Br. J. Pharmac. (1968), **34**, 499–507.

The interaction between angiotensin and sympathetic vasoconstriction in the isolated artery of the rabbit ear

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1. Infusions or injections of angiotensin into the isolated ear artery preparation of the rabbit produced only feeble vasoconstrictions and depressed the sensitivity of the preparation to sympathetic stimulation, tyramine and noradrenaline.

2. The anti-sympathetic effect of angiotensin was not prevented or reversed by noradrenaline or cocaine.

3. The vasoconstrictor response to angiotensin was usually markedly increased during sympathetic stimulation or other procedures which liberate endogenous noradrenaline (for example, infusion of tyramine), but not when the perfusion pressure was raised by increasing the flow rate or by infusing noradrenaline.

4. The vasoconstrictor action of tyramine was also potentiated by sympathetic stimulation.

5. The potentiation by sympathetic stimulation of the responses to angiotensin was prevented by reserpine, guanethidine and phentolamine in concentrations which abolished sympathetic vasoconstriction.

6. It is suggested that angiotensin facilitates the release of endogenous noradrenaline and owes part of its vasoconstrictor activity to this property.

Much evidence has accumulated in recent years suggesting an interaction between angiotensin and the sympathetic nervous system. McCubbin & Page (1963a, b) found that the pressor responses in anaesthetized dogs to procedures which caused a release of noradrenaline were enhanced during infusions of angiotensin. Benelli, Della Bella & Gandini (1964) found that administration of angiotensin specifically increased the response of the guinea-pig isolated vas deferens to sympathetic stimulation and that the pressor response to angiotensin in cats was decreased by blocking sympathetic ganglia with tetramethyl ammonium or nicotine. They concluded that angiotensin potentiates sympathetic stimulation by promoting a greater output of noradrenaline and that the vasoconstrictor response to an injection of angiotensin is partly due to the liberation of noradrenaline. Similar enhancement

of the responses to sympathetic stimulation by angiotensin has been reported in the cat isolated spleen (Benelli *et al.*, 1964 ; Thoenen, Hurlimann & Haefely, 1965 ; Hertting & Suko, 1966).

Other reports have also suggested that the vasoconstrictor activity of angiotensin is partly dependent on the sympathetic nervous system. Zimmerman (1962) showed that the vasoconstriction in the dog hind limb in response to angiotensin was reduced by sympathectomy and a similar observation was made in the rat by Laverty (1963). These observations have been extended to other vascular beds including the cutaneous (Zimmerman & Gomez, 1965), the mesenteric (McCubbin & Page, 1963a) and the vessels of the human hand (Scroop & Whelan, 1966). Further indirect evidence that the vasoconstrictor activity of angiotensin is partly mediated via the sympathetic nerves is afforded by its lack of activity in the human isolated umbilical artery, which is not sympathetically innervated (Gokhale, Gulati, Kelkar & Kelkar, 1966), and by its feeble constrictor activity in some isolated blood vessel preparations (McGregor, 1965 ; De la Lande & Rand, 1965).

The purpose of the present investigation was to test the hypothesis of Benelli *et al.* (1964) that angiotensin has both direct vasoconstrictor activity and an indirect action involving an increased release of noradrenaline from sympathetic nerve-endings. We have used the isolated central artery of the rabbit ear preparation described by De la Lande & Rand (1965) because it affords a convenient method of studying the interaction between angiotensin and vasomotor sympathetic nerves in a simplified *in vitro* system.

Methods

Central ear artery preparation of the rabbit

This preparation was set up as described by De la Lande & Rand (1965), except that the artery was suspended in air instead of in an organ-bath containing the perfusion solution. In this way drugs injected or infused into the lumen of the vessel could not produce an effect on the outside of the artery. The preparations were perfused with Krebs solution containing (g/l.): NaCl 7.7, KCl 0.34, $CaCl_2$ 0.30, KH_2PO_4 0.16, $MgSO_4$ 0.29, $NaHCO_3$ 2.1, dextrose 2, maintained at 37° C and continuously gassed with 95% oxygen and 5% carbon dioxide. Perfusion pressures were recorded by means of a mercury manometer (rat blood pressure type) or by using a blood pressure transducer (Devices/CEC type 4-327-L221) and a Devices M4 electronic recorder.

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

The vascular sympathetic nerves were stimulated by threading the artery through bipolar platinum electrodes (Burn & Rand, 1960) and delivering rectangular pulses from an electrode stimulator ; details of stimulation parameters are given in **Results**. In general, pulses were of supramaximal strength (20–50 V) of 1 msec duration, and at frequencies ranging from 1 to 50 pulses/sec. Stimulation was usually applied for 40 sec periods in every 4 min.

The drugs used were angiotensin (Hypertensin, Ciba), noradrenaline bitartrate, 5-hydroxytryptamine creatinine sulphate, tyramine HCl, phenylethylamine HCl,

reserpine (Serpasil, Ciba), guanethidine sulphate (Ismelin, Ciba), phentolamine (Rogitine, Ciba). The doses of noradrenaline are expressed as base ; all other doses are in terms of the salts.

Results

Effect of angiotensin and noradrenaline on perfusion pressure

The dose of angiotensin needed for a measurable vasoconstriction varied from 50 ng to 1 mg injected in a volume of 0.1 ml. but one in seven or eight preparations was insensitive even to 1 mg. If the same dose of angiotensin was repeated at intervals of not less than 10 min, the response to the second and subsequent injections was constant and smaller than the response to the first injection. Compared with noradrenaline, angiotensin was less potent on a weight basis and gave a less steep log dose-response curve as shown in Fig. 1. The relative potencies of angiotensin and noradrenaline on the isolated artery preparation contrast sharply with their relative pressor potencies in anaesthetized rabbits where angiotensin was 4 (one experiment) or 10 (one experiment) times more potent and the dose-response curves were almost parallel.

Infusions of angiotensin in concentrations up to 0.5 μg/ml. produced either a transient increase in perfusion pressure or were without effect.

Effect of infusions of angiotensin on the response to sympathetic stimulation, tyramine and noradrenaline

The addition of angiotensin to the perfusion fluid in concentrations less than 1 ng/ml. had no effect on the responses to sympathetic stimulation, noradrenaline or tyramine. In concentrations ranging from 1 to 500 ng/ml. the predominant effect of angiotensin was to impair the responses to sympathetic stimulation as well as those to noradrenaline and tyramine. In a few preparations the impairment was preceded by a short-lived enhancement of the sympathetic constriction.

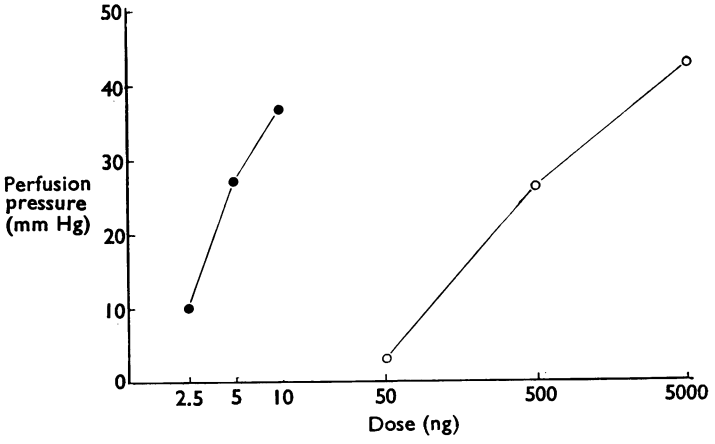

FIG. 1. Isolated artery preparation: dose-response curves to noradrenaline (left) and angiotensin (right), both administered intra-arterially. Perfusion pressure in this and subsequent figures recorded with a mercury manometer.

The anti-sympathetic effect of angiotensin was most marked with low frequencies of stimulation (1–4 pulses/sec) and still occurred in the presence of cocaine in a concentration (5 μg/ml.) which in other experiments virtually prevented the blocking action of guanethidine (2 μg/ml.). When angiotensin was removed from the perfusion fluid, the recovery of the responses to sympathetic stimulation occurred slowly and was not hastened in the presence of noradrenaline (100 ng/ml.).

Effect of angiotensin administered during sympathetic stimulation

In forty-six out of sixty-one experiments the constrictor response to angiotensin (50–500 ng) was markedly potentiated either in size or duration during sympathetic stimulation. In eight preparations the response to angiotensin was not increased during sympathetic stimulation but showed two peaks of vasoconstriction which occasionally became quite separate. In seven preparations, including those which were insensitive to 1 mg angiotensin, sympathetic stimulation did not potentiate the response to angiotensin.

The responses to intra-arterial noradrenaline (2.5–50 ng) were unaffected or reduced during sympathetic stimulation. Figure 2 illustrates an experiment in which angiotensin and noradrenaline were given before, during and after sympathetic stimulation at two frequencies. The response to angiotensin was markedly increased whereas that to noradrenaline was decreased during each period of sympathetic stimulation. The extent of the enhancement of the angiotensin response during

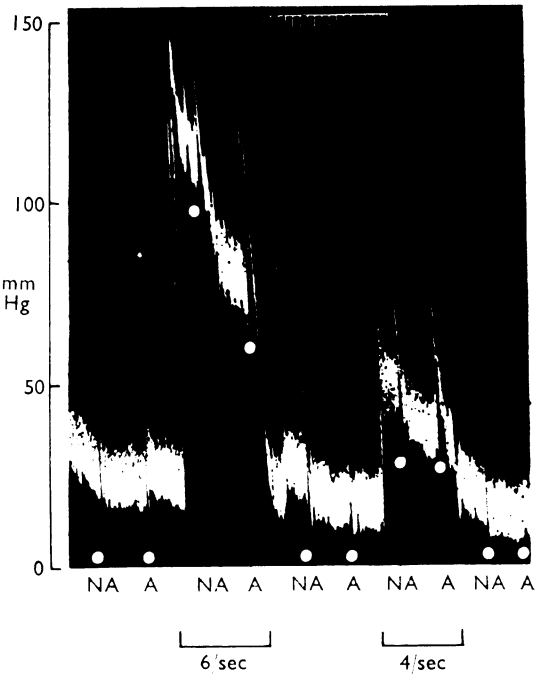

FIG. 2. Isolated artery preparation: Responses to intra-arterial injections of noradrenaline (2.5 ng) at NA and angiotensin (500 ng) at A. The response to angiotensin, but not that to noradrenaline, was enhanced during continuous sympathetic stimulation at 6 pulses/sec and at 4 pulses/sec. Stimulation with supramaximal strength pulses; time scale in min.

sympathetic stimulation varied in different experiments and at different frequencies of stimulation. In four experiments in which a two point dose-response curve to angiotensin was constructed before and during sympathetic stimulation it was found that the dose-response curves were parallel and that the enhancement of the responses was equivalent to increasing the angiotensin dose 50–100 times.

The enhanced responsiveness to angiotensin during sympathetic stimulation could be demonstrated several times in any one experiment but usually the potentiation became less pronounced possibly as a result of the sympathetic blocking action of angiotensin already described. The vasoconstrictor action of tyramine (10–50 μg) was also increased during sympathetic stimulation as shown in Fig. 3.

Effect of increased rate of perfusion and of vasoconstrictor drugs on responses to angiotensin

It was considered desirable to determine whether the constrictor effect of angiotensin was increased during the administration of other drugs and procedures which raised perfusion pressure.

Increased perfusion rate. When the perfusion pressure was increased from a resting level of 30–40 mm Hg to 80–100 mm Hg by increasing the rate of flow of the perfusion fluid the vasoconstrictor responses to both angiotensin and to noradrenaline were decreased but returned to control levels when the flow rate was returned to its original level.

Noradrenaline infusions. Infusions of noradrenaline (1–5 ng/ml.) were used to raise the perfusion pressure to similar levels to those produced by sympathetic stimulation at 5–10 pulses/sec. In most experiments the responses to angiotensin were unchanged but in a few experiments the responses were slightly increased or

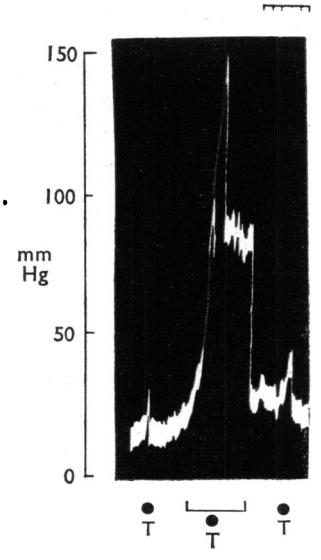

FIG. 3. Isolated artery preparation: experiment showing enhanced vasoconstriction in response to tyramine (15 μg) at T, during sympathetic stimulation (at bar) at a frequency of 2 pulses/sec with supramaximal strength pulses. Time scale in min.

decreased. In all these experiments injections of noradrenaline produced a smaller effect during noradrenaline infusions.

Indirectly acting sympathomimetic amines. The response to angiotensin was increased during an infusion of phenlethylamine or tyramine (1–6 μg/ml.), as shown in Fig. 4.

5-Hydroxytryptamine. This substance has been shown to increase the response to sympathetic stimulation and to vasoconstrictor agents in this preparation (De la Lande, Cannell & Waterson, 1966). In the present experiments an infusion of 5-hydroxytryptamine (20 ng/ml.) increased the responses to sympathetic stimulation, noradrenaline and angiotensin to a similar extent, thus confirming its non-specific sensitizing effect in this tissue.

Action of drugs affecting sympathetic mechanisms

Reserpine. (a) Pretreatment. Reserpine (0.25 mg/kg/day intravenously) was administered to seven rabbits for 1–4 days. Four preparations taken from rabbits pretreated in this way were completely unresponsive to sympathetic stimulation, although they showed a normal sensitivity to angiotensin. In none of these preparations was the vasoconstrictor activity of angiotensin increased during sympathetic stimulation. In five other preparations taken from these rabbits, however, a small sympathetic vasoconstriction remained and the vasoconstrictor response to angiotensin was markedly increased during sympathetic stimulation.

(b) Acute effects. The effect of adding reserpine (50–500 ng/ml.) to the perfusion fluid was similar to the effect of pretreatment with reserpine. Only in those preparations in which sympathetic stimulation caused no vasoconstriction was the potentiation of the response to angiotensin abolished. The constrictor effect of

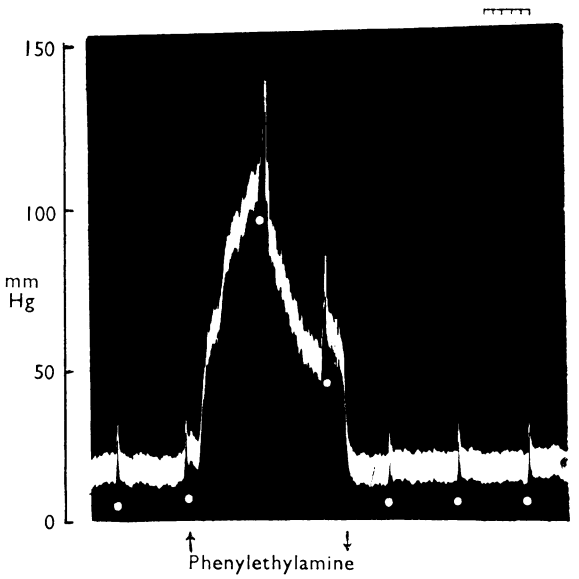

FIG. 4. Isolated artery preparation: Responses to 500 ng doses of angiotensin injected into the artery at 10 min intervals (at dots) before, during and after adding phenylethylamine 6 μg/ml. to the perfusion fluid. Time marker in min.

angiotensin in the absence of sympathetic stimulation was unaffected or slightly reduced after complete sympathetic blockade with acutely administered reserpine.

Guanethidine. The results obtained with this substance were essentially similar to those obtained with reserpine. Concentrations of guanethidine (2–6 μg/ml.) which completely abolished sympathetic vasoconstriction prevented the potentiation of the angiotensin responses during stimulation. The responses to angiotensin in the absence of sympathetic stimulation were, however, consistently increased after guanethidine. These observations are illustrated in Fig. 5. Initially, in this experiment the constrictor action of angiotensin was increased during sympathetic stimulation and returned to its control size afterwards. Guanethidine (4 μg/ml.) added to the perfusion fluid increased the vasoconstrictor response to angiotensin but prevented any further enhancement during sympathetic stimulation. The potentiation of the angiotensin responses by guanethidine occurred in preparations taken from animals treated with reserpine and was therefore independent of its blocking action on adrenergic neurones.

Phentolamine. Addition of phentolamine (50–250 ng/ml.) to the perfusion fluid abolished the constrictor responses to noradrenaline and sympathetic stimulation but did not affect those to angiotensin. Like the other adrenaline antagonists, phentolamine prevented the enhancement of the angiotensin constrictions by sympathetic stimulation.

Discussion

Angiotensin seems to interact with the sympathetic nervous system in several ways. Thus there is evidence which suggests that at least part of the pressor activity of angiotensin is mediated by the central nervous system (Bickerton & Buckley, 1961 ; Scroop & Whelan, 1966). Second, angiotensin increases the responses of

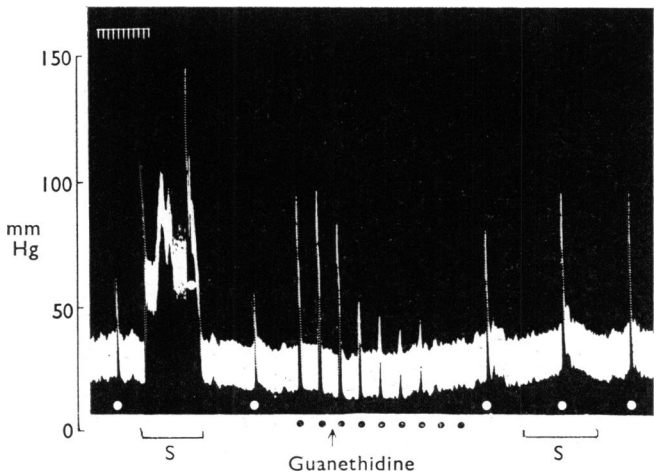

FIG. 5. Isolated artery preparation: Angiotensin (250 ng) was injected intra-arterially at the white dots and its constrictor effect was enhanced during continuous sympathetic stimulation with supramaximal strength pulses at 4 pulses/sec (at S). At the black dot intermittent sympathetic stimulation for 40 sec periods was applied at the same pulse frequency. Guanethidine (4 μg/ml.) was added to the perfusion fluid (at↑) and abolished the sympathetic constriction and also the enhancement of the angiotensin response during continuous stimulation. Time scale in min.

tissues to noradrenaline released from peripheral sympathetic nerves both in whole animals (McCubbin & Page, 1963a, b ; Zimmerman & Gomez, 1965) and in isolated tissues (Benelli *et al.*, 1964 ; Thoenen *et al.*, 1965 ; Hertting & Suko, 1966). Finally, in some vascular beds the vasoconstrictor action of angiotensin is dependent on an intact sympathetic innervation and is greatly reduced by procedures abolishing sympathetic tone (Zimmerman, 1962 ; Laverty, 1963 ; Benelli *et al.*, 1964).

We have used an isolated sympathetically innervated artery in order to examine the peripheral component of the angiotensin-sympathetic interaction. The initial experiments were designed to reveal a possible potentiation of sympathetic vasoconstriction by angiotensin. Instead we found that the only clear effect of adding angiotensin to the perfusion fluid was to reduce the constrictor responses to sympathetic stimulation as well as those to tyramine and noradrenaline. Angiotensin has been reported to cause some loss of noradrenaline from the walls of isolated arteries (Distler, Liebau & Wolff, 1965) but this is unlikely to be the mechanism of its sympathetic blocking action in our experiments for it was not reversed by adding noradrenaline to the perfusion fluid. The angiotensin block differed from that produced by guanethidine in that it still occurred in the presence of cocaine. This suggests that angiotensin acts directly on vascular smooth muscle where it causes an unspecific loss of sensitivity to constrictor agents. Such a mechanism could explain the finding of McCubbin & Page (1963a) that in a minority of experiments the pressor response of anaesthetized dogs to carotid occlusion was decreased during infusions of angiotensin.

We have confirmed the observation of De la Lande & Rand (1965) that the isolated artery preparation is relatively insensitive to angiotensin. In our experiments, however, the vasoconstrictor effect of angiotensin was greatly increased during sympathetic stimulation. Zimmerman (1962) made a similar observation in the dog's perfused hind limb preparation ; he showed that acute sympathectomy greatly reduced the vasoconstrictor activity of angiotensin but that sensitivity could be partly restored by sympathetic stimulation.

In our experiments angiotensin responses were not regularly increased by infusing noradrenaline and were diminished by increasing the perfusion pressure by increased flow rate. The effect of sympathetic stimulation on the angiotensin responses cannot therefore be attributable to the increased level of perfusion pressure. The angiotensin responses were, however, increased in the presence of amines which liberate noradrenaline suggesting that endogenously released noradrenaline is necessary for the enhancement of responses to angiotensin. Our results using drugs which modify sympathetic activity support this view ; abolition of the responses to sympathetic nerve stimulation by complete reserpinization or by guanethidine prevented the enhancement of the angiotensin responses during sympathetic stimulation.

The mechanism whereby angiotensin responses are increased during release of endogenous noradrenaline is not certain. One possibility is that the vascular sensitivity to angiotensin is increased in the presence of endogenously released noradrenaline. A more attractive explanation is suggested by the hypothesis of Benelli *et al.* (1964) that angiotensin normally releases noradrenaline and owes part of its vasoconstrictor activity to this action. The release of noradrenaline by other agents (for example, sympathetic stimulation) may facilitate or be facilitated by this action of angiotensin. This suggestion is supported by the fact that the response to

tyramine, a substance known to produce its effects through release of endogenous noradrenaline, is also potentiated during sympathetic stimulation. There are also other reasons which favour this explanation. First, it accounts for the fact that exogenous noradrenaline does not potentiate angiotensin vasoconstriction. Second, in some preparations the response to angiotensin consisted of two separate peaks; presumably the first was due to angiotensin itself and the second to the noradrenaline released by angiotensin. The enhanced single responses seen in other experiments are likely to have been due to the superimposition of the two phases. Third, phentolamine, which acts by blocking α-receptors, prevented the sympathetic enhancement of the response to angiotensin; the simplest interpretation of this result is that phentolamine prevented the action of the noradrenaline released by angiotensin.

These results suggest that angiotensin resembles tyramine in causing release of endogenous noradrenaline stores. Angiotensin (but not tyramine) has a direct action of its own, however, which is not dependent on intact noradrenaline stores and is therefore not abolished by drugs which render the noradrenaline stores inaccessible, such as cocaine, reserpine and guanethidine.

We are grateful to the Medical Research Council for a grant which financed part of this work and to Professor F. Gross of CIBA (Basle) Ltd. for a generous gift of angiotensin.

REFERENCES

BENELLI, G., DELLA BELLA, D. & GANDINI, A. (1964). Angiotensin and peripheral sympathetic nerve activity. *Br. J. Pharmac. Chemother.*, **22**, 211–219.

BICKERTON, R. K. & BUCKLEY, J. P. (1961). Evidence for a central mechanism in angiotensin induced hypertension. *Proc. Soc. exp. Biol. Med.*, **106**, 834–836.

BURN, J. H. & RAND, M. J. (1960). The relation of circulating noradrenaline to the effect of sympathetic stimulation. *J. Physiol., Lond.*, **150**, 295–305.

DE LA LANDE, I. S. & RAND, M. J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. exp. Biol. med. Sci.*, **43**, 639–659.

DE LA LANDE, I. S., CANNELL, V. A. & WATERSON, J. G. (1966). The interaction of serotonin and noradrenaline on the perfused artery. *Br. J. Pharmac. Chemother.*, **28**, 255–272.

DISTLER, A., LIEBAU, H. & WOLFF, H. P. (1965). Action of angiotensin on sympathetic nerve endings in isolated blood vessels. *Nature, Lond.*, **207**, 764–765.

GOKHALE, S. D., GULATI, O. D., KELKAR, L. V. & KELKAR, V. V. (1966). Effect of some drugs on human umbilical artery *in vitro*. *Br. J. Pharmac. Chemother.*, **27**, 332–346.

HERTTING, G. & SUKO, J. (1966). Influence of angiotensin, vasopressin or changes in flow rate on vasoconstriction, changes in volume and ^{3}H-noradrenaline release following postganglionic sympathetic nerve stimulation in the isolated cat spleen. *Br. J. Pharmac. Chemother.*, **26**, 686–696.

LAVERTY, R. (1963). A nervously-mediated action of angiotensin in anaesthetized rats. *J. Pharm. Pharmac.*, **15**, 63–68.

MCCUBBIN, J. W. & PAGE, I. H. (1963a). Neurogenic component of chronic renal hypertension. *Science (N.Y.)*, **139**, 210–211.

MCCUBBIN, J. W. & PAGE, I. H. (1963b). Renal pressor system and neurogenic control of arterial pressure. *Circulation Res.*, **12**, 553–559.

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

SCROOP, G. C. & WHELAN, R. F. (1966). A central vasomotor action of angiotensin in man. *Clin. Sci.*, **30**, 79–90.

THOENEN, H., HURLIMANN, A. & HAEFELY, W. (1965). The effect of angiotensin on the responses to postganglionic sympathetic stimulation of the cat spleen; lack of facilitation of norepinephrine liberation. *Med. Pharmac. exp.*, **13**, 379–387.

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

ZIMMERMAN, B. G. & GOMEZ, J. (1965). Increased response to sympathetic stimulation in the cutaneous vasculature in presence of angiotensin. *Int. J. Neuropharmac.*, **4**, 185–193.

(*Received April* 17, 1968)