Br. J. Pharmac. (1970), **39**, 414–427.

The effect of reserpine on the pressor responses to angiotensin in the conscious cat

M. D. DAY AND D. A. A. OWEN*

Pharmacological Laboratories, Department of Pharmacy, University of Aston in Birmingham, Birmingham 4

Summary

1. Blood pressure recordings have been made in conscious cats in an attempt to reveal a possible indirect component to the angiotensin pressor response.

2. Reserpine (50 to 250 μg/kg per day) caused a maximal reduction of about 50% in the pressor response to angiotensin whilst virtually abolishing the responses to tyramine and McN-A-343. Responses to noradrenaline were only slightly and transiently reduced.

3. Syrosingopine (0·5 mg/kg) and reserpine (250 μg/kg) reduced the responses to angiotensin, McN-A-343 and tyramine to much the same extent, but tetrabenazine only reduced the responses to all these agents in a dose (25 mg/kg) which probably had effects on the catecholamine stores of smooth muscle.

4. The reduction in the responses to angiotensin, tyramine and McN-A-343 by reserpine was partly reversed by tranylcypromine. Noradrenaline and (±)-dopa infusions were ineffective by themselves, but increased the effects of tranylcypromine in restoring the responses to angiotensin, tyramine and McN-A-343 after reserpine.

5. Infusion of α-methyldopa markedly increased the responses to angiotensin, tyramine and McN-A-343 after these had been reduced by reserpine.

6. The results suggest that the pressor response to angiotensin in the conscious cat is partly mediated by release of noradrenaline from peripheral neuronal stores.

Introduction

Intravenous infusions of angiotensin enhance the responses of the cardiovascular system of dogs (McCubbin & Page, 1963a, b; McCubbin, de Moura, Page & Olmsted, 1965; Page, Kaneko & McCubbin, 1966), man (Kaneko, Takeda, Nakajima & Ueda, 1966) and pithed rats (Day & Owen, 1969) to endogenous noradrenaline released by sympathetic nerve stimulation, ganglion stimulants and by indirectly acting sympathomimetic amines, but do not change the responses to exogenous noradrenaline. The precise mechanism of this interaction is not clear but Benelli, Della Bella & Gandini (1964) suggested that angiotensin may increase the release of noradrenaline from its neuronal stores. The results from direct testing of this hypothesis have been contradictory. An increased release of noradrenaline by sympathetic nerve stimulation in the presence of angiotensin was reported by

* Present address: Department of Pharmacology, Biological and Medical Research Division, Sandoz, Basle, Switzerland.

Zimmerman & Whitmore (1967) and by Zimmerman & Gisslen (1968), while Thoenen, Hurlimann & Haefely (1965) and Hertting & Suko (1966) detected no increase in their experiments.

On the isolated aortic strip preparation taken from several species angiotensin causes a contraction which is mediated, at least partly, by release of noradrenaline from endogenous stores (Distler, Liebau & Wolff, 1965a, b ; Liebau, Distler & Wolff, 1965, 1966 ; Suzuki & Matsumoto, 1966 ; Schumann & Guther, 1967). However, Day & Owen (1968) showed that in the isolated central artery from the rabbit ear, acute or chronic reserpine treatment to deplete noradrenaline stores did not reduce the vasoconstrictor response to angiotensin. This treatment did, however, prevent the usual increase in the angiotensin vasoconstrictor response which occurred during sympathetic stimulation in this preparation.

These results suggest that at least part of the vasoconstrictor effect of angiotensin *in vivo* and *in vitro* is mediated via an indirect action on the sympathetic nervous system. Previous studies using the intact cardiovascular system have not produced convincing evidence of an indirect component to the angiotensin pressor response in either the rat (Schmitt & Schmitt, 1968) or in man (Laurence & Nagle, 1963). However, Baum (1963) reported a reduction in the size of the angiotensin vasoconstrictor responses in the dog perfused hind limb after reserpine and Farr & Grupp (1967) showed that the cardio-accelerator action of angiotensin in the anaesthetized dog was mediated partly through an action on the cardiac sympathetic nerves.

It is likely that previous studies using whole animal preparations have been complicated by the use of anaesthetized (Baum, 1963 ; Farr & Grupp, 1967) or pithed (Schmitt & Schmitt, 1968) animals. The present study was undertaken to determine the sympathetic component, if any, of the pressor responses to angiotensin in the conscious unrestrained cat using reserpine and related substances to modify tissue catecholamine levels.

Methods

Conscious cats

The methods used for recording systemic arterial blood pressure and for the intravenous injection of drugs were essentially modifications of the methods developed by Thuransky (1966) and by Hall, Gomersall & Heneage (1967).

Female cats weighing 2·4 to 3·2 kg were used throughout and were trained, by frequent handling for at least 10 days before implantation of the cannulae, to sit quietly in the cage subsequently used for blood pressure recordings for periods of up to 3 h.

Implantation of arterial and venous cannulae. Anaesthesia was induced with halothane 3·5% in a mixture of 20% oxygen in nitrous oxide and maintained with 1·5% halothane in the same oxygen/nitrous oxide mixture.

An incision was made through the previously shaved ventral surface of the neck and the muscles overlying the trachea parted by blunt dissection to reveal the carotid arteries. The right carotid artery was cannulated with polyvinyl chloride tubing (Portland Plastics PP 90) previously filled with sterile 0·9% saline containing 10 units/ml heparin. The cannula was connected via a blood pressure transducer

to an electronic recorder (Devices M4) so that continuous measurement of blood pressure was possible during the positioning of the cannula. The length of the cannula inserted was measured off first such that the tip of the cannula lay in the aorta at the level of the xiphoid process. Continuous monitoring of the blood pressure during insertion of the cannula ensured that it did not become kinked or pass via the aortic valve into the left ventricle. The cannula was firmly tied into the artery and passed beneath the skin to emerge from a small incision on the back of the neck. A small branch of the right external jugular vein was cannulated with fine polyethylene tubing (Portland Plastics PP 30) and the tip of the cannula pushed into the main external jugular vein. The venous cannula was passed beneath the skin to emerge with the arterial cannula through a skin incision on the back of the neck. The ventral neck incision was closed at this stage with thread sutures and the animal placed face downwards on the operating table for the remainder of the operative procedure. The arterial cannula was then closed off close to the back of the neck and attached to a small Perspex valve of the type described by Hall *et al.* (1967). The valve was fixed to a rectangular Perspex base 3·8 cm long, 0·6 cm wide and 0·3 cm thick through which holes were drilled at each corner. The valve base was stitched firmly beneath the skin using thread sutures threaded through the valve base and the incision closed around the valve. The venous cannula was cut off so that it protruded about 8 cm from the skin through the valve incision and the end was closed with a dissecting pin.

At the end of the operative procedure chlorpromazine (1 mg/kg) was injected intramuscularly ; this substance reduced the rate of recovery of motor activity following the anaesthesia and also appeared to reduce the tendency of cats to scratch their wounds in the first few hours after the operation. Sodium benzylpenicillin (0·6 mg/kg) was injected intramuscularly immediately after the operation and thereafter daily for 4 days. During the first 7 postoperative days the arterial cannula was flushed through daily with 2 ml of saline containing heparin (50 units/ml) and subsequently this was repeated on alternate days and before the start of each recording session. The venous cannula was flushed out in the same way except that only 0·5 ml heparinized saline was used.

The cats were allowed at least 3 days for recovery after the operation, after which the process of training them to sit quietly during experiments and to accept intravenous injections without alarm began. In most cases the cats became sufficiently well trained to start experiments after a further 3 days.

Measurement of blood pressure and heart rate in conscious cats. For recording blood pressure the arterial valve cap was replaced by a threaded valve top (Hall *et al.*, 1967) which opened the valve and permitted continuous blood pressure measurement to be made. Arterial pressure was recorded by means of a pressure transducer (Devices/C.E.C. type 4-327-L221) connected to an electronic recorder (Devices M4). Heart rate was monitored by means of a Neilson tachygraph unit connected to the Devices recorder and triggered from the blood pressure pulse.

Intravenous drug injections. The intravenous cannula was connected by a broken off hypodermic needle to a 45 cm length of fine polyethylene tubing (Portland Plastics PP 30) and drugs dissolved in 0·9% sodium chloride solution were administered in a dose/volume not exceeding 0·4 ml and flushed in with a further 1 ml of saline. By using a dose volume of 0·4 ml or less the drug solution was retained

in the tubing until flushed in and thus the injection could be delayed on the few occasions when the cat became restless at the start of the injection.

Drugs used. α-methyldopa (Aldomet injection, Merck, Sharp & Dohme), angiotensinamide (Hypertensin, Ciba), chlorpromazine hydrochloride (Largactil injection, May & Baker Ltd.) (±)-dopa (Sigma), McN-A-343 (McNeil), (−)-noradrenaline hydrochloride (Sigma), pempidine tartrate (May & Baker Ltd.), reserpine (injection made by Halewood Chemical Company), syrosingopine (Aspro-Nicholas), tetrabenazine (Hoffman La-Roche), tranylcypromine sulphate (Smith, Kline & French Labs.), tyramine hydrochloride (Sigma).

The doses of noradrenaline quoted are expressed in terms of base ; all other doses are expressed in terms of the salts.

Syrosingopine and tetrabenazine were prepared for injection by dissolving the solid in three drops of lactic acid and 0·8 ml of ethanol and then making up to half volume with distilled water. The pH of this solution was about 2·5 and this was adjusted to pH 4·5 by the dropwise addition of a saturated aqueous solution of sodium bicarbonate. This solution was made up to volume with distilled water.

Results

Selection of doses and reproducibility of responses to intravenous pressor substances

Acute experiments. In addition to angiotensin, the other pressor agents used were noradrenaline, the indirectly acting amine tyramine and McN-A-343, a substance which exerts its pressor action by activation of sympathetic ganglia (Roszkowski, 1961 ; Levy & Ahlquist, 1962). In initial experiments doses of these pressor agents producing submaximal responses were established and these doses were used in all subsequent experiments. The doses selected were angiotensin (50 ng/kg and 100 ng/kg), noradrenaline (200 ng/kg), McN-A-343 (15 μg/kg) and tyramine (50 μg/kg). The responses to those doses of these substances were found to be as reproducible in conscious cats as in previous experiments using chloralosed cats. Figure 1 illustrates a typical experiment in which the responses to three consecutive doses of each control agent injected at 10 min intervals are plotted in the form of a histogram.

Chronic experiments. The responses in any one cat to all the pressor substances used were reproducible from day to day (Fig. 2). To reduce the variability of drug responses, each pressor agent was administered three times in every experiment and the mean taken. The mean response to any one pressor agent was usually found to be within ±5% of the mean response on another day and always within ±10%. This order of day to day reproducibility in responsiveness was shown by each of the five cats used in the present study.

Effect of reserpine on blood pressure and heart rate

Reserpine ((10 μg/kg)/day intravenously) for 1 to 7 days caused no detectable change in resting blood pressure or heart-rate. When the dose was increased to (50 μg/kg)/day for up to 7 days there was a reduction in both systolic and diastolic blood pressures of 20–30 mmHg (1 mmHg≡1·333 mbar) and a fall in heart-rate of 10 to 20 beats/min. With this dose of reserpine there was evidence of central nervous system depression ; the cats became lethargic and lost interest

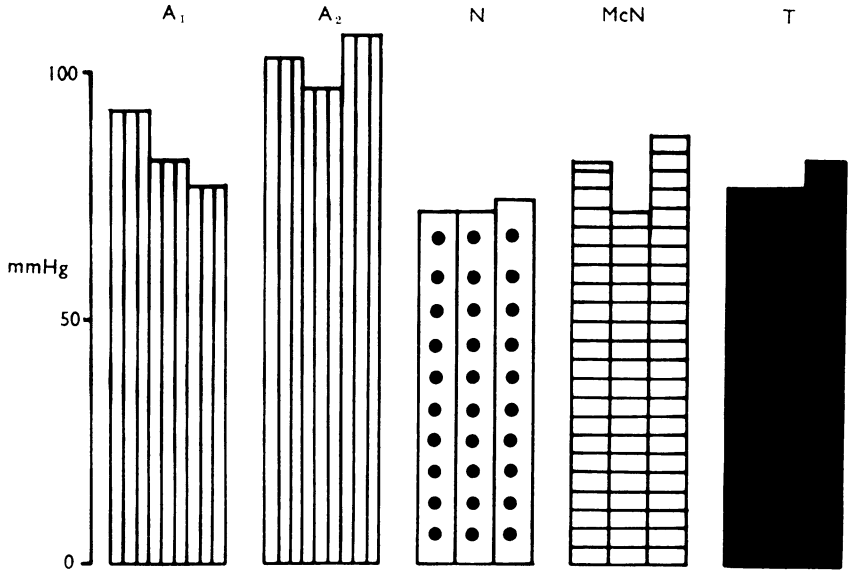

FIG. 1. Blood pressure in conscious cat. Histogram representing pressor responses to three consecutive intravenous doses of each of the following pressor agents at 10 min intervals: angiotensin 50 ng/kg (A_1) and 100 ng/kg (A_2), noradrenaline 200 ng/kg (N), Mc-A-343 15 μg/kg (McN) and tyramine 50 μg/kg (T).

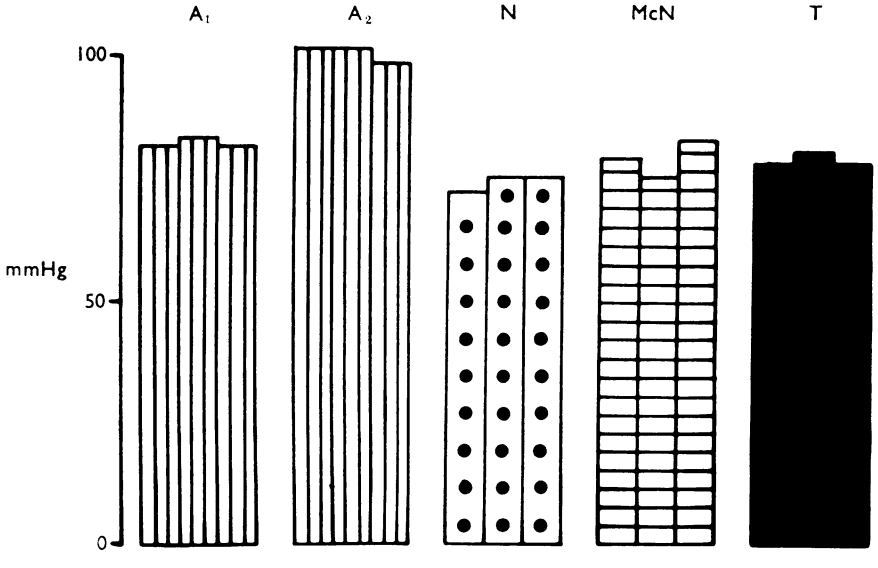

FIG. 2. Blood pressure in conscious cat. In the histogram each vertical column represents the mean of three consecutive pressor responses to each agent injected intravenously at 10 min intervals. The mean response to each agent was obtained on each of three consecutive days. Thus the height of the columns in each block of 3 represents the variation of the response from day to day. Angiotensin 50 ng/kg (A_1) and 100 ng/kg (A_2), noradrenaline 200 ng/kg (N), Mc-A-343 15 μg/kg (McN) and tyramine 50 μg/kg (T).

in their surroundings. In about half of these experiments the animals suffered mild diarrhoea, but this was not associated with any significant loss of body weight. Higher doses of reserpine ((100 and 250 μg/kg)/day) for 1 to 3 days reduced mean arterial blood pressure by up to 50 mmHg, heart-rate by up to 50 beats/min, caused marked diarrhoea in all experiments with an associated loss of body weight of up to 10% of control weight, and finally caused marked depression and relaxation of the nictitating membranes.

Effect of reserpine on pressor responses to angiotensin, noradrenaline, tyramine and McN-A-343

A single dose of reserpine (10 μg/kg) reduced the pressor response to tyramine by approximately 50% but did not affect the responses to the other pressor agents. Reserpine 50 μg/kg reduced the responses to all four control agents when these were tested 4 to 24 h later. However, when this dose of reserpine was administered daily for up to 7 days the responses to the various pressor agents were affected differently as illustrated in Fig. 3. The responses to both noradrenaline and angiotensin were similarly reduced 1 day after reserpine, but after 2 days the noradrenaline responses had returned to almost control level whereas those to angiotensin continued to decline until they reached a steady level of about 50% of control height after 3 days' treatment. The responses to both McN-A-343 and tyramine were markedly reduced 24 h after reserpine and thereafter did not significantly recover throughout the 7 days of this experiment. A large single dose of reserpine (250 μg/kg) caused a greater impairment of the noradrenaline responses and a long-lasting reduction in the responses to both McN-A-343 and tyramine. However, the responses to angiotensin were only reduced to the same extent as after the lowest effective dose (50 μg/kg) of reserpine.

In three other experiments the pressor responses to angiotensin (25–200 ng/kg) were reduced by reserpine (50–250 μg/kg) to a maximum of approximately 50% of control responses throughout the dose ranges of both substances.

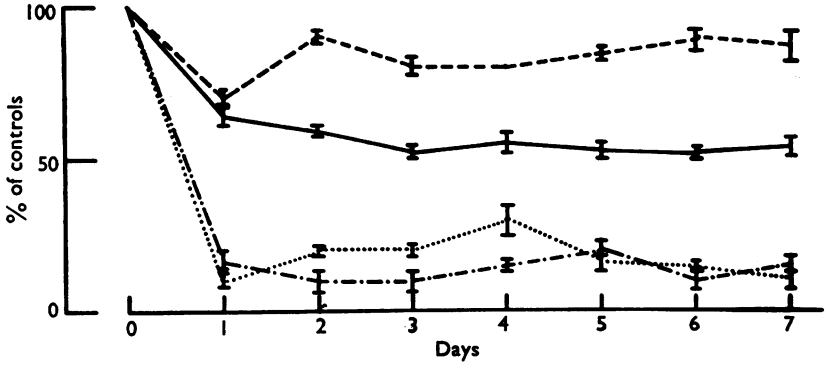

FIG. 3. Blood pressure in conscious cats. Reserpine (50 μg/kg intravenously) administered daily commencing at time 0. Mean daily pressor responses (expressed as percentage of responses in the absence of reserpine) to noradrenaline 200 μg/kg (– – –), angiotensin 50 ng/kg (——), McN-A-343 15 μg/kg (·····) and tyramine 50 μg/kg (– · – · –). Each point is the mean of results from three cats. Vertical bars, S.E. of mean.

Effect of tetrabenazine

Reserpine is known to deplete catecholamines from both central and peripheral noradrenaline storage sites and thus the site of its anti-angiotensin effect could be either central or peripheral. Tetrabenazine (1 to 25 mg/kg intraperitoneally), which depletes catecholamines predominantly from storage sites in the brain (Brodie, 1960), had no effect on blood pressure or heart rate and did not cause relaxation of the nictitating membranes or diarrhoea. In doses of 5 mg/kg or more, it caused depression of the central nervous system, but this was less well marked than after 250 μg/kg reserpine. At doses less than 5 mg/kg, tetrabenazine did not affect the pressor responses to any of the control drugs whilst at 5 mg/kg it slightly reduced the responses to tyramine alone. At 25 mg/kg tetrabenazine reduced the responses to all the control agents as illustrated in Fig. 4. The responses to tyramine were most reduced while those to noradrenaline were least affected and the responses to both McN-A-343 and angiotensin were reduced by about 50%. The maximal depression in all the control responses occurred about 6 h after tetrabenazine and sensitivity to all the control agents had returned by 24 h. The effect of tetrabenazine (25 mg/kg) on pressor responses closely resembled the effects of a small dose of reserpine and suggests that a peripheral rather than a central site of action is implicated for the effect of this dose of tetrabenazine on angiotensin.

Effect of syrosingopine

Six hours after syrosingopine (500 μg/kg intraperitoneally), which depletes peripheral catecholamine stores but has relatively little effect on brain amine levels (Brodie, 1960), there was a slight reduction in both systolic and diastolic blood pressures (15 to 20 mmHg) and a slight bradycardia (10 to 20 beats/min). This dose also caused a moderate relaxation of the nictitating membranes in each of the three cats tested, mild diarrhoea in two of them but no overt behavioural changes in any of them.

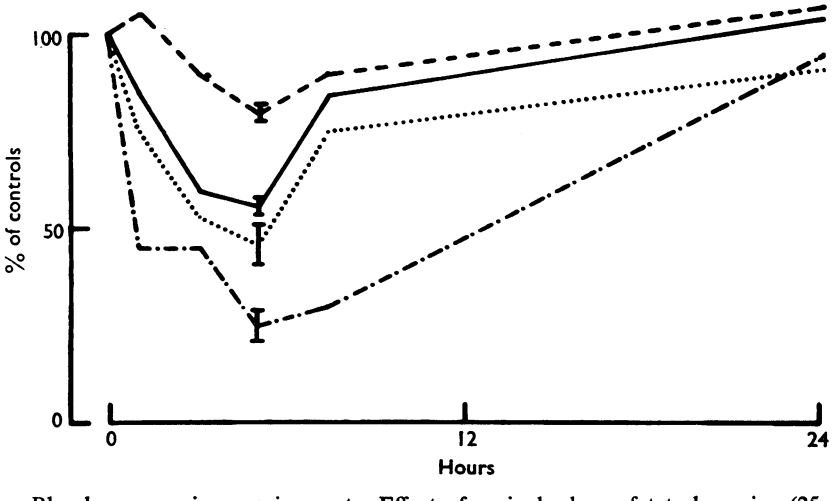

FIG. 4. Blood pressure in conscious cat. Effect of a single dose of tetrabenazine (25 mg/kg intraperitoneally) on the mean pressor responses (expressed as percentage of controls) to noradrenaline 200 ng/kg (– – –), angiotensin 50 ng/kg (——), McN-A-343 15 μg/kg (····) and tyramine 50 μg/kg (–·–·–). Vertical bars, S.E. of mean.

The effect of this dose of syrosingopine was to cause a rapid reduction of the responses to both McN-A-343 and angiotensin, the effect being most marked with McN-A-343 (Fig. 5). The maximum depression of the responses to each substance occurred after 6 h, then they recovered in a parallel fashion until full responsiveness returned after about 12 h. The responses to tyramine were initially slightly enhanced and subsequently reduced by a maximum of 50%. The responses to noradrenaline were virtually unaffected by syrosingopine.

Effect of reserpine in ganglion-blocked cats

Sedvall (1964) and Sedvall & Thorsen (1965) demonstrated two functional noradrenaline stores within peripheral sympathetic neurones using reserpine. Reserpine readily depleted the larger noradrenaline pool from the neurones while a smaller more tightly bound pool was only slowly released as a result of sympathetic nerve activity combined with a reserpine-induced failure of noradrenaline re-uptake into this pool. These workers demonstrated that the smaller "nerve" pool of noradrenaline could be protected from depletion by blockade of impulses in the post-ganglionic sympathetic nerves by sectioning the preganglionic nerve trunks. In order to determine which pool of noradrenaline was involved in the angiotensin pressor response we retested the effect of reserpine in ganglion-blocked cats. Three cats were given pempidine (5 mg/kg intravenously) every 8 h for 24 h before reserpine (50 μg/kg) and subsequently, at the same intervals, for 24 h after reserpine. This treatment with pempidine maintained a state of virtually complete ganglion blockade throughout the experiment as shown by a marked relaxation of the nictitating membranes and a complete absence of reflex bradycardia after the injection of any pressor agent.

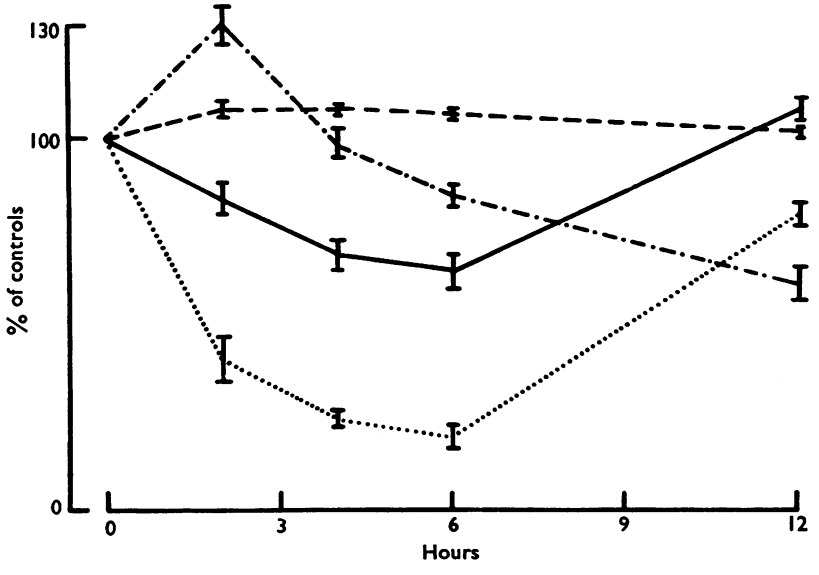

FIG. 5. Blood pressure in conscious cat. Effect of a single dose of syrosingopine (500 μg/kg intraperitoneally) on the mean pressor responses (expressed as percentage of controls) to noradrenaline 200 ng/kg (– – –), angiotensin 50 ng/kg (——), NcN-A-343 15 μg/kg (· · · ·) and tyramine 50 μg/kg (– · – · –). Vertical bars, S.E. of mean. Each point represents the mean responses from three separate cats.

In cats which had received pempidine alone the responses to all the pressor agents were increased, presumably due to the absence of inhibitory cardiovascular reflexes. Figure 6 illustrates the mean results from the same cats after reserpine (50 μg/kg). The responses to both tyramine and noradrenaline were depressed as in non-ganglion blocked cats. However, the responses to McN-A-343 and angiotensin were each reduced to the same extent but with a much more rapid onset and recovery than in cats given reserpine alone.

Reversal of the anti-angiotensin action of reserpine

In the following experiments attempts to reverse the anti-angiotensin effects of reserpine were carried out in cats pretreated with reserpine (50 μg/kg per day) for 7 days before the experiment in which the responses to angiotensin were reduced by approximately 50%, those to McN-A-343 and tyramine by about 80% and those to noradrenaline only slightly. Each drug was tried in three cats and the sensitivity to the control agents was retested 30 min after the infusion in each experiment.

Noradrenaline

Noradrenaline ((2 μg/kg)/min) was infused for periods of 1 to 2 h. It caused a sustained increase in mean blood pressure of about 20 mmHg and a slight bradycardia (10 to 20 beats/min) in each of the three experiments. Noradrenaline at this dose level had no effect on the responses to angiotensin and McN-A-343, caused a marginal increase in the responses to tyramine and a marginal decrease in the noradrenaline responses.

(±)-Dopa

(±)-Dopa ((20 μg/kg)/min) was infused for 1 to 2 h and had no effect on blood pressure, heart-rate or the pressor responses.

Tranylcypromine

The monoamine oxidase inhibiting drug tranylcypromine (5 mg/kg) caused a partial reversal of the effect of reserpine on the pressor agents when these were tested 16–20 h after treatment. This is illustrated in Fig. 7. After tranylcypromine

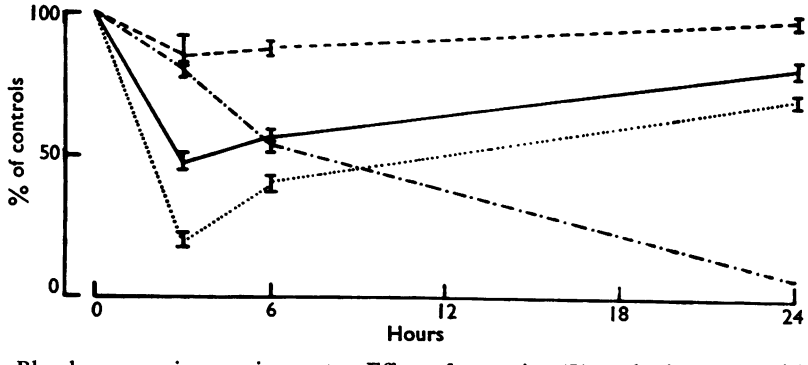

FIG. 6. Blood pressure in conscious cats. Effect of reserpine (50 μg/kg intravenously) in cats treated with pempidine (5 mg/kg intravenously every 8 h) started 24 h before reserpine (administered at time 0) and continued throughout the experiment. Noradrenaline 200 ng/kg (– – –), angiotensin 50 ng/kg (——), McN-A-343 15 μg/kg (·····) and tyramine 50 μg/kg (–·–·–). Results are the means taken from three separate cats. Vertical bars, S.E. of mean.

the responses to noradrenaline were increased above control level whilst those to angiotensin, McN-A-343 and tyramine were partly restored.

Effect of infusions of (±)-dopa and noradrenaline after tranylcypromine

The infusions of noradrenaline and (±)-dopa previously found to be virtually ineffective in reversing the action of reserpine on the control responses were repeated

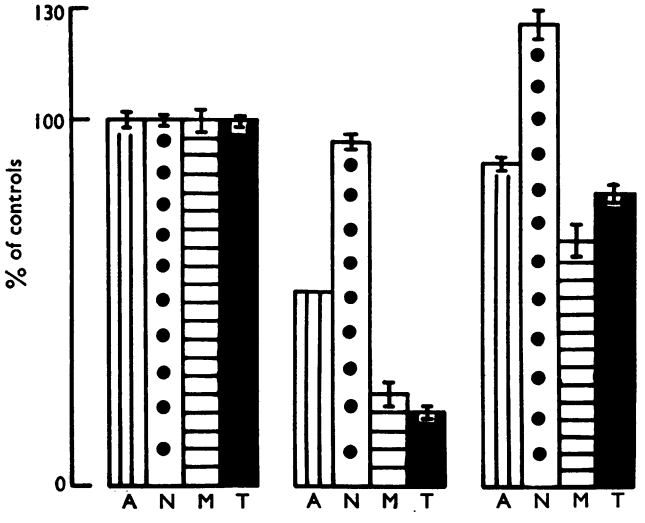

FIG. 7. Blood pressure in conscious cats. Mean pressor responses to angiotensin 50 ng/kg (A), noradrenaline 200 ng/kg (N), McN-A-343 15 μg/kg (M) and tyramine 50 μg/kg (T). Columns at left show control responses, centre columns responses 24 h after last dose of reserpine (50 μg/kg intravenously every day for 7 days) and at right responses 16 h after tranylcypromine (5 mg/kg intravenously). All panels are the mean results from three separate cats. Vertical bars, S.E. of mean.

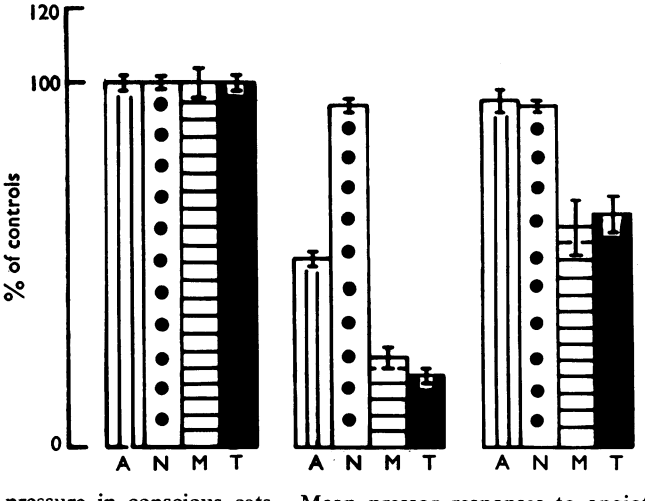

FIG. 8. Blood pressure in conscious cats. Mean pressor responses to angiotensin 50 ng/kg (A), noradrenaline 200 ng/kg (N), McN-A-343 15 μg/kg (M) and tyramine 50 μg/kg (T). Columns at left: control responses, centre columns: responses after 7 days' treatment with reserpine (50 μg/kg intravenously every day), and at right: responses 30 min after an intravenous infusion of α-methyldopa ((20 μg/kg)/min for 2 h). All columns are the mean results obtained from three separate cats. Vertical bars, S.E. of mean.

16 h after treatment with tranylcypromine. A combination of either noradrenaline or (±)-dopa with tranylcypromine caused a greater reversal of the effects of reserpine on the control responses than did any of these substances singly.

Infusion of α-methyldopa

In view of the apparent involvement of monoamine oxidase in the restorative action of noradrenaline and (±)-dopa after reserpine, α-methyldopa was used because it and its decarboxylated metabolites are immune to monoamine oxidase. α-Methyldopa ((20 μg/kg)/min for 2 h) was as effective as a combination of (±)-dopa and tranylcypromine in restoring the responses to McN-A-343, angiotensin and tyramine, as shown in Fig. 8.

Discussion

The initial experiments in which the day to day reproducibility of drug responses on the cardiovascular system of conscious cats were studied indicated that this preparation is well suited for studying interactions between drugs such as angiotensin and reserpine. This preparation offers several important advantages over studies in anaesthetized cats. Thus, long-term studies are possible in which animals serve as their own controls free from possible artefacts introduced by anaesthesia. Reserpine produced its characteristic effects on the pressor responses to tyramine and to sympathetic activation by McN-A-343 but at a dose (50 μg/kg) which caused no marked diarrhoea or loss in body weight as is associated with larger doses used in many previous studies (Withrington & Zaimis, 1961). 24 h after an intravenous dose of reserpine (50 μg/kg) there was a decrease in the sensitivity to all four control pressor agents, although this was much greater for tyramine and McN-A-343 than it was for angiotensin and noradrenaline. With daily administration of this dose of reserpine the responses to noradrenaline virtually recovered after 2 days while the responses to angiotensin continued to decline until they reached a steady level of about 50% control height. The responses to McN-A-343 and tyramine remained severely impaired throughout the experiment. The impairment of the angiotensin responses was consistent over a wide range of doses of both angiotensin (25 to 200 ng/kg) and reserpine (50 to 250 μg/kg). Although reserpine in doses greater than 50 μg/kg caused a greater and more persistent impairment of the responses to noradrenaline, tyramine and McN-A-343 the responses to angiotensin were reduced only to the same extent as after a 50 μg/kg dose, suggesting that a constant proportion of the angiotensin response is reserpine-sensitive.

Since reserpine is known to deplete biogenic amines from both brain and peripheral tissues and since angiotensin is reputed to have central as well as peripheral effects (Bickerton & Buckley, 1961) further experiments were performed to determine the site of the reserpine-sensitive part of the angiotensin pressor response. Tetrabenazine, which depletes catecholamines preferentially from brain stores (Brodie, 1960), had no effect on the pressor responses to any of the control agents in doses which caused central depression. However, larger doses affected the control responses in the same way as small doses of reserpine, suggesting that at these dose levels tetrabenazine may be acting peripherally. Syrosingopine, on the

other hand, is known to deplete catecholamines chiefly from peripheral stores and this substance produced a similar but more transient effect on control responses to angiotensin as did reserpine. The action of syrosingopine differed from that of reserpine in that it caused a much more rapid impairment of the responses to McN-A-343 than did reserpine. The responses to McN-A-343 were markedly impaired by syrosingopine at a time when tissue catecholamine levels, as judged by tyramine responses, were relatively unaffected. This suggests that syrosingopine may exert part of its effect on peripheral sympathetic neurones by a mechanism not shared by reserpine.

After reserpine, tetrabenazine and syrosingopine the onset of the block and recovery of the responses to angiotensin and McN-A-343 closely paralleled each other in time course although the McN-A-343 responses were more markedly reduced. The results suggest that peripheral catecholamine stores are involved in the acute pressor responses to angiotensin and further that the stores involved are those from which nerve impulses release noradrenaline. The results of Sedvall (1964) and Sedvall & Thorsen (1965) suggest that the " nerve " store of noradrenaline can be protected from depletion by reserpine by blocking post-ganglionic nerve activity. However, in our experiments using ganglion-blocked cats reserpine caused a more rapid reduction of the responses to McN-A-343 and angiotensin followed by a more rapid recovery than in control cats, whilst the responses to noradrenaline and tyramine were affected as in non-ganglion-blocked cats. The more rapid onset of the reserpine block was difficult to explain. However, as in previous experiments, the impairment of the responses to McN-A-343 and angiotensin followed an almost identical time-course.

The experiments in which noradrenaline and (±)-dopa were infused after reserpine provided further evidence in favour of an indirect component to the angiotensin pressor response. In the doses used neither noradrenaline nor (±)-dopa were effective in restoring the responses to tyramine, McN-A-343 or angiotensin. This is contrary to previous observations made in anaesthetized cats (Burn & Rand, 1958 ; Day & Rand, 1964) and is probably accounted for by the relatively low doses of noradrenaline and (±)-dopa used compared with previous studies. Tranylcypromine was partly effective in restoring the responses to the control agents after reserpine and this effect was increased by infusions of noradrenaline or (±)-dopa in doses previously found to be ineffective before inhibition or monoamine oxidase. In view of the apparent involvement of monoamine oxidase in maintaining the neuronal noradrenaline stores at a low level after reserpine, experiments were performed with α-methyldopa, which is unaffected by this enzyme. α-Methyldopa and its metabolites α-methyldopamine and α-methylnoradrenaline are known partly to reverse the reduction in the responses to tyramine and sympathetic stimulation in the reserpinized anaesthetized cat (Day & Rand, 1964). In the present experiments it was found that α-methyldopa was approximately equi-active with a mixture of (±)-dopa and tranylcypromine in restoring the responses to angiotensin, tyramine and McN-A-343 after reserpine.

The evidence presented, therefore, strongly suggests that the acute pressor action of angiotensin has both direct and indirect components. The indirect component which accounts for approximately half of the response is abolished by depletion of peripheral catecholamine stores with reserpine or syrosingopine. Tetrabenazine, which depletes central catecholamine stores only, reduced the pressor effect of

angiotensin in large doses which also reduced responses to tyramine and McN-A-343 and was, therefore, presumably acting peripherally. A significant central component to the angiotensin pressor response is also precluded by the finding that the pressor responses to angiotensin were increased after ganglion blockade. The reduction in the responses to angiotensin by reserpine and related substances more closely paralleled the onset and duration of the impairment of the McN-A-343 responses than of the tyramine responses, suggesting that it is the "nerve" pool from which angiotensin releases noradrenaline. Finally, the restorative effect on angiotensin pressor responses after reserpine by tranylcypromine, by noradrenaline and (±)-dopa after tranylcypromine and by α-methyldopa provide further evidence of an indirect sympathetic component in the acute pressor effect of angiotensin.

We are grateful to the following firms for gifts of drugs, Ciba Ltd. (angiotensinamide), Hoffman La-Roche (tetrabenazine), McNeil Labs. (McN-A-343) and Aspro-Nicholas (Syrosingopine). This work was supported by a grant from the Medical Research Council.

REFERENCES

BAUM, T. (1963). Vascular reactivity of reserpine-pretreated dogs. *J. Pharmac. exp. Ther.*, **141**, 30–35.

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.

BRODIE, B. B. (1960). Selective release of norepinephrine and serotonin by reserpine-like compounds. *Diseases of the Nervous System.* Suppl. to Volume 21, pp. 107–109. Irvington, N.J. : Physicians Postgraduate Press.

BURN, J. H. & RAND, M. J. (1958). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol., Lond.*, **144**, 314–336.

DAY, M. D. & OWEN, D. A. A. (1968). The interaction between angiotensin and sympathetic vasoconstriction in the isolated artery of the rabbit ear. *Br. J. Pharmac.*, **34**, 499–507.

DAY, M. D. & OWEN, D. A. A. (1969). Potentiation by angiotensin of responses to endogenously released noradrenaline in the pithed rat. *Archs int. Pharmacodyn. Thér.*, **179**, 469–479.

DAY, M. D. & RAND, M. J. (1964). Some observations on the pharmacology of α-methyldopa *Br. J. Pharmac. Chemother.*, **22**, 72–86.

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

DISTLER, A., LIEBAU, H. & WOLFF, H. P. (1965b). Untersuchungen uber der mechanismus des pressorichen effektes von angiotensin. *Verh. dt. Ges. inn. Med.*, **71**, 437–441.

FARR, W. & GRUPP, G. (1967). Sympathetically mediated effects of angiotensin on the dog heart *in situ*. *J. Pharmac. exp. Ther.*, **156**, 528–537.

HALL, G. H., GOMERSALL, J. C. R. & HENEAGE, E. (1967). A simple device for recording blood pressure or for intravenous injection of drugs in the conscious unrestrained cat. *Physiol. Behav.*, **3**, 205–206.

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

KANEKO, Y., TAKEDA, T., NAKAJIMA, K. & UEDA, H. (1966). Effect of angiotensin on the pressor response to tyramine in normotensive subjects and hypertensive patients. *Circulation Res.*, **19**, 673–680.

LAURENCE, D. R. & NAGLE, R. E. (1963). The effects of bretylium and guanethidine on the pressor responses to noradrenaline and angiotensin. *Br. J. Pharmac. Chemother.*, **21**, 403–413.

LEVY, B. & AHLQUIST, R. P. (1962). A study of sympathetic ganglionic stimulants. *J. Pharmac. exp. Ther.*, **137**, 219–228.

LIEBAU, H., DISTLER, A. & WOLFF, H. P. (1965). The noradrenaline releasing effect of angiotensin II in isolated blood vessels. *Acta endocr., Copenh.*, Suppl., 100, No. 138.

LIEBAU, H., DISTLER, A. & WOLFF, H. P. (1966). Untersuchungen zur indirekten sympathomimetischen wirkung von angiotensin an isolierten blutgefaben. *Klin. Wschr.*, **44**, 322–326.

MCCUBBIN, J. W., DE MOURA, R. S., PAGE, I. H. & OLMSTED, F. (1965). Arterial hypertension elicited by subpressor amounts of angiotensin. *Science, N.Y.*, **149**, 1394–1395.

MCCUBBIN, J. W. & PAGE, I. H. (1963a). Neurogenic component of chronic renal hypertension *Science, N.Y.*, **139**, 210–215.

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

PAGE, I. H., KANEKO, Y. & McCUBBIN, J. W. (1966). Cardiovascular reactivity in acute and chronic renal hypertensive dogs. *Circulation Res.*, **18**, 379–387.

PAGE, I. H. & OLMSTED, F. (1961). Hemodynamic effects of angiotensin, norepinephrine and bradykinin continuously measured in unanaesthetised dogs. *Am. J. Physiol.*, **201**, 92–97.

ROSZKOWSKI, A. P. (1961). An unusual type of sympathetic ganglion stimulant. *J. Pharmac. exp. Ther.*, **132**, 156–170.

SCHMITT, H. & SCHMITT, H. (1968). Modifications des effets hypertensuers de l'angiotensine par les agents adrenergigues et antidrenergigues. *Archs int. Pharmacodyn. Thér.*, **171**, 31–46.

SCHUMANN, H. J. & GUTHER, W. (1967). Untersuchungen zum wirkungmechanismus von angiotensin am isolierten aortenpraparatund am blutdruck von ratten und meerschweinchem. *Arch. Pharmak. exp. Path.*, **256**, 169–182.

SEDVALL, G. (1964). Short term effects of reserpine on noradrenaline levels in skeletal muscle. *Acta physiol. scand.*, **62**, 101–108.

SEDVALL, G. & THORSEN, J. (1965). Adrenergic transmission at vasoconstrictor nerve terminals partially depleted of noradrenaline. *Acta physiol. scand.*, **64**, 251–258.

SUZUKI, A. & MATSUMOTO, H. (1966). The mechanism of contracting action of angiotensin on the isolated aortic strip of rabbit. *Kobe J. med. Sci.*, **12**, 167–178.

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

THURANSKY, K. (1966). Continuous blood pressure measurement in non-anaesthetised animals. *Acta Physiol. Acad. Sci. Hung.*, **29**, 33–40.

WITHRINGTON, P. & ZAIMIS, E. (1961). The reserpine-treated cat. *Br. J. Pharmac. Chemother.*, **17**, 380–391.

ZIMMERMAN, B. G. & GISSLEN, J. (1968). Pattern of renal vasoconstriction and transmitter release during sympathetic stimulation in presence of angiotensin and cocaine. *J. Pharmac. exp. Ther.*, **163**, 320–329.

ZIMMERMAN, B. G. & WHITMORE, L. (1967). Effect of angiotensin and phenoxybenzamine on release of norepinephrine in vessels during sympathetic nerve stimulation. *Int. J. Neuropharmac.*, **6**, 27–38.

(*Received January* 21, 1970)