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Further evidence for a central hypotensive action of α-methyldopa in both the rat and cat

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Summary

1. α-Methyldopa (300 mg/kg i.p.) produced a fall in blood pressure in conscious genetic hypertensive rats. Pretreatment with intraventricular 6-hydroxydopamine prevented this hypotensive effect of α-methyldopa, whilst intravenous 6-hydroxydopamine reduced but did not prevent the hypotension.

2. The hypotensive effect of α-methyldopa was prevented or reversed by intraventricular injection of phentolamine (200 μg/rat).

3. Pressor responses obtained by stimulation of the entire sympathetic outflow in the Gillespie & Muir preparation, were unaffected by pretreatment with α-methyldopa (300 mg/kg i.p.).

4. Vasoconstrictor responses to periarterial nerve stimulation of the isolated renal artery preparation of the rat were markedly reduced by pretreatment with α-methyldopa. Furthermore, α-methylnoradrenaline was found to have one-eighth the vasoconstrictor potency of noradrenaline in this particular artery preparation.

5. Pressor responses obtained by stimulation of the posterior hypothalamus or midbrain reticular formation in the rat anaesthetized with urethane were markedly reduced by pretreatment with α-methyldopa. FLA-63, a selective dopamine-β-hydroxylase inhibitor, prevented the reduction of the pressor responses to hypothalamic stimulation produced by α-methyldopa.

6. Stimulation of the posterior hypothalamus in the anaesthetized cat caused both an increase in sympathetic nerve activity and a rise in blood pressure. These responses were markedly reduced 3–4 h after the injection of α-methyldopa (100 mg/kg i.v.).

7. These results strongly suggest that the central actions of α-methyldopa are important for its hypotensive effect, although a possible peripheral effect cannot be excluded.

Introduction

Day & Rand (1963, 1964) proposed that α-methyldopa exerts its hypotensive effect by the formation of α-methylnoradrenaline which was assumed to be a less potent transmitter than noradrenaline in the peripheral sympathetic nervous system. However, the finding that treatment with α-methyldopa produces only a moderate impairment of peripheral adrenergic transmission is strong evidence against a

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peripheral false transmitter mechanism (Haefely, Hürlimann & Thoenen, 1967; Henning & Svensson, 1968; Finch, 1971). Furthermore, α-methylnoradrenaline and noradrenaline have been reported to be equipotent pressor agents in both the anaesthetized dog and rat (Trinker, 1971).

Since α-methyldopa has a far greater hypotensive effect when administered into the vertebral artery than when given intravenously, a central site of action has been proposed (Henning & Van Zwieten, 1968). This theory is strengthened by the additional finding that decarboxylation of α-methyldopa within the central nervous system is essential for the hypotensive effect of the drug (Henning, 1969). The observation that perfusion of the cat brain with α-methyldopa produces a marked decrease in peripheral vascular resistance also supports a central mode of action of α-methyldopa (Ingenito, Barrett & Procita, 1970; Heise & Kroneberg, 1972).

The present study provides further evidence that the hypotension caused by α-methyldopa is predominantly due to a central mechanism of action.

Methods

Genetic hypertensive rats which originated as a hypertensive mutant of a Wistar strain (Okamoto & Aoki, 1963) were obtained by brother-sister mating. Blood pressure was measured by an indirect method (Gerold & Tschirky, 1968) and only animals with a systolic blood pressure of 190 mmHg and over were selected for experiments (1 mmHg≡1·333 mbar).

Measurement of blood pressure (direct method)

Rats were anaesthetized with halothane and a cannula implanted into the aorta according to the method of Popovic & Popovic (1960). Blood pressure was recorded 1–2 days later in conscious unrestrained rats with a Statham P23Dd pressure transducer connected to a Hellige He17 recorder.

Gillespie & Muir preparation

Pithed rat preparations were used for stimulation of the entire sympathetic outflow according to the method of Gillespie & Muir (1967). Stimulation at varying frequencies, 0·1 ms duration, at supramaximal voltage was applied for periods of 15 s and repeated every 10 minutes. Atropine (0·5 mg/kg) and tubocurarine (1 mg/kg) were given intravenously before commencement of stimulation.

Experiments on anaesthetized rats

Rats were anaesthetized with urethane (1·5 g/kg i.p.); the left carotid artery was then cannulated and connected to a pressure transducer. The animal's head was placed in a David Kopf stereotaxic apparatus, and a bipolar electrode (0·5 mm diameter, 0·5 mm uninsulated tip) was introduced through a small trephine hole. Coordinates (A=4·6, L=0·5, H=−2·5) for the posterior hypothalamus were those of the De Groot atlas (1963) and for the mid-brain reticular formation (A=1950 μm, L=1·0, H=0−1·0; König & Klippel, 1963). Centrally evoked pressor responses were obtained with stimulation of 10 V, 1 ms duration, at 6·4-50 Hz for periods of 10 s, delivered from a Grass S9 stimulator.

Isolated renal artery preparation

Rats were anaesthetized with ether and the left renal artery was cannulated from the aorta with a stainless steel cannula (No. 18 needle) and cut at the level of the kidney hilus. The isolated artery floated on the surface of a 50 ml organ bath which was filled with Krebs–Henseleit solution (37° C) and bubbled gently with 95% O_2 and 5% CO_2. The preparation was also perfused with oxygenated Krebs–Henseleit solution delivered from a Vario Perpex peristaltic pump. The flow rate was adjusted to 6 ml/min, which resulted in a basal perfusion pressure of 25–40 mmHg. After an equilibration period of 45 min, periarterial nerve stimulation was carried out with a specially designed fluid electrode placed around the artery. Supramaximal stimulation at varying frequencies, 1 ms duration for 20 s, was obtained with a Grass S4 stimulator. For dose-response curves, injections (0·1 ml) of noradrenaline were given into the perfusion system 2 cm from the renal artery. Increases in perfusion pressure were recorded with a Statham P23Dd pressure transducer.

Experiments on anaesthetized cats

Cats (2–3 kg) were anaesthetized with chloralose, 50 mg/kg i.p., and urethane, 250 mg/kg i.p.; the femoral artery and vein were then cannulated and a pressure transducer was connected to the arterial cannula. The animal's head was fixed in a David Kopf stereotaxic apparatus and a bipolar electrode introduced into the posterior hypothalamus using the following coordinates: A=8·5, L=1·0, H=−2·4 (Snider & Niemer, 1961). The right splanchnic and renal nerves were exposed through a dorsal incision and multifibre preparations of each nerve placed on bipolar platinum wire electrodes. The sympathetic discharges were then amplified and the resultant activities integrated every 30 seconds. In order to ensure that recordings of discharges were free from movement artifacts, the cats were immobilized with gallamine (5 mg/kg i.v.) and respired with a Palmer pump.

Drugs

Atropine sulphate (Siegfried, Switzerland), FLA-63 (Bis[4-methyl-1-homopiperazinyl-thiocarbonyl]), donated by A. B. Astra, Sweden, gallamine (Flaxedil, Rhône-Poulenc, France), 6-hydroxydopamine hydrobromide (synthesized by Dr. A. Langemann, F. Hoffman-La Roche & Co. Ltd.), α-methyldopa (synthesized by Dr. A. Langemann), α-methylnoradrenaline (Corbasil, Hoechst, Germany), (−)-noradrenaline (Fluka AB., Switzerland), phentolamine (Regitine, Ciba-Geigy, Switzerland). All drug weights refer to salt except those of noradrenaline and α-methylnoradrenaline. 6-Hydroxydopamine was given intravenously (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7) and was dissolved immediately before use in 0·001 N hydrochloric acid which had been bubbled with nitrogen. Animals were then used for experiments on days 8 or 9. Administration of intraventricular 6-hydroxydopamine (dissolved in 1% ascorbic acid) was carried out in conscious rats by the technique of Hayden, Johnson & Maickel (1966). Three doses of 6-hydroxydopamine (250 μg/rat) were given at 2-day intervals and the experiments were carried out 7 days later.

Results

Hypotensive effect of α-methyldopa in conscious rats

Conscious genetic hypertensive rats, in groups of 15 were used. The mean blood pressure was recorded directly from a catheter introduced into the aortic arch. One

hour after the injection of α-methyldopa (300 mg/kg i.p.) in these rats, a fall in blood pressure (40 mmHg) was observed and the maximal fall of 70 mmHg was obtained after four hours (Fig. 1). Administration of 6-hydroxydopamine (2 × 50 mg/kg and 2 × 100 mg/kg i.v.) in doses which are known to produce a peripheral sympathectomy (Thoenen & Tranzer, 1968), reduced but did not prevent the hypotensive effect of α-methyldopa (Fig. 1). However, in animals treated with intra-

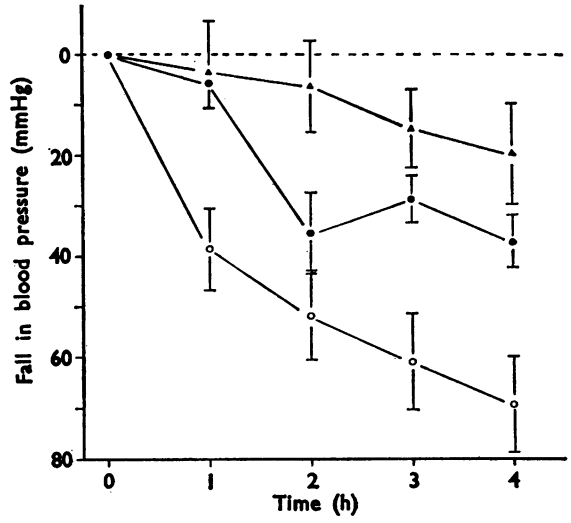

FIG. 1. Effect of α-methyldopa (300 mg/kg i.p.) on the mean arterial blood pressure of conscious genetic hypertensive rats. Controls (α-methyldopa alone) (○——○); rats pretreated with 6-hydroxydopamine, 2×50 mg/kg and 2×100 mg/kg intravenously (●——●); rats pretreated with intraventricular 6-hydroxydopamine, 3×250 μg (▲——▲). Vertical bars show S.E.M. (n=15 for all groups).

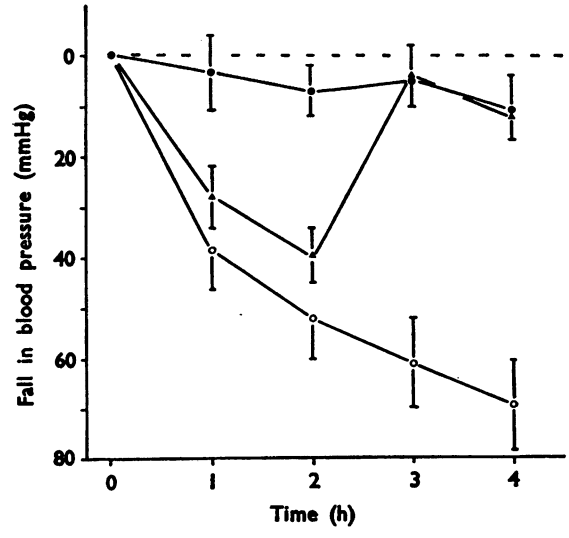

FIG. 2. Effect of α-methyldopa (300 mg/kg intraperitoneally) on the mean arterial blood pressure of conscious genetic hypertensive rats. Control (α-methyldopa alone) (○——○); rats given intraventricular phentolamine, 200 μg, 30 minutes before injection of α-methyldopa (●——●); rats given intraventricular phentolamine (200 μg), 2·5 h after the injection of α-methyldopa (▲——▲). Vertical bars show S.E.M. (n=15 for all groups).

ventricular 6-hydroxydopamine (3 × 250 μg), the hypotensive effect of α-methyldopa was almost completely prevented.

In order to study the central actions of α-methyldopa, cannulae were implanted into the lateral brain ventricle (Hayden *et al.*, 1966). An intraventricular injection of phentolamine (200 μg/rat), 30 min before the injection of α-methyldopa (300 mg/kg i.p.), completely prevented the hypotensive effect of the drug (Fig. 2). Furthermore, the same dose of phentolamine, given 2·5 h after the injection of

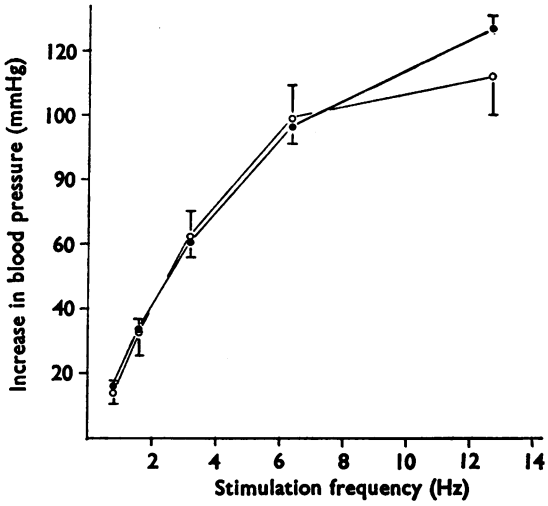

FIG. 3. Effect of α-methyldopa on the pressor response obtained in Gillespie & Muir preparations. Sympathetic outflow stimulated at supramaximal voltage, 0·1 ms duration at varying frequencies for periods of 15 seconds. Controls (○——○); rats pretreated with α-methyldopa (300 mg/kg intraperitoneally) 2·5 h before stimulation was started (●——●). Vertical bars show S.E.M. (n=10 for all groups).

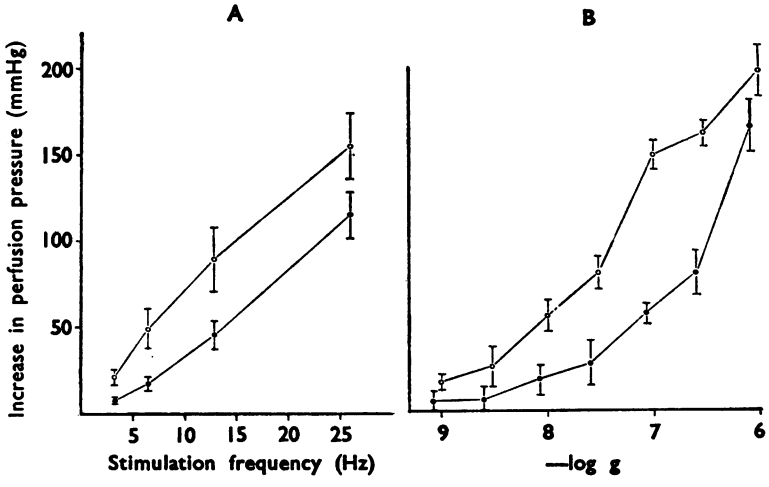

FIG. 4. Isolated perfused renal artery preparations from genetic hypertensive rats. (A) Effect of α-methyldopa on the vasoconstrictor responses obtained by periarterial nerve stimulation. Controls (upper line, ○——○); rats pretreated with α-methyldopa (300 mg/kg intraperitoneally) three hours before stimulation was started (●——●). (B) Effect of vasoconstrictor agents. Noradrenaline (upper line, ○——○); α-methylnoradrenaline (●——●). Vertical bars show S.E.M. (n=10 for all groups).

α-methyldopa to a different group of rats, reversed the hypotensive effect of α-methyldopa (Fig. 2). Intraventricular injections of phentolamine alone (50–200 μg/rat), after an initial short-lasting pressor effect, did not alter the resting blood pressure of conscious genetic hypertensive rats.

Effect of α-methyldopa on sympathetic nerve function

The action of α-methyldopa on peripheral sympathetic nerve function was investigated in the Gillespie & Muir preparation (1967). In untreated preparations, graded pressor responses were obtained with alterations in the frequency of stimulation (Fig. 3). Treatment with α-methyldopa (300 mg/kg i.p., 2·5 h beforehand) did not alter the magnitude of the pressor responses produced by sympathetic nerve stimulation.

The isolated renal artery was perfused with Krebs solution and the vasoconstrictor responses to periarterial nerve stimulation were recorded as increases in perfusion pressure (Fig. 4A). The preparations obtained from rats pretreated with α-methyldopa (300 mg/kg i.p., 2 h beforehand) showed a significant reduction in size of the vasoconstrictor responses to sympathetic nerve stimulation when compared with untreated control preparations.

The relative potency of noradrenaline and α-methylnoradrenaline as vasoconstrictor agents was compared in the isolated perfused renal artery preparations obtained from genetic hypertensive rats. The dose-response curves show that α-methylnoradrenaline has approximately one-eighth the vasoconstrictor potency of noradrenaline (Fig. 4B). Very similar results were obtained with preparations from rats pretreated with α-methyldopa (300 mg/kg i.p., 2 h before the start of the perfusion).

Effect of α-methyldopa on centrally induced pressor responses

Electrical stimulation of the mid-brain reticular formation induced an immediate rise in blood pressure. Graded responses were obtained by increasing the frequency of stimulation and maximal responses were observed at 50 Hz. Treatment of rats with α-methyldopa (300 mg/kg i.p.) markedly reduced the pressor responses to stimulation one hour after the administration of the drug (Fig. 5). This reduction in the pressor responses was maintained for the 4-hour duration of the experiments. In control experiments the responses showed only minimal variation over a similar time-period of the experiments.

Electrical stimulation of the posterior hypothalamus also induced a rise in blood pressure (Fig. 6) which was reduced by pretreatment with α-methyldopa (300 mg/kg i.p.). This reduction by α-methyldopa of the centrally evoked pressor responses could be prevented by pretreating the rats with a selective dopamine-β-hydroxylase inhibitor (FLA-63, 25 mg/kg i.p. ; Andén & Fuxe, 1971), 30 min before the administration of α-methyldopa.

Further studies were carried out in anaesthetized cats. The centrally evoked pressor responses produced by stimulation of the posterior hypothalamus were frequency-dependent (Fig. 7). These responses were due to increases in sympathetic nerve activity, which were recorded from both the splanchnic and renal nerves. Three to four hours after the injection of α-methyldopa (100 mg/kg i.v.) the pressor responses to stimulation were markedly reduced (90%) and the concurrent increases

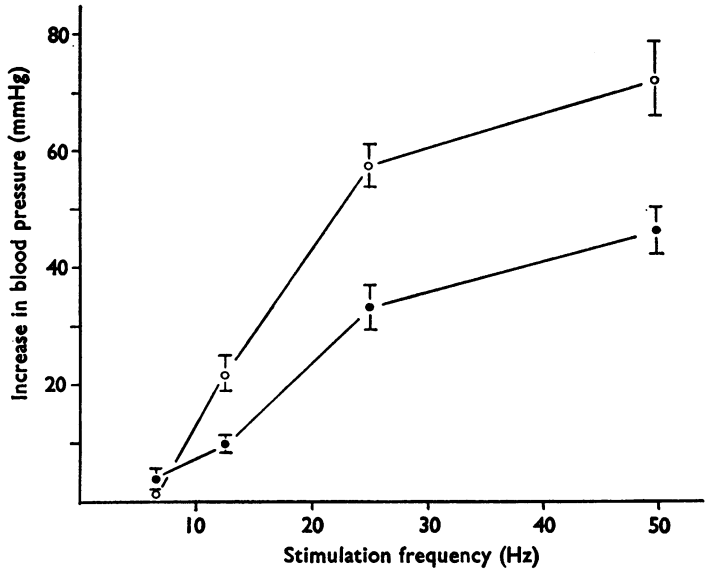

FIG. 5. Systemic blood pressure responses to stimulation of the mid-brain reticular formation in genetic hypertensive rats anaesthetized with urethane. Stimulations were carried out for periods of 10 s at intensities of 10 V, 1 ms duration at frequencies of 6·4-50 Hz. Controls (○——○); rats pretreated with α-methyldopa (300 mg/kg intraperitoneally) one hour before stimulation was started (●——●). Vertical bars represent S.E.M. (n=10 for each group).

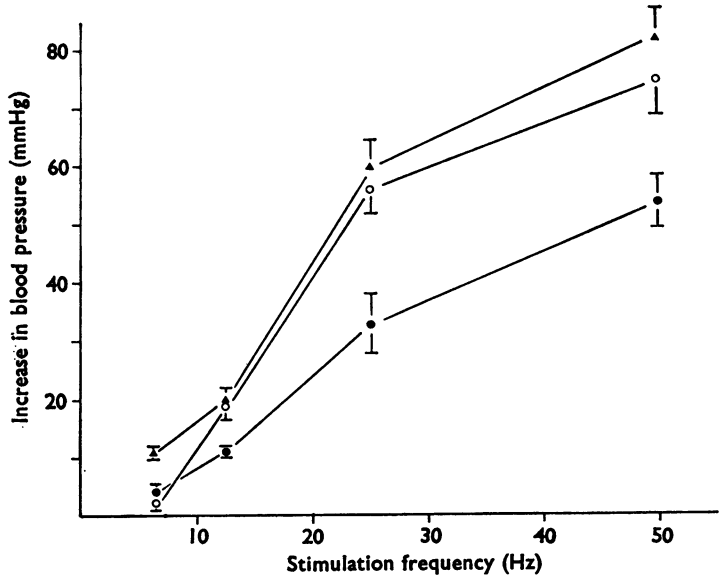

FIG. 6. Systemic blood pressure responses to stimulation of the posterior hypothalamus in urethane-anaesthetized genetic hypertensive rats. Stimulation was carried out for periods of 10 s at intensities of 10 V, 1 ms duration and at frequencies of 6·4-50 Hz. Controls (○——○); rats pretreated with α-methyldopa (300 mg/kg intraperitoneally) one hour before stimulation was started (●——●); rats pretreated with FLA-63 (25 mg/kg intraperitoneally) and α-methyldopa (300 mg/kg intraperitoneally) 1·5 and one hour respectively before start of stimulation (▲——▲). Vertical bars represent S.E.M. (n=10 for each group).

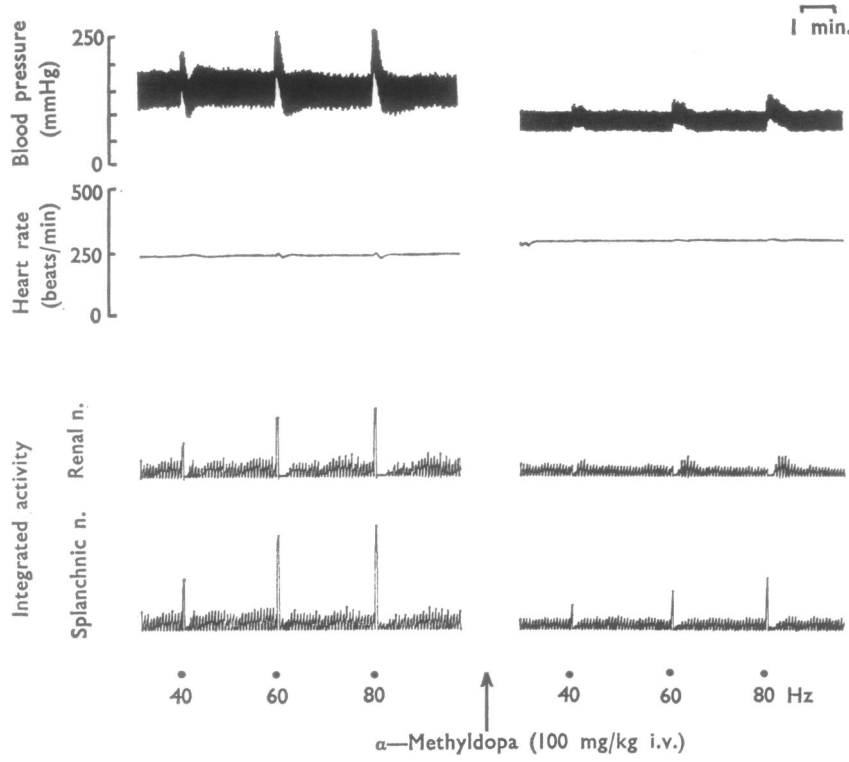

FIG. 7. Effects of α-methyldopa (100 mg/kg intravenously) on the cardiovascular responses elicited by stimulation of the posterior hypothalamus in a urethane-anaesthetized cat (2·4 kg). Stimulations were carried out for periods of 10 s at an intensity of 30 V, 0·1 ms duration at 40, 60 and 80 Hz. Effects on integrated sympathetic discharges from both the right splanchnic and renal nerves were recorded simultaneously.

in sympathetic nerve activity were also similarly depressed (Fig. 7). In control experiments no reduction in the pressor responses or sympathetic nerve activity were observed during the 4-hour period. However, only a slight decrease in the spontaneous sympathetic nerve activity was seen after the injection of α-methyldopa, and this could not account for the clear-cut hypotensive effect (Fig. 7).

Discussion

The results of the present study demonstrate the ability of α-methyldopa to lower arterial blood pressure in conscious, genetic hypertensive rats. This confirms previous findings in which other types of experimental hypertensive rats were used (Davis, Drain, Horlington, Lazare & Urbanska, 1963 ; Henning, 1969 ; Ayitey-Smith & Varma, 1970 ; Finch, 1971). Pretreatment of rats with 6-hydroxydopamine, in doses known to produce a peripheral sympathectomy (Thoenen & Tranzer, 1968 ; Finch & Leach, 1970), did not prevent the hypotensive effect of α-methyldopa, which is evidence against a false transmitter mechanism for α-methyldopa (Day & Rand, 1963, 1964). However, it is unlikely that an incomplete sympathectomy of blood vessels by 6-hydroxydopamine (Berkowitz, Spector & Tarver, 1972) could explain this persisting hypotensive effect of α-methyldopa, because a complete impairment of adrenergic nerve function and sympathectomy does take place with

the dose schedule of 6-hydroxydopamine used in these experiments (Finch, Haeusler, Kuhn & Thoenen, 1972). Therefore, either a direct action of α-methyldopa must be assumed (Ayitey-Smith & Varma, 1970) or that α-methyldopa acts centrally and reduces the impulse flow to the adrenal medulla which is not affected by 6-hydroxydopamine (Thoenen & Tranzer, 1968). Since the destruction of central adrenergic neurones with intraventricular 6-hydroxydopamine (Uretsky & Iversen, 1970) almost completely prevented the hypotensive effect of α-methyldopa, the latter hypothesis seems to be the more probable one.

The central actions of α-methyldopa were studied in more detail by the use of intraventricular injections of phentolamine. Since the hypotensive effect of α-methyldopa was both prevented and antagonized by these injections, it strongly suggests that central α-adrenoceptors are involved in the mechanism of action of α-methyldopa. It is unlikely that a peripheral α-blockade is involved since the pressor responses to intravenous noradrenaline in anaesthetized rats were unaffected by similar intraventricular injections of phentolamine (Finch, unpublished observations). If α-methyldopa does act by stimulation of central α-receptors, then this mechanism is only part of an integrated process since the regulatory control of blood pressure by central adrenergic mechanisms is known to be complex (Share & Melville, 1963; Gagnon & Melville, 1969; Brezenoff & Jenden, 1969; Philippu, Przuntek, Heyd & Burger, 1971). However, other drugs like α-methyldopa, such as L-DOPA (Henning & Rubenson, 1970; Osborne, Wenger & Willems, 1971) and clonidine (Kobinger, 1967; Schmitt & Schmitt, 1969), are also thought to lower blood pressure at least partially by a central mechanism which, in all probability, is noradrenergic in nature.

Pretreatment of rats with α-methyldopa, in doses which are known to be hypotensive, did not affect the magnitude of the pressor responses obtained by stimulation of the entire sympathetic outflow according to the method of Gillespie & Muir (1967). This observation is in agreement with the persistence of the α-methyldopa-induced hypotension after peripheral chemical sympathectomy. Moreover, both these observations question the importance of the formation of α-methylnoradrenaline in peripheral adrenergic nerves (Muscholl & Maître, 1963; Schümann & Grobecker, 1964) for the hypotensive effect of α-methyldopa. The findings obtained in the isolated perfused renal artery preparation, in which the vasoconstrictor responses to periarterial nerve stimulation were markedly reduced by pretreatment with α-methyldopa, would however lead to the opposite conclusion. Furthermore, with the same type of preparation, α-methylnoradrenaline was found to have one-eighth the vasoconstrictor potency of noradrenaline. Several workers have demonstrated a short-lasting impairment of peripheral adrenergic nerve function after treatment with α-methyldopa which supports the present findings with the renal artery preparation (Day & Rand, 1964; Haefely *et al.*, 1967; Malik & Muscholl, 1969; Salmon & Ireson, 1970). The apparent contradictory results obtained with the Gillespie & Muir preparation may be due to the fact that the cardiovascular effects rely partially on the heart and that α-methylnoradrenaline and noradrenaline may be equipotent in stimulating this organ. Support for this theory comes from the observation that α-methylnoradrenaline and noradrenaline are equipotent pressor agents in the anaesthetized dog, rabbit and pithed rat preparation (Trinker, 1971).

Electrical stimulation of various areas of the brain, such as the posterior hypothalamus and mid-brain reticular formation, elicits a rise in blood pressure via an

increased sympathetic nerve activity (Morpurgo & Morillo, 1962; Folkow & Rubinstein, 1966; Morpurgo, 1968; Baum & Shropshire, 1969). The present results show that centrally evoked pressor responses in the rat anaesthetized with urethane are markedly reduced by treatment with α-methyldopa. Also, the onset and duration of this effect coincides with the hypotension obtained with similar doses of α-methyldopa in conscious genetic hypertensive rats. Furthermore, pre-treatment with FLA-63, a selective dopamine-β-hydroxylase inhibitor, abolished this effect of α-methyldopa on the pressor responses produced by stimulation of the posterior hypothalamus. Therefore, the conversion of α-methyldopa to α-methyl-noradrenaline seems to be important for the inhibition by α-methyldopa of the centrally evoked pressor responses.

Further evidence for a central mechanism of action of α-methyldopa was obtained in experiments with cats. Stimulation of the posterior hypothalamus elicited an increase in the sympathetic nerve activity recorded from both the splanchnic and renal nerves and a rise in systemic blood pressure. Three to four hours after the injection of α-methyldopa the pressor responses to stimulation were markedly reduced. Since the increases in both pre- and post-ganglionic sympathetic nerve activity produced by hypothalamic stimulation were also strongly depressed, this inhibitory effect of α-methyldopa must be central in origin. However, it is difficult to explain why the spontaneous sympathetic nerve activity was not decreased after the administration of α-methyldopa, even though a hypotensive effect was obtained. Because of this observation and those with the renal artery preparation, a partial peripheral false transmitter mechanism for α-methyldopa cannot be completely ruled out.

In conclusion, the results indicate that the hypotensive effect of α-methyldopa requires intact central adrenergic neurones, probably for the conversion of α-methyldopa to α-methylnoradrenaline and also central α-adrenoceptors. Since no clear-cut correlation was found between impairment of peripheral adrenergic nerve function and α-methyldopa treatment, the results strongly suggest that the central actions of α-methyldopa are important for its hypotensive effect. At the same time, it is not denied that the formation and release of α-methylnoradrenaline from the peripheral sympathetic nerves may, to a limited extent, contribute to the hypotensive effect of α-methyldopa.

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