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ANGIOTENSIN AND PERIPHERAL SYMPATHETIC NERVE ACTIVITY

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On the isolated vas deferens of the guinea-pig angiotensin potentiated strongly the height of contractions due to electrical stimulation of the hypogastric nerve; it did not affect the responses to noradrenaline and acetylcholine, nor did it elicit any contraction when given alone. Angiotensin likewise potentiated the responses of the cat spleen to nerve stimulation, but it also induced by itself strong contractions of the organ and reduction of the venous outflow. In experiments on the arterial blood pressure of anaesthetized and spinal cats, in which sympathetic postganglionic transmission was temporarily blocked by nicotine or tetramethylammonium, pressor responses to angiotensin were strongly reduced. As with some ganglion-stimulating drugs, the pressor responses, enhanced after a second series of nicotine injections, were reduced to the control level by hexamethonium. These findings indicate the involvement of peripheral sympathetic nerves in the action of angiotensin: the hypothesis is advanced that angiotensin acts at the peripheral nerve endings by promoting a greater output of noradrenaline.

It is generally assumed that angiotensin owes its pharmacological properties to a direct stimulation of peripheral smooth muscle. Growing experimental evidence, however, suggests that the muscular activity is not the sole mechanism involved in the action of the polypeptide : the hypothesis of a nerve-mediated influence playing some role requires serious consideration (Bickerton & Buckley, 1961 ; Buckley, Bickerton, Halliday & Kato, 1963 ; Laverty, 1963). To test this possibility and to collect more information on the mechanism of action of angiotensin, experiments were undertaken both *in vitro* and *in vivo*.

One of the preparations employed was the sympathetically innervated vas deferens of the guinea-pig, electrically stimulated over a wide frequency range so as to uncover possible potentiating effects of angiotensin on transmission of nerve impulses. Additional evidence was obtained with the cat isolated splenic nerve-spleen preparation by recording contractions and venous outflow simultaneously.

Pressor activity of angiotensin was also investigated *in vivo* on anaesthetized and spinal cats, after excluding the peripheral sympathetic pathway by "depolarizing" blocking doses of tetramethylammonium and nicotine.

METHODS

Hypogastric nerve–vas deferens preparation

Guinea-pigs, weighing 600 to 700 g, were used. The preparation, set up according to Huković (1961), was mounted in a 100 ml. organ-bath containing Krebs solution at 32° C, through which a mixture of 95% oxygen and 5% carbon dioxide was passed. The hypogastric nerve was held in

stimulating platinum electrodes, submerged in the organ-bath and connected to an electronic stimulator. Stimulation was carried out at 2 min intervals, at a frequency of 10 to 50 shocks/sec, the stimuli being rectangular, of 0.5 msec duration and from a constant voltage source at 3 to 5 V. Contractions were recorded by an isotonic writing lever (load 2 g) with a magnification of four-times. For the experiments in which the direct effects of acetylcholine and noradrenaline on the vas deferens were examined, a 20 ml. organ-bath was used.

Isolated splenic nerve–spleen preparation

For the setting up of the preparation, a method analogous to that described by Brandon & Rand (1961) was followed. Cats of either sex, weighing 2 to 3 kg, were used. Animals were anaesthetized with ether and the spleen with its hilum was removed. The main splenic artery and vein were cannulated and the splenic nerve isolated and dissected free. The preparation was suspended in a 500 ml. organ-bath at 33° C so that its longitudinal contractions could be measured with a writing lever (ratio 1 : 25) on a kymograph. The splenic artery was connected to a 5 l. Mariotte bottle, a cannula being interposed for direct injection into the spleen. The spleen was perfused with Krebs solution containing polyvinylpyrrolidone (1 g/100 ml.), the perfusion fluid pressure being 70 to 90 cm of water. The splenic venous outflow was measured with a Thorp impulse counter. The splenic nerve was placed on platinum electrodes submerged in the organ-bath and connected to an electronic stimulator. Stimulation was applied at 3 or 4 min intervals, at a frequency of 10 to 50 shocks/sec, the stimuli being rectangular, of 1 or 2 msec duration, from a constant voltage source at 1.5 to 3.5 V. Drugs were injected directly into the spleen through the arterial cannula, either in a single dose or by perfusion at constant rate.

In vivo *recording of the systemic arterial pressure*

Cats of either sex, weighing about 3 kg, were anaesthetized with chloralose (80 mg/kg intravenously). Blood pressure of heparinized animals was recorded from a carotid artery with a mercury manometer. Drugs were injected into an external jugular vein through a cannula. Some experiments were carried out on spinal cats, prepared according to Burn (1952), or after bilateral adrenalectomy performed by ligation of all connections between the glands and their surroundings.

The following drugs were used: synthetic angiotensin (Ipertensina, Ciba), acetylcholine chloride, nicotine bitartrate, hexamethonium bromide, tetramethylammonium chloride and noradrenaline base. With the exception of noradrenaline the doses given refer to the salts.

RESULTS

Guinea-pig hypogastric nerve–vas deferens preparation

When assaying angiotensin on the responses of the vas deferens to hypogastric nerve stimulation, we observed an increase in the height of contractions which proved to be dose- and frequency-dependent, and similar to that described for physostigmine by Burn & Weetman (1963). The potentiating effect occurred promptly, being apparent 1 min after administration of angiotensin, and reached its peak in 3 to 5 min, that is in two or three stimulation periods. The effect slowly disappeared upon washing, but remained unaltered for 30 to 40 min if angiotensin was left in contact with the preparation (Fig. 1, *a*). After normal responses had been again obtained, the same dose of angiotensin was far less effective (Fig. 1, *b*) ; the tachyphylaxis varied from preparation to preparation but was always appreciable, unless 60 to 90 min elapsed between the two administrations.

As shown in Table 1, which summarizes the results, a close dose-effect relationship was seen in all instances with doses from 0.001 to 1 μg/ml. when a low stimulus frequency, 15 shocks/sec, was applied. At higher frequencies the minimal angiotensin

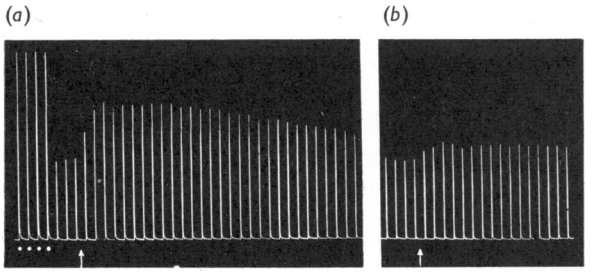

Fig. 1. Contractions of the isolated vas deferens of the guinea-pig in response to hypogastric nerve stimulation. Each stimulation was 100 shocks, given either at 50 shocks/sec (at dots) or at 15 shocks/sec, every 2 min. (*a*) shows enhanced responses to the low frequency stimulation in the presence of angiotensin (0·01 μg/ml., added to the bath at the arrow); (*b*) is with the same preparation as in (*a*), after washing, and shows that the same dose of angiotensin (at the arrow), 1 hr later, potentiated the contractions only slightly.

dose required to show the potentiating effect was far higher ; thus an inverse ratio between potentiation and stimulus frequency was observed.

At this stage we carried out a second series of experiments to elucidate the nature of the angiotensin-induced enhancement : for this purpose direct stimulation with acetylcholine and noradrenaline was applied. We confirmed that the vas deferens is completely unresponsive to angiotensin alone, even in doses as high as 5 μg/ml., except for occasional spontaneous rhythmic motility, as already seen after hypertensin (Picarelli, Branco & Valle, 1951). No effect was seen with angiotensin concentrations from 0.001 to 5 μg/ml., when 1 μg/ml. of acetylcholine or 5 μg/ml. of noradrenaline was used to stimulate the preparation.

TABLE 1

INFLUENCE OF ANGIOTENSIN ON CONTRACTIONS OF THE VAS DEFERENS AT HIGH AND LOW STIMULUS FREQUENCIES

Values are means of contraction heights as percentages of previous controls, with numbers of observations in parentheses. For each stimulus frequency 100 shocks were given

Concentration of angiotensin (μg/ml.)	Contraction after angiotensin (%) for stimulus frequency (shocks/sec)		
	15	30	50
0·001	49 (4)	11 (3)	5 (4)
0·005	70 (4)	17 (3)	8 (2)
0·01	113 (5)	23 (3)	16 (4)
0·1	147 (4)	64 (4)	35 (3)

Cat isolated splenic nerve-spleen preparation

Angiotensin (0.005 to 0.5 μg), directly injected into the splenic artery, caused an immediate contraction of the organ and reduction of the venous outflow (Fig. 2). When the same dose of angiotensin was injected repeatedly at 15 to 60 min intervals, a marked tachyphylaxis involving both contractions and outflow was seen. The speed of application appeared to affect the responses considerably, especially when low doses were injected ; contraction of the spleen failed sometimes to develop, although

the venous outflow was always reduced. At higher doses, a spontaneous rhythmic motility of the parenchymal as well as of the vascular muscle apparatus was sometimes observed (Fig. 2).

In view of these findings we studied the influence of angiotensin on the responses of the spleen to splenic nerve electrical stimulation, care being taken that low angiotensin doses were injected slowly to avoid a direct response.

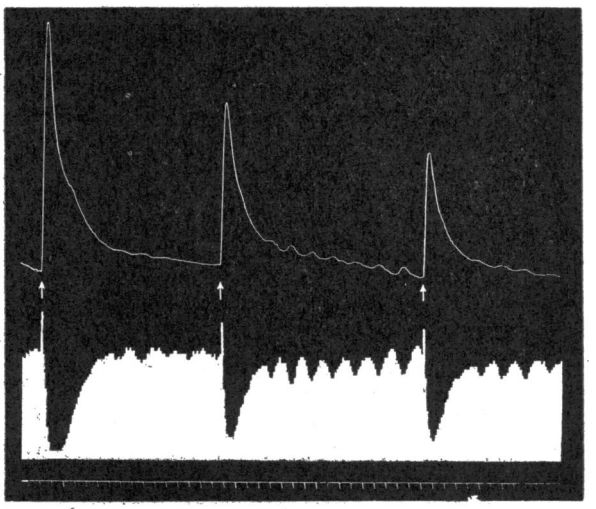

Fig. 2. Isolated and perfused spleen preparation of the cat. Upper tracing: longitudinal contractions of the spleen; lower tracing: splenic venous outflow. At each arrow, 0.1 μg of angiotensin was injected into the splenic artery. It evoked strong contractions of the spleen and great reductions of the outflow; both effects got progressively reduced upon the second and third administrations. Note the spontaneous rhythmic motility of the spleen, becoming most evident after the second treatment with angiotensin. Time marks, 1 min.

The results of eleven experiments may be briefly summarized. Angiotensin (0.001 to 0.5 μg), slowly introduced into the splenic artery, potentiated the responses to electrical stimulation to a degree varying from 12 to 37% (Fig. 3). The effect was most apparent in the response immediately following the drug, quickly disappearing, and occurring to a reduced extent upon repeated administrations at short intervals. Only in a few preparations could it still be observed after two or three responses. As far as intensity of the effect is concerned, neither did the dose of angiotensin nor the stimulus frequency seem to have as much influence as for the vas deferens.

Additional experiments on the possible direct influence of angiotensin on the responses evoked by noradrenaline injection into the splenic artery gave variable results : responses appeared unaffected in three preparations, reduced in one, and potentiated in another. As may be seen in Fig. 4, the potentiating effect became more evident the higher the angiotensin dose introduced.

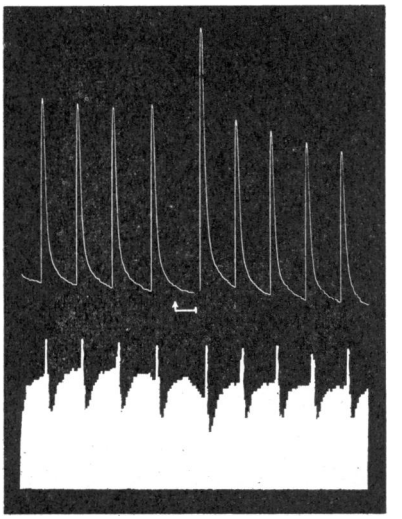

Fig. 3. Cat splenic nerve—isolated and perfused spleen preparation. Upper tracing: longitudinal contractions of the spleen in response to electrical stimulation of the splenic nerve (30 shocks/sec for 3 sec every 3 min); lower tracing: splenic venous outflow. At the arrow and line, 0.005 μg of angiotensin in 0.2 ml. was slowly introduced into the splenic artery, and considerably potentiated the response to splenic nerve stimulation.

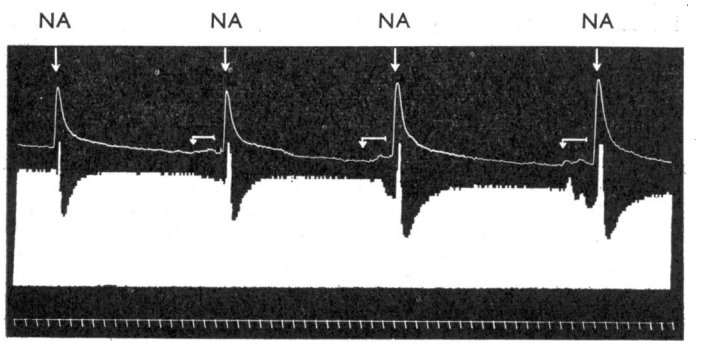

Fig. 4. Cat isolated and perfused spleen preparation. Upper tracing: longitudinal contractions of the spleen in response to 1 μg doses of noradrenaline (NA); lower tracing: splenic venous outflow. At the arrows and lines, progressively increasing doses of angiotensin (0.015, 0.045 and 0.09 μg) were injected successively. The responses to noradrenaline appear slightly potentiated, the size of potentiation depending on the dose of angiotensin. Time marks, 1 min.

Angiotensin-induced pressor responses in vivo

In further experiments we intended to examine the effects of angiotensin *in vivo* after a "ganglion block by depolarization" which, according to Paton & Perry (1953) and to Jones, Gomez Alonso de la Sierra & Trendelenburg (1963), follows administration of tetramethylammonium or nicotine in high doses.

Both normal and spinal cats were used. Once two equal pressor resposes to the same angiotensin dose had been obtained, tetramethylammonium or nicotine was injected in increasing amounts until there was no further response of the blood pressure to successive introduction of the "depolarizing" drug. Administration of angiotensin now gave significantly reduced pressor responses (Figs. 5 and 6). The reduction appeared more pronounced and less easily reversible after tetramethylammonium than after nicotine. During the phase of "non-depolarizing" ganglion block following a second series of nicotine injections, a subsequent addition of angiotensin always elicited enhanced pressor responses.

Like the observations of Trendelenburg (1961) on the enhancement of the pressor responses to histamine and pilocarpine under similar experimental conditions, hexamethonium (2 to 5 mg/kg intravenously) abolished the potentiating effect of nicotine on the angiotensin responses (Fig. 7).

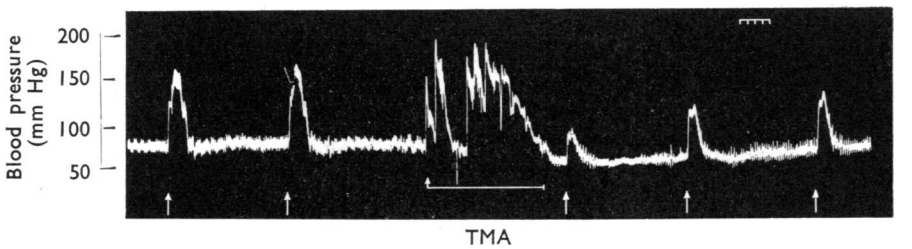

Fig. 5. Spinal cat, 2.5 kg; record of arterial blood pressure showing pressor responses to angiotensin during "depolarizing" ganglion block by tetramethylammonium. At the arrows, 1 μg of angiotensin was injected intravenously. During the horizontal line, 2.7 mg/kg of tetramethylammonium (TMA) was given in divided doses. The pressor response to angiotensin immediately following this treatment was greatly reduced. The reduction appears reversible upon repeated angiotensin administrations. Time marks, 1 min.

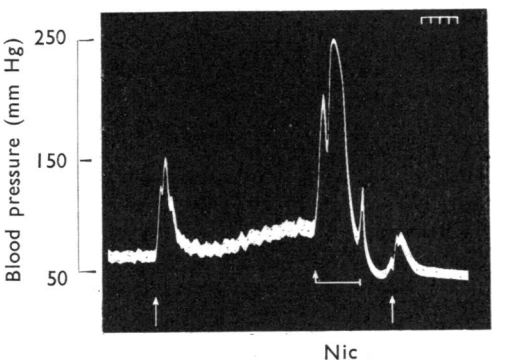

Fig. 6. Spinal cat, 3.6 kg; record of arterial blood pressure showing pressor responses to angiotensin during "depolarizing" ganglion block by nicotine. At the arrows, 0.5 μg of angiotensin was injected intravenously. During the horizontal line, 7.3 mg/kg of nicotine (Nic) was given in divided doses. The pressor response to angiotensin after treatment with nicotine appears much reduced. Time marks, 1 min.

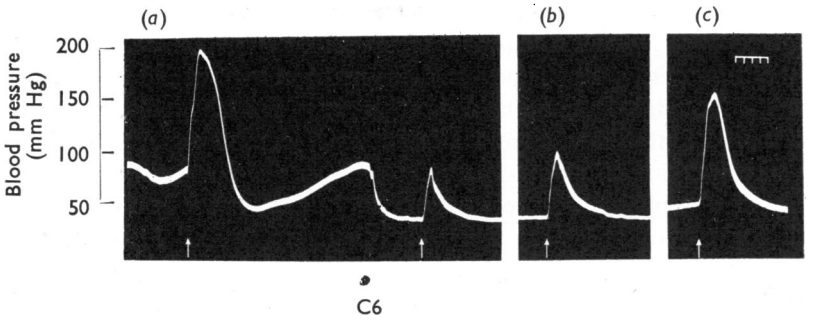

Fig. 7. Same preparation as for Fig. 6, showing enhanced pressor responses to angiotensin (at the arrows, 0.5 μg, intravenously) after the second series of nicotine injections (total dose of nicotine, 4.8 mg/kg). In (*a*) hexamethonium (5 mg/kg, intravenously at C6) abolished the potentiating effect of nicotine on the responses to angiotensin. (*b*) and (*c*) show pressor responses to angiotensin 20 and 50 min respectively after administration of hexamethonium. Time marks, 1min.

A comparable reduction by nicotine was obtained in bilaterally adrenalectomized animals : it is of interest to point out that the pressor responses to angiotensin obtained immediately after adrenalectomy and before treatment with nicotine appeared, in three out of four animals, to be 7 to 23% smaller than the initial controls.

DISCUSSION

According to recent work (Bickerton & Buckley, 1961 ; Buckley *et al.*, 1963), angiotensin administration to the isolated perfused head of a dog causes a neurogenic vasoconstriction in the trunk and limbs of the animal. Furthermore Laverty (1963) observed in the perfused innervated hind-limb of a rat anaesthetized with chloralose a nerve-mediated vasoconstriction or vasodilation (depending on the dose), either of which was abolished by section of the nerves leading to the hind-limb. In agreement with the former workers, Laverty suggested that the vasoconstriction may be due in part to a stimulation of central sympathetic structures as well as to a direct action on arterial smooth muscle. Little is known of the influence of angiotensin on the autonomic peripheral system. Robertson & Rubin (1962) reported that drugs which interfere with cholinergic transmission (ganglion blocking and antiacetylcholine drugs as well as botulinum toxin) are able to alter significantly the typical stimulation by angiotensin of the rabbit or guinea-pig ileum.

After investigating the positive inotropic and chronotropic actions of angiotensin on the isolated heart, Beaulnes (1963) concluded that they are at least partially due to a release of catechol amines. Similarly, an adrenaline-releasing effect of angiotensin on cat adrenal glands was described by Feldberg & Lewis (1963).

Our results seem to provide further evidence for a direct effect of angiotensin on peripheral sympathetic transmission : low concentrations (10^{-7} to 10^{-9}) greatly increased the height of contractions of the vas deferens in response to electrical stimulation of the hypogastric nerve, whereas no direct action of the polypeptide was observed on this preparation. The enhancement was more pronounced at a low frequency of stimulation (15 shocks/sec), but still occurred, although to a less extent,

at higher frequencies. On the contrary, angiotensin even in high concentrations failed to potentiate the responses of the vas deferens to noradrenaline and acetylcholine. We conclude from this last finding that angiotensin probably acts by increasing the release of noradrenaline due to stimulation of the hypogastric nerve. The mechanism through which angiotensin would promote such an effect has still to be elucidated. A number of observations, however, make us believe that it is different from that described for physostigmine by Burn & Weetman (1963). First, physostigmine increases the noradrenaline released by low stimulus frequencies, as does angiotensin, but decreases it for high frequencies whereas angiotensin does not ; secondly, an acetylcholine-like or anticholinesterase activity of angiotensin may be excluded in view of the lack of any direct stimulating effect on the vas deferens as well as the failure of the polypeptide to potentiate the contractions in response to acetylcholine.

Results on the isolated splenic nerve–spleen preparation resemble those on the vas deferens except for the direct stimulating effect of angiotensin on the spleen, even at the very low concentration of a few thousandths of a microgram. Our observations do not permit an understanding of the noradrenaline-angiotensin interaction on this preparation. For a possible explanation it should be taken into account that the angiotensin-induced vasoconstriction may be responsible for a different diffusion rate of noradrenaline into the organ.

The hypothesis that angiotensin affects peripheral sympathetic transmission by promoting a greater output of noradrenaline seems to be consistent with the findings *in vivo* : in fact, the altered pressor response to angiotensin during the "depolarizing" ganglion block by high doses of nicotine or tetramethylammonium points to a similarity between angiotensin and several ganglion-stimulating drugs, such as McN–A–343, 3-acetoxy-1-benzyl-1-methylpyrrolidinium bromide (AHR 602) (Jones *et al.*, 1963), pilocarpine and histamine (Trendelenburg, 1961). In addition, the observation that responses to angiotensin are decreased but not abolished points to a bimodal mechanism of action responsible for the systemic blood pressure increase induced by the polypeptide, that is, angiotensin exerts indirect nerve-mediated effects along with direct peripheral activity on vascular muscle.

Analogy between the actions of angiotensin and some ganglion-stimulating drugs may be adduced also from the fact that increased responses occur during the late phase of "non-depolarizing" block, following the second administration of nicotine. This angiotensin potentiation, however, is quite different from that observed after giving ganglion-blocking agents, since it is abolished by hexamethonium. Similar results have been reported for pilocarpine and histamine (Trendelenburg, 1961).

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