A NERVOUSLY-MEDIATED ACTION OF ANGIOTENSIN IN ANAESTHETISED RATS

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In rats under chloralose anaesthesia, angiotensin in large doses caused a nervously-mediated vasoconstriction in the vascularly-isolated innervated hind limb, whereas in small doses it caused a nervously mediated peripheral vasodilatation. Noradrenaline over an equipressor dose range caused only a nervously-mediated peripheral vasodilatation. These peripheral responses were abolished by section of the nerves leading to the hind limb.

Angiotensin, a pressor polypeptide formed *in vivo* by the action of renin on plasma globulins, has been prepared in a pure form (Rittel, Iselin, Kappeler, Riniker and Schwyzer, 1957), resulting in renewed interest in its pharmacology, Experimental studies using the biologically prepared and, more recently, synthetic material have elucidated many of its reactions (Braun-Menendez, 1956; Page and Bumpus, 1961).

During a comparative study of the actions of synthetic and biologically prepared angiotensin and noradrenaline on rats (Laverty, 1960) it was found that large doses of angiotensin caused a nervously-mediated vasoconstriction in peripheral blood vessels. This observation, here investigated further, suggests some modification of the usual view that "angiotensin has a strong peripheral vasoconstrictor action and no action on the central nervous system" (Page and Bumpus, 1961). A comparable effect of angiotensin on the central nervous system of dogs has also been observed recently (Bickerton and Buckley, 1961).

Methods

The hind limb of a rat (300–380 g.) anaesthetised with chloralose (60 mg./kg.) was isolated from the circulation of the remainder of the animal at the level of the inguinal ligament by cutting the muscular tissue, leaving only the femur, the femoral and sciatic nerves and the femoral vein intact (Field and Laverty, 1958). This isolated innervated hind limb was then perfused through the femoral artery, with blood taken from the opposite femoral artery, by means of a constant output pump (Field, de Graaf and Wallis, 1958). In the experiments investigating the nervously-mediated effects of drugs given to the remainder of the animal, on the hind-limb peripheral resistance, a delay coil of 6 ml. volume was interposed between the animal and the pump, to separate the neurogenic effects from direct effects of the blood-borne drug on the hind-limb blood vessels.

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In some experiments the isolated innervated hind limb was perfused with blood taken from a separate donor rat (Field and Laverty, 1958) instead of from the animal that supplied the hind limb. This ensured that drugs given to the remainder of the recipient animal could only exert an action on the blood vessels of the isolated hind limb through the nervous system.

The trachea and jugular vein of the recipient rat were cannulated in all experiments. The drugs being tested were given intravenously by the jugular vein into the main circulation of the animal or directly into the blood perfusing the hind limb by injection into the femoral cannula. Drugs used were noradrenaline bitartrate (Levophed, Winthrop; dose given in terms of the free base), synthetic angiotensin (CIBA prep. 19990*a*, hypertensin-val$_5$-amide; dose as pure compound) and biologically prepared angiotensin (Angiotonin, Lilly; dose in units).

Pressures were measured by small-volume manometers. Since the output of the perfusion pump was constant (Field and others, 1958), the peripheral resistance of the perfused hind limb was measured directly as the perfusion pressure.

Results

Small doses of angiotensin (0·1–0·5 μg.) were administered intravenously into the main circulation of a rat anaesthetised with chloralose. The rises in the animal's blood pressure due to the angiotensin were accompanied simultaneously by small falls in the peripheral resistance of the

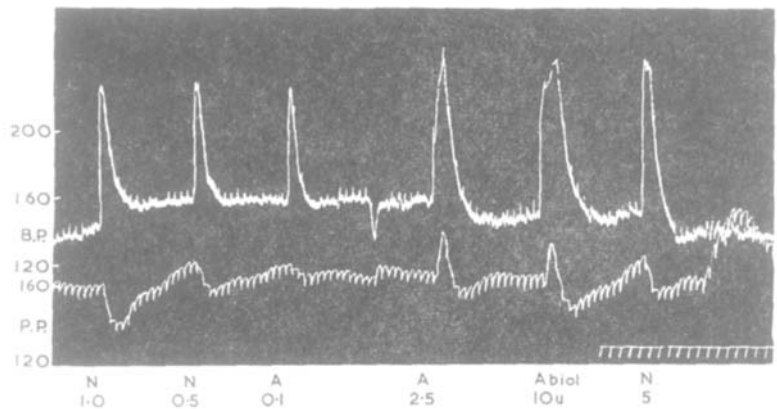

FIG. 1. Effects of synthetic angiotensin (A), noradrenaline (N) and biologically prepared angiotensin (A biol) given intravenously in varying doses, on the blood pressure (B.P.) and the hind-limb perfusion pressure (P.P.) of a rat anaesthetised with chloralose. The innervated vascularly-isolated hind-limb was perfused with blood at a constant rate so that changes in perfusion pressure directly represent changes in peripheral resistance. There was at least a 6 min. delay between the time the drug was given intravenously and the time the drug reached the hind limb in the perfusing blood. Time in min.; dosages in μg. or biological units.

Increasing the dose of angiotensin converted the immediate effect on the hind limb from vasodilatation to vasoconstriction; increasing the dose of noradrenaline merely increased the immediate vasodilatation.

blood vessels of the blood-perfused innervated hind limb, as shown by falls in the hind-limb perfusion pressure. An increase in the dose of angiotensin to 1 μg. or more changed the response in the hind limb to an immediate vasoconstriction (Fig. 1). Small doses of noradrenaline (0·5–1·0 μg.) caused slight vasodilatation in the hind limb; increasing the dose of noradrenaline caused only a slightly increased vasodilatation (Fig. 1). In all instances, the hind-limb effect was apparent within 5 min. of the drug being given to the remainder of the animal, i.e., before the drug could reach the hind limb in the perfusing blood. The immediate vasoconstriction produced by angiotensin was sometimes accompanied by respiratory depression and increased voluntary muscular movement.

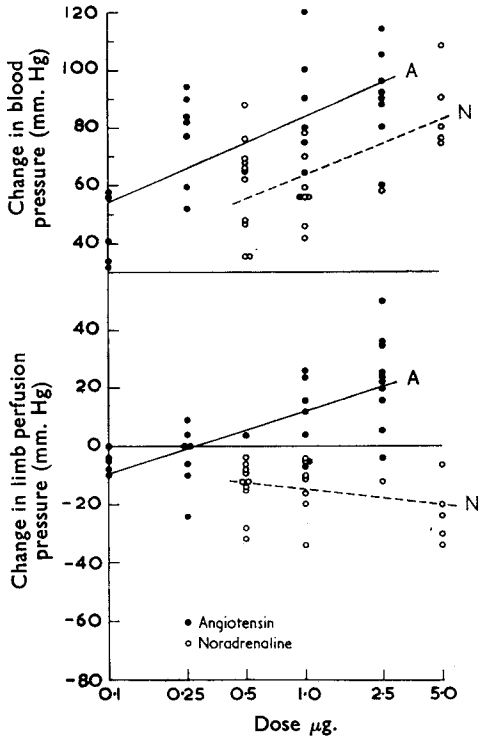

FIG. 2. Dose-response curves for the direct blood pressure effects and the nervously-mediated effects on the hind-limb perfusion pressure of angiotensin (A) and noradrenaline (N) for 14 experiments.

Fig. 2 summarizes the results of 14 experiments, showing the mean values, in each rat, of the response of the blood pressure and the response of the hind-limb perfusion pressure to differing doses of angiotensin and noradrenaline administered to the main circulation of the rat. The regression coefficients of the lines shown in Fig. 2 are statistically significant except for the regression with dose of the hind-limb response to noradrenaline. There is a highly significant difference statistically between the

slopes of the regression lines of the hind-limb effects of angiotensin and noradrenaline. The results demonstrate a marked difference between angiotensin and noradrenaline in their indirect actions on the blood vessels of the perfused hind limb. Biologically prepared angiotensin was also capable of producing a similar immediate vasoconstriction (Fig. 1).

Section of the nerves connecting the hind limb to the remainder of the animal totally abolished the immediate effects of both angiotensin and noradrenaline (Fig. 3), showing that these immediate effects were mediated solely through the nervous system.

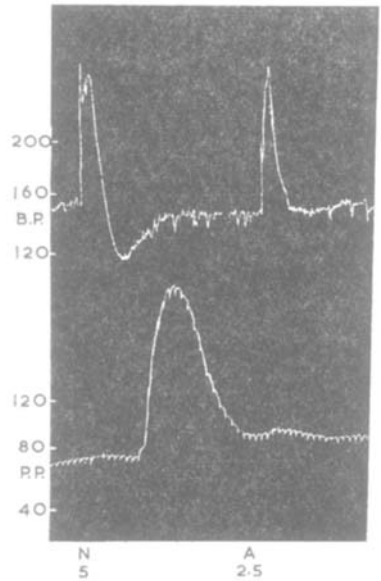

FIG. 3. Effect of synthetic angiotensin and noradrenaline on the blood pressure (B.P.) and the perfusion pressure (P.P.) in the *denervated* vascularly-isolated hind limb of a rat anaesthetised with chloralose. Time in min.; dosages in μg.

Drugs were given intravenously and reached the hind limb in the perfusing blood approximately 6 min. later. Thus the rise in blood pressure preceded the direct effect on the hind limb. There was no immediate effect on the limb after the nerves supplying it had been cut (compare Fig. 1).

Clamping the carotid arteries in three single animal experiments reduced only slightly the neurogenic vasodilatation produced by noradrenaline and did not prevent the nervously-mediated peripheral vasoconstriction induced by large doses of angiotensin.

In five experiments the hind limb of one rat was perfused with blood from a second animal to ensure the complete isolation of the circulation through the hind limb. Angiotensin and noradrenaline were given into the main circulation of the first animal as a prolonged infusion. Noradrenaline thus administered caused a nervously-mediated vasodilatation in the hind limb during the infusion, whereas angiotensin caused a small vasodilatation or a vasoconstriction in the hind limb (Fig. 4).

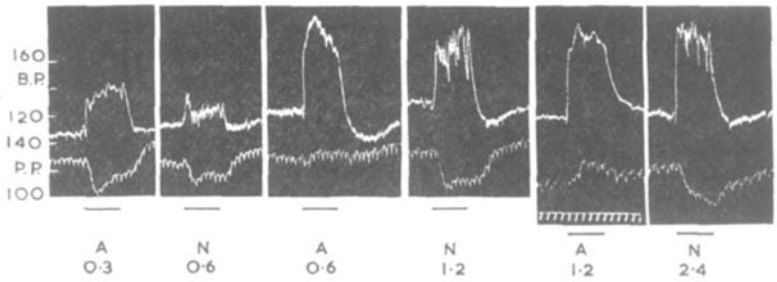

FIG. 4. Effects of prolonged intravenous infusions of synthetic angiotensin (A) and noradrenaline (N) on the blood pressure (B.P.), and on the perfusion pressure (P.P.) in the innervated vascularly-isolated hind limb, of a rat anaesthetised with chloralose. In this experiment the blood perfusing the hind limb came from a separate donor rat, thus preventing any drug given to the recipient animal from reaching the hind limb. Time in min.; dosages in μg./min.

Increasing the dose of angiotensin converted the nervously-mediated effect on the hind-limb perfusion pressure from vasodilatation to vasoconstriction, whereas increasing the dose of noradrenaline merely increased the vasodilatation.

DISCUSSION

The ability of angiotensin to produce a nervously-mediated peripheral vasoconstriction represents a new aspect of its pharmacology in rats, and suggests that angiotensin may have other actions than those of a purely peripheral vasoconstrictor. The vasoconstriction was abolished by nerve section (Fig. 3) which strongly suggests that it was mediated through the nervous system. Attempts to show vascular anastomoses through the femur or other tissues by means of dye were unsuccessful; if the vasoconstriction were due to anastomotic leaks to the hind limb from the remainder of the recipient animal, it would be expected that a similar vasoconstriction would occur with noradrenaline. In fact, it is the marked difference between the neurogenic effects of angiotensin and noradrenaline, both drugs being regarded mainly as peripheral vasoconstrictors, that is of great interest. Bickerton and Buckley (1961) have shown that angiotensin administered to the isolated head of a dog causes a neurogenic vasoconstriction in the vascularly-isolated remainder of the animal, which suggests that this nervously-mediated action of angiotensin is not restricted to one species.

The nervously-mediated peripheral vasoconstriction following angiotensin administration is not due to the rise in systemic blood pressure, as it was not observed following corresponding blood pressure rises due to noradrenaline (Fig. 2). However, angiotensin may cause a more marked cerebral vasoconstriction with consequent anoxia, since angiotensin and noradrenaline differ in their relative potencies in different vascular beds (Gross and Turrian, 1960). The muscular and respiratory effects of large doses of angiotensin may be consistent with this, though Mandel and Sapirstein (1962) have recently shown that angiotensin in low dosage had little effect on cerebral blood flow in rats. Another possible site of the difference between the actions of angiotensin and noradrenaline is the

carotid sinus; noradrenaline causes reflex hypotension when applied directly (Heymans and Delaunois, 1953) whereas angiotensin does not (McCubbin, Page and Bumpus, 1957; Bianchi, de Shaepdryver, de Vleeschhouwer and Preziosi, 1960). Attempts to alter any carotid sinus effects by carotid occlusion were inconclusive.

Thus it appears that angiotensin in large doses through an action probably on the central nervous system causes a nervously-mediated peripheral vasoconstriction. The determination of the exact site of action needs further experiments, preferably on larger animals than the rat.

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