Br. J. Pharmac. (1970), **40**, 186–193.

Mechanism of the hypotensive action of methyldopa in normal and immunosympathectomized rats

E. AYITEY-SMITH* AND D. R. VARMA

Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada

Summary

1. The antihypertensive action of methyldopa was investigated in the light of the prevailing false sympathetic neurotransmitter hypothesis of Day & Rand (1963). Immunosympathectomized and normal animals were used for the investigation.

2. Methyldopa in a single injection significantly decreased the blood pressure in control hypertensive, immunosympathectomized normotensive, and normal rats. Guanethidine did not decrease the blood pressure of immunosympathectomized rats ; it decreased the blood pressure of control and control hypertensive rats.

3. The hypotensive effect of methyldopa was not antagonized by adrenoceptor and ganglion blocking agents or by the inhibition of dopamine β-oxidase.

4. High doses of methyldopa produced less hypotension than relatively low doses. This might be due to an antagonism of the hypotensive effect by methylnoradrenaline, which is formed from methyldopa.

5. The antihypertensive action of methyldopa could not be correlated with any change in aortic sodium or potassium.

6. It is concluded that methyldopa does not exert its hypotensive effect by producing a weakly active false sympathetic neurotransmitter.

Introduction

Day & Rand (1963) proposed that methyldopa (α-methyldihydroxyphenylalanine) lowered the blood pressure by producing a weakly active false sympathetic neurotransmitter. This hypothesis is inconsistent with the observations and suggestion of other workers (Mohammed, Gaffney, Yard & Gomez, 1968 ; Nickerson, 1965 ; Zaimis, 1965). It was recently shown in this laboratory that methyldopa exerted its usual antihypertensive effect in immunosympathectomized rats implanted with DOCA pellets and given a 1% solution of NaCl to drink (Varma, 1967). These results suggested that the antihypertensive action of methyldopa cannot be explained by the "false neurotransmitter" hypothesis of Day & Rand (1963). However, immunosympathectomy does not lead to complete destruction of the entire peripheral sympathetic system (Levi-Montalcini & Angeletti, 1962 ; Iversen, Glowinski & Axelrod, 1966 ; Zaimis, Berk & Callingham, 1965) and it has since been found that a more complete destruction of the sympathetic innervation to the cardiovascular system can be produced than was achieved during our earlier studies. The

* Present address: Department of Pharmacology, University of Ottawa, Ottawa, Ontario.

current study was therefore undertaken to further investigate the mode of action of methyldopa using rats with more intensive immunosympathectomy as well as rats in which peripheral sympathetic control was abolished by acute drug administration. The results of the present study support the earlier conclusion that the antihypertensive action of methyldopa cannot be explained solely by the hypothesis of Day & Rand (1963).

Methods

Experiments were performed on Sprague-Dawley rats of either sex (250–350 g) using a similar experimental procedure to that described in the preceding paper (Ayitey-Smith & Varma, 1970). All immunosympathectomized rats used in this study were injected subcutaneously with 12,000 units of antiserum to nerve-growth factor once daily for 2 days after birth and are similar to those described by Ayitey-Smith & Varma (1970) as " total " immunosympathectomized rats. Arterial pressure (1 mmHg$\equiv$1·333 mbar) was recorded directly from the femoral artery in rats under chloralose-urethane anaesthesia (50 mg/kg chloralose plus 350 mg/kg urethane); an electrocardiogram (lead II) was recorded to determine the heart rate. Drugs were injected acutely either intravenously through a cannula inserted into the femoral vein or intraperitoneally.

The effect of methyldopa on the blood pressure of normal, DOCA-NaCl hypertensive and renal hypertensive rats was studied. DOCA-NaCl hypertension was produced by subcutaneous implantation of 40 mg DOCA pellets and maintaining the rats on 1% sodium chloride. Renal hypertension was produced by constriction of one renal artery and removal of the contralateral kidney. In some normal control, DOCA-NaCl hypertensive and renal hypertensive rats, methyldopa was injected sub-acutely for 4 days and its effect on the systolic pressure, which was measured indirectly by the tail cuff method, and aortic sodium and potassium were subsequently determined. Details of the method used are given in the preceding paper (Ayitey-Smith & Varma, 1970). The details of the dose schedule are given with the results. Differences between two means were compared according to Student's t test (Steel & Torrie, 1960) and were considered statistically significant when $P<0{\cdot}05$.

The following agents were used: antiserum to nerve-growth factor (61,000 units/ml of bovine antiserum to nerve-growth factor); desoxycorticosterone acetate (DOCA) (K & K Labs, N.Y.); guanethidine (Ismelin, Ciba); hexamethonium (Poulenc Ltd., Montreal); 3-hydroxybenzyloxyamine; methyldopa ((±)-α-methyldopa, Aldomet, Merck Sharp & Dohme); (±)-methylnoradrenaline ((±)-cobefrine HCl, Sterling-Winthrop); phenoxybenzamine (SK & F); propranolol (Inderal, Ayerst Labs.).

Results

The hypotensive effects of methyldopa and guanethidine in anaesthetized normal and immunosympathectomized rats, both groups on a DOCA-NACl regime, are presented in Fig. 1. Only the normal rats developed hypertension; and they had a mean arterial pressure of 152±2·1 mmHg. Immunosympathectomized rats on the other hand did not develop hypertension and had a mean arterial pressure of 87±6·4 mmHg (under chloralose-urethane anaesthesia). However, methyldopa

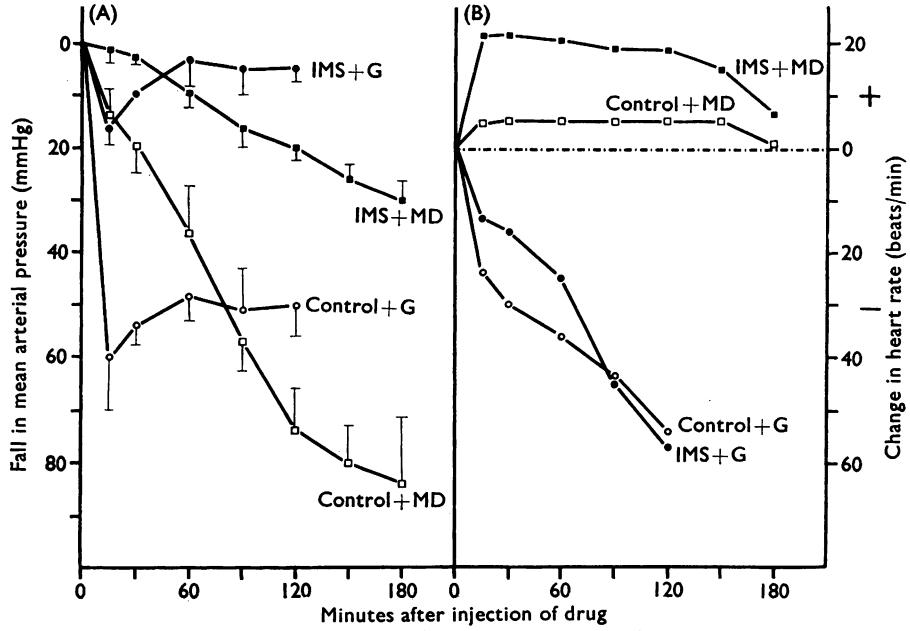

FIG. 1. Effect of methyldopa and guanethidine on the mean arterial pressure (A) and the heart rate (B) of DOCA-NaCl hypertensive (control) rats (○, □) and of DOCA-NaCl normotensive immunosympathectomized rats (IMS) (●, ■) under chloralose-urethane anaesthesia. Methyldopa (MD, 200 mg/kg) or guanethidine (G, 20 mg/kg) was injected intraperitoneally. There were four or five rats in each group. Vertical lines represent one half the S.E.

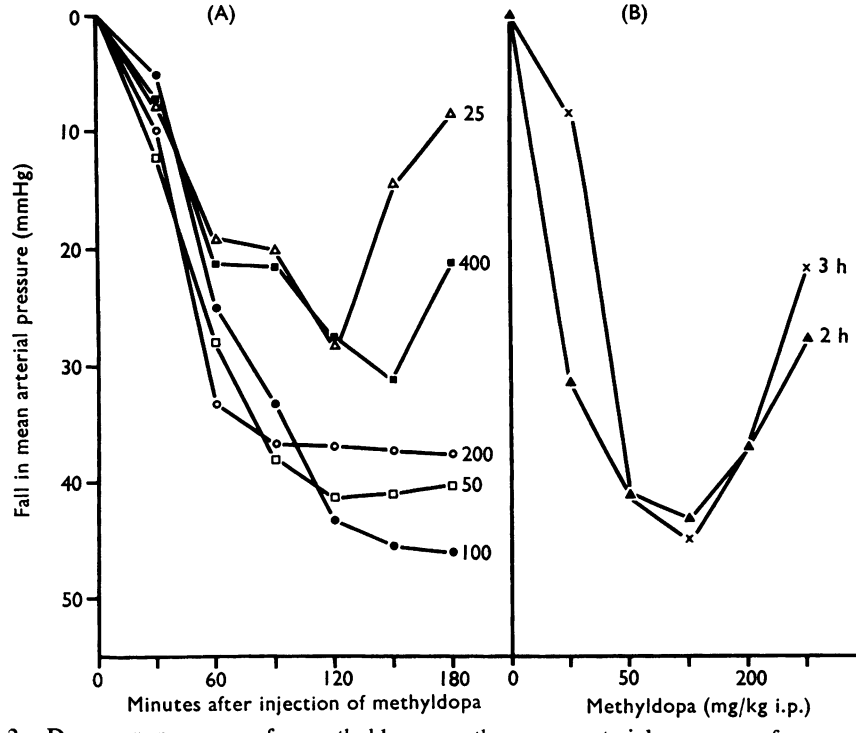

FIG. 2. Dose-response curve for methyldopa on the mean arterial pressure of normal rats under chloralose-urethane anaesthesia. (A), Time course of the hypotensive effect of intraperitoneal injection of methyldopa in doses (mg/kg) shown by numbers against the curves. (B), Hypotensive effect of various doses of methyldopa at 2 and 3 h after injection. Values in (B) are obtained from those in (A). There were three to five rats in each group and only one dose was injected in each rat.

(200 mg/kg, intraperitoneally) caused a significant fall in blood pressure in both these groups, regardless of their initial arterial pressure. Although the net hypotensive effect of methyldopa was greater in the hypertensive rats than in the immunosympathectomized normotensive rats, the percentage decrease in the arterial pressure in the two groups was not significantly different.

Unlike methyldopa, guanethidine (20 mg/kg, intraperitoneally) caused a significant fall in the arterial pressure of the hypertensive rats only. The hypotensive effect of guanethidine in the immunosympathectomized rats was of brief duration; it was associated with a decrease in heart rate and the blood pressure returned to the initial level in less than 60 minutes. The heart rate of both groups of rats increased after methyldopa and decreased after guanethidine.

The dose-response curve of methyldopa on the blood pressure of anaesthetized normal rats is shown in Fig. 2. Each animal was given only one intraperitoneal injection of methyldopa. A dose of 100 mg/kg produced the maximum hypotensive effect. Three hours after the administration of methyldopa, the hypotensive effect of 400 mg/kg was significantly less than that of 100 mg/kg and was not significantly different from that of 25 mg/kg. Since the high doses of methyldopa caused a smaller hypotensive effect, the dose-response curve to methyldopa was bell-shaped.

The effect of methyldopa on the mean arterial pressure of anaesthetized normal rats after blockade of adrenoceptors and ganglionic receptors is shown in Fig. 3.

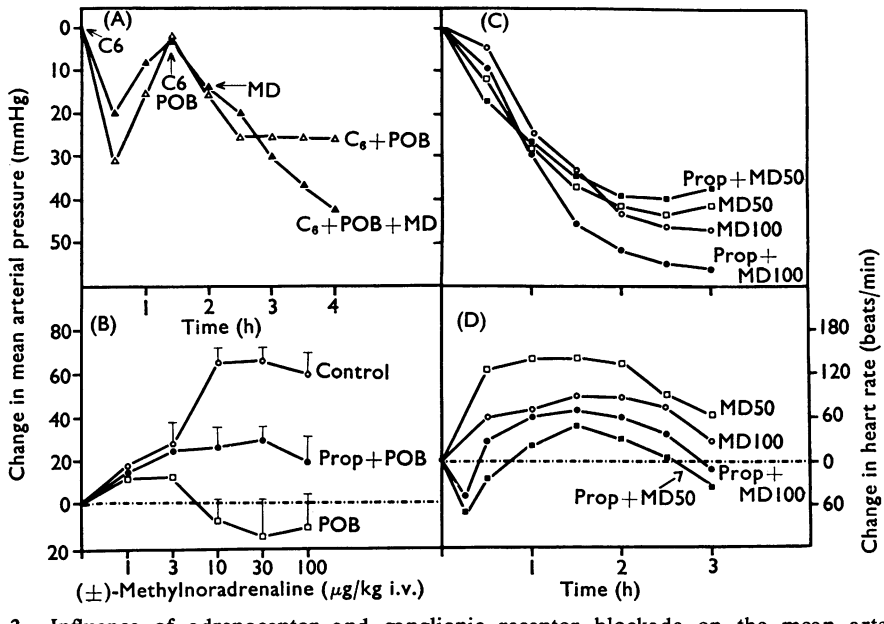

FIG. 3. Influence of adrenoceptor and ganglionic receptor blockade on the mean arterial pressure and the heart rate of normal rats under chloralose-urethane anaesthesia. All agents were injected intraperitoneally except methylnoradrenaline which was injected intravenously. There were three to six rats in each group. (A), Time of injection of drugs is shown by the arrows. Doses of methyldopa (MD), hexamethonium (C_6) and phenoxybenzamine (POB) were 200, 5 and 2·5 mg/kg, respectively. (B), Phenoxybenzamine (2·5 mg/kg) was injected 30 min before and propranolol (Prop, 2·5 mg/kg) 15 min before beginning the dose-response curve to methylnoradrenaline. (C), Propranolol (2·5 mg/kg) was injected 15 min before administration of 50 or 100 mg/kg methyldopa. (D), Same as in (C) showing changes in heart rate (beats/min).

Injection of methyldopa 200 mg/kg after phenoxybenzamine (2·5 mg/kg, intraperitoneally) plus hexamethonium (10 mg/kg given in two divided doses of 5 mg/kg each) caused a significant decrease in arterial pressure. To test whether the hypotensive effect of methyldopa was due to stimulation of β-adrenoceptors, methyldopa was injected in doses of 50 or 100 mg/kg to normal rats after treatment with 2·5 mg/kg of propranolol. The hypotensive effect of methyldopa in the rats given propranolol was not different from its effect in untreated controls. Propranolol did, however, decrease the positive chronotropic effect of methyldopa (Fig. 3D). On the other hand, the hypotensive effect of methylnoradrenaline, which was observed only after injection of phenoxybenzamine, was abolished by propranolol (Fig. 3B). The hypotensive effect of methyldopa (100 mg/kg) was not reduced by pretreatment of normal rats with 200 mg/kg intraperitoneally of 3-OH-benzyloxyamine (a dopamine β-oxidase inhibitor) when injected 30 min before methyldopa. This dose of 3-OH-benzyloxyamine which had a negative chronotropic action reduced the blood pressure by 23 ± 10·4 mmHg within 60 min after administration, but the blood pressure and heart rate returned to control levels after the 90 min period of observation (Fig. 4).

Injection of methyldopa 200 mg/kg daily for 4 days reduced the systolic pressure (measured indirectly) of unanaesthetized DOCA-NaCl hypertensive rats from 174 ± 6·3 mmHg to 111 ± 6·2 mmHg, of renal hypertensive rats from 191 ± 12·3

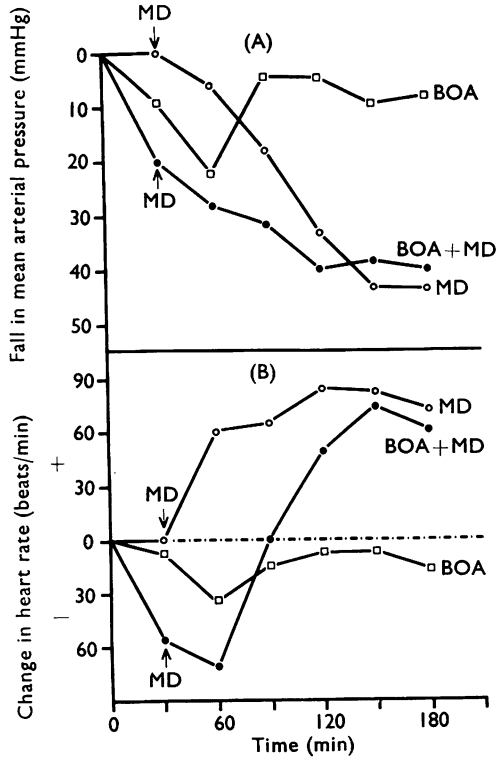

FIG. 4. Influence of 3-hydroxybenzyloxyamine (BOA) on the effect of methyldopa on the blood pressure (A) and heart rate (B) of normal rats under chloralose-urethane anaesthesia. Methyldopa (100 mg/kg, intraperitoneally) was injected at the arrows. BOA was injected at 0 time. Each group consisted of three or four rats.

mmHg to 135 ± 3·5 mmHg, and of normal controls from 130 ± 5·0 mmHg to 106 ± 4·4 mmHg. Aortic sodium and potassium levels were measured in these rats but the effect of methyldopa was inconsistent (Fig. 5). Thus, compared with untreated DOCA-NaCl hypertensive rats, methyldopa caused a significant increase in the aortic sodium and potassium of DOCA-NaCl hypertensive rats ; but no significant changes were seen in the other two groups when compared with their untreated counterparts.

Discussion

These studies demonstrate that methyldopa can cause a significant hypotensive effect in rats made hypertensive by DOCA-NaCl treatment or by renal artery constriction, as well as in normotensive rats. The greater reduction in blood pressure of hypertensive than of normotensive rats is apparently related to the initial blood pressure. The observation that methyldopa also caused a significant reduction in the blood pressure of immunosympathectomized rats whereas guanethidine failed to do this but merely reduced the blood pressure in the hypertensive rats, strongly suggests that the hypotensive action of methyldopa is not due to a reduction in the sympathetic tone. If the hypotensive effect of methyldopa were due to inhibition of the residual sympathetic system in the immunosympathectomized rats, one would have expected a hypotensive effect with guanethidine. The evidence that immunosympathectomy was successfully performed was presented in the preceding

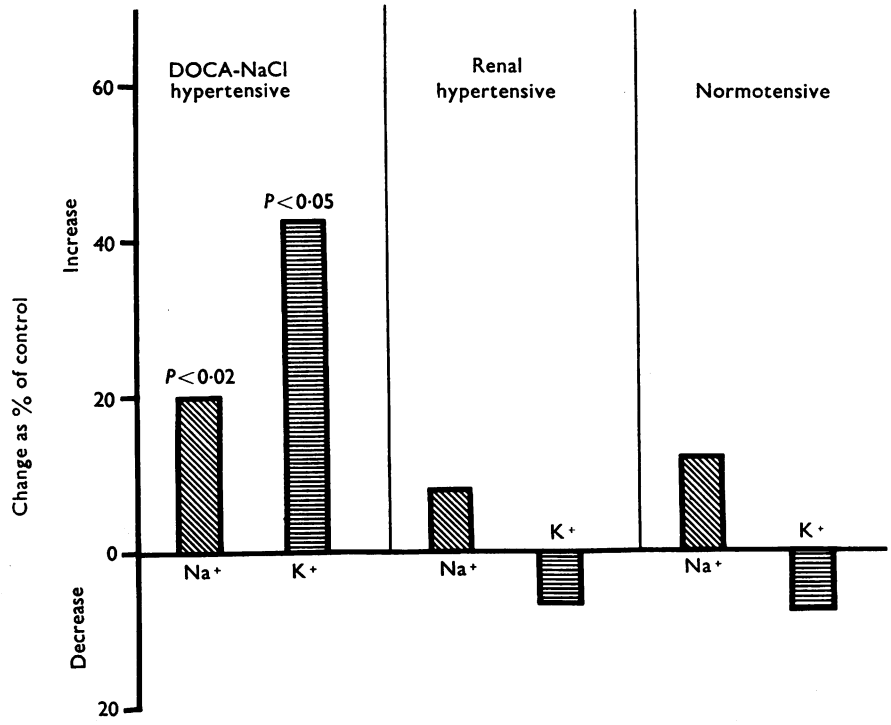

FIG. 5. Effect of chronic administration of methyldopa on aortic sodium and potassium of different groups of rats. Methyldopa (200 mg/kg) was injected intraperitoneally daily for 4 days before the animals were killed. There were three to five rats in each group. The values are expressed as the percentage of the values found in the untreated counterparts for each group. Significant changes are shown by *P* values on the top of the histograms.

paper (Ayitey-Smith & Varma, 1970). These results show that the hypotensive effect of methyldopa cannot be explained solely by the false neurotransmitter hypothesis of Day & Rand (1963). They are in agreement with the observations of Mohammed *et al.* (1968) who showed that methyldopa, given intravenously for 3 to 5 days, reduced the vascular resistance without reducing the sympathetic tone.

It can be argued that methyldopa is converted into methylnoradrenaline in the residual sympathetic nerve terminal of immunosympathectomized rats and that its hypotensive effect is exerted by circulating methylnoradrenaline. This argument is untenable, for the hypotensive effect of methylnoradrenaline in anaesthetized normal rats was observed only after phenoxybenzamine and was completely blocked by propranolol. Propranolol, on the other hand, failed to block the hypotensive effect of methyldopa.

Furthermore, since a high dose of methyldopa (400 mg/kg) produced a smaller hypotensive effect than a lower dose (100 mg/kg), it appears that methylnoradrenaline which is formed from methyldopa tends rather to antagonize the hypotensive effect of methyldopa. These observations plus the fact that the hypotensive effect of methyldopa is present after blockade of α-adrenoceptors and after inhibition of the synthesis of methylnoradrenaline from methyldopa by 3-hydroxybenzyloxyamine (Nikodijevic, Creveling & Udenfriend, 1963) are inconsistent with the hypothesis of Day & Rand (1963). According to this hypothesis, the hypotensive effect of methyldopa is exerted by the replacement of noradrenaline in sympathetic nerves by newly synthesized methylnoradrenaline which is a weak neurotransmitter.

Since the hypotensive effect of methyldopa is retained after blockade of α-adrenoceptors, it is clear that methyldopa does not act by blocking α-adrenoceptors. Since the hypotensive effect of methyldopa was not reduced by propranolol, it is unlikely that this effect is produced by stimulation of β-adrenoceptors. This observation agrees with that of Muscholl & Rahn (1966) who reported that pronethalol did not abolish the hypotensive effect of methyldopa. On the other hand, the results of Holtz & Palm (1967) and of Brunner, Hedwall & Meier (1965) suggest that the hypotensive effect of methyldopa may be exerted through an action on β-adrenoceptors. However, the suggestion of Holtz & Palm (1967) is based mainly on studies with methylated amines ; and Brunner *et al.* (1965) observed an antagonism by pronethalol of the effects of very low but not of high doses of methyldopa. In our studies the effect of a dose as low as 50 mg/kg of methyldopa was not reduced by 2·5 mg/kg of propranolol.

There is some evidence that the hypotensive effect of methyldopa is produced by the decarboxylation of methyldopa rather than by methyldopa *per se* (Gessa, Costa, Kuntzman & Brodie, 1962 ; Udenfriend & Zaltzman-Nirenberg, 1962 ; Levine & Sjoerdsma, 1964). As discussed earlier, our results suggest that methylnoradrenaline is unlikely to account for the hypotensive effect of methyldopa. There remains the possibility that methyldopamine might be responsible for the hypotensive effect of methyldopa. The results of this study are inconclusive in this respect. Regardless of the nature of the active form of methyldopa, these results indicate that the hypotensive effect of methyldopa is unlikely to be due to inhibition in the sympathetic control of blood vessels. It can be inferred that the hypotensive effect of methyldopa is largely due to direct action on the blood vessels. However, we have not directly tested this suggestion. Zaimis (1965) has also suggested that the hypotensive effect of methyldopa may be due to a direct action on blood vessels. The

studies on the effect of methyldopa on aortic sodium and potassium do not show any consistent change which could be correlated with an effect on the blood pressure.

In conclusion, these studies show that the hypotensive effect of methyldopa cannot be solely explained on the basis of the "false neurotransmitter" hypothesis of Day & Rand (1963) since methyldopa exerted its usual hypotensive effect in immunosympathectomized rats and in normal rats after adrenoceptor or ganglionic receptor blockade and after inhibition of dopamine β-oxidase. The hypotensive effect of methyldopa could not be correlated with its effect on aortic sodium.

We thank Dr. R. K. Richards of Abbott Laboratories, Chicago, for kindly supplying us with the bovine antiserum to nerve growth factor; Ciba, Dorval, Quebec, for Ismelin; Poulenc Ltd., Montreal, Quebec, for hexamethonium; Warner-Lamber, N.J., for 3-hydroxybenzyloxyamine; SK & F, Montreal, Quebec, for phenoxybenzamine; Ayerst Labs., Montreal, Quebec, for Inderal; and Merck Sharp & Dohme of Canada for Aldomet. This work was supported by a grant from the Quebec Heart Foundation and the Medical Research Council of Canada.

REFERENCES

AYITEY-SMITH, E. & VARMA, D. R. (1970). An assessment of the role of sympathetic nervous system in experimental hypertension using normal and immunosympathectomized rats. *Br. J. Pharmac.*, **40**, 175–185.

BRUNNER, H., HEDWALL, P. R. & MEIER, M. (1965). Influence of an adrenergic beta-receptor blocking agent on the effect of various hypotensive agents in the hypertensive rat. *Experientia*, **21**, 231–232.

BUHS, R. P., BECK, J. L., SPETH, O. C., SMITH, J. L., TRENNER, N. R., CANNON, P. J. & LARAGH J. H. (1964). The metabolism of methyldopa in hypertensive human subjects. *J. Pharmac. exp. Ther.*, **143**, 205–214.

DAY, M. D. & RAND, M. J. (1963). A hypothesis for the mode of action of alpha-methyldopa in relieving hypertension. *J. Pharm. Pharmac.*, **15**, 221–224.

DAY, M. D. & RAND, M. J. (1967). Mode of action of methyldopa. *J. Pharm. Pharmac.*, **19**, 395–396.

GESSA, G. I., COSTA, E., KUNTZMAN, R. & BRODIE, B. B. (1962). On the mechanism of norepinephrine release by alpha-methyl-meta-tyrosine. *Life Sci., Oxford*, **1**, 353–360.

HENNING, M. (1967). Blood pressure and noradrenaline levels after treatment with alpha-methyldopa, alpha-methyldopamine and alpha-methyl-m-tyrosine. *J. Pharm. Pharmac.*, **19**, 775–779.

HOLTZ, P. & PALM, D. (1967). On the pharmacology of alpha-methylated catecholamines and the mechanism of the antihypertensive action of alpha methyldopa. *Life Sci., Oxford*, **6**, 1847–1857.

IVERSEN, L. L., GLOWINSKI, J. & AXELROD, J. (1966). The physiologic disposition and metabolism of norepinephrine in immunosympathectomized animals. *J. Pharmac. exp. Ther.*, **151**, 273–284.

LEVI-MONTALCINI, R. & ANGELETTI, P. U. (1962). Noradrenaline and monoaminoxidase content in immunosympathectomized animals. *Int. J. Neuropharmac.*, **1**, 161–164.

LEVINE, R. J. & SJOERDSMA, A. (1964). Dissociation of the decarboxylase-inhibiting and norepinephrine-depleting effects of alpha-methyldopa, alpha-ethyldopa, 4-bromo-3-hydroxybenzyloxyamine and related substances. *J. Pharmac. exp. Ther.*, **146**, 42–47.

MOHAMMED, S., GAFFNEY, T. E., YARD, A. C. & GOMEZ, H. (1968). Effect of methyldopa, reserpine, guanethidine on hindleg vascular resistance. *J. Pharmac. exp. Ther.*, **160**, 300–307.

MUSCHOLL, E. & RAHN, K. H. (1966). Nachweis von alpha-methylnoradrenalin im harn von hypertonikern wahrendeiner behandlung mit alpha-methyldopa. *Klin. Wschr.*, **44**, 1412–1413.

NIKODIJEVIC, B., CREVELING, C. R. & UDENFRIEND, S. (1963). Inhibition of dopamine beta-oxidase *in vivo* by benzyloxyamine and benzylhydrazine analogs. *J. Pharmac. exp. Ther.*, **140**, 224–228.

NICKERSON, M. (1965). Drugs inhibiting adrenergic nerves and structures innervated by them. In *The Pharmacological Basis of Therapeutics*, 3rd ed., ed. Goodman, L. S. and Gilman, A., p. 572. New York: Macmillan.

STEEL, R. G. D. & TORRIE, J. H. (1960). *Principles and Procedures of Statistics*, 1st ed. New York: McGraw-Hill Book Company.

UDENFRIEND, S. & ZALTZMAN-NIRENBERG, P. (1962). On the mechanism of the norepinephrine release produced by α-methyl-meta-tyrosine. *J. Pharmac. exp. Ther.*, **138**, 194–199.

VARMA, D. R. (1967). Antihypertensive effect of methyldopa in metacorticoid immunosympathectomized rats. *J. Pharm. Pharmac.*, **19**, 61–62.

ZAIMIS, E. (1965). On the mechanism of action of hypotensive drugs. *Proc. R. Soc. Med.*, **56**, 1067–1070.

ZAIMIS, E., BERK, L. & CALLINGHAM, B. A. (1965). Morphological, biochemical and functional changes in the sympathetic nervous system of rats treated with nerve growth factor-antiserum. *Nature, Lond.*, **206**, 1220–1222.

(*Received February* 6, 1970)