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The role of the sympathetic nervous system in the cardiovascular responses to angiotensin in the pithed rat

L. FINCH AND G. D. H. LEACH

School of Studies in Pharmacology, University of Bradford, Bradford 7, Yorkshire

1. Angiotensin (200–500 ng, intravenously) produced a biphasic pressor response accompanied by reflex bradycardia in rats anaesthetized with sodium pentobarbitone. In pithed rats and in anaesthetized animals treated with a ganglion blocking agent there was a similar pressor response but an increase in the heart rate which was coincident with the second phase of the pressor response. In pithed preparations an increase in cardiac contractile force accompanied the heart rate changes.

2. Acute or chronic adrenalectomy did not alter the pressor responses and chronotropic effect of angiotensin in the pithed rat. Propranolol abolished the chronotropic and inotropic action of angiotensin, but left the biphasic pressor response unaltered. Bethanidine, or pretreatment with reserpine, altered the pressor response to a simple rise, and also abolished the chronotropic action of angiotensin. Phentolamine also abolished the second pressor component. Desmethylimipramine produced a marked potentiation of the cardiovascular effects of angiotensin at all dose levels tested (10–500 ng).

3. It is concluded that in the rat only large doses of angiotensin are capable of indirectly stimulating the sympathetic nerves either at the site of their ganglionic cells or their post-ganglionic nerve ending.

For many years angiotensin has been considered to exert its cardiovascular effects chiefly as a result of its direct constrictor action on arteriolar smooth muscle. In recent years, however, this view has been modified by the observations that angiotensin enhances the pressor response to agents releasing endogenous noradrenaline (McCubbin & Page, 1963), releases catecholamines from the adrenal medulla (Feldberg & Lewis, 1964) and from the peripheral adrenergic nerves (Benelli, Della Bella & Gandini, 1964; Distler, Liebau & Wolff, 1965), and stimulates the sympathetic ganglia (Lewis & Reit, 1965; Trendelenburg, 1966).

Angiotensin also has been shown to exert a cardioaccelerator action in the dog (Krasney, Paudler, Hogan, Lowe & Youmans, 1966; Farr & Grupp, 1967) and in the rat (Hughes, 1968) after the prevention of bradycardia by ganglion blocking drugs. In these species the effect of angiotensin on the heart rate can be abolished by β-adrenoreceptor blocking drugs. The pressor response to angiotensin has been

shown by Farr & Grupp (1967) and by Ross & White (1966) to be biphasic. The latter workers showed that in the cat the second pressor phase was modified by adrenalectomy. In the pithed rat preparation, however, the pressor responses to angiotensin have been shown not to be significantly mediated via the release of noradrenaline nor to be altered by adrenalectomy (Schmitt & Schmitt, 1968). Schümann & Güther (1967) using the rat and guinea-pig isolated aortic strip preparation demonstrated the existence of both a direct and an indirect mechanism of action of angiotensin. This present study is an attempt to clarify the role of the sympathetic nervous system in the cardiovascular responses of angiotensin in the rat.

Methods

Male C.S.E. rats (250–400 g) were used throughout. Those animals pretreated with reserpine were given 5 mg/kg intraperitoneally 6 hr before the experiment. All animals were anaesthetized with sodium pentobarbitone (60 mg/kg) given intraperitoneally. Pithed rats were prepared by the method of Shipley & Tilden (1947) and were maintained on artificial respiration by a Palmer miniature ideal pump using a stroke volume of 1 ml./100 g body weight at a rate 50 strokes/min. The systemic blood pressure was recorded from the common carotid artery using a Bell and Howell pressure transducer (Type 4-326-L212) and a Devices pen recorder. The heart rate was recorded using the method of Clarke, Hiscoe, Hulley, Jackson & Leach (1966). The contractile force of the heart was measured, in pithed preparations only, with a strain gauge (Basile microtransducer Type 1·5/120LB11) sutured to the wall of the left ventricle and the signals fed into a single channel carrier amplifier (New Electronic Products type 2506), the output of which was fed into a final amplifier of the pen recorder. Drugs were injected into the femoral vein in a volume not greater than 0·1 ml., and washed in with 0·2 ml. saline. Some injections were made into the aorta through a polythene cannula inserted into the carotid artery. In these preparations the blood pressure was recorded from the iliac artery. Intravenously injected sodium heparin (2,000 u./kg) was given as an anticoagulant. In experiments using acutely adrenalectomized preparations the glands were removed 1 hr beforehand under halothane anaesthesia. Chronic adrenalectomy was carried out 2 weeks previously and 0·9% w/v NaCl substituted for the normal drinking water during the first week, after the operation. Animals were used at the end of the second week.

Drugs

All stock solutions of drugs were diluted in 0·9% w/v NaCl solution before use. Noradrenaline acid tartrate (Hoechst) and isoprenaline sulphate (B.D.H.), calculated as base, were stored in 0·01N HCl. The following drugs were calculated as salt: mecamylamine hydrochloride (Merck, Sharp & Dohme); reserpine phosphate (Ciba) dissolved in 20% ascorbic acid; desmethylimipramine (Geigy), phenoxybenzamine (S.K.F.) dissolved in equal parts of 95% alcohol and propylene glycol; bethanidine sulphate (Burroughs Wellcome); propranolol (I.C.I.); tyramine hydrochloride (B.D.H.); phentolamine mesylate (Ciba); dimethylphenylpiperazinium iodide (Aldrich) and vasopressin (Parke Davis).

Val-5-angiotensin 11 amide (Ciba) was dissolved in saline immediately before use.

Results

Action of angiotensin on the blood pressure and heart rate in the anaesthetized rat

The intravenous administration of low doses of angiotensin (10 ng–100 ng) to rats anaesthetized with sodium pentobarbitone (60 mg/kg) produced a rise in blood pressure without significant alteration of the resting heart rate. Larger doses (200–500 ng) produced a biphasic pressor response accompanied by a reflex bradycardia which was abolished by bilateral vagotomy. The administration of mecamylamine (2·0–10 mg/kg intravenously), in doses sufficient to abolish the cardiovascular responses to dimethylphenylpiperazium (DMPP) (20 μg), potentiated the angiotensin pressor responses obtained at all of the dose levels tested (10–500 ng). The responses to the larger doses (200–500 ng) obtained after mecamylamine showed an increase in heart rate, the onset of which coincided with that of the secondary phase of the pressor response and occurred 15–20 sec after the start of the first pressor phase.

Action of angiotensin in the pithed rat

The typical cardiovascular responses to intravenous injections of angiotensin in the pithed rat preparation can be seen in Fig. 1, and are essentially similar to those observed in the anaesthetized rat after treatment with a ganglion blocking agent. Intra-arterial injections of angiotensin (200–500 ng) directly into the aorta produced much smaller increases in heart rate than did intravenous injections, but the biphasic pressor responses were quantitatively similar by either route of injection. Angiotensin (200–500 ng intravenously) was also seen to produce an increase in the force of cardiac contraction, this effect coinciding with the chronotropic action.

Actions of adrenoreceptor blocking agents on responses to angiotensin in the pithed rat

Both phentolamine (5 mg/kg) and phenoxybenzamine (7·5 mg/kg) in doses that blocked the pressor response to injected noradrenaline (50 ng) reduced or abolished

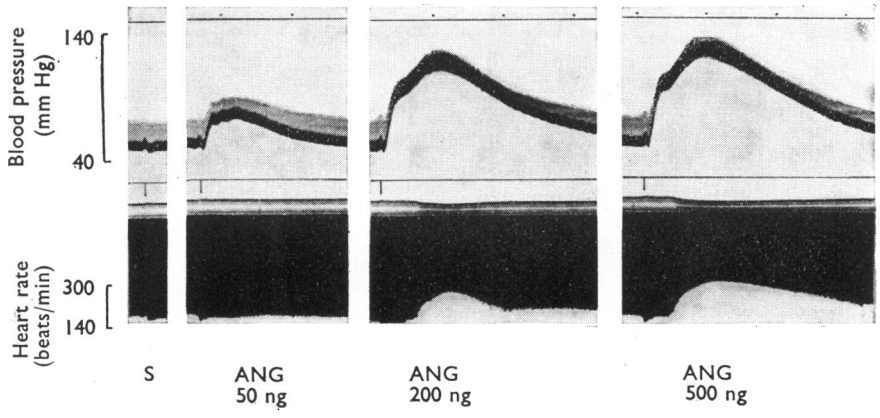

FIG. 1. Pithed rat. Blood pressure and heart rate responses to angiotensin (ANG). S, Injection of 0·2 ml. saline. Time marks, 1 min.

the secondary phase of the pressor response to large doses of angiotensin (200–500 ng) (Fig. 2A). The magnitude of the initial phase was also seen to be increased in a majority of cases, so that the height of the overall pressor effect was maintained. The chronotropic response to angiotensin was still observed after α-adrenoceptor blockade.

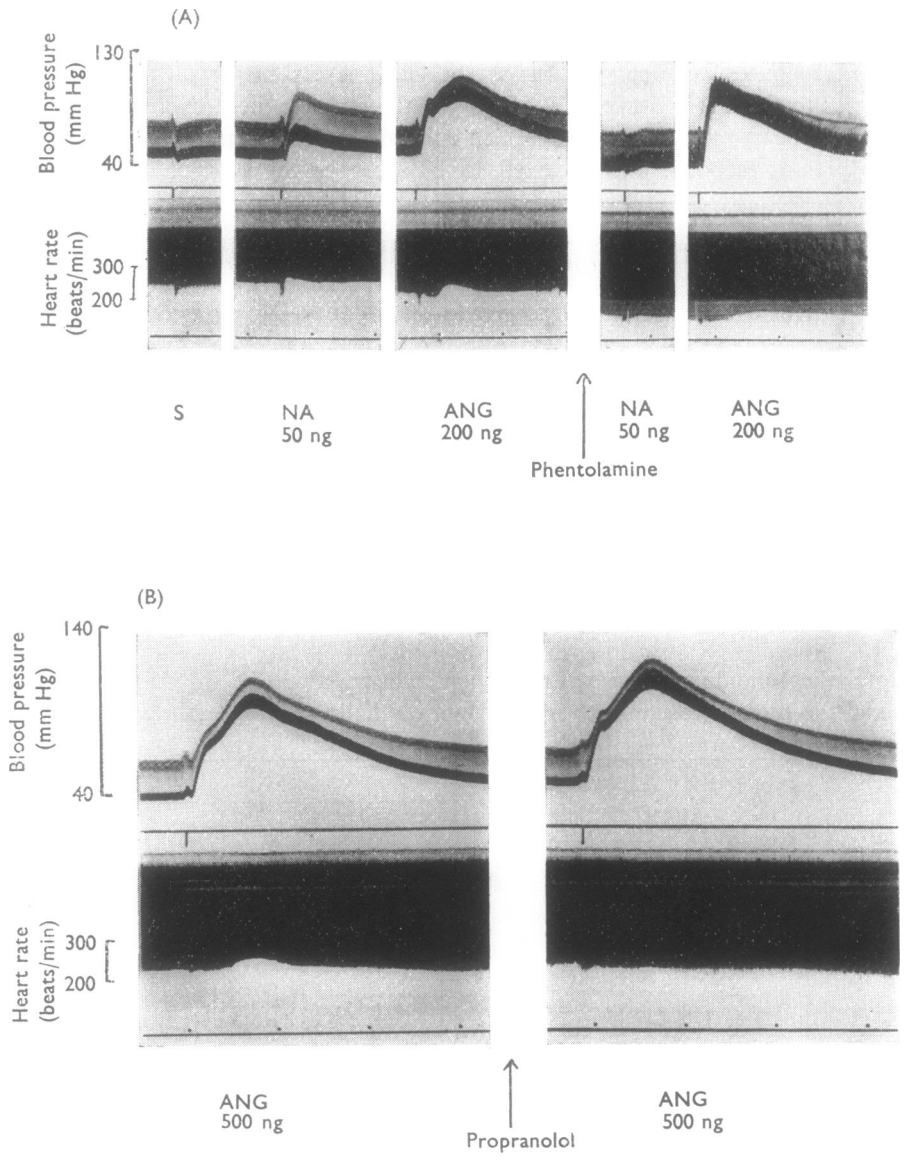

FIG. 2. Pithed rats. Blood pressure and heart rate responses to angiotensin (ANG) and noradrenaline (NA). A: Before and after intravenous injection of phentolamine (5 mg/kg); B: before and after intravenous injection of propranolol (300 μg/kg). S, Injection of 0·2 ml. saline. Time marks, 1 min.

Propranolol (300 μg/kg) in doses that reduced or abolished the chronotropic and inotropic effects to both isoprenaline (10 ng), and angiotensin (200–500 ng) did not alter the magnitude of the biphasic pressor response (Fig. 2B).

Actions of an adrenergic neurone blocking agent on responses to angiotensin in the pithed rat

The adrenergic neuronal blocking agent, bethanidine sulphate (0·5–2 mg/kg) changed the complex angiotensin response (200–500 ng) to that of a simple pressor response, similar in its onset to the first part of the original biphasic pressor response and was not accompanied by any marked increase in heart rate (Fig. 3). At the range of angiotensin doses tested (10–500 ng) the pressor responses were seen to be potentiated by bethanidine and this effect could mask any existing biphasic response. However, noradrenaline responses (50–200 ng) after bethanidine were potentiated to a greater degree than the increases seen to angiotensin.

Effect of catecholamine depletion and desmethylimipramine in pithed rats

The response of the pithed rat preparation to angiotensin (10–500 ng) was not significantly altered after acute or chronic adrenalectomy. Pretreatment with reserpine (5 mg/kg) 6 hr previously, which abolished cardiovascular responses to tyramine (25 μg), still produced normal pressor responses to noradrenaline (50–200 ng) when administered after completing the test sequences of angiotensin. In these conditions the compound angiotensin response (200–500 ng) was changed to a simple pressor response which resembled the first pressor phase of the response seen in normal pithed preparations. Reserpine pretreatment also abolished the positive chronotropic action of angiotensin seen at these higher dose levels (Fig. 4A).

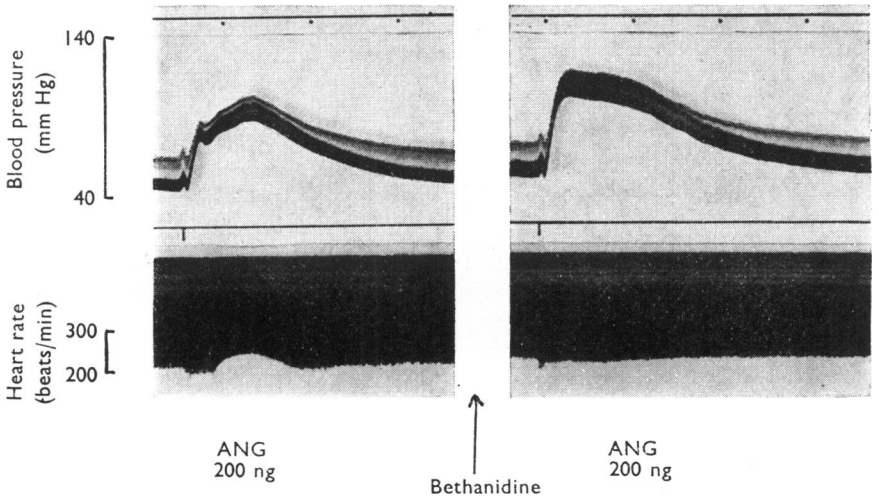

FIG. 3. Pithed rat. Blood pressure and heart rate responses to angiotensin (ANG) before and after intravenous injection of bethanidine (0·5 mg/kg). Time marks, 1 min.

Desmethylimipramine (0·5 mg/kg intravenously), which produced a 150–200% potentiation in both the magnitude and duration of the responses to noradrenaline (50–200 ng), produced a less marked potentiation (40–60%) in these parameters to all the angiotensin dose levels tested (10–500 ng) (Fig. 4B). The responses to vasopressin (1–4 m-u./kg) were potentiated to a similar degree to those seen with angiotensin. At this dose level, desmethylimipramine (0·5 mg/kg) produced a rise in blood pressure (10–20 mm Hg) and an increase in heart rate (40–70 beats/min) which persisted throughout the experiment. Reserpine pretreatment (5 mg/kg) reduced the desmethylimipramine potentiating action on the angiotensin responses.

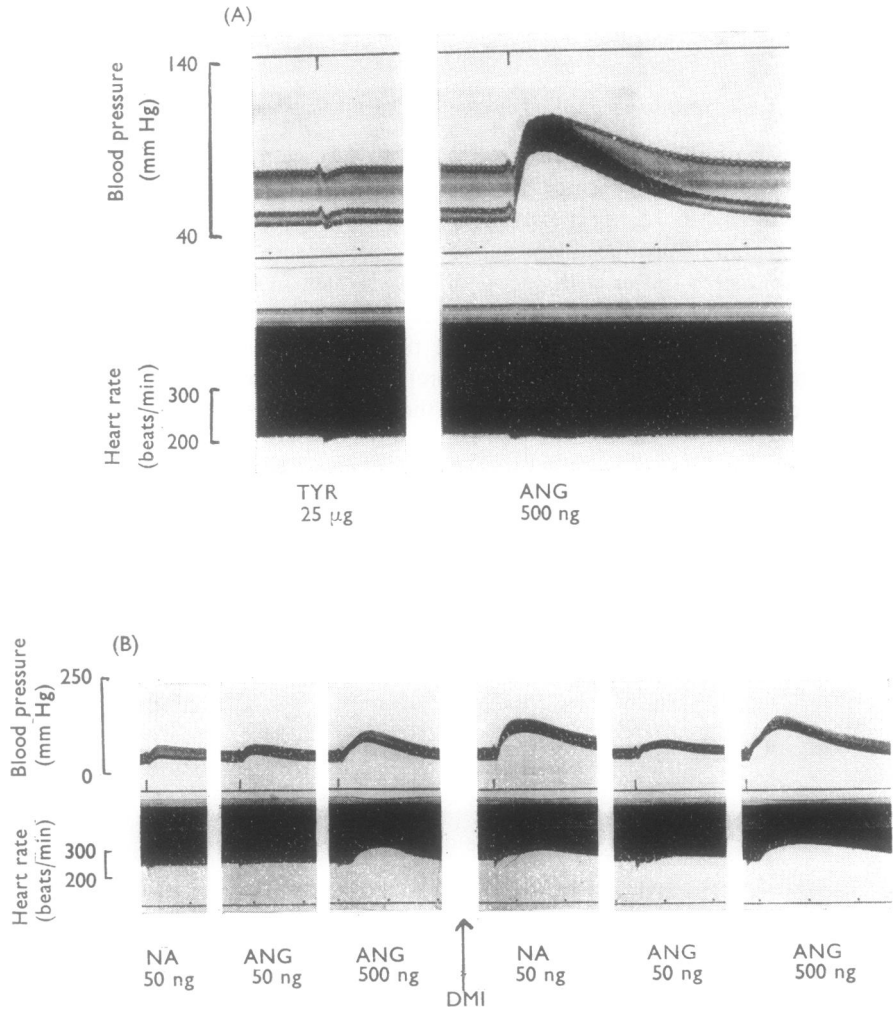

FIG. 4. Pithed rats. Blood pressure and heart rate responses to angiotensin (ANG), noradrenaline (NA) and tyramine (TYR). A: Pretreatment with reserpine 6 hr previously. B: Before and after intravenous injection of desmethylimipramine (DMI) (0·5 mg/kg). Time marks, 1 min.

Discussion

In the experiments reported in this paper, it was found that the responses to angiotensin obtained in pithed preparations varied, both in character and magnitude, from simple pressor effects at low doses of 10–100 ng, to biphasic pressor responses at higher doses (200–500 ng). An accompanying chronotropic effect was always seen with large doses of angiotensin ; the onset of the effect coincided with that of the secondary pressor phase, but its duration, although dose dependent (Fig. 1), appeared to be independent of the total pressor response. The chronotropic action of angiotensin was abolished by bethanidine, reserpine pretreatment and propranolol, suggesting that it was mediated by sympathetic nervous activity involving β-adrenoreceptor sites. Since the increase in heart rate was observed in the pithed, adrenalectomized and anaesthetized preparations treated with a ganglion blocking agent, the response is probably not the result of centrally mediated effects, nor of the release of catecholamines from the adrenal medulla. The responses to angiotensin administered intra-arterially, in which the passage through the heart and lungs was initially by-passed, showed that approximately equipressor doses of angiotensin produced much smaller increases in heart rate. It therefore seems likely that the chronotropic action of angiotensin is due to stimulation of adrenergic nerves to the heart either at the level of the ganglia or at the sympathetic nerve endings. These conclusions are in partial agreement with those reported by Hughes (1968) for the urethane anaesthetized rat and also those of Farr & Grupp (1967) for the dog in which they suggested the site of stimulation by angiotensin occurs at the sympathetic ganglia.

The biphasic pressor response to angiotensin occurred in the pithed rat, in the ganglion blocked anaesthetized rat, in adrenalectomized preparations. It was still present in pithed preparations treated with propranolol despite the reduction of the accompanying chronotropic and inotropic response. This suggests, therefore, that the cardiac effects of angiotensin do not contribute greatly to the biphasic response. Treatment with phentolamine, bethanidine and pretreatment with reserpine modified the response to one of a simple pressor effect with a more rapid rise to its maximum although its duration was unaltered. In pithed preparations treated with bethanidine the overall enhancement seen in the responses to both angiotensin and noradrenaline may mask the underlying biphasic response. However, failure to observe any changes in the potentiated effects at lower angiotensin doses leaves the matter undecided. As intra-arterial injections did not alter the characteristics seen in the intravenous pressor response, it would seem likely that the secondary pressor phase of the compound angiotensin response is caused by stimulation of adrenergic nerves, involving α-receptors at vascular sites other than those of the heart and lungs.

The action of desmethylimipramine (DMI) in potentiating angiotensin responses throughout its entire dose range in something of an anomaly, although the extent was only approximately one-third of that obtained for equivalent pressor doses of noradrenaline. The results described in this paper are supported in part by those obtained by Hughes (1968) and Schmitt & Schmitt (1968) in which the authors found that the size of the pressor response in the rat was not reduced by adrenalectomy, phentolamine, bretylium or propranolol. Pals (1968) reported that bretylium facilitated the responses to angiotensin infusions and concluded that this was due to residual sympathomimetic effects of bretylium. Potentiation of angiotensin responses after DMI was observed in the dog and it was suggested that this

potentiation occurred by a different mechanism from that of noradrenaline (Kaumann, Zuberbühler & Taquini, 1964). However, Peach & Ford (1968) showed that DMI potentiated the increased plasma catecholamine concentrations observed in the dog after infusions of angiotensin. Our experiments suggest that the enhanced angiotensin responses seen in the pithed rat after DMI may not necessarily be the result of noradrenaline potentiation, and might be the result of unspecific effects as suggested by the fact that vasopressin is also potentiated by DMI.

From evidence presented it seems that some adrenergic interaction is involved in the overall cardiovascular responses to angiotensin. The actual mechanism may be the result of stimulation of the sympathetic ganglia, sympathetic nerve ending or by other sympathetic activity. The interaction is unlikely to be pre-ganglionic in origin as indicated by the results obtained with mecamylamine unless angiotensin acts as a non-nicotinic ganglion stimulant (Trendelenburg, 1966). The possibility of stimulation at the sympathetic nerve endings is more likely and is supported by the evidence of Peach & Ford (1968). Although evidence for some degree of sympathetic contribution has been obtained the amount is unlikely to be great in the rat, as shown by the necessity for large doses of angiotensin to demonstrate sympathetic effects.

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