PHARMACOLOGICAL REVIEWS
Vol. 25, No. 3

Printed in U.S.A.

Effects of Angiotensin on the Central Nervous System*

WALTER B. SEVERS AND ANNE E. DANIELS-SEVERS

Department of Pharmacology, The Milton S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania

I. Introduction 415
A. General comments 415
B. Methodological considerations 416
II. Presence of angiotensin in the brain 419
A. Distribution of peripherally administered angiotensin 419
B. Components of the renin-angiotensin system in brain 421
III. Experiments demonstrating centrally mediated cardiovascular effects of angiotensin II 421
A. Intravascular administration 421
1. Isolated perfused head studies 421
2. Effects of administration of angiotensin into the cranial circulation 422
B. Angiotensin administration into cerebrospinal fluid 423
C. Proposed sites of central cardiovascular effects of angiotensin 423
1. Midbrain 423
2. Medulla 432
3. Hypothalamus 432
D. Influence of angiotensin on sympathetic nerve activity and its effect on baroreceptor reflexes 433
IV. Effects of centrally administered angiotensin on hydration 434
A. Dipsogenic effects 434
B. Pituitary effects of angiotensin 438
C. Significance of the effect of angiotensin on hydration 439
V. Cholinergic, adrenergic, and macromolecular effects of angiotensin in the central nervous system 441
A. Cholinergic 441
B. Adrenergic 442
C. Macromolecular effects 443
VI. Central angiotensin effects in man 444
VII. Summary and conclusions 445
VIII. Addendum 445

I. Introduction

A. General Comments

RENIN, released from the kidney, cleaves angiotensin I from plasma α_2 globulins. Angiotensin I is converted to angiotensin II† by an enzyme present in particularly high concentration in the lung (116, 117). Other organs contain renin (or a renin-like enzyme) (114) and an enzyme capable of

* This study was supported by Grant #HL-13063 from the National Heart and Lung Institute.
† When unspecified, angiotensin refers to the octapeptide, angiotensin II.

converting angiotensin I to angiotensin II (33, 168). However, as its name implies, renin is regarded as a renal enzyme, the word being given by Tigerstedt and Bergman (164) to a saline extractable pressor material from rabbit kidney. It is therefore logical that the roles of renin and angiotensin have been studied extensively in hypertension of renal origin. The simultaneous discovery of angiotensin by Page and Helmer (124) and Braun-Menendez *et al.* (21) and the subsequent availability of pure material have established the peptide as the most potent endogenous vasopressor substance known. However, by itself this effect of angiotensin (or renin) is insufficient to explain most hypertensive states including chronic renal hypertension.

Many earlier scientists recognized this point. For example, Dock and colleagues (42–44), who subjected control and renal hypertensive rats to decerebration and pithing, reported that the blood pressure of both groups fell to identical levels, although vascular smooth muscle was sensitive to direct vasoconstriction by epinephrine or posterior pituitary extract. Similar neuraxis destruction in control and chronic renal hypertensive dogs also produced immediate equality of blood pressures. Hence, as Dock and colleagues pointed out, "the renal pressor hormone does not act directly on the arteries" (43). Similar conclusions from experiments with chronic renal hypertensive dogs were reached by Glenn *et al.* (76) and with rats by Ogden and colleagues (120, 121). Pickering (132), who studied the circulation of the human hand during acute and chronic nephritis, provided early evidence for a difference in mechanism between these two stages of hypertension in man.

If the renin-angiotensin system is involved in hypertensive states where abnormal amounts of circulating vasoconstrictor material are not found, then the mechanism requires further definition. Many lines of research have been pursued. One of these, perhaps less well known than others, concerns interactions of angiotensin with the central nervous system (CNS). Some highlights of this research are listed in table 1. In 1961, Bickerton and Buckley (15) first demonstrated that angiotensin could produce a centrally-mediated hypertensive response. Subsequently, the literature concerning interactions between angiotensin and the CNS has greatly expanded. Differences of opinion exist concerning the role of angiotensin in the brain. Two quotations, taken from the recent literature, illustrate this diversity of opinion. Levy and Ahlquist (102) state: "Most workers agree that while angiotensin exerts a potent peripheral effect it has no effect on the central nervous system." On the other hand, Ferrario *et al.* (56) conclude, "It appears that a significant portion of the hypertensive effect of circulating angiotensin is mediated through a direct action on the brainstem."

This review discusses: a) difficulties associated with the study of angiotensin interaction in the brain; b) observed central angiotensin effects; and c) their possible significance. Although this review is concerned with central angiotensin effects, our intent is not to minimize the importance of the many peripheral effects of the peptide. Clearly, angiotensin has marked effects on the smooth muscle of the blood vessels, intestine, and uterus as well as cardiac muscle; it increases adrenal cortical and medullary secretions and interacts with the sympathetic nervous system at ganglia and nerve terminals. The reader is referred to a group of excellent reviews for broad coverage of peripheral angiotensin effects (95, 111, 123, 125, 131).

B. Methodological Considerations

A prime difficulty in studying central, as well as peripheral, angiotensin activity is that the biochemistry of its interaction with tissues is unclear. Angiotensin was long thought to be an intravascular hormone, being synthesized and degraded in blood. However, Hodge *et al.* (84), with the superfusion technique, showed that angiotensin was cleared rapidly from many vascular

TABLE 1

Some highlights of the angiotensin-CNS field

Research Finding	Reference(s)
First demonstration of a central effect of angiotensin: hypertensive response in cross-circulated dogs	Bickerton and Buckley, 1961 (15)
First suggestion that vertebral arterial infusion of angiotensin caused a greater hypertensive response than intravenous administration	Dickinson and Lawrence, 1963 (39)
Localization of a central site (midbrain) where angiotensin produced a hypertensive response after administration into ventricular cerebrospinal fluid	Severs *et al.*, 1966 (151)
Evidence that a medullary site (area postrema) produces the hypertensive response due to vertebral arterial angiotensin infusions	Scroop and Lowe, 1969 (144); Joy and Lowe, 1970 (90)
Suggestion that angiotensin may have central hypertensive effects in man	Johnsson *et al.*, 1965 (86); Scroop and Whelan, 1966 (146)
Early demonstrations that centrally administered angiotensin causes changes in drinking behavior	Booth, 1968 (19); Hendler and Blake, 1969 (82); Daniels *et al.*, 1969 (35); Epstein *et al.*, 1969 (53)
Early suggestions that centrally administered angiotensin may cause antidiuretic hormone release	Andersson *et al.*, 1970 (7); Severs *et al.*, 1970 (155); Mouw *et al.*, 1971 (110)
First demonstrations that renin and angiotensin are endogenous to brain	Fischer-Ferraro *et al.*, 1971 (58); Ganten *et al.*, 1971 (70, 71)
Positive correlation between blood pressure of essential hypertensive humans and cerebrospinal fluid concentration of an angiotensin I-like peptide	Finkielman *et al.*, 1972 (57)

beds, including that supplied by the carotid artery. The disappearance of the peptide *en passant* through tissue was far more rapid than could be accounted for by blood catabolism. What happens to angiotensin as it passes through a tissue phase in terms of distribution, binding and/or accumulation, retention and catabolism is not well established [for a review, see (95)]. For example, for proper interpretation, studies of the fate of labeled angiotensin would have to involve isolation, identification, and localization of labeled species in pure form with high recovery. Furthermore, since angiotensin is a reactive molecule, it would be necessary to evaluate the biological significance of products differing from the starting material. To our knowledge, such a thorough analysis of the interaction of angiotensin with any tissue has not been achieved. Hence, it is difficult to determine what type of access angiotensin may have to the brain.

CNS studies with angiotensin are even more difficult because of the additional problem imposed by the blood-brain barrier (BBB). The tightly packed endothelium of brain capillaries poses a formidable barrier to the passage of many molecules (46). From a purely physicochemical point of view, it would seem unlikely that an octapeptide carrying a negative charge at plasma pH would cross this barrier. However, in spite of the above difficulties, the possibility of penetration cannot be automatically dismissed. It is of interest in this regard that renin and angiotensin appear to increase protein leakage from the vascular compartment (94). Moreover, the BBB appears to be deficient in several brain regions such as the neurohypophysis, tuber cinereum, area postrema, pineal body, intercolumnar tubercle (176), and circumventricular organs (46).

Penetration of angiotensin into cerebrospinal fluid (CSF) must also be considered as a mechanism which can initiate CNS effects. Some evidence is available to suggest that

angiotensin may cross the blood-CSF barrier (172), and it is clear that exogenous peptide produces marked effects when injected into ventricular CSF. The presence of neurons in the ependyma of cerebral ventricles has been established by light and electron-microscopy (22, 173), and neuronal processes appear to project into the cerebral ventricles of many vertebrates. These anatomical observations have led to the concept that intraventricular nerve processes are receptors which could monitor CSF composition or pressure.

In addition to the problems concerned with the access of angiotensin to the brain and/or CSF, research concerned with the centrally mediated cardiovascular effects of angiotensin must deal with differentiating central from multiple peripheral sites where the peptide could alter the cardiovascular system. One approach has been the utilization of vascularly isolated, neurally intact head perfusions. When appropriate measures are taken to prevent blood leakage from the head circulation to the periphery, centrally administered angiotensin will not reach peripheral sites of action. However, cross-circulated or extracorporally perfused head circulation preparations have a number of limitations. They require reasonably deep anesthesia and rather extensive surgery. Moreover, drugs administered into the head circulation may produce cardiovascular effects secondary to the release of vasoactive substances from the cranial vasculature or by altering pressure/flow relationships that result in cardiovascular readjustments (*cf.* 112 for methodological limitations).

Another approach to the demonstration of centrally mediated cardiovascular effects of angiotensin involves infusion of the peptide into an artery supplying the brain. Most studies of this type compare the magnitude and temporal aspects of observed responses with intravenous infusions at the same dose rate. Although this approach has been extremely useful, it is still difficult to eliminate the possibility that such arterial infusions cause local CNS ischemia or redistribution of blood flow within the cranial circulation.

Injection or infusion of angiotensin into CSF has also been utilized in research designed to detect angiotensin-CNS-cardiovascular interaction. It is necessary in this type of experimental approach to eliminate leakage of the peptide into the peripheral circulation as the cause of any observed effect. This is most frequently done by making additional injections after destruction of part of the neuraxis. This type of control, however, does not establish a central effect of the peptide since the lesion may simply prevent an afferent neural effect caused by peptide which leaks into the circulation. It is also theoretically possible that nerve sections to eliminate endogenous sympathetic tone could antagonize a peripheral effect of peptide which leaks from the CSF to cause a potentiation of sympathetic tone.

In addition to the problem of access of angiotensin to central structures and the methodological problems involved in distinguishing central from peripheral effects of the peptide, it is evident that many "classical" variables are of importance. Some of these variables and an example of each are: a) Anesthesia—intraventricular (IVT) injections of angiotensin are pressor in conscious rats (155) but not in rats anesthetized with chloralose or barbiturate (147); urethane inhibits the sympathetic component of the cardiovascular effects of angiotensin in rats (171). b) Sex—central pressor activity is markedly reduced in anesthetized cats which are pregnant or lactating (149). c) Species—IVT angiotensin is pressor in anesthetized cats (151) but not rats (147). d) Sensitivity—significant differences in central angiotensin pressor effects exist between groups of cats but not within groups (34). e) Route of administration—intravertebral infusion of angiotensin, but not intracarotid, produces hypertension (90, 161). f) Duration of contact—pressor effects of "subpressor" intravenous angiotension infusions are seen after a time lag of 1 to 3 days (106). g) Tachyphylaxis—repetitive IVT injections of angiotensin produce diminished pressor responses in conscious rabbits (138). h) Interaction with other substances—prostaglandin E_1 po-

pathetic discharge to the cardiovascular system. Further investigation (24, 81) showed that: a) the angiotensin effect was not produced by central injection of other vasopressor substances; b) denervation of the carotid sinus-body complex did not alter angiotensin activity; and c) the central angiotensin response had no temporal correlation with the response to hypoxia.

With both the donor-perfused dog cross circulation preparation and extracorporeal perfusion of the isolated dog head, Benetato *et al.* (13) independently confirmed the existence of central angiotensin pressor activity. These investigators found that the blood pressure response to intracarotid angiotensin (1–4 μg/kg) was abolished or reversed by prior reserpinization (2 mg/kg, i.p., 12 hr prior). Since central infusion of norepinephrine to reserpinized animals restored the central pressor effect of angiotensin, the peptide may have acted by central adrenergic mechanisms. Intracarotid administration of large doses (10 mg/kg) of chlorpromazine did not influence angiotensin activity, thereby leading Benetato *et al.* (13) to postulate a medullary site of action for angiotensin.

Barrett *et al.* (9) developed a method for the extracorporeal perfusion of the CNS in the chloralose-anesthetized cat. The technical considerations of the methodology indicate that the brain circulation was vascularly isolated from the periphery while the CNS control of the cardiovascular system remained intact. Addition of angiotensin (0.5 μg/ml) to the brain perfusion circuit (with carotid arterial inflow) caused a significant increase in peripheral blood pressure only if the cats were deafferented by sectioning cranial nerves IX–XII (see section III,D for further discussion of this research).

2. *Effects of administration of angiotensin into the cranial circulation.* Evidence suggests that continuous intravenous infusions of subpressor amounts of angiotensin can produce sustained hypertension in dogs (106). Dickinson and colleagues (39–41) demonstrated that vertebral arterial infusion of low doses of angiotensin to unanesthetized rabbits produced greater hypertensive responses than intravenous administration of the same doses. To our knowledge, a similar differential responsiveness has not been demonstrated between the carotid artery and intravenous routes although large intracarotid doses produce centrally mediated pressor responses in isolated head preparations (see above). Some details concerning administration of angiotensin into the cranial vasculature are summarized in table 2. The following points emerge from this literature.

a. The majority of investigations confirm that intravertebral angiotensin is more effective in producing hypertension than peptide administration by the intravenous (23, 30, 37, 55, 65, 103, 138, 143, 145, 162, 163, 179, 180) or intracarotid (30, 55, 161, 163) route. The negative reports may be associated with anesthetic inhibition of angiotensin actions, difficulty in analyzing central *versus* peripheral effects due to temporal factors, or other variables. All experiments with unanesthetized animals have detected hypertensive responses to low-dose infusions of angiotensin into the vertebral artery.

b. Pressor responses to angiotensin administered by way of the vertebral artery have been observed in rabbits (30, 39, 41, 138, 179, 180), cats (23, 37, 93, 165), and dogs (55, 65, 73, 74, 87, 90, 103, 143–145, 161, 163).

c. Hypertensive responses produced by vertebral infusions of angiotensin have been sustained up to 7 days (65).

d. Dickinson and Lawrence (39) originally interpreted the effects of angiotensin administered by way of the vertebral artery as being due to cerebral vasoconstriction leading to relative ischemia of vasomotor centers. Most of the later literature suggests that angiotensin exerts a CNS effect which is not secondary to vasoconstriction or ischemia (55, 56, 138).

e. The hypertensive response to intravertebral angiotensin in mongrel dogs is due to a sympathetically produced rise in peripheral resistance with little or no change in cardiac output or rate (56). The response in greyhound dogs (which are bred for endurance)

tensive response to vertebral arterial angiotensin strongly suggest peptide interaction with the CNS.

B. Components of the Renin-Angiotensin System in Brain

Fischer-Ferraro *et al.* (58) and Ganten *et al.* (71) independently reported results of investigations showing that renin and angiotensin are endogenous to brain. Their data suggest that angiotensin may be locally formed and could thus exert effects on the CNS. Both groups isolated an enzyme from dog brain which, when incubated with homologous plasma substrate, formed a polypeptide with separation characteristics and biological properties similar to angiotensin. An angiotensin-like peptide fraction was also isolated. Ganten *et al.* (71) were able to extract a protein fraction from brain which would serve as renin substrate, and Fischer-Ferraro *et al.* (58) identified renin and angiotensin in rat and in dog brain. Since both groups reported that previous nephrectomy did not alter their data, brain renin and angiotensin in these experiments were not of renal origin.

Ganten *et al.* (70) studied the distribution of renin in eight regions of dog brain and did not detect localization. Subcellular fractionation revealed that renin activity was concentrated in synaptosome preparations (108). Fischer-Ferraro *et al.* (58), however, reported that angiotensin in dog brain is concentrated in the brainstem and especially in the hypothalamus. Both groups observed that most angiotensin in brain is angiotensin I. Considering this, it is of interest that little, if any, attention has been directed at the possibility of angiotensin I entering the brain. Crude rat brain homogenate, like angiotensin converting enzyme in the periphery, is capable of liberating His-Leu from benzyloxy-carbonyl-Pro-Phe-His-Leu and Hippuryl-His-Leu (32, 140). Yang and Neff (177) described a dipeptide hydrolase in rat brain associated with the mitochondrial fraction after subcellular fractionation. The enzyme had broad substrate specificity and resembled converting enzyme in the periphery in several respects, including a chloride ion requirement, inhibition by synthetic *Bothrops jararaca* nonapeptide, and various metalloenzyme inhibitors. The enzyme was detected in most brain regions, but the striatum, cerebellum, and pituitary were particularly rich sources.

The studies described above document that brain contains renin, renin substrate, angiotensin I, converting enzyme, and angiotensin II. Little is known about the regulation and function of the renin-angiotensin system in brain, but it seems plausible that central effects of exogenous angiotensin could mimic those of locally formed angiotensin. Like most tissues, the brain contains enzymes capable of catabolizing angiotensin (2, 105). One hydrolytic enzyme has the characteristics of a neutral proteinase (105), and a purified arylamidase (2) has been isolated from pig brain which possesses high activity against Ile^5 angiotensin II.

III. Experiments Demonstrating Centrally Mediated Cardiovascular Effects of Angiotensin II

A. Intravascular Administration

1. Isolated perfused head studies. Bickerton and Buckley (15) provided the first evidence that angiotensin could produce centrally mediated hypertensive responses. These investigators transversely divided the circulation of a recipient dog between C-2 and C-4 and perfused the isolated head with blood from a donor dog. By use of radioisotopes they verified that the head circulation was completely separated from that of the trunk. Under these conditions, intracarotid administration of angiotensin (0.2–4 μg/kg) to the isolated recipient head produced an increase in blood pressure of 12 to 50% as recorded from the peripheral circulation of the recipient. Since blockade of peripheral *alpha* adrenergic receptors by intravenous piperoxan antagonized the pressor response, centrally injected angiotensin probably increased sym-

brain. The data do appear to indicate that the brain (or cranial vasculature) accumulates angiotensin and/or metabolic products. Unfortunately, the biological significance of these observations cannot be determined.

The distribution in mice of ^{14}C-angiotensin 5 min after intravenous injection was examined by Volicer and Loew (172) in experiments in which inulin, plasma, and angiotensin spaces were determined. The volume of distribution of angiotensin was significantly greater than plasma volume but significantly less than the volume of distribution of inulin. These data indicate that angiotensin was not distributed uniformly within the extravascular space to which it was shown to have access. Sephadex chromatography of plasma and brain supernatant after addition of carrier angiotensin showed the radioactivity peak separating with the carrier angiotensin, as assessed by rat blood pressure assay. The angiotensin fraction contained 83.5 and 95% of the initial radioactivity of plasma and brain supernatant, respectively. Radioautographic examination of the brain demonstrated label in the lateral and third ventricles with some label penetrating into the surrounding brain parenchyma. Interestingly, no label was located in the fourth ventricle. The authors suggest that angiotensin enters the CSF after intravenous administration and may stimulate structures surrounding the ventricles.

While the low specific activity of ^{14}C-angiotensin necessitated the use of a dose of 70 μg/kg for sufficient labeling in the above experiments, mice are relatively resistant to angiotensin, and the i.v. LD50 is approximately 40 times the dose used (172). Moreover, the plasma volume of brain in the angiotensin-treated mice was not abnormal, thereby suggesting that no major intracranial hemorrhage occurred. The high dose of angiotensin and the distribution data at only one point in time (5 min) limit the interpretation of the experiment in physiological terms. However, it is noteworthy that radioactivity from brain was associated with the angiotensin peak after Sephadex separation and bioassay.

Richardson and Beaulnes (134) published an important paper dealing with the distribution of ^{14}C-angiotensin and angiotensin coupled to horseradish peroxidase or cytochrome *c* which can be histochemically localized. After intravenous injection of ^{14}C-angiotensin (10 μg/kg) and ^{3}H-inulin, mice were killed at 0.5, 5, and 15 min for determination of the carbon/tritium ratio in various tissues. Each of the six tissues examined had a ratio of 1:1 after 0.5 min. At 5 and 15 min, the liver accumulated angiotensin and/or metabolites so that the ratio rose to about 15. At 5 and 15 min, the ratio in the brain was significantly elevated to 2.3 and 3.6, respectively. There was no evidence of ^{14}C-accumulation by kidney, lung, heart, or diaphragm. In studies with angiotensin coupled to horseradish peroxidase or cytochrome *c*, mice were given pressor doses of the complex equivalent to 2 to 20 μg/kg of angiotensin. Equipotent doses of free and complexed angiotensin had similar onset-duration relationships in several test systems. Although it remains unproved, the complex was probably not split after *in vivo* administration. The histochemical observations in this study relevant to the CNS involved observations of the choroid plexus. The authors stated that "Vesicles containing the reaction product are seen in the cells of the choroid plexus 5 min after the injection, and some of those vesicles are seen near the villous border but none are seen to be emptying into the ventricular side. The reaction product is also seen in the extracellular space surrounding the blood vessels" (134).

Collectively, the available data are compatible with the possibility that circulating angiotensin and/or metabolites enter and influence the brain. Technical limitations of the above experiments do not allow unequivocal conclusions concerning the penetration or distribution of circulating angiotensin within the CNS. However, studies to be discussed (section III,A) which show the hyper-

tentiates the hypertensive response to intravertebral angiotensin (79). Because of these variables, we have generally included doses, routes of administration and temporal factors in the description of the experiments referred to in this review.

II. Presence of Angiotensin in the Brain

A. Distribution of Peripherally Administered Angiotensin

Most tissues (except lung) remove angiotensin from arterial blood (16, 119, 167). With the superfusion bioassay method, Hodge *et al.* (84) demonstrated that about 50% of angiotensin infused into a carotid artery of anesthetized dogs was removed from the blood during one passage through the head. Although the dose of angiotensin used was large (2 μg/min), the experiments clearly suggest some angiotensin-tissue interaction since disappearance by blood catabolism would require 10 to 20 circulation times. The fate of the angiotensin removed from the cranial circuit remains a matter of conjecture since the disposition of the peptide passing through a vascular, glial, and/or neuronal tissue phase is largely unknown.

Ganten *et al.* (70, 71) studied renin activity in CSF obtained by cisternal puncture in control dogs and in dogs with high plasma renin activity (due to sodium restriction, homologous renin infusion, or Goldblatt hypertension). CSF was also assayed for angiotensin in control dogs and in dogs infused with angiotensin (0.2 μg/kg/min, i.v., for 1 hr). Since renin and angiotensin were not found in the CSF, the authors suggest that renin and angiotensin do not cross the BBB. However, the negative findings with respect to angiotensin in cisternal CSF do not resolve the question of angiotensin penetration into the brain. Considering the low endogenous level of angiotensin in brain (2.7 ng/g, see section II, B) it is possible that small quantities of peptide may have been undetected in CSF. It is also possible that the site of CSF sampling (suboccipital cisternal puncture) was not optimal, since radioautographs of mouse brain after intravenously administered angiotensin detected label in the lateral and third ventricle but not in the fourth ventricle (172, see below).

The disposition of randomly labeled ^{3}H-angiotensin, which was chemically and radiochemically homogeneous, was studied by Bumpus *et al.* (25, 97). When infused into 200-g rats at a rate of 4.6 μg/min, angiotensin "... raised the blood pressure to very high levels and soon produced tachyphylaxis." The rats were sacrificed immediately after infusion or 30 min later to determine the distribution of tritium. Of the 10 tissues examined, the adrenal, kidney, and uterus accumulated much more tritium than other tissues immediately after the infusion. The brain had the lowest value but no data were provided on the identity of the labeled species. Thirty minutes after the infusion radioactivity declined in lung, adrenal, spleen, uterus, and salivary gland; it was unchanged or slightly elevated (less than 2-fold) in heart, kidney, liver, and skeletal muscle. Interestingly, radioactivity in the brain increased 14-fold, although on electrophoresis it was shown to have a different mobility than the starting angiotensin.

When these experiments were repeated in rats nephrectomized 18 to 24 hr earlier, radioactivity was distributed more randomly in the various tissues. The liver had accumulated the most label, whereas the brain had the least label immediately after the infusion. Thirty minutes after the infusion, radioactivity declined in every tissue except the brain, in which it had increased about 4-fold. At this time, the brain was the most radioactive organ with the exception of the liver. Similar to the experiments with intact rats the electrophoretic mobility of label in the brain was different from the starting material. The authors suggested that the altered distribution pattern of tritium in nephrectomized animals may be related to extensive edema, with angiotensin being distributed in body water. Because of the need to use a high dose of angiotensin and the general problems with studies utilizing labeled peptides these data do not resolve the problem of whether angiotensin penetrates into the

is principally associated with a rise in cardiac output and rate. Vagotomy abolishes most of the effect of angiotensin in this breed, and the residual activity can be eliminated by adrenergic neuron blockade (103). Since the human myocardium is under vagal dominance, these data from greyhounds may be of importance in man (166).

B. Angiotensin Administration into Cerebrospinal Fluid

Administration of exogenous angiotensin into CSF produces a centrally-mediated hypertensive response in many species including the dog (75, 150), cat (34, 38, 80, 115, 150, 151, 158), rat (154, 155), rabbit (138), and goat (5). Some of the available data are summarized in table 3. All experiments involving administration of angiotensin into subarachnoid CSF have produced negative data, even though large amounts (over 10 μg) of the peptide were injected (14, 138, 150). Most experiments in which angiotensin was injected into the cerebral ventricles did produce pressor activity. Therefore, it appears that the active site(s) is reached from ventricular, but not subarachnoid spaces. The inability of large amounts of subarachnoid angiotensin to increase blood pressure suggests that angiotensin does not exit from the CSF to the general circulation in appreciable amounts. Multiple lines of evidence indicate that pressor responses after IVT injections are not due to systemic absorption of the peptide. As examples, the hypertensive activity is abolished in anesthetized cats (158) and dogs (150) by cervical spinal section; IVT doses produce greater hypertensive effects than the same doses given intravenously to unanesthetized rabbits (138) or anesthetized cats (158); and activity is abolished in unanesthetized hypophysectomized, ganglion-blocked rats (155).

The concentration of angiotensin needed to produce pressor activity at an active site after IVT administration is unknown. Rates of diffusion, penetration, and inactivation, as well as dilution by endogenous CSF, make such a determination formidable, if not impossible. In unanesthetized rats, however, IVT injection of 1 ng of angiotensin will elevate mean blood pressure by 19 ± 2 mm Hg (155). Ten nanograms appear to be the threshold dose for pressor activity after a single injection in anesthetized cat ventriculocisternal perfusion experiments (158).

C. Proposed Sites of Central Cardiovascular Effects of Angiotensin

1. Midbrain. Studies involving injections of angiotensin into the lateral ventricles of the cat (being perfused from the lateral ventricle to the cisterna magna) have identified the midbrain as the area in which the peptide initiates peripheral hypertensive responses. The following data substantiate this hypothesis. Cervical spinal transection, cerveau isolé transection and electrolytic transection of the anterior midbrain all block the pressor response to angiotensin (150, 151). Direct injection of the peptide (500 ng) into the posterior hypothalamus (151) or topical application of up to 50 μg of angiotensin onto the occipital or parietal cortex (147) did not change blood pressure. Cannulation of the cerebral aqueduct significantly inhibits the activity of IVT angiotensin injections (151). Angiotensin added to the perfusate as it emerges from the cerebral aqueduct is nonpressor (150). Hence, the active site initiating central angiotensin cardiovascular effects is reached as the peptide passes through the cerebral aqueduct. Deuben and Buckley (38) provided strong evidence to substantiate this point. During ventriculocisternal perfusion in cats, angiotensin was injected IVT and at two levels within the cerebral aqueduct. The lateral ventricle perfusion carried the small-volume injections made in the aqueduct caudally towards the cistern. In one series of cats IVT injections of angiotensin produced pressor effects equivalent to those obtained by injecting angiotensin into the aqueduct at a plane 6 mm anterior to Horsley-Clarke zero. Similar injections into the aqueduct at a 4 mm anterior plane produced significantly less pressor activity

TABLE 2

*Summary of studies on effects of infusions of angiotensin into the cranial circulation**, †*

Species	Route	Dose of ANG	Duration of Infusion	Maximum Increase in Mean Blood Pressure	Comments
				mm Hg	
I. Dog					
A. Mongrel					
1. Unanesthetized					
Sweet *et al.* (163)	v.a.	10 ng/kg/min	5 days	25	Maximum reached after 3 days; greater pressor responses with larger
	c.a.	10 ng/kg/min	5 days	None	v.a. doses ANG
	i.v.	10 ng/kg/min	5 days	None	No pressor responses from v.a. norepinephrine
					Reserpine pretreatment blocked effect of v.a. ANG
Fukiyama *et al.* (65)	v.a.	1, 10 μg/kg/day	7 days	14, 24	Maximum reached after 4–5 days
	i.v.	1, 10 μg/kg/day	7 days	5, 10	Effect of v.a. ANG significantly greater than i.v. ANG
					Pressure increased during sleep
2. Anesthetized					
a. Pentobarbital					
Zimmerman (181)	v.a.	1, 10 μg	S.I.	19, 49	Pressor response attributed to peripheral vasoconstriction after recirculation of ANG
	c.a.	2, 10 μg	S.I.	19, 36	No differences between pentobarbital and chloralose-urethane anesthesia were noted
Sweet and Brody (161)	v.a.	10 ng/kg/min	5 min	10	Reflex vasodilatation in the hindlimb was significantly reduced by v.a. ANG at a dose which had no effect i.v.
					Pressure significantly increased by v.a. ANG but not by c.a. or i.v. ANG
Ferrario *et al.* (55)	v.a.	1 ng/kg/min	5 min	16	1–10 ng/kg/min ANG ineffective when given c.a. or i.v., but raised pressure when given v.a.
		20 ng/kg/min	5 min	38	
		48 ng/kg/min	5 min	53	20–50 ng/kg/min ANG raised pressure by all routes; v.a. responses significantly higher than c.a. or i.v.
	c.a.	1 ng/kg/min	5 min	0	
	i.v.	1 ng/kg/min	5 min	0	Norepinephrine, 2–6 or 600–800 ng/kg/min, had same effect by all routes;
		20 ng/kg/min	5 min	8	therefore, cerebral ischemia is unlikely explanation for effects
		48 ng/kg/min	5 min	30	
Gildenberg (73, 74)					Area postrema postulated site of action of ANG
b. Morphine-chloralose					
Fukiyama (64)	v.a.	3.5–9.5 ng/kg/min	2 min	27	Guanethidine pretreatment inhibits vertebral response
					Activity of splanchnic and renal, but not cardiac nerves increased
	i.v.	3.5–9.5 ng/kg/min	2 min	10	

Werning *et al.*	c.a.	5 ng/kg/min	30 min	0	High dose ANG increased urinary epinephrine
(172a)	c.a.	1 μg/kg/min	30 min	70	
Sweet *et al.* (162)	v.a.	2–6 ng/kg/min	3 min	19	Vertebral response can be antagonized by Sar[1], Ile[8]-angiotensin
	i.v.	2–6 ng/kg/min	3 min	0	
Gildenberg *et al.* (75)	v.a.	3.4 ng/kg/min	3 min	21	Dose ineffective i.v. Area postrema ablation abolishes response
B. Greyhound Anesthetized—morphine-chloralose					
Scroop and Lowe (145)	v.a.	8 ng/min	5 min	30	Pressor effect not seen on c.a. administration
	i.v.	500 ng/min	5 min	30	High i.v. doses required to evoke effects of ANG seen with low v.a. doses
	v.a.	8 ng/min	10 min	30	Pressor effect due entirely to a rise of cardiac output
		32 ng/min	10 min	50	Pressor effect unaffected by bethanidine or propranolol but reduced or abolished by vagotomy or atropine
	i.v.	333 ng/min	10 min	28	
		250 ng/min	10 min	43	Conclude that principal effect of v.a. ANG is due to withdrawal of vagal tone
Lowe and Scroop (103)	v.a.	2 ng/min	5 min	10	Cardiovascular response to v.a. infusion of ANG differs in character and dose-response relationships from that to i.v. infusions
		32 ng/min	5 min	20	
		125 ng/min	5 min	35	At 32 ng/min, response characterized by tachycardia, increased B.P. and C.O., fall of central venous pressure; no change in total peripheral resistance
	i.v.	2 ng/min	5 min	0	
		32 ng/min	5 min	10	
		125 ng/min	5 min	20	Considered unlikely that the ANG response is due to a reduction of blood flow to spinal or medullary centers
Scroop and Lowe (144)	v.a.	32 ng/min	5 min	25	Response to v.a. ANG not significantly altered by sympathetic nerve block by a) bretylium tosylate; b) bethanidine; c) cervical cord section at C 4–6; or d) β-block with propranolol Tachycardia abolished and pressor response reduced by vagotomy or atropine Remaining pressor response abolished by sympathetic nerve block by bethanidine Major cardiovascular effect of ANG attributed to withdrawal of vagal tone to the heart
Joy and Lowe (90)	v.a.	8 ng/min	5 min	20	Clamping of basilar artery between upper pons and pyramidal decussation did not modify effect of v.a. ANG
	c.a.	8 ng/min	5 min	None	Cervical spinal section at C 1–2 reduced, but did not abolish response
	Basilar (cephalad)	8 ng/min	5 min	None	Reduced response was abolished by vagotomy Local infusions of ANG into the small arteries supplying the medulla produced a response similar to v.a. ANG Medulla suggested as site of action

TABLE 2—*Continued*

Species	Route	Dose of ANG	Duration of Infusion	Maximum Increase in Mean Blood Pressure	Comments
				mm Hg	
Joy (87)	v.a.	32 ng/min	5 min	30	Study of intramedullary connections of the area postrema which are involved in the response to ANG Response decreased by unilateral ablation of area postrema When both vagi were blocked by cooling, residual sympathetic response was unmodified by unilateral ablation of area postrema Midcollicular transection of midbrain did not alter response Conclude that the angiotensin-area postrema system is a neurohumoral vasomotor center afferent pathway
Scroop *et al.* (143)	v.a.	32 ng/min	5 min	20	Ablation of area postrema blocked effect of v.a. ANG, caused significant reduction in pressor response to i.v. ANG Response to equipressor dose of norepinephrine was not significantly modified Suggests that this central effect of ANG on the area postrema contributed to the cardiovascular response to *endogenous* ANG
	i.v.	500 ng/min	5 min	50	
II. Cat					
Anesthetized—Chloralose (or immobilized, under local anesthesia					
Suga *et al.* (159)	v.a.	25–300 ng/kg	S.I.		Pressor response to ANG caused a decrease in splanchnic nerve discharge and an increase in spontaneous discharge in the vagus nerve similar to that induced by equipressor doses of norepinephrine No consistent changes seen in EEG recorded from hypothalamus or medulla oblongata No potentiation of pressor response to electrical stimulation of pressor areas was seen during ANG infusion
	i.v.	25–300 ng/kg	S.I.		
	v.a.	5–500 ng/kg/min			
	i.v.	5–500 ng/kg/min			
Buckley (23) and Deuben (37)	v.a.	1–10 ng/kg/min	<10 min	22	Infusions of ANG, v.a., produced greater ($P < .001$) pressor responses than i.v. infusions Suggest that ANG crosses the BBB to activate neural pressor mechanisms
	i.v.	1–10 ng/kg/min	<10 min	3	
Gyang *et al.* (79)	v.a.	0.5–10 ng/kg/min	≃5 min	31	Response to v.a. ANG potentiated by adding prostaglandin E_1 to infusion
	i.v.	0.5–10 ng/kg/min	≃5 min	6	
Ueda *et al.* (165)	v.a.	2 μg	S.I.	25	Integrated unit activities recorded extracellularly from the area postrema increased after v.a. ANG

Keim and Sigg (93)	v.a.	0.01 μg	S.I.	~30	Increase in spontaneous preganglionic cervical sympathetic nerve activity seen after 0.001–1.0 μg ANG was injected v.a.
	v.a.	0.1 μg	S.I.	~40	Response showed tachyphylaxis, was independent of pressor response
					No change in enhancement of sympathetic discharges in response to hypothalamic stimulation was seen after v.a. ANG
III. Rabbit					
A. Anesthetized (pentobarbital) Cranston *et al.* (30)	v.a.	23–230 ng/min	5 min		Greater rise in pressure with a more rapid onset seen after v.a. ANG than after i.v. ANG
	i.v.	23–230 ng/min	5 min		
	c.a.	23–230 ng/min	5 min		Tachycardia seen after v.a. ANG, whereas bradycardia seen after intraventricular or i.v. ANG
					At higher doses, i.v. ANG gave greater pressor effect than v.a. ANG
					At all doses i.v. ANG gave a greater pressor effect than c.a. ANG
Yu and Dickinson (180)	v.a.	0.04, 0.09 μg/kg	1 min	45, 65	Slow rise in blood pressure mediated by sympathetic nervous system
	i.v.	0.04, 0.09 μg/kg	1 min	25, 45	Rise is almost entirely blocked by bethanidine
	Aortic	0.04, 0.09 μg/kg	1 min	15, 40	Increase in sympathetic tone is attributed to a central neurogenic action of ANG
					Rise is greater when ANG is given v.a. than i.v. or aortic
					These effects were apparent in the anesthetized rabbit only after extensive cerebral artery ligation and reduction of blood pressure by hemorrhage
					Constriction of cerebral arteries to the vasomotor center by ANG is postulated to mediate the central neurogenic action
B. Unanesthetized					
Dickinson and Lawrence (39)					Have observed a much greater systemic pressor response when ANG was infused into cerebral, rather than general, circulation
Yu and Dickinson (179)	v.a.	0.0075–0.005 μg/kg/min		6–45	Increased cerebrovascular resistance postulated as mechanism
					Pressor response to ANG is enhanced when ANG is infused v.a. rather than i.v.
	i.v.	0.002–0.05 μg/kg/min		0–25	
					Neurogenic component thought to be at least partly mediated indirectly by vasoconstriction of hindbrain circulation
Dickinson and Yu (41)	v.a.	0.033 μg/kg/min	5 hr	25	Infusions of 10 min duration were made every 30 min for 5 hr
					No evidence found to implicate adrenal hormone stimulation or renal sodium and water retention in the progressive pressor response to ANG
					Response almost completely prevented by adrenergic neuron blockade
					Suggested that autonomic nervous system mediated action of ANG
					Response thought largely due to upward resetting of baroreceptors
					Suggest that ANG causes constriction of vasomotor center blood vessels rather than directly affecting neurons

TABLE 2—*Continued*

Species	Route	Dose of ANG	Duration of Infusion	Maximum Increase in Mean Blood Pressure	Comments
				mm Hg	
Rosendorff *et al.* (138)	v.a.	45 ng/min	5 min	25	ANG infusions, v.a., caused tachycardia and a larger increase in blood pressure than the same dose, i.v.
		115 ng/min	5 min	30	
		230 ng/min	5 min	30	At all dose levels, c.a. infusions had less effect than i.v. infusions
	i.v.	45 ng/min	5 min	5	Previous ligation of one vertebral artery necessary for infusions of ANG to have an effect
		115 ng/min	5 min	25	
		230 ng/min	5 min	25	States that it is probable that the central effects of ANG are not secondary to vasoconstrictor properties but are due to a more specific action on angiotensin receptors
Yu and Dickinson (180)	v.a.	0.0008–0.02 μg/kg/min	2 hr	12–32	Slow rise of blood pressure due to ANG infusion is almost entirely blocked by bethanidine; a smaller rise develops immediately and hardly changes over 3 days
	i.v.	0.002–0.05 μg/kg/min	2 hr	0–25	Rise of pressure is greater when ANG is introduced into the hindbrain circulation than into the distal aorta or vein

* The abbreviations used are: ANG, angiotensin II; v.a., vertebral artery; c.a., carotid artery; i.v., intravenous; S.I., single injection.

† Data shown are representative of that presented in papers cited and are sometimes estimated from tracings but do not necessarily include all experiments reported by the authors.

TABLE 3

Summary of studies on effects of administration of angiotensin (ANG) into the cerebrospinal fluid

Species	Dose	Maximum Increase in Mean Blood Pressure	No. of Animals	Comments
		mm Hg		
I. Intraventricular administration				
A. Cat—anesthetized*				
Nashold *et al.* (115)	?, 10 μg	?	?	Animals anesthetized with pentobarbital ANG evoked a marked pressor response in all concentrations, decreased rate of respiration and increased body temperature Transection of mesencephalon at level of superior colliculus abolished pressor response
Smookler *et al.* (158)	0.005 μg	3	2	Effects of ANG included hypertension, tachycardia and contraction of the nictitating membrane Effects blocked by C_1 section of spinal cord Suggest that physiological levels of brain norepinephrine are necessary for the ANG effect Reductions or increases in brain norepinephrine decrease the response to ANG
	0.01 μg	14	5	
	0.05 μg	15	5	
	0.1 μg	34	4	
	0.5 μg	36	3	
	2.0 μg	36	28	
Severs *et al.* (151)	4 μg	50–75	27	Suggest that ANG response is sympathetic in nature Direct injection of ANG into posterior hypothalamus failed to produce pressor responses Blockade of response by *cerveau isolé* section indicated involvement of suprapontine structures Lack of response when perfusion was confined to the lateral and third ventricles by cerebral aqueduct cannulation suggested that the activity was of midbrain origin Possibility of bulbar afferent mechanisms noted
Severs *et al.* (150)	4 μg	~54	10	Midbrain lesions reduced pressor activity to 6 mm Hg ($P < .01$) Doses of 10 μg ANG applied at the termination of the cerebral aqueduct produced pressor response of only 6 mm Hg indicating that direct activation of afferent or efferent bulbar mechanisms was not responsible for response
Daniels and Buckley (34)	4 μg	54	36	Significant differences were seen between animals in their response to IVT ANG, but there was no significant difference between 4 responses obtained in each animal Elevated CSF calcium concentrations had little effect on the response but perfusion with acalcemic CSF inhibited pressor activity

TABLE 3—*Continued*

Species	Dose	Maximum Increase in Mean Blood Pressure	No. of Animals	Comments
		mm Hg		
Deuben and Buckley (38)	4 μg	~40	12	Data suggest that ANG produces its centrally mediated pressor response *via* the subnucleus medialis Marked pressor effects obtained from injection of ANG into the cerebral aqueduct anterior to the subnucleus medialis; responses were significantly lower when ANG was given caudal to the area Bilateral lesions in the subnucleus medialis inhibited the pressor response
Hageman *et al.* (80)	0.75–3 μg	25–35	18	Pyr(3)A1a[6] derivative possessed 48% of angiotensin II central pressor activity
B. Dog—anesthetized*				
Bianchi *et al.* (14)	5–10 μg/kg	?	?	Bradycardia, extrasystole and symptoms of coronary impairment are produced Only a moderate increase of heart rate occurs in vagotomized or atropinized animals
Kaneko *et al.* (91)	5–100 units	?	5	Animals anesthetized with morphine-pentobarbital Both vagus-sympathetic depressor trunks were cut before injections were made Heart rate was little affected, and there was "usually no decrease of arterial pressure" Carotid occlusion response was decreased about 26% within 20 min after injection
Severs *et al.* (150)	4 μg	59	4	No pressor effect obtained with doses up to 10 μg after section of spinal cord at C_1
Cuparencu *et al.* (31)	60–120 μg/kg	20–60	4	Pressure changes not interpreted to be significant Lag time associated with leakage into peripheral circulation Probable inactivation of large amounts of ANG in CSF and nerves No changes were seen in responses to acetylcholine, adrenaline, vagal stimulation, carotid occlusion; ECG and respiration patterns were also unchanged
Gildenberg *et al.* (75)	186 ng	21	7	Response blocked by midbrain transection but not by area postrema ablation
C. Rabbit—unanesthetized				
Rosendorff *et al.* (138)	0.115 μg	~20	7	Successive IVT injections caused progressively smaller responses, even when 1 hr was allowed between injection Response to IVT injection of ANG was greater than effects of much larger total doses given i.v. Response blocked by C_2 section of the spinal cord

TABLE 3—*Continued*

Species	Dose	Maximum Increase in Mean Blood Pressure	No. of Animals	Comments
		mm Hg		
D. Rat—unanesthetized				
Severs *et al.* (155)	0.001 μg	19	12	Pressor effect enhanced by hexamethonium given peripherally, inhibited by hexamethonium given centrally
	0.010 μg	24	5	
	0.025 μg	27	8	
	0.1 μg	30	6	Hypophysectomy reduces pressor effect by about 50% and ganglionic blockade abolishes the remaining activity
	0.5 μg	34	8	
	1.0 μg	39	8	Lesions in supraoptic nuclei decrease effect to values obtained in hypophysectomized rats
				IVT ANG also produces a neurogenic thirst which can be markedly inhibited by anticholinergic drugs
				Suggest that ANG releases antidiuretic hormone
Severs *et al.* (154)	0.5 μg	30	>80	Effects of adrenergic blocking drugs were assessed
				IVT phentolamine prevented ANG-induced drinking, sympathetic stimulation and vasopressin release
				Variable effects with different *beta* blockers
				Local anesthetics inhibited pressor and drinking effect
E. Goat—unanesthetized				
Andersson *et al.* (5)	0.8 ng/kg/min	20	4	Infusions into the third ventricle caused the most pronounced hypertensive effect when ANG was infused with hypertonic saline (increase of 30 mm Hg)
				The hypertensive response correlated in mganitude and duration with the antidiuretic and natriuretic response
II. Subarachnoid administration				
A. Dog—anesthetized				
Severs *et al.* (150)	20 μg	0	3	No pressor response with doses up to 20 μg
Bianchi *et al.* (14)	7 mg/kg	0	?	No influence on vasomotor centers on intracisternal administration
B. Rabbit—unanesthetized				
Rosendorff *et al.* (138)	115 ng	0	2	No cardiovascular effects were detectable when ANG was injected into the subarachnoid space over the cerebral cortex

* Animals were anesthetized with chloralose unless otherwise indicated in comments.

($P < .01$). Subsequent replacement of the aqueduct cannula to the original 6 mm plane fully restored the response to angiotensin.

Enoch and Kerr (49, 50) reviewed the neuroanatomy of cardiovascular integration in the midbrain. The data by Deuben and Buckley (38) suggested that the subnucleus medialis was a likely site of action to explain

their observations. To test this possibility the response to IVT angiotensin was measured before and after producing lesions in the subnucleus medialis. Since the lesions inhibited the pressor response to angiotensin, this midbrain site was postulated to be the region at which angiotensin initiates central pressor activity. Regional injections of angiotensin to other midbrain cardiovascular zones (deep tegmental nucleus of Olszewski and Baxter) were non-pressor, indicating a degree of specificity in the action of angiotensin.

2. Medulla. In investigations in which angiotensin has been administered intravertebrally to dogs, studies on the site of action have consistently implicated the area postrema of the medulla as the responsive region. Evidence for this hypothesis includes: a) Distribution of intravertebrally administered dye as a marker shows that the infusions always reached the medulla when angiotensin pressor activity was seen; when angiotensin action was absent, dye distribution did not reach appropriate medullary regions (90). However, the distribution of dye does not necessarily identify all sites to which angiotensin may have penetrated. b) The area postrema has no BBB so the likelihood of angiotensin penetration at this region is presumably greater than in regions which do appear to have a well developed barrier. c) Various ligations of cerebral vasculature and selective administration of angiotensin into the basilar artery to regulate the distribution of angiotensin appear to document the correlation between good medullary blood distribution and angiotensin activity (89, 90). d) Cervical spinal section inhibits the response to intravertebrally administered angiotensin, whereas mesencephalic transections do not alter peptide responsiveness (56). e) Lesions in the areas postrema (88) abolishes pressor activity of angiotensin after intravertebral administration; local cooling (56) of these structures can reversibly inhibit the peptide effects. f) Regional injections of angiotensin directly into the area postrema produce pressor activity, and area postrema units are activated by intravertebral angiotensin in anesthetized cats (165). It is also of interest that ablation of the area postrema reduces the pressor activity of intravenous angiotensin but not that of norepinephrine (143). Gildenberg *et al.* (75) recently demonstrated in dogs that both the midbrain and the area postrema respond to angiotensin depending on the route of administration. Intravertebral pressor responses were abolished by area postrema ablation but not by midbrain transection; on the other hand, pressor responses to IVT angiotensin were abolished by midbrain transection but not by ablation of the area postrema.

3. Hypothalamus. Dutta *et al.* (45) have reported that angiotensin injections (2.5–10 μg) into the ventromedial hypothalamus of chloralose-anesthetized cats produced pressor responses in 54% of the animals. The site of angiotensin deposition produced pressor activity when stimulated electrically. Considering the time lag before the peak effect occurred (mean = 6 min) and the rapidity (<1 min) with which such a peak is reached after IVT or intra-aqueductal injections during ventricular perfusions (38), there is a possibility that CSF flow in these experiments may have transported angiotensin to other periventricular regions. Data of Hendler and Blake (82) provide further evidence for a hypothalamic site. Implantation of angiotensin crystals into the anterior, ventromedial, and posterolateral hypothalamus of pentobarbital-anesthetized rats increased blood pressure 38, 22, and 11 mm Hg, respectively. Although these data are from only 2 to 4 rats at each site and no temporal information on the pressor response was given, the diffusion of angiotensin was probably minimal. In experiments by Hendler and Blake implants of angiotensin in the anterior hypothalamus caused altered drinking behavior over a 1-hr period while ventromedial implantation did not. Since these sites are only 1mm apart in rats, rapid diffusion of angiotensin probably did not occur. However, injections of angiotensin in the poste-

rior hypothalamus (0.5 μg) (151) or regional injections at multiple hypothalamic loci (0.1–1.0 μg) (165) were non-pressor in chloralose-anesthetized cats. Thus, the evidence for initiation of cardiovascular effects by angiotensin at hypothalamic levels remains equivocal. On the other hand, there appears to be general agreement that some hypothalamic sites are responsible for angiotensin-induced changes in drinking behavior (section IV). These findings indicate the need for further evaluation of the potential angiotensin-hypothalamic-cardiovascular mechanisms.

D. Influence of Angiotensin on Sympathetic Nerve Activity and Its Effect on Baroreceptor Reflexes

Systemic administration of angiotensin to animals with a neurally intact, vascularly isolated, vascular bed can cause neurogenic vasoconstriction in the isolated vascular bed. The rat hindquarter (100), cat mesenteric (20), and femoral (9) beds may be cited as examples. Angiotensin has also been shown to increase electrical activity of a number of nerves such as the renal nerve in rabbit (1) and dog (64), and the splanchnic (64, 142) and inferior cardiac nerves in dogs (142). Such data are compatible with the interpretation that angiotensin may increase central sympathetic outflow, thereby raising peripheral vascular resistance.

However, recording nerve activity or the resistance of isolated vascular beds does not always show such an angiotensin effect (109, 181). The reasons for this inconsistency are not fully known. At least two variables should, however, be considered, namely the nature of the nerves from which recordings are made, and the influence of baroreceptor reflex mechanisms on neural activity and vascular resistance. As an example of interpretive problems with nerve recording experiments, the effects of angiotensin on superior cervical sympathetic nerve (CSN) discharge are illustrative. The following data have been reported: intravenous angiotensin inhibits spontaneous CSN discharge (109); the CSN is inhibited during the central pressor response after IVT angiotensin (148); spiking of the CSN increases after intravertebral angiotensin, but reflex inhibition can occur (93). Some investigators have interpreted the inhibition of spiking on the CSN to indicate that angiotensin does not increase central sympathetic outflow (109). However, since the central pressor effect of angiotensin can be produced along with CSN inhibition, the peptide may be activating only central cardiovascular (as opposed to non-cardiovascular) sympathetic pathways (148). Aars and Akre (1) have pointed out that vasoconstrictor fibers form only a small part of the CSN and that vasoconstrictor effects of angiotensin could be masked by electrical activity of other fibers. However, spiking of the CSN after intravertebral angiotensin always preceded vasopressor responses but could be inhibited reflexly (93). These data leave open the possibility that sympathetic fibers in the CSN, not associated with vasoconstriction, are activated by angiotensin.

The influence of baroreceptor reflexes on experiments designed to demonstrate central cardiovascular angiotensin effects are further illustrated by experiments of Barrett *et al.* (9). As discussed in section III A, these investigators developed a method to perfuse extracorporeally the cat brain through the carotid arteries and the transverse venous sinuses. A separate system was utilized simultaneously to perfuse a vascularly-isolated, neurally-intact hindlimb. The carotid sinus areas were perfused by the cerebral circuit, and bilateral cervical vagotomy eliminated afferent baroreceptor information associated with change in the systemic blood pressure. Addition of angiotensin to the cerebral perfusion circuit in these preparations did not produce a centrally mediated hypertensive response. However, after 15 min a neural vasoconstriction was observed in the hindlimb. When these experiments were repeated in animals in which cranial nerves IX–XII were sectioned, the addition of angiotensin to the cerebral perfusion circuit produced a clear central hypertensive response and a more rapid and intense neural

vasoconstriction in the isolated hindlimb. The authors suggested that in intact animals increased cerebral perfusion pressure activated baroreceptor mechanisms which masked central angiotensin effects. In debuffered animals, however, there was no relationship between cerebral perfusion pressure and the peripheral hypertensive response.

In addition to potential baroreceptor inhibition of neurally-mediated cardiovascular effects of angiotensin, the peptide appears to be capable of antagonizing reflex vasodilation. Sweet and Brody (161) found that intravertebral administration of angiotensin to anesthetized dogs inhibited baroreceptor-induced reflex vasodilation in the hindlimb. These effects of angiotensin were observed at a dose (10 ng/kg/min) which was ineffective when given intravenously or into a carotid artery. The authors suggest that the inhibition of reflex vasodilation is not due to alteration of sympathetic tone but to inhibition of neurally-mediated vasodilatory mechanisms (possibly histaminergic) (12). The importance of the observations by Sweet and Brody (161) in this regard should be emphasized. There is general agreement that resetting of cardiovascular reflex mechanisms probably contributes to hypertensive pathophysiology, and the data by Sweet and Brody (161) suggest that angiotensin could be involved in the reset mechanism. Other investigators have speculated that central angiotensin effects could include baroreceptor alterations (56, 172).

IV. Effects of Centrally Administered Angiotensin on Hydration

A. Dipsogenic Effects

A growing body of literature indicates that administration of angiotensin into the CNS produces marked changes in hydration (reviews, 59, 61). Many species respond to CNS injections of angiotensin by increasing water intake (61) even without an apparent initiating stimulus, *i.e.*, hypovolemia or hyperosmolality (133). Such consummatory behavior is generally associated with a short latency (seconds to minutes), requires small amounts of angiotensin (0.5 ng at some hypothalamic sites or 1 ng IVT to rats), and appears to be quite specific (52, 155). In fact, feeding behavior in hungry rats is suppressed in favor of drinking after central administration of angiotensin (107). Furthermore, rats will drink quinine solutions after injection of the peptide into preoptic areas (137).

Epstein *et al.* (52) microinjected angiotensin into various regions of rat brains to localize the site of angiotensin activity. They observed that large doses (2–4 μg) of locally injected angiotensin cause changes in drinking behavior at the nucleus accumbens, septum, preoptic area, anterior and lateral hypothalamic areas, ventromedial nucleus, and the amygdala. Sites which were unresponsive even to large doses of angiotensin included the cerebellum, midbrain tegmentum, dorsal hippocampus, posterior hypothalamus, caudate nucleus and the frontal cortex. Similar regional analysis with 10% or less of the above doses of angiotensin indicated that the most sensitive dipsogenic sites were located in the septum, anterior hypothalamus and medial preoptic areas. In another study (82) the 1-hr fluid intake was increased after crystalline implants of angiotensin at anterior, but not ventromedial or posterolateral, hypothalamic sites. The possibility of such angiotensin activity in the anterior hypothalamus is supported by the observation that the peptide increases oxidative metabolism in this brain region (54). Moreover, activity of mitochondrial Na-K ATPase of rat hypothalamus is increased by angiotensin (78). The central origin of the drinking response is indicated by the fact that the minimal effective intravenous doses of angiotensin were at least 1000 times the minimal effective intracranial dose (52). According to Fitzsimons (59), since the subfornical body lies outside of the BBB, circulating angiotensin could reach sensitive diencephalic sites at this level of integration. This suggestion is analogous to proposing the area postrema

(outside the BBB) as the site mediating the hypertensive response to intravertebral angiotensin.

Additional studies by Fitzsimons (60) demonstrated that active angiotensin drinking sites were not being stimulated in a nonspecific manner. Thus, kallikrein, bradykinin, vasopressin, oxytocin, epinephrine, or aldosterone did not cause significant changes in drinking behavior. Various fragments and derivatives of angiotensin were similarly ineffective as dipsogens. On the other hand, renin, synthetic tetradecapeptide substrate, and angiotensin I were all effective as thirst-inducing substances (60). It is possible that conversion to angiotensin II accounts for the drinking behavior produced by these substances, as inhibition of brain converting enzyme (by IVT administration of synthetic *Bothrops jararaca* nonapeptide, SQ 20,881) blocks the drinking effect of angiotensin I but not of angiotensin II (153). This possibility is supported by the observation that intracranial injections of anti-angiotensin II serum (but not control serum) antagonize the changes in drinking produced by intracranial angiotensin II or renin substrate (51). In contrast, other investigators found that SQ 20,881 was inactive (160). They suggest that angiotensin I may stimulate either angiotensin II, or angiotensin I receptors.

Several investigators have attempted to identify potential neurotransmitters associated with angiotensin-induced drinking with various drugs as pharmacological antagonists. Some of the available data is summarized in table 4. The following conclusions can be drawn from the literature: a) Localized injections of atropine do not inhibit drinking produced by angiotensin injected at the same site (29, 62, 72, 77, 160). On the other hand, drinking after IVT angiotensin is inhibited by IVT administration of atropine and other anticholinergic substances (155). These observations suggest that angiotensin does not initiate drinking by a cholinergic mechanism but secondary cholinergic neuron(s) may participate in the overall effect. In monkeys, there is a close anatomic similarity between cholinergic and angiotensin sensitive thirst areas (113). b) Some evidence suggests that *beta* adrenergic receptors in the CNS participate in drinking behavior (101). Central *beta* receptors are probably not associated with angiotensin because regional injection of *d*-propranolol is as effective as *l*-propranolol in antagonizing angiotensin activity (62) and IVT treatment with some other potent *beta* receptor blockers does not antagonize the effects of IVT angiotensin (154). c) The effects of centrally administered *alpha* receptor blocking agents are unclear. IVT injections of phentolamine completely antagonize drinking responses to IVT angiotensin in rats without producing behavioral abnormalities (154). On the other hand, intrahypothalamic phentolamine inhibited intrahypothalamic angiotensin only at toxic doses in the same species (62). Injections of phentolamine into medial septal areas did not inhibit drinking produced by angiotensin at the same site (29). Since IVT phentolamine undoubtedly was distributed to many more anatomical loci, an adrenergic mechanism may exist at a site other than the specific areas tested. d) Intrahypothalamic administration of 6-hydroxydopamine inhibited the drinking response to angiotensin injected at the same site (62). The compound did not, however, antagonize cholinergic drinking produced by carbachol. These data suggest that an aminergic mechanism is involved in the initiation of angiotensin drinking. Inhibition of drinking by haloperidol is compatible with this idea (62) and suggests a possible dopaminergic mechanism. The haloperidol effect was not, however, observed by other investigators (160). The inconsistency may be due to differences in injection sites and/or pretreatment times. However, since local anesthetics are also inhibitory to drinking behavior, transmitters deduced by drug inhibition must be regarded as tentative rather than conclusive (154).

TABLE 4

*Summary of studies on effects of pharmacologic antagonists on drinking induced by central administration of angiotensin II**

Antagonist		Angiotensin		Effect on Angiotensin Induced Drinking	Reference
Dose	Route, time	Dose	Site		
Atropine sulfate, peripheral					
10 mg/kg	IP, 15 min prior	1.5 μg	LPO, LHA	23% Mean reduction	17
20 mg/kg	IP, 15 min prior	1.5 μg	LPO, LHA	70% Mean reduction	17
5 mg/kg	SC, 20 min prior	2 μg	LPO	Inhibition 75%, $P < .001$	77
5 mg/kg	IP, 15 min prior	0.5 μg	IVT	Inhibition, $P < .005$	155
Atropine sulfate, central					
1, 10 μg	POR, 10–15 min prior	100 ng	POR	No effect	62
100 μg	POR, 10–15 min prior	100 ng	POR	Inhibition, $P < .05$	62
20–50 μg	LPO, 5–10 min prior	2 μg	LPO	No effect	77
417 ng	MS, MPO, 1 min prior	100 ng	MS, MPO	No effect	160
2 μg	MS, 30 min prior	1 μg	MS	No effect	29
15–20 μg	LSA, 10 min prior	15–20 μg	LSA	No effect	72
100 μg	IVT, 15 min prior	0.5 μg	IVT	Abolition	155
10 μg	IVT, 15 min prior	0.5 μg	IVT	91% Inhibition	147†
Atropine methyl nitrate, peripheral					
5 mg/kg	SC, 5–10 min prior	2 μg	LPO	No effect	77
Atropine methyl nitrate, central					
15–20 μg	LSA, 10 min prior	15–20 μg	LSA	No effect	72
Chlorpromazine, peripheral					
2 mg/kg	SC, 5–10 min prior	2 μg	LPO	Inhibition 86%, $P < .001$	77
Chlorpromazine, central					
10 μg	LPO, 5–10 min prior	2 μg	LPO	Increase, $P < .005$	77
Chlorpromazine methyl iodide, peripheral					
2 mg/kg	SC, 5–10 min prior	2 μg	LPO	No effect	77
Reserpine, peripheral					
3 mg/kg	IP, 20 hr and 3 hr prior	0.5 μg	IVT	Abolition	155
Phentolamine, peripheral					
15 mg/kg	IA, 15 min prior	0.5 μg	IVT	Inhibition, $P < .001$	154
Phentolamine, central					
50 μg	IVT, 15 min prior	0.5 μg	IVT	Inhibition, $P < .001$	154
10, 20, 40 μg	POR, 10–15 min prior	100 ng	POR	No effect	62
11.3 μg	MS, 60 min prior	1 μg	MS	No effect	29

TABLE 4—*Continued*

Antagonist		Angiotensin		Effect on Angiotensin Induced Drinking	Reference
Dose	Route, time	Dose	Site		
Tolazoline, peripheral					
8.5 mg/kg	SC, 5–10 min prior	2 μg	LPO	No effect	77
15 mg/kg	IA, 15 min prior	0.5 μg	IVT	Inhibition, $P < .05$	147
Tolazoline, central					
50 μg	IVT, 15 min prior	0.5 μg	IVT	Inhibition, $P < .001$	147
20 μg	LPO, 5–10 min prior	2 μg	LPO	Inhibition 64%, $P < .005$	77
Phenoxybenzamine, peripheral					
3 mg/kg	IV, 20 hr prior plus	0.5 μg	IVT	No effect	155
2 mg/kg	IP, 3 hr prior				
Haloperidol, central					
2.5, 5, 10 μg	POR, 10–15 min prior	100 ng	POR	Inhibition with all 3 doses, $P < .05$	62
5 μg	MS-MPO, 1 min prior	50 ng	MS-MPO	No effect	160
6-Hydroxydopamine, central					
8 μg	POR, 24 hr prior	100 ng	POR	Abolition in 10 out of 15	62
Propranolol, peripheral					
6 mg/kg	SC, 5–10 min prior	2 μg	LPO	No effect	77
7.5 mg/kg	IA, 15 min prior	0.5 μg	IVT	Inhibition, $P < .01$	154
Propranolol, central					
50 μg	IVT, 15 min prior	0.5 μg	IVT	Inhibition, $P < .01$	154
10, 20 μg	LPO, 10–15 min prior	100 ng	POR	No effect	62
40 μg	LPO, 10–15 min prior	100 ng	POR	Inhibition, $P < .05$ (local anesthesia?)	62
20 μg	LPO, 5–10 min prior	2 μg	LPO	No effect	77
10.4 μg	MS, 60 min prior	1 μg	MS	No effect	29
DCI, peripheral					
10 mg/kg	IA, 15 min prior	0.5 μg	IVT	Inhibition, $P < .01$	154
DCI, central					
50 μg	IVT, 15 min prior	0.5 μg	IVT	Inhibition, $P < .01$	154
LB-46, peripheral					
10 mg/kg	IA, 15 min prior	0.5 μg	IVT	No effect	154
LB-46, central					
6.7 μg	IVT, 15 min prior	0.5 μg	IVT	No effect	154
MJ-1999, peripheral					
2 mg/kg	IA, 15 min prior	0.5 μg	IVT	No effect	154
MJ-1999, central					
50 μg	IVT, 15 min prior	0.5 μg	IVT	Inhibition, $P < .01$	154

TABLE—4 *Continued*

Antagonist		Angiotensin		Effect on Angiotensin Induced Drinking	Reference
Dose	Route, time	Dose	Site		
D(−) INPEA, peripheral					
5 mg/kg	IA, 15 min prior	0.5 μg	IVT	Inhibition, $P < .01$	154
10 mg/kg	IA, 15 min prior	0.5 μg	IVT	Inhibition, $P < .01$	154
D(−) INPEA, central					
50 μg	IVT, 15 min prior	0.5 μg	IVT	No effect	154
Procaine, central					
50 μg	IVT, 15 min prior	0.5 μg	IVT	Inhibition, $P < .05$	154
Tetracaine, central					
50 μg	IVT, 15 min prior	0.5 μg	IVT	Inhibition, $P < .01$	154
Hexamethonium, peripheral					
10 mg/kg	IA, 10 min prior	0.5 μg	IVT	Inhibition, $P < .01$	155
Hexamethonium, central					
100 μg	IVT, 15 min prior	0.5 μg	IVT	Inhibition, $P < .001$	155
Methysergide, central					
10 μg	MS, 10 min prior	1 μg	MS	No effect	29

* The abbreviations used are: IA, intraarterial; IP, intraperitoneal; IV, intravenous; IVT, intracerebroventricular; LHA, lateral hypothalamic area; LPO, lateral preoptic area; LSA, lateral septal area; MPO, medial preoptic area; MS, medial septal area; POR, preoptic region; SC, subcutaneous.

† n = 6; methodology as in reference 154.

B. Pituitary Effects of Angiotensin

In addition to causing marked changes in drinking behavior, administration of angiotensin into the CNS also appears to release antidiuretic hormone (ADH) from the neurohypophysis. Several lines of evidence have been obtained for this effect of the peptide. a) Infusion of angiotensin into the third ventricle of unanesthetized goats causes changes in drinking behavior, volume retention, and natriuresis; similar effects are produced by infusion of hypertonic sodium chloride. These effects are potentiated by combining the two stimuli and they do not occur after production of experimental diabetes insipidus (3, 4, 6, 7). Furthermore, administration of hypertonic NaCl in the third ventricle increases bioassayable ADH in CSF (130). b) IVT injection of angiotensin to unanesthetized rats produces a pressor response and changes in drinking behavior. Significant inhibition of the pressor response occurs after hypophysectomy or bilateral destruction of the supraoptic nuclei (155). Since the supraoptic nuclei may be primarily associated with the production of ADH (8, 47), these data suggest that angiotensin released ADH after IVT injection. Furthermore, IVT injection of angiotensin into hydrated rats produced volume retention, natriuresis, and kaliuresis (152). c) Microiontophoretic application of angiotensin onto units in the supraoptic nuclei increase their activity. This effect is not caused by non-specific stimulation since neurons elsewhere in brain are unresponsive (118). d) Infusions of angiotensin into the carotid artery of conscious dogs or perfusion of the peptide through the cerebro-

ventricles of anesthetized dogs increase plasma ADH, as measured by bioassay (104, 110). e) Addition of angiotensin (10^{-10} M) to *in vitro* posterior pituitary incubations increases the spontaneous release of vasopressin (66).

IVT injection of angiotensin in unanesthetized rats increases plasma and adrenal corticosterone within an hour. This effect is pituitary-mediated since it is not observed in hypophysectomized animals. Thus, angiotensin may release adrenocorticotrophic hormone (ACTH) (36). If the steroidogenic effect was produced by ACTH, the mechanism could have been due to a direct effect of angiotensin or a secondary release of ACTH associated with the pressor response and/or ADH release, both of which release ACTH (170, 178). Circulating angiotensin can release ACTH (141). A Boolean model of ACTH release (67) predicted that angiotensin should act at a site earlier than the anterior pituitary, presumably at the median eminence. Infusion of angiotensin (1–2 μg/min) to dogs subjected to brain removal except for a pituitary-median eminence island released much more ACTH compared to similar infusions in hypophysectomized dogs or dogs undergoing brain removal except for the anterior pituitary (67). These data are compatible with the interpretation that circulating angiotensin acts centrally to produce ACTH release.

C. Significance of the Effect of Angiotensin on Hydration

Thirst and vasopressin release are vital to the maintenance of proper hydration. They are complimentary mechanisms in that they both increase body water. Furthermore, both are classically regarded as being stimulated by hyperosmolality or hypovolemia. The broad topic of the regulation of body fluids is well reviewed by Share and Claybaugh (156). Only data associated with CNS-angiotensin effects are dealt with here. Research on angiotensin-CNS-hydration interactions is relatively new and no firm conclusions on their significance can be drawn from the data now available. One reason for this involves relationships between the brain and renal renin-angiotensin systems. The brain system appears to be independent of the renal system, yet both are associated with hydration changes. Under what conditions do each of these systems operate independently, in concert, or in opposition? Furthermore, do intracerebral injections of angiotensin activate cells responsive to brain and/or renally derived angiotensin? Clearly, the two systems must normally be in close communication to preserve the overall well being of the organism. As an example of the complexity of these problems, *centrally* administered angiotensin initiates thirst associated with osmotic factors (133), whereas hypovolemic thirst involves the kidney, and, more specifically, the *renal* renin-angiotensin system (61). Since nothing is known of the relationship between the brain and kidney renin-angiotensin systems, the following discussion includes viewpoints of various laboratories where centrally mediated effects of angiotensin on hydration have been studied.

Severs *et al.* (152) injected angiotensin IVT (0.5 μg) to rats prehydrated with water by stomach tube. Compared with the response in the same rats in a control experiment, the peptide caused significant volume retention for 90 min. After this interval angiotensin-treated rats excreted more sodium and potassium than the control group. Since angiotensin caused thirst, volume retention, natriuresis, and kaliuresis in hydrated rats, it was suggested that the IVT injection of the peptide caused the brain to perceive a state of hyperosmolality. The resultant effects of the peptide would then reflect expected compensatory mechanisms. This hypothesis was further investigated in normally hydrated rats that received IVT infusions (0.1 μg/min) for 5 hr (133). Water consumption and volume retention produced by angiotensin infusion were consistently (for 5 hr) associated with 13 ml of excess volume compared with control-infused rats. Since solute balance of both

groups was always equal, the retained volume suggested a 5% dilution of body water. If rats had both 0.9% saline and water available for drinking, about half of the animals preferred to drink saline when angiotensin was infused. The saline-drinking rats ingested and retained much more volume than either water-drinking or control rats. Although these rats retained more solute than control rats, it was an insufficient amount to make the excess volume isosmotic. Whereas rats who drank only water stopped drinking before 90 min, the saline-preferring rats drank almost continuously during the 5 hr of angiotensin infusion. These data suggest that ingestion and retention of water satiate the initial angiotensin stimulus since the excess water is sustained during infusion and thirst does not reappear. Because the very marked volume expansion produced by saline ingestion and fluid retention was insufficient to terminate angiotensin-induced thirst, dilution rather than expansion of body fluids probably corrects the initial stimulus produced by the peptide. Centrally administered angiotensin presumably caused an osmotic, rather than a hypovolemic, thirst drive. In an experiment in which angiotensin was injected into the hypothalamus, both saline and water inhibited or satiated thirst within 15 min (136). Since the thirst stimulus decayed rapidly even without drinking, it appears that in short-term experiments saline inhibits, rather than satiates, angiotensin thirst.

Andersson and colleagues (3–7) reported that angiotensin, dissolved in slightly hypotonic NaCl, infused into the third ventricle in goats stimulates drinking behavior and ADH release, whereas administration of the peptide in hypertonic solutions of glucose or sucrose had little or no effect. Infusion of small volumes of hypertonic NaCl into the third ventricle also produced drinking behavior and ADH release, and this response was noticeably augmented by addition of angiotensin to the infusion. Since sodium ions penetrate cells poorly, the effect of hypertonic NaCl infusion would appear to be associated with water efflux from osmoregulator cells which, according to Verney (169), would initiate ADH release. Based on the ADH releasing effects of several hypertonic solutions infused by the carotid and ventricular routes, Andersson and colleagues postulated that the sensitive receptors are not activated by osmotic factors *per se* but by changes in CSF [Na^+]. These receptors are presumably close to the third ventricle, regulate thirst and ADH release, and may not be under the influence of an effective BBB. Angiotensin may stimulate these receptors by: a) facilitating transependymal Na^+ movement from CSF into brain tissue; b) sensitizing the receptors to existing sodium concentration of brain extracellular fluid; or c) facilitating passage of sodium ions into receptor cells. Infusions of iso- and hypotonic solutions of monosaccharides into the lateral ventricles of goats inhibit the dipsogenic, antidiuretic and natriuretic effects of intracarotid infusions of hypertonic NaCl (122); this suggests that blood-borne osmotic stimuli may be related to these periventricular Na^+ receptors since presumably the IVT infusions kept CSF [Na^+] low.

Considering Andersson's hypothetical sodium receptors it is of interest that renin release from incubated canine renal cortical slices was observed to vary directly with the [Na^+] of the incubation medium. Osmotic changes at constant [Na^+] failed to affect renin release (63).

Claybaugh and Share (27) investigated the role of angiotensin in the ADH release associated with non-hypotensive hemorrhage in anesthetized dogs. The experimental design involved ligation of the right renal vasculature and placement of a snare around the left renal vasculature. Hemorrhage-induced increases in plasma ADH and renin activity (PRA) were measured when the snare was a) non-occlusive and b) occlusive to the renal vessels. In the non-occlusive state, hemorrhage increased plasma ADH and renin activities. When the snare occluded the vessels, increases in plasma ADH were more marked although PRA was

unchanged. Thus, in response to a reduction in blood volume, the renin-angiotensin system (of the kidney) did not appear to be required for ADH release. Other data from the same laboratory (28) demonstrated that intravenous angiotensin infusions (10–60 ng/kg/min), or carotid artery infusions (10 ng/kg/min), did not change plasma ADH in hydrated anesthetized dogs. Another study from Share's laboratory (157) was designed to determine whether angiotensin affected ADH release in response to an osmotic stimulus. Anesthetized hydrated dogs received an intravenous infusion of hypertonic sodium chloride solution with or without a simultaneous intracarotid infusion of angiotensin (10 ng/kg/min) in slightly hypotonic saline. Angiotensin infusion alone had no effect on plasma ADH. In control dogs receiving intravenous hypertonic saline, a rectilinear relationship was observed between plasma ADH and plasma osmolality. Intracarotid angiotensin increased the slope of this relationship suggesting that the ADH releasing mechanism was responsive to a lower osmotic threshold. It was concluded that the peptide, *via* the carotid artery, can potentiate the release of ADH in response to rising plasma osmolality. Experiments of Kozlowski *et al.* (99) are complementary to this hypothesis. These investigators found that intravenous angiotensin (50 ng/min) decreased the thirst threshold of dogs receiving intravenous 5% NaCl. It would be of interest to determine if these effects of angiotensin involved CNS sodium receptors as described by Andersson (3).

Fitzsimons (59, 61) has reviewed the role of the kidney in extracellular (hypovolemic) thirst. This complex thirst drive was produced in rats with many experimental models, and all known causes of extracellular thirst increased renin release. Studies with nephrectomized rats suggest that both renal and extrarenal factors participate in extracellular thirst to varying degrees depending on the model used. Thus, thirst produced by intraperitoneal injections of hyperoncotic colloids is little affected by nephrectomy, whereas thirst after aortic constriction above the renal arteries, or renal arterial constriction, is abolished by nephrectomy. Thirst initiated by caval ligation or hemorrhage is intermediately affected by nephrectomy. According to Fitzsimon's model (59), extracellular hypovolemia activates afferent pathways from the low pressure side of the circulatory system (also arterial baroreceptors in severe cases) which stimulate thirst centers in the CNS. Renin is released from the kidney by a) increased efferent sympathetic activity as a result of the same afferent pathways, and by b) hemodynamic factors associated with hypovolemia. Increased amounts of circulating angiotensin presumably cause further drinking by an effect on thirst areas of the diencephalon. The peptide also assists extracellular dehydration by causing sodium retention through aldosterone release.

Because of the limitations mentioned at the beginning of this section the physiological and/or pathophysiological significance of these observations cannot be precisely stated. A significant negative correlation occurs between brain renin activity and brain water, sodium, potassium, calcium, and magnesium (69). Exogenous administration of angiotensin into CSF produces thirst, ADH release, natriuresis, and kaliuresis (3, 152). Peripheral infusion of subpressor and mildly pressor doses of angiotensin, in general, produce a reduction both in urine flow and sodium excretion (18). Collectively, however, the available data suggest that in addition to its adrenocortical, intrarenal, and hemodynamic effects, angiotensin produces hydration changes by an action on the CNS.

V. Cholinergic, Adrenergic, and Macromolecular Effects of Angiotensin in the Central Nervous System

A. Cholinergic

Angiotensin has been shown to increase acetylcholine output at cholinergic terminals in the periphery (129, 135). Some data indicate that the peptide also affects central

cholinergic components. Thus, intravenous administration of angiotensin to mice (0.3–30 μg/kg, minimal/maximal effective doses) elevated total brain acetylcholine (11). Acetylcholine was measured by bioassay with the frog rectus abdominus. The maximal effect was observed after only 10 min. The authors suggested that angiotensin either increased the synthesis and/or storage of acetylcholine or inhibited its destruction. Elie and Panisset (48) found that angiotensin released acetylcholine into cortical cups placed on the parietal cortex of encephale isolé cats. The acetylcholine was measured by bioassay in eviscerated cats. Identification of the vasoactive substance as acetylcholine was based on the following: a) physostigmine was required in the media bathing the cortex; b) atropinization of the assay cat antagonized the vascular response; and c) samples were inactivated by alkalinization and exposure to room temperature. Angiotensin, applied topically (10^{-9} M), or microinjected (1 ng/0.1 μl) 1 mm deep into the cortex produced significant increases in acetylcholine output. Addition of angiotensin to brain homogenates (10^{-11}–10^{-3} g/ml) had no effect on cholinesterase activity measured by a gazometric method. The authors suggest that angiotensin increases spontaneous acetylcholine output by altering cholinergic release mechanisms.

B. Adrenergic

Peripheral angiotensin interaction with the sympathetic nervous system is well established, especially at the level of postganglionic terminals. At this site, there is good evidence that the peptide increases sympathetic activity by facilitating release (182), inhibiting reuptake (96), and increasing synthesis (139) of norepinephrine. Based on drug inhibition data the central pressor (13, 158) and drinking (62, 154) effects of angiotensin may involve adrenergic mechanisms. Literature suggesting adrenergic interaction of angiotensin in the CNS is difficult to evaluate since various authors utilize various routes of administration, doses, and temporal parameters, whereas basic information relative to the uptake, accumulation and inactivation of the peptide is incomplete or lacking. Thus, while angiotensin interaction at adrenergic terminals in peripheral test systems can be demonstrated at levels which are probably "physiological" (96, 182), the question of what constitutes a "physiological" experiment in the CNS is unknown. Since endogenous brain angiotensin seems to be distributed in a manner similar to norepinephrine (58), and since renin activity is localized in synaptosomes (108), angiotensin may affect central adrenergic terminals in a manner similar to its effects on peripheral adrenergic terminals.

Effects on endogenous brain norepinephrine. With doses of angiotensin which produced pressor responses, neither infusion into the *in situ* cat brain preparation (9) nor repetitive injections into perfused cat ventricles (34) altered norepinephrine levels in brain regions which contain high endogenous concentrations. Other investigators found that administration of 1 μg of angiotensin IVT to mice (26) or 0.5 μg/kg i.v. to rats (92) significantly reduced whole brain norepinephrine content. The maximal reduction (50%) in mouse brain occurred after 1 hr, and recovery was observed by 2 hr. The reduction in rats was observed at the three times measured (3, 10, and 20 min). Brain dopamine levels were not changed in the rat experiments. Regional analysis of the rat brain showed that hypothalamic, but not striatal, norepinephrine was decreased.

In vitro effects. Palaic and Khairallah (126) preincubated slices of brain, thoracic aorta, and spleen with and without angiotensin (20 μg/ml) followed by incubation with ^{14}C-norepinephrine. Angiotensin significantly reduced the radioactivity accumulated by all three tissues. Based on this and *in vivo* experiments described below, Palaic and Khairallah suggested that angiotensin has a general effect of inhibiting tissue norepinephrine uptake.

Janowsky *et al.* (85) added angiotensin (10–100 μg/ml) to rat brain synaptosomes incubated with ^{3}H-norepinephrine and ob-

served decreased uptake of isotope. The peptide had no effect on the efflux of ^{3}H from synaptosomes pre-equilibrated with ^{3}H-norepinephrine. Analysis of synaptosome pellets and supernatant in uptake and efflux experiments showed that angiotensin did not significantly change the percentage of ^{3}H-norepinephrine in the samples. Thus, the apparent inhibitory effect of angiotensin on norepinephrine uptake is not likely to be secondary to alterations in ^{3}H-norepinephrine metabolism. The peptide did, however, produce small but significant changes in the metabolites in supernatant samples. In uptake experiments, angiotensin decreased the amount of ^{3}H-normetanephrine. In efflux experiments, the peptide increased the amount of ^{3}H-deaminated catechols and decreased the quantity of ^{3}H-deaminated-O-methylated catechol metabolites. Angiotensin does not appear to directly affect monoamine oxidase or catechol-O-methyltransferase activity in peripheral tissues (10). Limited information is available concerning peptide effects on these enzymes in brain. *In vitro* addition of angiotensin to rat brain homogenates does not alter catechol-O-methyltransferase activity (10). One investigator reported that intravenous angiotensin reduces whole brain and hypothalamic monoamine oxidase activity (92). In the experiments of Janowsky *et al.* (85) with brain synaptosomes, interesting data were obtained relative to the inactivation of angiotensin by brain tissue. Addition of angiotensin to 5 ml of synaptosome suspension to make a final concentration in the media of 10^{-4} M (100 μg/ml) resulted in an immediate 42% decrease in pressor material measured by bioassay. Considering that the 5 ml of suspension represented synaptosomes from 1/20th of a rat brain, the inactivation process must have an enormous capacity. Similarly, Barth (10) reported that incubation of crude rat brain homogenate with angiotensin results in rapid disappearance of the peptide. These observations typify the types of problems encountered when trying to determine dose or concentration factors in CNS-angiotensin research.

In vivo effects. Palaic and Khairallah (127) studied angiotensin interaction with central adrenergic neurons with a ventriculo-cisternal perfusion method in anesthetized rats. The simultaneous perfusion of angiotensin (8–800 ng/min) and ^{3}H-norepinephrine, followed by a washout perfusion, showed that the peptide decreased the amount of isotope accumulated by the brain and in the washout fluid. Although the amount of tritium in the washout fluid was decreased, the rate of washout was similar to control values. Furthermore, supernatants from brain homogenates prepared after the washout perfusion showed that angiotensin did not change the norepinephrine/metabolite ratio. The data suggest that angiotensin inhibits the norepinephrine uptake mechanism. The biologically inactive angiotensin diamide did not produce this effect. Similar experiments in which ^{3}H-norepinephrine was perfused through the brain and followed by angiotensin perfusion in the washout period indicated that the peptide did not affect norepinephrine release. Additional perfusion experiments with ^{3}H-norepinephrine-equilibrated brains showed that stimulation of the central end of the cut vagus increased norepinephrine and decreased acid metabolites in the brain effluent, suggesting a shift of catecholamines to extracellular sites (128). This effect was potentiated when the ventriculo-cisternal perfusion contained angiotensin. Hence, angiotensin probably blocks reuptake of norepinephrine released endogenously by electrical stimulation. Palaic and Khairallah (127) suggested that angiotensin acts on the cell membrane amine pump, probably by altering ion movements. Some support for an ionic requirement is provided by the observation that central angiotensin pressor activity is inhibited by hypocalcemia (34).

C. Macromolecular Effects

Recently, data have been obtained demonstrating that angiotensin exerts effects on macromolecular systems of many tissues (98).

In vivo experiments in which angiotensin (45 ng/kg/min) was infused intravenously to rats after an intravenous injection of ^{3}H-thymidine showed that the peptide increased the specific activity and content of DNA in areas of the heart and kidney. Similar experiments where ^{3}H-uridine was utilized demonstrated that angiotensin significantly increased the incorporation of uridine into RNA of spleen, liver, brain, and regions of heart and kidney. A significantly increased RNA content was also found. Interestingly, these effects were measured only 2 hr after injection of the isotopic precursors. Angiotensin appears to stimulate the synthesis of aldosterone and norepinephrine by increasing protein synthesis in slices of adrenal glands and atria, respectively (139). The mechanism or significance of these macromolecular effects of angiotensin cannot be assessed at this time. However, it is possible that they may be partly associated with tyrosine hydroxylase synthesis.

VI. Central Angiotensin Effects in Man

Data suggest that the cardiovascular effects produced by angiotensin-CNS interaction in animals also occur in man. No data are yet available to determine whether the central effects of angiotensin on salt and water balance occur in man. However, renal mechanisms have been implicated in human thirst (59). Moreover, human brain contains renin (68); in rats, brain renin activity correlated with brain hydration and electrolytes (69).

Johnsson *et al.* (83, 86) found that intravenous infusions of angiotensin (2–3 μg/min) or norepinephrine (17–20 μg) reduced blood flow in the human hand. Local intra-arterial injections of phenoxybenzamine to one hand blocked the effect of both vasopressors only in the treated hand. Intra-arterial injection of guanethidine antagonized the effects of angiotensin, but not norepinephrine, on hand blood flow. These data suggest that angiotensin produced elevated resistance in the hand circulation by a sympathetic mechanism. Johnsson and colleagues (83, 86) investigated angiotensin activity further by infusing the peptide intravenously in patients while recording venous pressure in an arm occluded by a suprasystolic cuff. Venous pressure was increased by angiotensin in these experiments. Since the peptide did not reach the test arm because of the suprasystolic cuff, the indirect action of angiotensin must have occurred at a site proximal to sympathetic postganglionic terminals. Independently, Whelan and colleagues (146, 174, 175) made similar observations that intravenous angiotensin effects on hand blood flow could be antagonized by *alpha* receptor or adrenergic neuron blockade. Furthermore, these investigators showed that angiotensin effects on hand flow were absent after nerve block and in patients with idiopathic autonomic degeneration, sustained brachial plexus avulsion, surgical sympathectomy, and, in one patient, chronic cervical spinal damage. The circulation of the foot was found to respond to angiotensin in a manner similar to the hand. However, hand and foot circulations are not representative of all vascular beds. The forearm flow, for example, appears more sensitive to direct angiotensin effects (146). Evaluation of the direct *versus* the indirect effects of angiotensin on other regional circulations in man has, to our knowledge, not been clarified. Although the data did not clearly differentiate a ganglionic from a central site of angiotensin activity, a central mechanism was postulated because: a) no clinical evidence of widespread autonomic stimulation was observed; b) the sympathetic effect of angiotensin was absent in the cord-damaged patient although ganglia were intact; and (c) different sensitivities of various vascular beds to indirect angiotensin effects could be more precisely integrated by the brain.

Ueda *et al.* (166) injected angiotensin into the vertebral arteries of human volunteers. Their data showed that angiotensin (1–2.5 μg) produced greater rises in systolic and diastolic blood pressures than aortic injec-

tion of the same doses. Whereas aortic injections produced bradycardia, vertebral administration of angiotensin generally elevated heart rate. Finally, Finkielman *et al.* (57) obtained data suggesting that angiotensin interaction in the CNS may be relevant to human essential hypertension. These investigators state: "A pressor polypeptide was isolated from the cerebrospinal fluid of normotensive and hypertensive patients. Pharmacologically it behaves like angiotensin I. A very significant correlation ($r = 0.83, P < 0.001$) was found between the concentration of this polypeptide and the blood pressure of essential hypertensive patients."

Such clinical data, taken with many animal studies, suggest the possibility that central angiotensin effects may be involved in hypertensive pathophysiology.

VII. Summary and Conclusions

There is general agreement that the biological effects of angiotensin are complex. Although angiotensin is often regarded as a renal hormone and is associated with vasoconstriction and aldosterone release, the presence of components of the renin/angiotensin system in many tissues suggests a broader role for the peptide. The brain is one organ which contains and responds to angiotensin. When angiotensin reaches appropriate brain structures, a rise in blood pressure, thirst, and antidiuretic hormone release ensue. Technical problems limit precise analysis of whether endogenous angiotensin exerts these effects. There are several ways in which angiotensin can interact with the central nervous system. a) Much literature suggests that circulating angiotensin may influence brain structures outside of the BBB. b) A smaller amount of data suggests that circulating angiotensin could reach ventricular cerebrospinal fluid. c) Angiotensin may be produced locally and released at responsive brain sites.

It would be premature to assign any physiological or pathophysiological role to central angiotensin effects. On the other hand, it is reasonable to propose that they may contribute to the maintenance of some hypertensive states and disorders of fluid/electrolyte balance. How these central effects of angiotensin are integrated with the peripheral effects of the peptide is unknown. It is of interest that sodium and calcium are frequently identified as requirements for the activity of the peptide. Angiotensin may be a local regulator of the movement of these ions. Much research will be necessary to evaluate the above possibilities.

VIII. Addendum

The following papers have come to our attention after submission of the original manuscript. Minnich *et al.* (Union Méd. Can. **102:** 903–906, 1973) reported that angiotensin injections into the cerebroventricles of rats increase dopamine levels in the hypothalamus, pons-medulla, striatum and cerebellum. Norepinephrine levels in the hippocampus and cerebellum were also increased. Goldstein *et al.* (J. Neurochem. **19:** 2451–2452, 1972) reported on the presence and partial purification of angiotensinase activity in rat and dog brain. Fukiyama (Jap. Heart J. **14:** 135–139, 1973) reported that stimulation of the carotid sinus nerve of dogs causes less bradycardia and hypotension when angiotensin is infused through the vertebral arteries.

Acknowledgement. The authors appreciate the professional help given by the Hershey Medical Center Library Staff.

REFERENCES

1. Aars, H. and Akre, S.: Effect of angiotensin on sympathetic nerve activity. Acta Physiol. Scand. **74:** 134–141, 1968.
2. Abrash, L., Walter, R. and Marks, N.: Inactivation studies of angiotensin II by purified enzymes. Experientia (Basel) **27:** 1352–1353, 1971.
3. Andersson, B.: Thirst—and brain control of water balance. Amer. Sci. **59:** 408–415, 1971.
4. Andersson, B. and Eriksson, L.: Conjoint action of sodium and angiotensin on brain mechanisms controlling water and salt balance. Acta Physiol. Scand. **81:** 18–29, 1971.
5. Andersson, B., Eriksson, L. and Fernandez, O.: Reinforcement by Na^+ of centrally mediated hypertensive response to angiotensin II. Life Sci. **10:** 633–638, 1971.
6. Andersson, B., Eriksson, L. and Oltner, R.: Further evidence for angiotensin sodium interaction in central control of fluid balance. Life Sci. **9:** 1091–1096, 1970.

7. Andersson, B. and Westbye, O.: Synergistic action of sodium and angiotensin on brain mechanisms controlling fluid balance. Life Sci. 9: 601-608, 1970.
8. Barker, J. L., Crayton, J. W. and Nicoll, R. A.: Noradrenaline and acetylcholine responses of supraoptic neurosecretory cells. J. Physiol. (London) 218: 19-32, 1971.
9. Barrett, J. P., Ingenito, A. J. and Procita, L.: Influence of the carotid sinus on centrally mediated peripheral cardiovascular effects of angiotensin II. J. Pharmacol. Exp. Ther. 176: 692-700, 1971.
10. Barth, C.: Aktivität der catechol-ortho-methyltransferase unter angiotensin-einfluß und beim DOCA—hochdruck der ratte. Z. Klin. Chem. Klin. Biochem. 8: 379-382, 1970.
11. Beaulnes, A. and Ling, G. M.: Effect of angiotensin and bradykinin on brain acetylcholine. Fed. Proc. 27: 471, 1968.
12. Beck, L.: Histamine as the potential mediator of active reflex dilatation. Fed. Proc. 24: 1298-1310, 1965.
13. Benetato, Gr., Haulica, I., Uluitu, M., Bubuianu, E., Mocodean, J., Stefanescu, P. and Suhaciu, G.: The central nervous action of angiotensin on aldosterone secretion and electrolytic balance. Int. J. Neuropharmacol. 3: 565-570, 1964.
14. Bianchi, A., DeSchaepdryver, A. F., DeVleeschhouwer, G. R. and Preziosi, P.: On the pharmacology of synthetic hypertensine. Arch. Int. Pharmacodyn. Ther. 124: 21-44, 1960.
15. Bickerton, R. K. and Buckley, J. P.: Evidence for a central mechanism in angiotensin-induced hypertension. Proc. Soc. Exp. Biol. Med. 106: 834-836, 1961.
16. Biron, P., Meyer, P. and Panisset, J. C.: Removal of angiotensins from the systemic circulation. Can. J. Physiol. Pharmacol. 46: 175-178, 1968.
17. Blass, E. M. and Chapman, H. W.: An evaluation of the contribution of cholinergic mechanisms to thirst. Physiol. Behav. 7: 679-686, 1971.
18. Bock, K. D., Brown, J. J., Lever, A. F., Robertson, J. I. S. and Masson, G. M. C.: Effects of renin and angiotensin on excretion and distribution of water and salts. *In* Renal Hypertension, ed. by I. Page and J. McCubbin, Year Book Medical Publishers, Inc., Chicago, 1968.
19. Booth, D. A.: Mechanism of action of norepinephrine in eliciting an eating response on injection into the rat hypothalamus. J. Pharmacol. Exp. Ther. 160: 336-348, 1968.
20. Bourdois, P. and Panisset, J. C.: Mesenteric nerve stimulation by intracarotid administration of angiotensin. Rev. Can. Biol. 27: 167-169, 1968.
21. Braun-Menendez, E., Fasciolo, E., Leloir, L. F. and Munoz, J. M.: The substance causing renal hypertension. J. Physiol. (London) 98: 283-298, 1940.
22. Brightman, M. W. and Palay, S. L.: The fine structure of ependyma in the brain of the rat. J. Cell Biol. 19: 415-439, 1963.
23. Buckley, J. P.: Actions of angiotensin on the central nervous system. Fed. Proc. 31: 1332-1337, 1972.
24. Buckley, J. P., Bickerton, R. K., Halliday, R. P. and Kato, H.: Central effects of peptides on the cardiovascular system. Ann. N. Y. Acad. Sci. 104: 299-310, 1963.
25. Bumpus, F. M., Smeby, R. R., Page, I. H. and Khairallah, P. A.: Distribution and metabolic fate of angiotensin II and various derivatives. Can. Med. Ass. J. 90: 190-193, 1964.
26. Capek, R., Masek, K., Sramka, M., Krsiak, M. and Svec, P.: The similarities of the angiotensin and bradykinin action on the central nervous system. Pharmacology 2: 161-170, 1969.
27. Claybaugh, J. R. and Share, L.: Role of the renin-angiotensin system in the vasopressin response to hemorrhage. Endocrinology 90: 453-460, 1972.
28. Claybaugh, J. R., Share, L. and Shimizu, K.: The inability of infusions of angiotensin to elevate the plasma vasopressin concentration in the anesthetized dog. Endocrinology 90: 1647-1652, 1972.
29. Covian, M. R., Gentil, C. G. and Antunes-Rodrigues, J.: Water and sodium chloride intake following microinjections of angiotensin II into the septal area of the rat brain. Physiol. Behav. 9: 373-377, 1972.
30. Cranston, W. I., Lavery, H., Lowe, R. D. and Rosendorff, C.: The central pressor effect of angiotensin in the rabbit. J. Physiol. (London) 198: 30P-31P, 1968.
31. Cuparencu, B., Tisca, I., Safta, L., Csutak, V. and Mocan, R.: The influence of intraventricularly administered angiotensin II, bradykinin and eledoisin upon arterial blood pressure, respiration and electrocardiogram. Acta Biol. Med. Ger. 27: 435-441, 1971.
32. Cushman, D. W. and Cheung, H. S.: Concentrations of angiotensin-converting enzyme in tissues of the rat. Biochim. Biophys. Acta 250: 261-265, 1971.
33. Cushman, D. W. and Cheung, H. S.: Studies *in vitro* of angiotensin-converting enzyme of lung and other tissues. *In* Hypertension—1972, ed. by J. Genest and E. Koiw, Springer-Verlag, New York, 1972.
34. Daniels, A. E. and Buckley, J. P.: Relation of the centrally evoked pressor effect of angiotensin II to the calcium ion. Eur. J. Pharmacol. 4: 152-164, 1968.
35. Daniels, A. E., Ogden, E. and Vernikos-Danellis, J.: Effects of centrally administered angiotensin II in the unanesthetized rat. Physiologist 12: 205, 1969.
36. Daniels-Severs, A., Ogden, E. and Vernikos-Danellis, J.: Centrally mediated effects of angiotensin II in the unanesthetized rat. Physiol. Behav. 7: 785-787, 1971.
37. Deuben, R. R.: Studies on the central nervous system component of the angiotensin induced pressor response. Ph.D. Thesis, University of Pittsburgh, Pittsburgh, 1969.
38. Deuben, R. R. and Buckley, J. P.: Identification of a central site of action of angiotensin II. J. Pharmacol. Exp. Ther. 175: 139-146, 1970.
39. Dickinson, C. J. and Lawrence, J. R.: A slowly developing pressor response to small concentrations of angiotensin—its bearing on the pathogenesis of chronic renal hypertension. Lancet 1: 1354-1356, 1963.
40. Dickinson, C. J. and Yu, R.: The progressive pressor response to angiotensin in the rabbit. J. Physiol. (London) 190: 91-99, 1967.
41. Dickinson, C. J. and Yu, R.: Mechanisms involved in the progressive pressor response to very small amounts of angiotensin in conscious rabbits. Circ. Res. 21: suppl. 2, 157-163, 1967.
42. Dock, W.: Vasoconstriction in renal hypertension abolished by pithing. Amer. J. Physiol. 130: 1-8, 1940.
43. Dock, W. and Rytand, D. A.: Absence of vasoconstrictor substance in blood of rats with renal hypertension. Proc. Soc. Exp. Biol. Med. 32: 374-375, 1934.
44. Dock, W., Shidler, F. P. and Moy, B.: Vasomotor center essential in maintaining renal hypertension. Amer. Heart J. 23: 513-521, 1942.
45. Dutta, S. N., Davis, D. A., Booker, W. M. and Pradhan, S. N.: Responses to microinjections of angiotensin into the hypothalamus of cats. Neuropharmacology 10: 231-236, 1971.
46. Duvernoy, H. and Koritke, J. G.: Contribution a l'etude de l'angioarchitectonie des organes circumventriculaires. Arch. Biol. 75: suppl., 849-904, 1964.
47. Dyball, R. E. J. and Koizumi, K.: Electrical activity in the supraoptic and paraventricular nuclei associated with neurohypophysial hormone release. J. Physiol. (London) 201: 711-722, 1969.
48. Elie, R. and Panniset, J.: Effect of angiotensin and atropine on the spontaneous release of acetylcholine from cat cerebral cortex. Brain Res. 17: 297-305, 1970.
49. Enoch, D. M. and Kerr, F. W. L.: Hypothalamic vasopressor and vesicopressor pathways I. Arch. Neurol. 16: 290-306, 1967.
50. Enoch, D. M. and Kerr, F. W. L.: Hypothalamic vasopressor and vesicopressor pathways II. Arch. Neurol. 16: 307-320, 1967.
51. Epstein, A. N., Fitzsimons, J. T. and Johnson, A. K.:

Prevention by angiotensin II antiserum of drinking induced by intracranial angiotensin. J. Physiol. (London) **230**: 42P–43P, 1973.

52. Epstein, A. N., Fitzsimons, J. T. and Rolls, B. J. (neé (Simons): Drinking induced by injection of angiotensin into the brain of the rat. J. Physiol. (London) **210**: 457–474, 1970.
53. Epstein, A. N., Fitzsimons, J. T. and Simons, B. J.: Drinking caused by the intracranial injection of angiotensin into the rat. J. Physiol. (London) **200**: 98P–100P, 1969.
54. Fernandez, L. A.: Effects of renin and angiotensin on water intake. Medicina (Buenos Aires) **32**: 63–67, 1972.
55. Ferrario, C. M., Dickinson, C. J. and McCubbin, J. W.: Central vasomotor stimulation by angiotensin. Clin. Sci. (London) **39**: 239–245, 1970.
56. Ferrario, C. M., Gildenberg, P. L. and McCubbin, J. W.: Cardiovascular effects of angiotensin mediated by the central nervous system. Circ. Res. **30**: 257–262, 1972.
57. Finkielman, S., Fischer-Ferraro, C., Diaz, A., Goldstein, D. J. and Nahmod, V. E.: A pressor substance in the cerebrospinal fluid of normotensive and hypertensive patients. Proc. Nat. Acad. Sci. U.S.A. **69**:3341–3344, 1972.
58. Fischer-Ferraro, C., Nahmod, V. E., Goldstein, D. J. and Finkielman, S.: Angiotensin and renin in rat and dog brain. J. Exp. Med. **133**:353–361, 1971.
59. Fitzsimons, J. T.: The physiology of thirst: A review of the extraneural aspects of the mechanisms of drinking. *In* Progress in Physiological Psychology, vol. 4, ed. by E. Stellar and J. Sprague, pp. 119–201, Academic Press, New York, 1971.
60. Fitzsimons, J. T.: The effect on drinking of peptide precursors and of shorter chain peptide fragments of angiotensin II injected into the rat's diencephalon. J. Physiol. (London) **214**: 293–303, 1971.
61. Fitzsimons, J. T.: Thirst. Physiol. Rev. **52**: 468–561, 1972.
62. Fitzsimons, J. T. and Setler, P. E.: Catecholaminergic mechanisms in angiotensin-induced drinking. J. Physiol. (London) **218**: 43P–44P, 1971.
63. Forgacs, I. and Gaal, K.: Effect of sodium and osmotic concentration on renin release by renal cortical slices *in vitro*. Acta Physiol. Acad. Sci. Hung. **40**: 139–144, 1971.
64. Fukiyama, K.: Central action of angiotensin and hypertension-increased central vasomotor outflow by angiotensin. Jap. Circ. J. **36**: 599–602, 1972.
65. Fukiyama, K., McCubbin, J. W. and Page, I. H.: Chronic hypertension elicited by infusion of angiotensin into vertebral arteries of unanesthetized dogs. Clin. Sci. (London) **40**: 283–291, 1971.
66. Gagnon, D. J., Cousineau, D. and Boucher, P. J.: Release of vasopressin by angiotensin II and prostaglandin E_2 from the rat neurohypophysis in vitro. Life Sci. **12**: 487–497, 1973.
67. Gann, D. S.: Parameters of the stimulus initiating the adrenocortical response to hemorrhage. Ann. N. Y. Acad. Sci. **156**: 740–755, 1969.
68. Ganten, D., Boucher, R. and Genest, J.: Renin activity in brain tissue of puppies and adult dogs. Brain Res. **33**: 557–559, 1971.
69. Ganten, D., Kusumoto, M., Constantopoulos, G., Ganten, U., Boucher, R., and Genest, J.: Iso-renin, electrolytes and catecholamines in dog brain: possible interrelationship. Life Sci. **12**: 1–8, 1973.
70. Ganten, D., Marquez-Julio, A., Granger, P., Hayduk, K. Karsunky, K. P., Boucher, R. and Genest, J.: Renin in dog brain. Amer. J. Physiol. **221**: 1733–1737, 1971.
71. Ganten, D., Minnich, J., Granger, P., Hayduk, K., Brecht, H. M., Barbeau, A., Boucher, R. and Genest, J.: Angiotensin-forming enzyme in brain tissue. Science **173**: 64–65, 1971.
72. Giardina, A. R. and Fisher, A. E.: Effect of atropine on drinking induced by carbachol, angiotensin and isoproterenol. Physiol. Behav. **7**: 653–655, 1971.
73. Gildenberg, P. L.: Localization of a site of angiotensin vasopressor activity in the brain. Physiologist **12**: 235, 1969.
74. Gildenberg, P. L.: Site of angiotensin vasopressor activity in the brainstem. Fed. Proc. **30**: 432, 1971.
75. Gildenberg, P. L., Ferrario, C. M., and McCubbin, J. W.: Two sites of cardiovascular action of angiotensin II in the brain of the dog. Clin. Sci. (London) **44**: 417–420, 1973.
76. Glenn, F., Child, C. G. and Page, I. H.: Effect of destruction of spinal cord on hypertension artificially produced in dogs. Amer. J. Physiol. **122**: 506–510, 1938.
77. Goldman, W., Lehr, D. and Frank, E.: Pharmacologic characterization of the dipsogenic property of angiotensin II (ANG). Fed. Proc. **31**: 573, 1972.
78. Gutman, Y., Shamir, Y., Glushevitzky, D. and Hochman, S.: Angiotensin increases microsomal (Na^+-K^+)-ATPase activity in several tissues. Biochim. Biophys. Acta **273**: 401–405, 1972.
79. Gyang, E. A., Deuben, R. R. and Buckley, J. P.: Interaction of prostaglandin E_1 and angiotensin II on centrally mediated pressor activities in the cat. Proc. Soc. Exp. Biol. Med. **142**: 532–537, 1973.
80. Hageman, W. E., Hofmann, K., Ertel, R. J. and Buckley, J. P.: Some aspects of the pharmacology of valyl[5]-β-(pyrazolyl-3-)-L-alanyl[8]-angiotensin II. J. Pharmacol. Exp. Ther. **168**: 295–302, 1969.
81. Halliday, R. P. and Buckley, J. P.: Central hypertensive effects of angiotensin II. Int. J. Neuropharmacol. **1**: 43–47, 1962.
82. Hendler, N. H. and Blake, W. D.: Hypothalamic implants of angiotensin II, carbachol, and norepinephrine on water and NaCl solution intake in rats. Commun. Behav. Biol. **4**: 41–48, 1969.
83. Henning, M. and Johnsson, G.: Interference of phenoxybenzamine and guanethidine with the vasoconstrictor responses of noradrenaline and angiotensin II in the hand. Acta Pharmacol. Toxicol. **25**: 373–384, 1967.
84. Hodge, R. L., Ng, K. K. F. and Vane, J. R.: Disappearance of angiotensin from the circulation of the dog. Nature (London) **215**: 138–141, 1967.
85. Janowsky, D. S., Davis, J. M., Fann, W. E., Freeman, J., Nixon, R. and Michelakis, A. A.: Angiotensin effect on uptake of norepinephrine by synaptosomes. Life Sci. **11**: 1–11, 1972.
86. Johnsson, G., Henning, J. and Ablad, B.: Studies on the mechanism of the vasoconstrictor effect of angiotensin II in man. Life Sci. **4**: 1549–1554, 1965.
87. Joy, M. D.: The intramedullary connections of the area postrema involved in the central cardiovascular response to angiotensin II. Clin. Sci. (London) **41**: 89–100, 1971.
88. Joy, M. D. and Lowe, R. D.: Evidence that the area postrema mediates the central cardiovascular response to angiotensin II. Nature (London) **228**: 1303–1304, 1970.
89. Joy, M. D. and Lowe, R. D.: Abolition of the central cardiovascular effects of angiotensin by ablation of the area postrema. Clin. Sci. (London) **39**: 3P, 1970.
90. Joy, M. D. and Lowe, R. D.: The site of cardiovascular action of angiotensin II in the brain. Clin. Sci. (London) **39**: 327–336, 1970.
91. Kaneko, Y., McCubbin, J. W. and Page, I. H.: Mechanism by which serotonin, norepinephrine and reserpine cause central vasomotor inhibition. Circ. Res. **8**: 1228–1234, 1960.
92. Kawamura, H.: A study of hypertension. The effects of angiotensin II on the MAO activity and roles of calcium ion on the catecholamine contents in the rat hypothalamus and striatum. Jap. Circ. J. **36**: 349–362, 1972.
93. Keim, K. L. and Sigg, E. B.: Activation of central sympathetic neurons by angiotensin II. Life Sci. **10**: 565–574, 1971.
94. Khairallah, P. A. (ed.): Vascular reactivity: Experi-

mental and clinical. *In* Renal Hypertension, ed. by I. H. Page and J. W. McCubbin, pp. 193–194, Year Book Medical Publishers, Inc., Chicago, 1968.

95. KHAIRALLAH, P. A.: Pharmacology of angiotensin. *In:* Kidney Hormones, ed. by J. W. Fisher, pp. 129–171, Academic Press, New York, 1971.
96. KHAIRALLAH, P. A.: Action of angiotensin on adrenergic nerve endings: inhibition of norepinephrine uptake. Fed. Proc. **31:** 1351–1357, 1972.
97. KHAIRALLAH, P. A., PAGE, I. H., BUMPUS, F. M. AND SMEBY, R. R.: Angiotensin II: Its metabolic fate. Science **138:** 523–525, 1962.
98. KHAIRALLAH, P. A., ROBERTSON, A. L. AND DAVILA, D.: Effects of angiotensin II on DNA, RNA, and protein synthesis. *In* Hypertension—1972, ed. by J. Genest and E. Koiw, pp. 212–220, Springer-Verlag, Berlin, 1972.
99. KOZLOWSKI, S., DRZEWIECKI, K. AND ZURAWSKI, W.: Relationship between osmotic reactivity of the thirst mechanism and the angiotensin and aldosterone blood levels in dogs. Acta Physiol. Pol. **23:** 417–425, 1972.
100. LAVERTY, R.: A nervously mediated action of angiotensin in anesthetized rats. J. Pharm. Pharmacol. **15:** 63–68, 1963.
101. LEHR, D., MALLOW, J. AND KRUKOWSKI, M.: Copious drinking and simultaneous inhibition of urine flow elicited by beta-adrenergic stimulation and contrary effect of alpha-adrenergic stimulation. J. Pharmacol. Exp. Ther. **158:** 150–163, 1967.
102. LEVY, B. AND AHLQUIST, R. P.: Adrenergic drugs. *In* Drill's Pharmacology in Medicine, ed. by J. R. DiPalma, pp. 666, McGraw-Hill, New York, 1971.
103. LOWE, R. D. AND SCROOP, G. C.: The cardiovascular response to vertebral artery infusions of angiotensin in the dog. Clin. Sci. (London) **37:** 593–603. 1969.
104. MALVIN, R. L.: Possible role of the renin-angiotensin system in the regulation of antidiuretic hormone secretion. Fed. Proc. **30:** 1383–1386, 1971.
105. MATSUNAGA, M., SAITO, N., OGINA, K., TAKAYASU, M. AND KIRA, J.: Acid angiotensinase, renin, and acid adenosine triphosphatase in rat kidney lysosomes. Jap. Circ. J. **32:** 137–143, 1968.
106. MCCUBBIN, J. W., SOARES DEMOURA, R., PAGE, I. H. AND OLMSTED, F.: Arterial hypertension elicited by subpressor amounts of angiotensin. Science **149:** 1395–1396, 1965.
107. MCFARLAND, D. J. AND ROLLS, B.: Suppression of feeding by intracranial injections of angiotensin. Nature (London) **236:** 172–173, 1972.
108. MINNICH, J. L., GANTEN, D., BARBEAU, A. AND GENEST J.: Subcellular localization of cerebral renin-like activity. *In* Hypertension—1972, ed. by J. Genest and E. Koiw, pp. 432–435, Springer-Verlag, Berlin, 1972.
109. MORRISON, J. F. B. AND PICKFORD, M.: Effects of angiotensin and noradrenaline on discharge in fibres of the cervical sympathetic nerve in cats and dogs. Brit. J. Pharmacol. **41:** 674–682, 1971.
110. MOUW, D., BONJOUR, J., MALVIN, R. L. AND VANDER, A.: Central action of angiotensin stimulating ADH release. Amer. J. Physiol. **220:** 239–242, 1971.
111. MULROW, P. J. AND GOFFINET, J. A.: The renin-angiotensin system. *In* Physiology of the Human Kidney, ed. by L. G. Wesson, pp. 465–520, Grune & Stratton, New York, 1969.
112. MURRAY, R., BECK, L., RONDELL, P. A. AND BOHR, D. F.: A study of the central action of ganglionic blocking agents. J. Pharmacol. Exp. Ther. **127:** 157–163, 1959.
113. MYERS, R. D., HALL, G. H. AND RUDY, T. A.: Drinking in the monkey evoked by nicotine or angiotensin II microinjected in hypothalamic and mesencephalic sites. Pharmacol. Biochem. Behav. **1:** 15–22, 1973.
114. NAHMOD, V. E., FISCHER-FERRARO, C., FINKIELMAN, S., DIAZ, A. AND GOLDSTEIN, D. J.: Renin and angiotensin in extra-renal tissues. Medicina, (Buenos-Aires) **32:** (suppl. 1, 43–47, 1972.
115. NASHOLD, B. S., MANNARINO, E. AND WUNDERLICH, M.: Pressor-depressor blood pressure responses in the cat after intraventricular injection of drugs. Nature (London) **193:** 1297–1298, 1962.
116. NG, K. K. F. AND VANE, J. R.: Conversion of angiotensin I to angiotensin II. Nature (London) **216:** 762–766, 1967.
117. NG, K. K. F. AND VANE, J. R.: Fate of angiotensin I in the circulation. Nature (London) **218:** 144–150, 1968.
118. NICOLL, R. A. AND BARKER. J. L.: Excitation of supraoptic neurosecretory cells by angiotensin II. Nature New Biol. **233:** 172–174, 1971.
119. OELKERS, W., DUSTERDIECK, G. AND MORTON, J. J.: Arterial angiotensin II and venous immunoreactive material before and during angiotensin infusion in man. Clin. Sci. (London) **43:** 209–218, 1972.
120. OGDEN, E.: Extra-renal sequel to experimental renal hypertension. Bull. N. Y. Acad. Med. **23:** 643–660, 1947.
121. OGDEN, E., COLLINGS, W. D. AND SAPIRSTEIN, L. A.: A change of mechanism in the course of hypertension of renal origin. *In* Experimental Hypertension, a special publication of N. Y. Acad. Sci. **3:** 153–167, 1946.
122. OLSSON, K.: On the importance of CSF Na^+ concentration in central control of fluid balance. Life Sci. **11:** 397–402, 1972.
123. PAGE, I. H. AND BUMPUS, F. M.: Angiotensin. Physiol. Rev. **41:** 331–390, 1961.
124. PAGE, I. H. AND HELMER, O. M.: A crystalline pressor substance (angiotonin) resulting from the reaction between renin and renin-activator. J. Exp. Med. **71:** 29–42, 1940.
125. PAGE, I. H. AND MCCUBBIN, J. W. (eds.): Renal Hypertension, Year Book Medical Publishers, Inc., Chicago, 1968.
126. PALAIC, D. AND KHAIRALLAH, P. A.: Inhibition of noradrenaline uptake by angiotensin. J. Pharm. Pharmacol. **19:** 396–397, 1967.
127. PALAIC, D. AND KHAIRALLAH, P. A.: Effect of angiotensin on uptake and release of norepinephrine by brain. Biochem. Pharmacol. **16:** 2291–2298, 1967.
128. PALAIC, D. AND KHAIRALLAH, P. A.: Inhibition of norepinephrine reuptake by angiotensin in brain. J. Neurochem. **15:** 1195–1202, 1968.
129. PANISSET, J. C.: Effect of angiotensin on the release of acetylcholine from preganglionic and postganglionic nerve endings. Can. J. Physiol. Pharmacol. **45:** 313–318, 1967.
130. PAVEL, S. AND COCULESCU, M.: Arginine vasotocin-like activity of cerebrospinal fluid induced by injection of hypertonic saline into the third cerebral ventricle of cats. Endocrinology **91:** 825–827, 1972.
131. PEART, W. S.: The renin-angiotensin system. Pharmacol. Rev. **17:** 143–182, 1965.
132. PICKERING, G. W.: Observations on mechanism of arterial hypertension in acute nephritis. Clin. Sci. (London) **2:** 363–372, 1936.
133. RADIO, G. J., SUMMY-LONG, J., DANIELS-SEVERS, A. AND SEVERS, W. B.: Hydration changes produced by central infusion of angiotensin II. Amer. J. Physiol. **223:** 1221–1226, 1972.
134. RICHARDSON, J. B. AND BEAULNES, A.: The cellular site of action of angiotensin. J. Cell Biol. **51:** 419–432, 1971.
135. ROBERTSON, P. A. AND RUBIN, D.: Stimulation of intestinal nervous elements by angiotensin. Brit. J. Pharmacol. **19:** 5–12, 1962.
136. ROLLS, B. J. AND JONES, B. P.: Cessation of drinking following intracranial injections of angiotensin in the rat. J. Comp. Physiol. Psychol. **80:** 26–29, 1972.
137. ROLLS, B. J., JONES, B. P. AND FALLOWS, D. J.: A comparison of the motivational properties of thirst induced by intracranial angiotensin and by water deprivation. Physiol. Behav. **9:** 777–782, 1972.
138. ROSENDORFF, C., LOWE, R. D., LAVERY, H. AND CRANSTON, I.: Cardiovascular effects of angiotensin mediated by the central nervous system of the rabbit. Cardiovasc. Res. **4:** 36–43, 1970.
139. ROTH, R. H.: Action of angiotensin on adrenergic nerve

endings: enhancement of norepinephrine biosynthesis. Fed. Proc. 31: 1358–1364, 1972.

140. Roth, M., Weitzman, A. F. and Piquilloud, Y.: Converting enzyme content of different tissues of the rat. Experientia (Basel) 25: 1247, 1969.
141. Schally, A. V., Carter, W. H., Hearn, I. C. and Bowers, C. Y.: Determination of CRF activity in rats treated with monase, dexamethasone and morphine. Amer. J. Physiol. 209: 1169–1174, 1965.
142. Schmitt, H. and Schmitt, H.: Increased activity in sympathetic nerves induced by angiotensin. Rev. Can. Biol. 27: 255–257, 1968.
143. Scroop, G. C., Katic, F., Joy, M. D. and Lowe, R. D.: Importance of central vasomotor effects in angiotensin-induced hypertension. Brit. Med. J. 1: 324–326, 1971.
144. Scroop, G. C. and Lowe, R. D.: Efferent pathways of the cardiovascular response to vertebral artery infusions of angiotensin in the dog. Clin. Sci. (London) 37: 605–619, 1969.
145. Scroop, G. C. and Lowe, R. D.: Central pressor effect of angiotensin mediated by the parasympathetic nervous system. Nature (London) 220: 1331–1332, 1968.
146. Scroop, G. C. and Whelan, R. F.: A central vasomotor action of angiotensin in man. Clin. Sci. (London) 30: 79–90, 1966.
147. Severs, W. B.: Unpublished data.
148. Severs, W. B. and Buckley, J. P.: Spontaneous activity of the superior cervical nerve following central administration of angiotensin II. Pharmacology 3: 187–190, 1970.
149. Severs, W. B., Daniels, A. E. and Buckley, J. P.: The effect of pregnancy on the central sympathetic components in angiotensin-induced hypertension. J. Pharm. Pharmacol. 18: 475–476, 1966.
150. Severs, W. B., Daniels, A. E. and Buckley, J. P.: On the central hypertensive effect of angiotensin II. Int. J. Neuropharmacol. 6: 199–205, 1967.
151. Severs, W. B., Daniels, A. E., Smookler, H. H., Kinnard, W. J. and Buckley, J. P.: Interrelationship between angiotensin II and the sympathetic nervous system. J. Pharmacol. Exp. Ther. 153: 530–537, 1966.
152. Severs, W. B., Daniels-Severs, A., Summy-Long, J. and Radio, G. J.: Effects of centrally administered angiotensin II on salt and water excretion. Pharmacology 6: 242–252, 1971.
153. Severs, W. B., Summy-Long, J. and Daniels-Severs, A.: Effect of a converting enzyme inhibitor (SQ 20,881) on angiotensin-induced drinking. Proc. Soc. Exp. Biol. Med. 142: 203–204, 1973.
154. Severs, W. B., Summy-Long, J., Daniels-Severs, A. and Connor, J. D.: Influence of adrenergic blocking drugs on central angiotensin effects. Pharmacology 5: 205–214, 1971.
155. Severs, W. B., Summy-Long, J., Taylor, J. S. and Connor, J. D.: A central effect of angiotensin: release of pituitary pressor material. J. Pharmacol. Exp. Ther. 174: 27–34, 1970.
156. Share, L. and Claybaugh, J. R.: Regulation of body fluids. Annu. Rev. Physiol. 34: 235–260, 1972.
157. Shimizu, K., Share, L. and Claybaugh, J. R.: Potentiation by angiotensin II of the vasopressin response to an increasing plasma osmolality. Endocrinology 93: 42–50, 1973.
158. Smookler, H. H., Severs, W. B., Kinnard, W. J. and Buckley, J. P.: Centrally mediated cardiovascular effects of angiotensin II. J. Pharmacol. Exp. Ther. 153: 485–494, 1966.
159. Suga, T., Manger, W. M. and Reich, T.: Effect of angiotensin II on activity of the sympathetic nervous system. Physiologist 14: 240, 1971.
160. Swanson, L. W., Marshall, G. R., Needleman, P. and Sharpe, L. G.: Characterization of central angiotensin II receptors involved in the elicitation of drinking in the rat. Brain Res. 49: 441–446, 1973.
161. Sweet, C. S. and Brody, M. J.: Central inhibition of reflex vasodilatation by angiotensin and reduced renal pressure. Amer. J. Physiol. 219: 1751–1758, 1970.
162. Sweet, C. S., Ferrario, C. M., Khosla, M. C. and Bumpus, F. M.: Antagonism of peripheral and central effects of angiotensin II by (1-sarcosine, 8-isoleucine) angiotensin II. J. Pharmacol. Exp. Ther. 185: 35–41, 1973.
163. Sweet, C. S., Kadowitz, P. J. and Brody, M. J.: Arterial hypertension elicited by prolonged intravertebral infusion of angiotensin II in conscious dog. Amer. J. Physiol. 221: 1640–1644, 1971.
164. Tigerstedt, R. and Bergman, P. G.: Niere und kreislauf. Skand. Arch. Physiol. 8: 223, 1898.
165. Ueda, H., Katayama, S. and Kato, R.: Area postrema-angiotensin-sensitive site in brain. Advan. Exp. Biol. Med. 17: 109–116, 1972.
166. Ueda, H., Uchida, Y., Ueda, K., Gondaira, T. and Katayama, S.: Centrally mediated vasopressor effect of angiotensin II in man. Jap. Heart J. 10: 243–247, 1969.
167. Vane, J. R.: The release and fate of vasoactive hormones in the circulation. Brit. J. Pharmacol. 35: 209–242, 1969.
168. Vane, J. R.: Sites of conversion of angiotensin I. *In* Hypertension—1972, ed. by J. Genest and E. Koiw, pp. 523–532, Springer-Verlag, New York, 1972.
169. Verney, E. B.: The antidiuretic hormone and the factors which determine its release. Proc. Roy. Soc. Ser. B Biol. Sci. 135: 25–106, 1947.
170. Vernikos-Danellis, J.: The pharmacological approach to the study of the mechanisms regulating ACTH secretion. *In* Pharmacology of Hormonal Polypeptides and Proteins, ed. by N. Beck, L. Martin and R. Paoletti, Plenum Press, New York-London, 1968.
171. Volicer, L. and Loew, C. G.: The effect of urethane anesthesia on the cardiovascular action of angiotensin II. Pharmacology 6: 193–201, 1971.
172. Volicer, L. and Loew, C. G.: Penetration of angiotensin II into the brain. Neuropharmacology 10: 631–636, 1971.
172a. Werning, C., Shönbeck, M., Ziegler, W. H., Baumann, K., Gysling, E., Weidmann, P. and Siegenthaler, W.: Die Adrenalin- und Noradrenalinausscheidung während Angiotensin II-infusionen in die Arteria carotis beim narkotisierten Hund. Klin. Wochenschr. 47: 640–647, 1969.
173. Westergaard, E.: The fine structure of nerve fibers and endings in the lateral cerebral ventricles of the rat. J. Comp. Neurol. 144: 345–354, 1972.
174. Whelan, R. F.: Angiotensin. *In* Control of the Peripheral Circulation in Man, pp. 5–26, Charles C Thomas, Springfield, Ill., 1967.
175. Whelan, R. F., Scroop, G. C. and Walsh, J. A.: Cardiovascular actions of angiotensin in man. Amer. Heart J. 77: 546–565, 1969.
176. Wilson, C. M. W. and Brodie, B. B.: The absence of blood-brain barrier from certain areas of the central nervous system. J. Pharmacol. Exp. Ther. 133: 332–334, 1961.
177. Yang, H. Y. T. and Neff, N. H.: Distribution and properties of angiotensin converting enzyme of rat brain. J. Neurochem. 19: 2443–2450, 1972.
178. Yates, F. E., Russell, S. M., Dallman, M. J., Hedge, G. A., McCann, S. M. and Dhariwall, A. P. S.: Potentiation by vasopressin of corticotropin release induced by corticotropin-releasing factor. Endocrinology 88: 3–15, 1971.
179. Yu, R. and Dickinson, C. J.: Neurogenic effects of angiotensin. Lancet 2: 1276–1277, 1965.
180. Yu, R. and Dickinson, C. J.: The progressive pressor response to angiotensin in the rabbit. The role of the sympathetic nervous system. Arch. Int. Pharmacodyn. Thér. 191: 24–36, 1971.
181. Zimmerman, B. G.: Evaluation of peripheral and central components of action of angiotensin on the sympathetic nervous system. J. Pharmacol. Exp. Ther. 158: 1–10, 1967.
182. Zimmerman, B. G., Gomer, S. K. and Liao, J. C.: Action of angiotensin on vascular adrenergic nerve endings: facilitation of norepinephrine release. Fed. Proc. 31: 1344–1350, 1972.