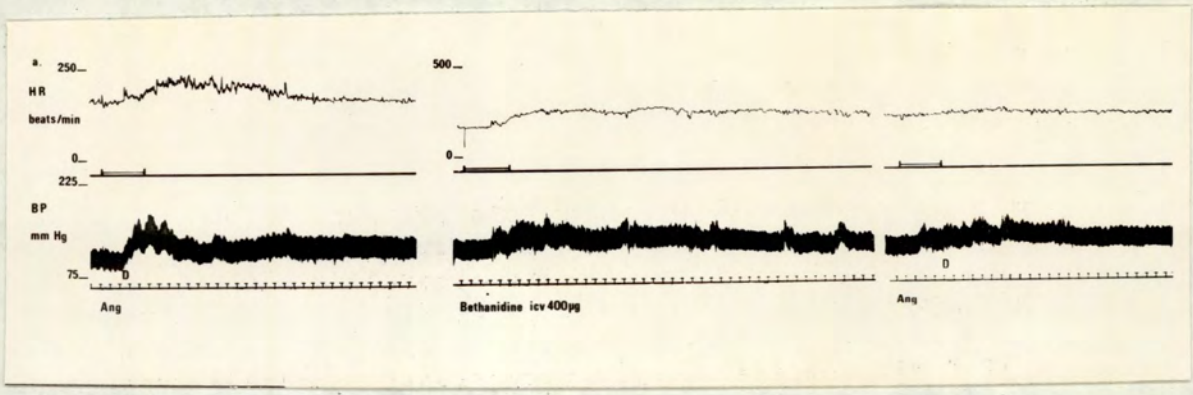


Fig. 4:5 Conscious cat. The effect of iv administration of β-adrenoceptor blocking agents on the cardiovascular responses elicited by icv angiotensin II (1μg). 'D' indicates drinking.
Upper trace. Propranolol (5mg kg^{-1})
Lower trace. Sotalol (5mg kg^{-1})
Each blocker was given 1h before the second dose of angiotensin.



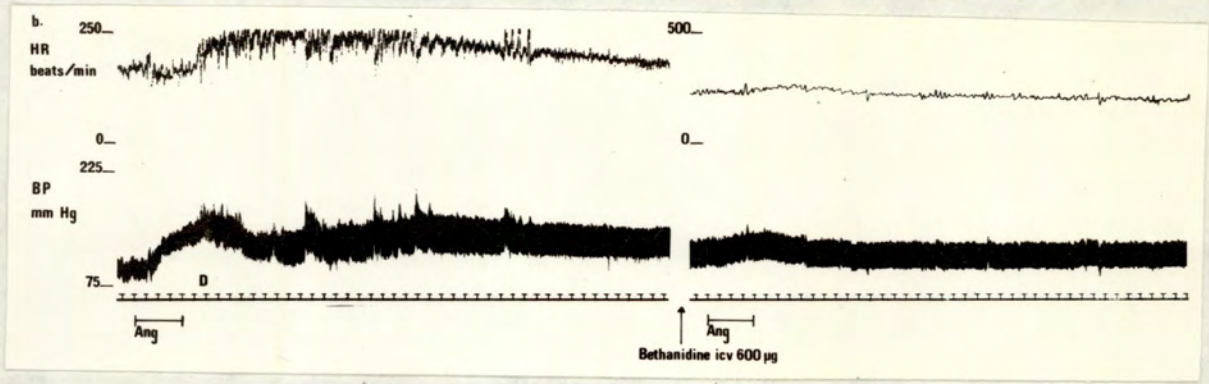


Fig. 4:6 Conscious cat. The effect of icv administration of bethanidine on the cardiovascular responses elicited by icv angiotensin II (1µg). 'D' indicates drinking.

a) Bethanidine (400µg)
b) Bethanidine (600µg)

Each blocker was given 1h before the second dose of angiotensin.

Intraventricular administration of phentolamine (500μg), DL-propranolol (450μg) or pindolol (500μg) all reduced the pressor response to icv angiotensin II (Fig. 4:7). These results must be viewed with caution since each of these antagonists also reduced drinking. The pressor response was not significantly affected by icv administration of either tolazoline (600μg) or atropine methylnitrate (200μg) neither of which reduced drinking.

The effect of icv infusion of hog renin

Hog renin, 0.1 - 0.25 GbU, infused into the lateral ventricle caused increases in blood pressure. The size of the response varied with each animal. Fig. 4:8a illustrates a response elicited by 0.25 GbU icv, the pressure began to rise 20 minutes after the infusion started, reached a maximum after 50 minutes and began to decline after 80 minutes. The response did not occur after prior icv infusion of Sar^1Ala^8angiotensin II (Fig. 4:8b).

The effect of icv administration of angiotensin II analogue antagonists on the cardiovascular response to icv angiotensin II

Administration of either Sar^1Ala^8angiotensin II icv (5μg) or Sar^1Ile^8angiotensin II icv (5μg) occasionally caused small pressor responses (5 - 10mm Hg). The pressor response to icv angiotensin II (1μg) was reduced 30 minutes after administration of either analogue. The inhibitory effect of Sar^1Ala^8angiotensin II was short lasting, the response to centrally administered angiotensin II could be elicited 150 minutes after the analogue. The effect of Sar^1Ile^8angiotensin II was more prolonged, but the effect of icv angiotensin II had returned when the cat was retested on the following day. These effects are illustrated in Fig. 4:9.

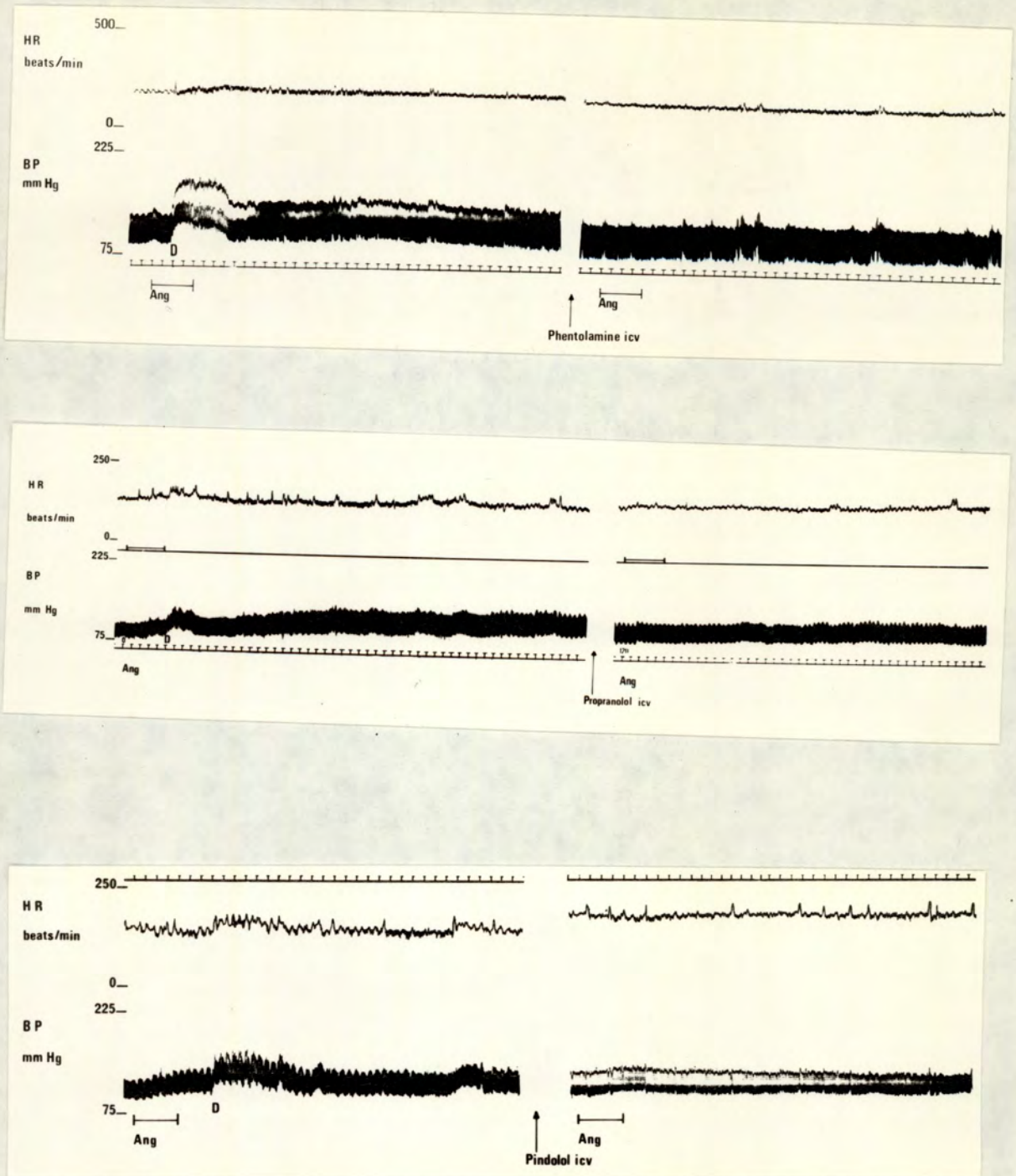
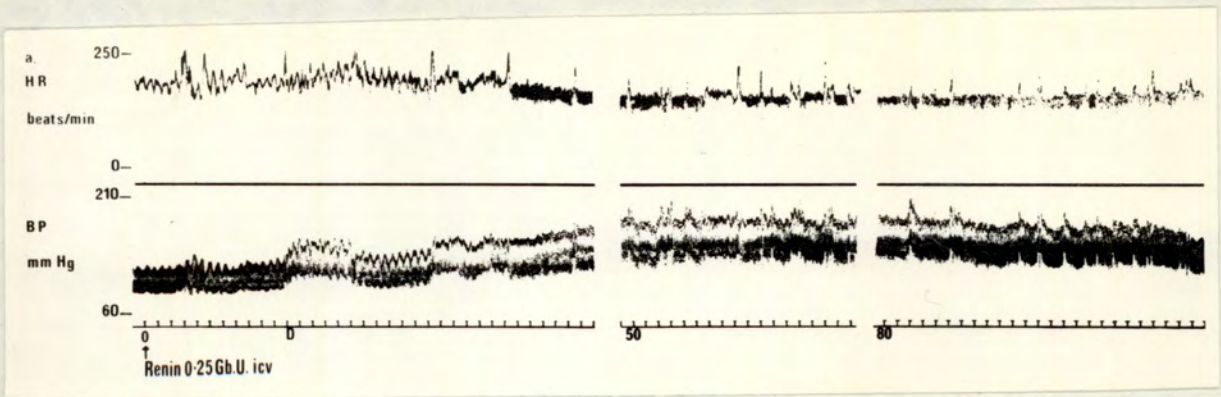


Fig. 4:7 Conscious cat. The effect of icv administration of adrenoceptor blocking agents on the cardiovascular responses elicited by icv angiotensin II (1μg). 'D' indicates drinking.

Upper trace. Phentolamine (500μg)
Middle trace. Propranolol (450μg)
Lower trace. Pindolol (500μg)

Each blocker was given 1h before the second dose of angiotensin.



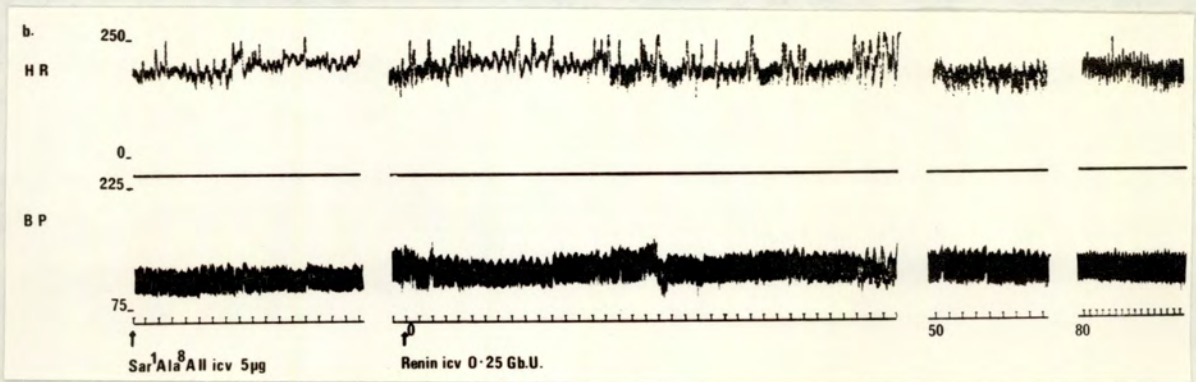


Fig. 4:8 Conscious cat. The effect of icv administration of Sar^1Ala^8 angiotensin II (5µg) on the cardiovascular actions elicited by icv renin (0.25 GbU). 'D' indicates drinking.

a) Control

b) 30 minutes after Sar^1Ala^8 angiotensin II.

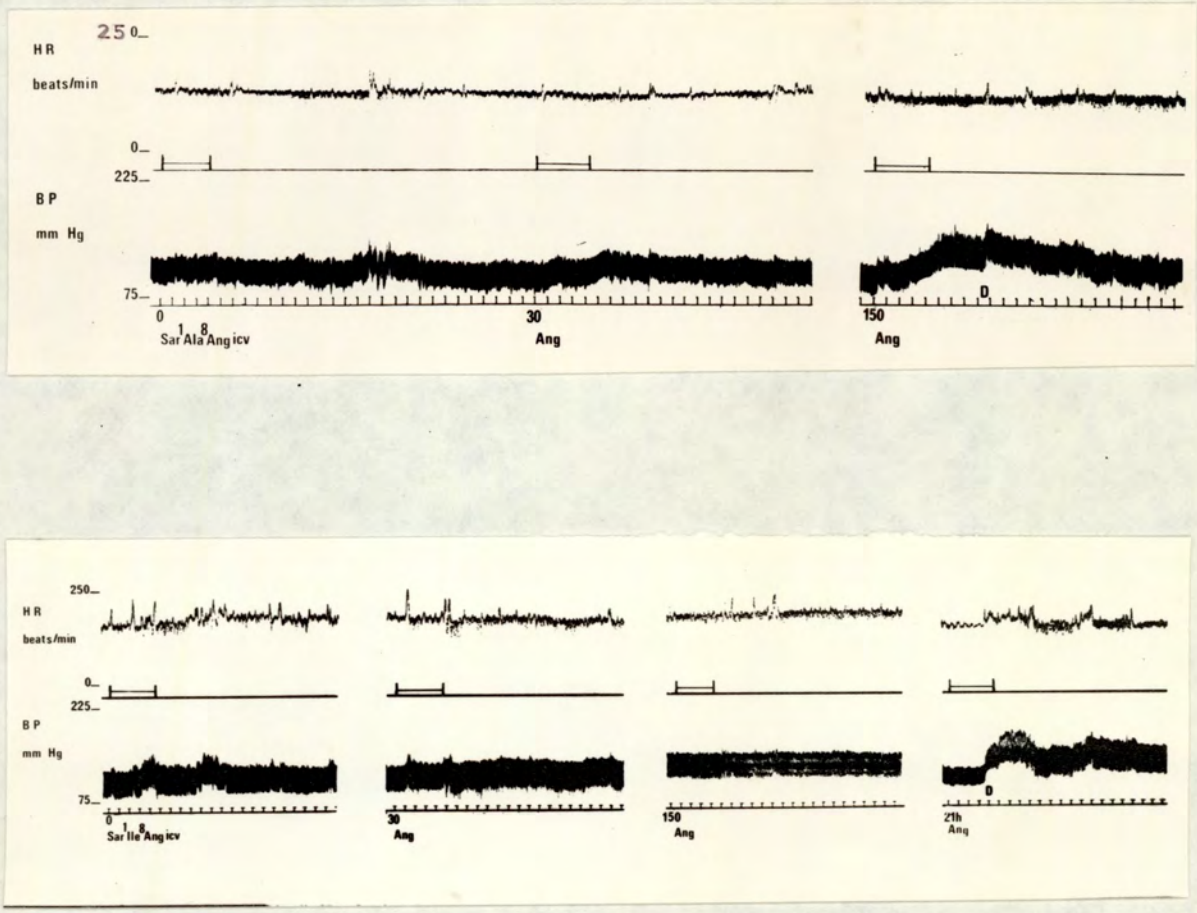


Fig. 4:9 Conscious cat. The effect of icv administration of angiotensin II analogues on the cardiovascular effects elicited by icv angiotensin II (1μg). 'D' indicates drinking.
Upper trace. Sar^1Ala^8angiotensin II (5μg)
Lower trace. Sar^1Ile^8angiotensin II (5μg)

The effect of icv administration of angiotensin II in the conscious rabbit and the use of autonomic blocking drugs to analyse the response

The rabbits used in this study did not drink following icv angiotensin II. This may have been due to the dose used being subthreshold or that the rabbit was disturbed by novel surroundings. This absence of a dipsogenic response was useful since it allowed the cardiovascular effects of icv angiotensin II to be studied in isolation.

In initial experiments the relative pressor activities of icv and iv infusions of angiotensin II were compared. When a dose of 500ng angiotensin II was infused icv over 4 minutes it elicited a sustained pressor response between 20 and 30mm Hg which lasted about 30 minutes. This response could be elicited by a similar infusion given 2h later. An icv infusion of 0.9% sodium chloride solution did not cause a rise in blood pressure. A pressor response of similar magnitude could be elicited by infusing twice the dose of angiotensin II (1μg) iv in 4 minutes. The response to iv angiotensin II differed from that to icv angiotensin II since it declined rapidly when the infusion ended. The greater response elicited by icv angiotensin II suggests that the effect was not due to the peptide leaking into the peripheral circulation.

Intravenous injection of either hexamethonium (7.5mg Kg^{-1}) or pempidine (5mg Kg^{-1}) reduced the pressor effect of icv angiotensin II (500ng) in three animals. The response to iv angiotensin II (1μg) was unaffected after ganglion blockade. However, neither the response to icv nor that to iv angiotensin II

was significantly reduced after systemic injection of bethanidine ($5mg\,Kg^{-1}$) or phentolamine ($5mg\,Kg^{-1}$). These results are illustrated in the traces in Fig. 4:10.

The involvement of central adrenergic mechanisms in the response to angiotensin II was investigated using α- and β-adrenoceptor antagonists given icv. Phentolamine ($500\mu g$) icv caused a marked reduction of the pressor response to icv angiotensin II given 1h later and a higher dose (1mg icv) abolished the response (Fig. 4:11). However tolazoline (1mg icv) caused only a small reduction in the response to icv angiotensin II. Propranolol ($500\mu g$) did not significantly reduce the response to icv angiotensin II but when the dose was increased to 1mg the response was reduced by 50% (Fig. 4:11).

Discussion

In the first systematic study of the centrally mediated cardiovascular effects of icv angiotensin II in the anaesthetised cat, dose-related pressor responses were demonstrated (Smookler et al, 1966). The threshold dose was 10ng and a maximum increase in blood pressure occurred with 100ng, although a dose of $2\mu g$ was necessary to obtain a maximum duration of the response. In the conscious cat the primary cardiovascular effects of icv angiotensin II were complicated by secondary cardiovascular changes associated with drinking. In most cats a prolonged pressor response occurred which continued after drinking ceased. This response was evident at a dose of 100ng and a maximum effect was obtained with doses of 2 to $4\mu g$. The differences in the dose-response relationship in anaesthetised and conscious cats may be due to the different

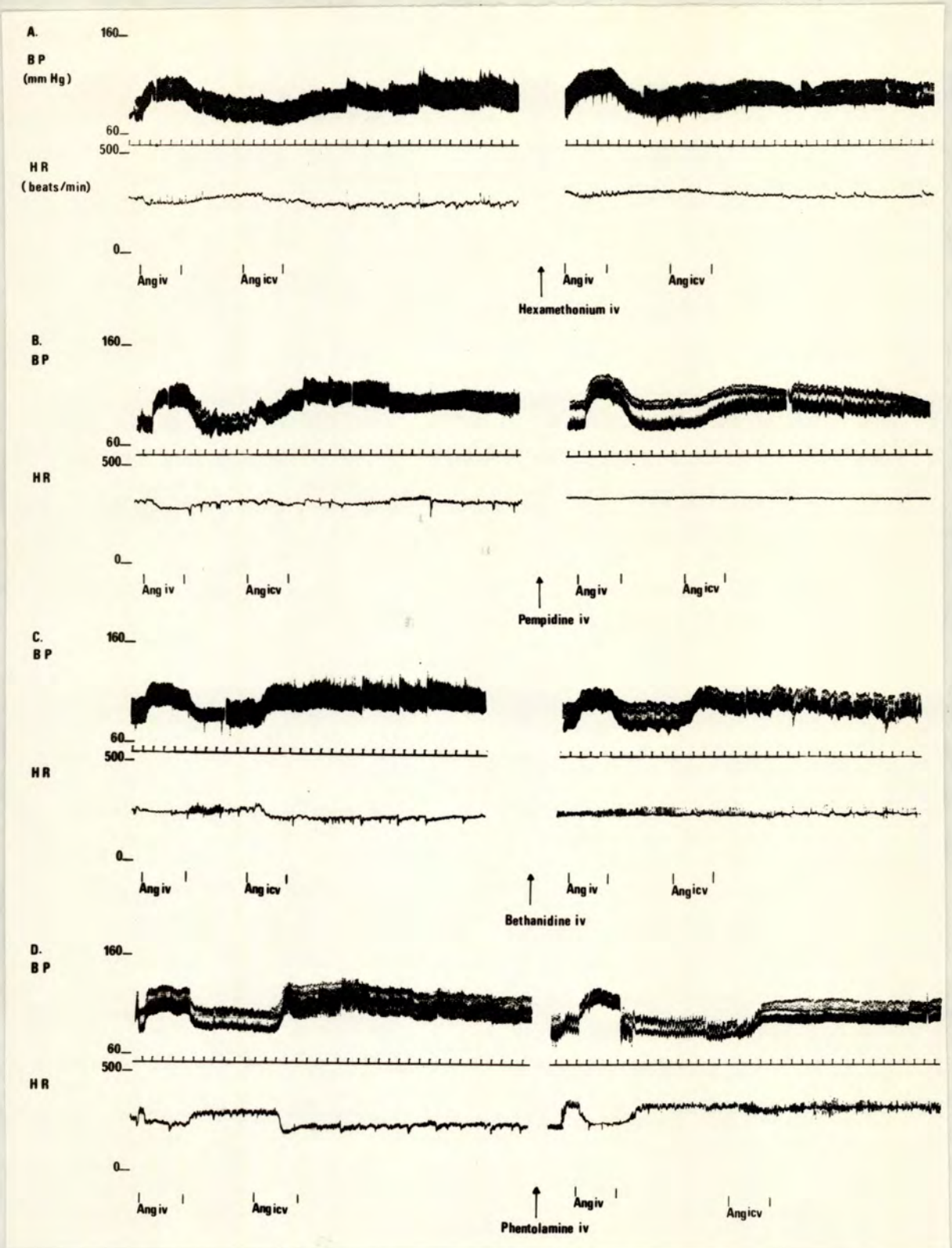


Fig. 4:10 Conscious rabbit. The effect of iv administration of autonomic blocking agents on the cardiovascular responses elicited by angiotensin II iv (1μg) or icv (500ng).

A. Hexamethonium (7.5mg kg^{-1}) B. Pempidine (5mg kg^{-1})
C. Bethanidine (5mg kg^{-1}) D. Phentolamine (5mg kg^{-1})

Each blocker was given 1h before the second dose of angiotensin.

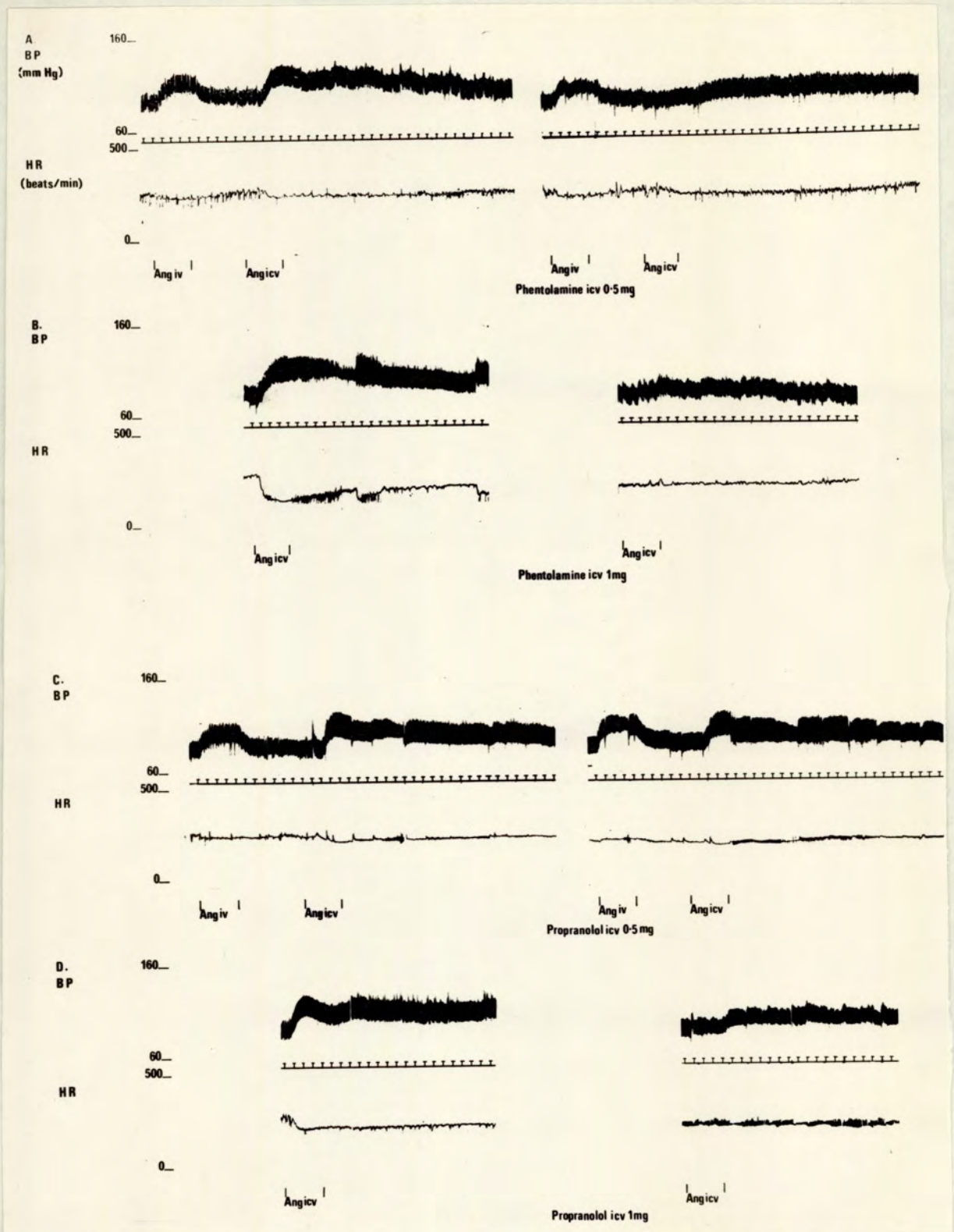


Fig. 4:11 Conscious rabbit. The effect of icv administration of autonomic blocking agents on the cardiovascular responses elicited by angiotensin II iv (1μg) or icv (500ng).

A. Phentolamine (0.5mg) B. Phentolamine (1mg)
C. Propranolol (0.5mg) D. Propranolol (1mg)

Each blocker was given 1h before the second dose of angiotensin.

techniques used. In anaesthetised animals the cerebral ventricles were perfused with artificial CSF, thus administered angiotensin would rapidly reach its site of action. Alternatively the anaesthetic may have inhibited baroreceptor reflexes which oppose the pressor responses in the conscious cat. It is also interesting to note that the threshold for cardiovascular effects seemed to be greater than that for dipsogenic actions.

The pressor response in anaesthetised cats was usually accompanied by tachycardia (Smookler et al, 1966). Drinking behaviour caused an increase in heart rate in the conscious cat and after drinking the heart rate remained elevated or did not fall below base-line. In some experiments the tachycardia began before the cat started to drink. When large doses of angiotensin II were given icv reflex bradycardia was often observed, Smookler et al (1966) reported a similar finding.

The results of experiments involving C_1 spinal section and systemic administration of α- or β-adrenoceptor blocking drugs indicated that peripheral adrenergic mechanisms mediated the response in the cat (Smookler et al, 1966; Severs et al, 1966). In the conscious cat the effects of submaximal icv doses of angiotensin II (1μg) were attenuated after systemic ganglion blockade, adrenergic neurone blockade or α-adrenoceptor blockade. However the effects of β-adrenoceptor blockade were not consistent. The pressor response was reduced after propranolol but not after sotalol, this might be due to a central effect of propranolol.

Both reduction or increases in brain catecholamines attenuated the centrally mediated pressor response to angiotensin II in

anaesthetised cats (Smookler et al, 1966). This group proposed that angiotensin II required "physiologic levels" of noradrenaline in the brain to exert its maximum effects. In the conscious cat central administration of bethanidine, phentolamine or propranolol reduced the pressor response to angiotensin II icv. These results also suggest that the response involves central adrenergic mechanisms.

Infusions of renin icv also caused a pressor response. The response was probably mediated by angiotensin released by the action of renin on a brain substrate. This view was supported by results indicating that central pretreatment with an angiotensin II analogue antagonist reduced the response to renin. Angiotensin II analogue antagonists also attenuated the pressor response to icv angiotensin II confirming previous reports in the anaesthetised cat (Soloman, 1972) and conscious rat (Schölkens, 1974).

Rosendorff et al (1970) previously described the pressor response to icv angiotensin II in the conscious rabbit. The magnitude and duration of the pressor response were similar in this study, although the dose used was four times greater than that of Rosendorff et al (1970). The results also confirmed their observation that the pressor response to icv angiotensin II was greater than that produced by a larger total dose given intravenously. In contrast to the above report no tachyphylaxis to the central action of angiotensin II was observed in this study. This was probably due to using a longer dose interval.

Since C_2 spinal section abolished the angiotensin II-induced

pressor response in the anaesthetised rabbit the effect appeared to be mediated entirely by the sympathetic nervous system (Rosendorff et al, 1970). This conclusion was based on evidence obtained from an experiment in one rabbit which survived this treatment. Two other rabbits died after spinal section thus the above result must be viewed with caution. In the conscious rabbit the pressor response to icv angiotensin II was reduced, but not abolished, by ganglion blockade and unaffected by systemic adrenergic neurone or α-adrenoceptor blockade. The central administration of phentolamine and a large dose of propranolol attenuated the centrally mediated effect of angiotensin II. It is unlikely that these results are due to leakage from the brain since the pressor response was reduced by central administration of antagonists. A possible explanation is that the pressor response is mediated by two mechanisms, the sympathetic nervous system and a humoral mechanism . Severs et al (1970) obtained very similar results in the conscious rat and suggested that the humoral response was mediated by release of vasopressin. They found that systemic administration of ganglion blockers, α- or β-adrenoceptor blockers did not markedly reduce the pressor effects of icv angiotensin II. Central administration of phentolamine or lesion of the supraoptic nucleus reduced the angiotensin induced pressor response (Severs et al, 1970; 1971).

GENERAL DISCUSSION

During the last 15 years the potent pharmacological effects of angiotensin II on central neurones have been convincingly demonstrated but their physiological or pathological function has not been adequately defined. In most early studies angiotensin II was either injected intracerebrally, thus by-passing the blood brain barrier, or was administered into the cerebral circulation. The significance of this work was not fully realised since the intravascular doses were large and resulted in plasma angiotensin levels in excess of those found during pathological situations. It was thus considered that at normal plasma angiotensin levels, the peptide did not penetrate the CNS. The possible physiological significance of these centrally mediated effects was emphasised in later experiments when it was established that administration of nanogram doses of angiotensin into the vertebral arteries elicited a hypertensive response (Lowe & Scroop, 1969). Another development which focused attention on the CNS effects was the discovery of an independent renin-angiotensin system in the brain (Ganten, Marquez-Julio et al, 1971). This led to speculation that angiotensin was generated within the CNS and acted locally on angiotensin-sensitive neurones.

There are two major functions which are influenced by central administration of angiotensin, these are body fluid regulation and the control of the cardiovascular system. In this thesis emphasis has been placed on the former function, in particular the effects on water intake, and only a minor portion has been devoted to cardiovascular effects. Fitzsimons (1969) created a revival of interest in a renal dipsogen when he isolated a substance from the rat renal cortex which induced drinking when injected

intraperitoneally into other rats. He could not distinguish this substance from renin using conventional techniques and proposed that the renin-angiotensin system participated in drinking behaviour. Subsequently the dipsogenic effect of systemic and central infusion of angiotensin II in the rat and central infusion of the peptide in other species was demonstrated. The stimulus for Fitzsimons' investigations was an earlier observation that drinking in response to certain procedures which imitate depletion of the ECF was attenuated in nephrectomised rats. Ligation of the inferior vena cava in nephrectomised rats was found to be almost ineffective as a dipsogenic stimulus implying that it was wholly dependent on a renal mechanism (Fitzsimons, 1964). In contrast injection of a hyperoncotic solution (PEG) into the interstitium was equally effective in nephrectomised and intact rats (Fitzsimons, 1961) implying that the kidneys were not essential for the dipsogenic effect. Fitzsimons suggested that there were two mechanisms which mediated "hypovolaemic" drinking, a neural mechanism and a humoral one. He considered that the sensory input to the brain from volume receptors in the vasculature activated central drinking mechanisms and also initiated reflex release of renin. Angiotensin liberated might enter the brain having either a direct dipsogenic action or facilitating the actions elicited by neural sensory input.

The above hypothesis was based on the effects of nephrectomy but has been corroborated by studies in which plasma renin activity (PRA) was measured. Leenen & Stricker (1974) found that plasma renin levels increased after either caval ligation or subcutaneous injection of PEG. A close correlation between PRA and water intake occurred

after PEG but not after caval ligation, thus the relationship between angiotensin and drinking behaviour is quite complex. These results also indicate that the renin-angiotensin system may mediate drinking elicited by hyperoncotic solutions. Blass, Nussbaum & Hanson (1974) have also proposed a significant renal contribution in PEG-induced drinking. They reported that baseline water intake in nephrectomised rats is greater than in intact ones. Thus they contend that this increase should be subtracted from total intake in response to PEG and this would reveal a significant drinking deficit in nephrectomised rats.

Other workers have used similar techniques to demonstrate a renin-angiotensin component in the dipsogenic response to hypotensive drugs. Systemic administration of hydralazine or phentolamine in the rat caused increases in plasma renin which correlated well with increased water intake. These effects were both abolished by nephrectomy (Meyer et al, 1971, Peskar et al, 1970). Increased plasma angiotensin levels are not entirely responsible for increased drinking since other drugs, such as metaraminol, which increase PRA but do not cause hypotension are not dipsogens (Leenen & Stricker, 1973). A renin dependent mechanism has also been proposed to account for the dipsogenic effects of isoprenaline.

An alternative view of the role of angiotensin in drinking has been advanced by Severs and his colleagues (Radio, Summy-Long, Daniels-Severs & Severs, 1972, Severs & Daniels-Severs, 1973). They found that during continuous central infusion of angiotensin II in the rat, animals would drink 0.9% saline for up to 5h but stopped drinking after 90 minutes when given water. Since dilution of the

body fluids appeared to inhibit drinking but expansion of the fluid volume did not, they considered that angiotensin-induced drinking was associated with intracellular fluid depletion.

In initial studies in the present investigation it was established that icv administration of angiotensin II in the cat also elicited a dipsogenic response. There were two significant aspects of this observation, firstly the cat in normal water balance does not readily drink water. Secondly specific water drinking behaviour in the cat had not previously been initiated by central administration of drugs. When other components of the renin-angiotensin system were administered icv in the cat they also elicited drinking, thus in this respect this species behaved similarly to the rat.

Fitzsimons' hypothesis requires that systemically liberated angiotensin II reaches angiotensin-sensitive regions in the brain. The evidence from biochemical and histochemical studies has proved inconclusive except when high doses were used. Fitzsimons & Simons (1969) did show that drinking was elicited in the rat by intravenous infusion of very high doses of angiotensin II. More recently Hsiao & Epstein (1973) demonstrated a dipsogenic effect using iv infusions of much lower doses of both the octapeptide and the decapeptide. In the experiments in the cat iv infusions of angiotensin I or II each elicited drinking, the doses used were similar to those of Hsiao & Epstein (1973). Although these observations appear to support Fitzsimons' hypothesis it must be emphasised that the doses were very large when considered in terms of their vasopressor activity in the cat. The effect of sudden prolonged increases in arterial pressure

on small capillaries in the brain has not been assessed. It is possible that this stress causes damage resulting in a breakdown of the blood brain barrier. Another observation which was not consistent with the hypothesis was that intravenous administration of renin was only weakly dipsogenic. This ineffectiveness may however be due to rapid removal by peripheral vascular tissue of the angiotensin liberated.

An important advance in the pharmacology of angiotensin has been the introduction of specific competitive blocking agents. It was proposed to use these agents to investigate the role of the renin-angiotensin system in various types of drinking behaviour. The first step in this investigation was to determine whether these inhibitors would block the effects of administration of exogenous components of the renin-angiotensin system. This study also enabled the elucidation of the mode of action of these various components. Since the peripheral actions of renin are mediated by release of angiotensin it was considered that this process also occurred in the CNS. This view was favoured by the observation that the latency of response to icv renin was longer than that to icv angiotensin. In the cat central administration of pepstatin, a renin-renin substrate inhibitor, specifically prevented the dipsogenic effect of renin, thus confirming the above opinion. Use of a converting enzyme inhibitor indicated that angiotensin I had little intrinsic central dipsogenic activity since after central pretreatment drinking in response to the decapeptide was markedly reduced. It was thus concluded that angiotensin II was the principal dipsogenic component. This conclusion was strengthened by results obtained using competitive

inhibitors of angiotensin II. Central administration of modified analogues of the octapeptide, which were known to be antagonists of the systemic effects of angiotensin II, caused reversible inhibition of drinking elicited by renin, angiotensin I and II in the cat. Similar results to those summarised above were obtained concurrently using the rat by Epstein et al (1974). In addition these workers extended their study by using renin substrate as a dipsogen. They showed that pepstatin partially reduced drinking elicited by renin substrate thus demonstrating that the inhibitor is effective against brain isorenin.

In certain experiments with angiotensin II analogues in the rat potent central dipsogenic activity was observed (Swanson et al, 1973). The compounds used in that study were substituted at position 8 only. In the cat analogues substituted in positions 1 and 8 were very weak central dipsogens but one compound substituted at position 8 only had a slightly greater action. A subsequent examination showed that the analogues used were also weak systemic vasopressor agonists in the conscious cat. Thus it was suggested that the central dipsogenic responses observed in the cat were due to weak partial agonist activity.

An important observation in the present study was that drinking elicited by intravenous administration of either angiotensin I or II was prevented by central administration of an angiotensin II antagonist. This is the first direct evidence that systemically administered angiotensin acts on central receptors. Previously this view was based on the circumstantial evidence that the central threshold dipsogenic dose was less than the systemic one. A central site of

action for systemically injected angiotensin II has also been demonstrated by making lesions in discrete brain areas. Lesions in the subfornical organ (Simpson, 1973) or the lateral preoptic area (LPO) (Peck, 1973) of the rat were also found to attenuate the dipsogenic effect of iv angiotensin II. The results of these studies are open to criticism since destruction of brain tissue could prevent drinking in a non-specific manner. Indeed, it has previously been reported that lesions in the rat LPO prevent drinking elicited by systemic injection of hypertonic saline (Blass & Epstein, 1971).

Investigation of the effect of central administration of a converting enzyme inhibitor on the dipsogenic effect of systemic angiotensin in the cat revealed another interesting result. Angiotensin I-induced drinking was reduced but angiotensin II-induced drinking was unaffected. Complementary observations in experiments in the rat have been reported, thus central administration of the same inhibitor (SQ 20881) reduced drinking elicited by ip renin (Lehr et al, 1973). These results implied that a significant proportion of an exogenous dose of angiotensin I enters the brain before being converted to angiotensin II. Endogenous angiotensin I may also undergo this process since peripheral inhibition of converting enzyme increases the dipsogenic response to some stimuli and this increase can be prevented by central administration of SQ 20881 (Lehr et al, 1973, Summy-Long & Severs, 1974).

Angiotensin II acting in the brain appeared to be the final link in drinking behaviour induced by the renin-angiotensin system. It was thus argued that the involvement of renin and angiotensin in various dipsogenic stimuli could be confirmed by studying them in

animals pretreated centrally with an angiotensin II antagonist. In the preliminary studies presented in this thesis the stimuli selected were systemic injection of either hypertonic saline or PEG solution. The first stage was to establish that these stimuli, which were standard experimental methods for inducing drinking in the rat, were also effective in the cat. Initial experiments established that reliable dipsogenic responses could be elicited by each stimulus. There was no reduction in the cumulative dipsogenic response to hypertonic saline in cats treated centrally with the angiotensin II antagonist. This result indicated that it was unlikely that angiotensin mediated the response. In contrast to this clear-cut result the experiments using PEG as a stimulus were difficult to interpret. In the first hour after presentation of water, cumulative water intake in animals treated with the antagonist was slightly reduced but after 2h the intake was normal. This initial decrease could be evidence that a small renin-angiotensin component was involved, however more experiments are necessary to determine this. In a similar more extensive analysis in the rat, Summy-Long & Severs (1974) found that central or systemic administration of SQ 20881 or $Sar^{1}Ala^{8}$angiotensin II did not affect the dipsogenic response to systemic administration of hypertonic saline or PEG solution. The failure to demonstrate a renin-angiotensin component may have been due to using insufficient amounts of the antagonist to inhibit endogenous angiotensin. Alternatively when the humoral mechanism is inhibited, a neural mechanism may compensate for this deficiency. It has already been mentioned that a similar compensatory mechanism may mediate PEG-induced drinking in nephrectomised rats

(Fitzsimons, 1972).

Most of the evidence obtained using the rat supports the view that drinking induced by β-adrenoceptor agonists is mediated by the renin-angiotensin system. The dipsogenic effect of centrally or systemically administered isoprenaline was markedly reduced in nephrectomised rats (Fisher, 1973; Meyer et al, 1971) and systemic administration of that amine caused increases in PRA (Meyer et al, 1971). The alternative viewpoint is that β-adrenoceptors in the brain directly activate drinking mechanisms. This hypothesis was proposed by Leibowitz (1971) but has been criticized since she injected large doses of α- or β-adrenoceptor antagonists (80μg) into the rat hypothalamus. Although it is likely that isoprenaline-induced drinking in the rat is renin dependent some anomalies still remain. Lehr (1973) has pointed out that angiotensin and isoprenaline given systemically have opposing actions on blood pressure and vascular volume. He has also reported that depletion of renal renin content by giving DOCA did not reduce isoprenaline-induced drinking (Lehr et al, 1973). Another discrepancy is the report that in the rat central pretreatment with 6OH-dopamine did not affect the dipsogenic response to ip isoprenaline (Stricker & Zigmond, 1974) whilst the response to ic angiotensin II was significantly reduced (Fitzsimons & Setler, 1971). In contrast to the rat, isoprenaline-induced drinking in the dog may not be dependent on renin since it occurs in nephrectomised animals (Fitzsimons & Szczepanska-Sadowska, 1974).

In the cat systemically administered β-adrenoceptor agonists were only weak dipsogens. The magnitude of the response was less

than that in either the rat or dog and drinking did not appear to be dose-related. Centrally administered isoprenaline was a more effective dipsogenic stimulus than a similar dose given systemically which suggested that a β-adrenoceptor mechanism in the brain might be implicated. Brophy & Levitt (1974) have also reported that isoprenaline injected into the brain of the cat has greater dipsogenic potency than other catecholamines. These results suggest that further detailed study of the central dipsogenic actions of β-adrenoceptor agonists in the cat is warranted.

A brief attempt to examine the role of renin in the response to isoprenaline was made utilising an angiotensin II analogue antagonist given icv. The results could be explained by proposing that the response to systemic administration of isoprenaline involved renin whilst that to icv isoprenaline did not. Studies by other groups in the rat yielded inconsistent results. Systemic infusion of an angiotensin II antagonist did not affect drinking elicited by iv isoprenaline (Tang & Falk, 1974) whereas systemic infusion of angiotensin II antiserum reduced drinking (Abdelaal et al, 1974b). These differences probably reflect the different modes of action of the two agents. The angiotensin II analogue probably blocks receptors in the brain and thus is dependent on an adequate dose entering the CNS. In contrast antiserum binds angiotensin II, reducing the plasma level and preventing significant quantities of the agonist entering the brain.

The exact location of the site of action of angiotensin in the brain is controversial. In early studies in the rat the lateral preoptic area and septum were identified as the most sensitive

regions (Epstein et al, 1970). This opinion was later questioned when it was discovered that the sensitivity to angiotensin II depended on the cannula traversing the cerebral ventricles (Johnson, 1973). It was suggested that the peptide leaked along the cannula track into the ventricles and acted there. The most suitable periventricular structure was the subfornical organ, an area outside the blood brain barrier. This region was subsequently shown to be very sensitive to angiotensin II and its destruction attenuated drinking elicited by injection of angiotensin at other central sites (Simpson & Routtenberg, 1973). Whilst the results in this thesis do not directly bear on this problem, the evidence that drinking in the cat can be elicited with very low icv doses of angiotensin II is consistent with a periventricular site of action. Recently results have been published suggesting that the subfornical organ is not the only site of action (Black, Kurcharczyk & Mogenson, 1974). These workers found that a unilateral lesion of the lateral hypothalamus reduced drinking elicited by angiotensin II applied to the ipselateral preoptic region. They suggest that there are angiotensin receptors in the preoptic area which have direct connections to the ipselateral hypothalamus. The results of a neuroanatomical study in the cat indicated that a wide range of structures were sensitive to angiotensin II (Sturgeon & Levitt, 1974). The structures included several with connections to the lateral hypothalamus and in addition icv administration was effective. These results also support the idea that there is more than one region of the brain which is sensitive to the dipsogenic action of angiotensin II. There is also a region in the brain which appears to inhibit the action of

angiotensin. Lesions in the septal nuclei in the rat result in an exaggerated drinking response to angiotensin but not to other stimuli (Blass et al, 1974).

The mode of action of angiotensin in the brain is also obscure, the peptide may act on pre- or post-synaptic receptors to release neurotransmitters or may alter membrane permeability to certain ions. Andersson (1971) proposed that angiotensin interacts with sodium ions to elicit drinking. He believes that the change in CSF sodium ion concentration is the major dipsogenic stimulus and that angiotensin sensitises central receptors by altering their permeability to sodium. His hypothesis was based on experiments in the goat which demonstrated a synergistic dipsogenic effect of icv infusion of angiotensin and hypertonic saline. In the rat some workers have observed a positive interaction between angiotensin and sodium ions whilst others have not. When experiments similar to those of Andersson were performed using the cat, potentiation of angiotensin-induced drinking occurred when the peptide was dissolved in hypertonic saline. However the mechanism involved may have been different to that in the goat since hypertonic saline alone did not have a central dipsogenic action in the cat. Fitzsimons (1970) has suggested that angiotensin sensitises the CNS to several types of dipsogenic stimulus, thus subthreshold stimuli may combine to elicit a response. It would be useful to examine the interaction between central administration of angiotensin and several different osmotic and hypovolaemic stimuli in the cat.

Evidence obtained in experiments using the rat indicated that both central cholinergic and adrenergic mechanisms were associated with drinking behaviour. Angiotensin II was known to affect release

or reuptake of neurotransmitters at peripheral synapses and also to alter levels of these substances in brain tissue. Thus the hypothesis was developed that the dipsogenic effects of angiotensin might be mediated by release of endogenous brain neurotransmitters (Fitzsimons, 1972). In order to test the hypothesis several different groups have studied the effects of central administration of autonomic blocking drugs on drinking initiated by ic angiotensin II. One problem associated with this type of approach is that it is difficult to determine the site of action of the inhibitor. A drug could act on the afferent mechanism by blocking the action of transmitters released by angiotensin or could act distally to the integrating mechanism blocking efferent neurones. In some experiments in the rat this has been controlled by comparing the effect of a blocker on the action of angiotensin and on the action of another dipsogen, usually carbachol. The results in the rat were not always consistent but the majority of the evidence supports the view that adrenergic neurones in the brain are involved in the response to angiotensin II. However, in the monkey there is evidence against a catecholaminergic mechanism (Sharpe & Swanson, 1974) and evidence in favour of a cholinergic mechanism (Myers et al, 1973). Thus it appears that, depending on species, different neurochemical mechanisms may be involved in the dipsogenic response to angiotensin.

In the cat it was not possible to control experiments by comparing the effect of the antagonist on another dipsogen. The results indicated that cholinergic neurones were not involved in angiotensin-induced drinking but did support a role for central catecholaminergic neurones. Thus the cat appeared to be similar to

the rat. A consistent reduction in angiotensin-induced drinking in the cat was obtained after central administration of β-adrenoceptor antagonists and this reduction was not related to local anaesthetic activity. This may indicate that central β-adrenoceptor mechanisms are involved and this is interesting since evidence in this thesis and from Brophy & Levitt (1974) indicated that central administration of isoprenaline in the cat caused drinking. Two groups have presented evidence that central dopaminergic mechanisms may be involved in the drinking response to angiotensin in the rat (Fitzsimons & Setler, 1971; Fisher, 1973). Some evidence in this thesis supports a similar role for dopamine in the cat. Administration of either haloperidol or pimozide reduced drinking elicited by icv angiotensin II but dopamine itself was not an effective dipsogen. Another inconsistent finding was that icv administration of the catecholamine depleting agent, 6OH-dopamine, did not initially affect angiotensin-induced drinking in the cat. In future studies it would be useful to re-examine angiotensin-induced drinking in cats pretreated with drugs which deplete brain amines.

Studies in the cat also indicated that a peripheral cholinergic pathway was involved in the response to angiotensin II. It seems likely that the mechanism involved may be common to other dipsogenic stimuli since results in the rat indicate that "salt arousal of drinking" and water deprivation-induced drinking are also reduced by systemic cholinoceptor blockade.

The relationship between the cerebral and renal renin-angiotensin systems is an important aspect of this topic. Severs & Daniels-Severs (1973) speculated that these two systems may mediate different types

of dipsogenic stimulus; the central system, ICF depletion and the renal one, ECF depletion. Alternatively the cerebral system may act as a fine control of body fluid balance by influencing water intake and excretion. The renal system may not have a physiological role but may be activated during pathological conditions and the angiotensin released may augment the effect of that released centrally. There has been little direct research into this problem but it has been found that brain isorenin levels vary inversely with brain tissue water (Ganten, Kusumoto, Constantopoulos, Ganten, Boucher & Genest, 1973). In earlier studies they had shown that brain isorenin activity was altered by systemic administration of steroid hormones and changes in electrolyte balance (Ganten, Minnich et al, 1971). This supports the view that angiotensin acts as a local hormone whose release is stimulated by changes in body fluid balance. A future approach to this problem will be the development of selective renin inhibitors which are specific for either brain isorenin or the renal enzyme. Pepstatin does not appear to fulfil this function since it inhibits both enzymes in vivo. The synthesis of peptides with differing amino acid structures around a Leu - Leu bond may yield suitable inhibitors.

Investigations into the centrally mediated cardiovascular effects of angiotensin II have developed from two different approaches. In the first angiotensin II was administered via the vascular system thus mimicking the effects of angiotensin formed in the systemic circulation. Intracerebral administration of angiotensin II either into the CSF or brain tissue was the second approach. When applied in this manner the peptide may act at sites which are penetrated by systemic angiotensin but in addition may mimic angiotensin II

formed within the CNS.

In this thesis the second method was adopted and angiotensin was infused into the lateral cerebral ventricles of conscious cats or rabbits. The characteristics of the cardiovascular responses elicited by icv infusion of angiotensin II in the anaesthetised cat have been summarised by Buckley (1972). Briefly, they consisted of a dose-related pressor effect which was sometimes accompanied by tachycardia. The responses were mediated by the peripheral sympathetic-adrenal system and appeared to be initiated by an interaction of angiotensin II with central adrenergic neurones. In the conscious cat the centrally mediated cardiovascular responses to angiotensin II were difficult to interpret due to secondary effects caused by drinking. In retrospect it would have been better to study the dipsogenic and cardiovascular effects of angiotensin II separately. There are however problems when this is attempted since the cat becomes restless presumably due to a motivation to seek water. The present study did confirm that icv angiotensin II initiated a dose-related vasopressor response in the cat. The threshold dose in the conscious animal appeared to be higher than in the anaesthetised cat which may indicate that reflexes inhibit the response. In most animals systemic administration of ganglion blockers, an adrenergic neurone blocker or an α-adrenoceptor blocker reduced the pressor response. This supported the view that the sympathetic nervous system mediated the effects. The centrally mediated pressor response was also reduced by central administration of either an adrenergic neurone blocker, an α- or a β-adrenoceptor blocker. This also confirmed the result obtained by Buckley's group (Smookler et al,

1966). It is difficult to analyse the type of receptor involved in the response to angiotensin II icv since catecholamines themselves elicit complex effects depending on dose and the site of injection.

The recent introduction of angiotensin II antagonists has enabled the specific blockade of angiotensin II receptors. Soloman (1972) first studied the action of icv Sar^{1}Ala^{8}angiotensin II in the anaesthetised cat, finding that it prevented the pressor response to icv angiotensin II. This observation was confirmed in the present study. In addition this analogue was used to analyse the pressor response elicited by icv renin. The analogue prevented the response indicating that renin acted indirectly by releasing angiotensin within the CNS.

In view of the difficulty in the cat of separating the direct cardiovascular actions from those secondary to drinking, some experiments were performed in rabbits. The rabbit has been reported to drink following icv angiotensin II (Fitzsimons, 1972) but at the doses used in the present study no drinking occurred. This species seemed to be useful since Rosendorff et al (1970) found that the pressor response to icv angiotensin II was greater than that to a higher dose given iv. This result was confirmed in this thesis despite the use of larger doses than Rosendorff et al. On the basis of spinal section experiments the above group concluded that the central response in the rabbit was also mediated by sympathetic neurones. This view was contradicted by the results described here. These indicated that the effect was partially reduced by ganglion blockade but unaffected by adrenergic neurone or α-adrenoceptor blockade. Large icv doses of either phentolamine or propranolol

prevented the pressor response to icv angiotensin II indicating that the effect was not due to leakage of the peptide into the systemic circulation. The most plausible explanation is that the response was mediated by release of a neurohumoral substance from the brain. A possible candidate is vasopressin since its release by angiotensin II is well documented. This peptide has also been implicated in the central pressor effect produced by angiotensin II in the conscious rat (Severs et al, 1970). The actions of icv phentolamine and propranolol do not necessarily indicate the involvement of central adrenergic neurones since at the doses used local anaesthetic effects may occur.

The contribution of these centrally mediated cardiovascular effects in the development of hypertension has not been defined. In certain types of hypertension plasma angiotensin levels are similar to those obtained after intravertebral artery infusion of angiotensin II. Thus it is possible that in these cases a central component is present. An experiment which is germane to this point was performed by Scroop, Katic, Joy & Lowe (1971). They showed that destruction of the area postrema in the greyhound reduced the pressor response to iv angiotensin II but not to iv noradrenaline. This was corroborated by a similar experiment in the rabbit (Joy & Lewis, 1971). An alternative possibility is that hypertension is initiated by increased levels of angiotensin II formed within the CNS. In such cases there would be no increase in plasma angiotensin II. Evidence which supports this idea has been presented by Finkielman et al (1972). These workers showed that hypertensive patients had much higher levels of an angiotensin-like peptide in their CSF than normotensive

subjects.

Icv or intravertebral administration of angiotensin II analogues have been found to block the cardiovascular effects of similarly administered angiotensin II (Soloman, 1972; Sweet, Ferrario, Khosla & Bumpus, 1973). This suggests that a possible approach to the problem may be to examine the acute antihypertensive effects of centrally administered angiotensin II antagonists during various stages of hypertension.

Another function in which these central effects may be involved is the response to acute haemorrhage. Katic, Joy, Lavery, Lowe & Scroop (1971) showed that the homeostatic cardiovascular adjustment to haemorrhage in the greyhound was abolished by ablation of the area postrema.

The results described in this thesis have provided further evidence that renin and angiotensin have potent central pharmacological actions. The central dipsogenic effect has been analysed using peptide inhibitors and autonomic blocking drugs with the aim of providing a basis for physiological studies. In addition to its dipsogenic effect, angiotensin II acts centrally to release ADH and may also stimulate salt intake. These observations suggest that the cerebral renin-angiotensin system may be a physiological regulator of body fluid balance. These central actions are complemented by peripheral actions since angiotensin II promotes sodium and water retention at the kidney both directly and by releasing aldosterone. The contribution of systemic angiotensin to centrally mediated effects is obscure but development of specific peptide inhibitors with different actions on cerebral and renal renin

will aid future studies. Centrally mediated cardiovascular effects of angiotensin also contribute to the homeostatic response to haemorrhage. This also supports the view that the central effects of angiotensin are primarily involved in the maintainance of body fluids.

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