PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 129–134 (1974)

Peripheral Vascular Actions of α-Methyl-DOPA and Its Mode of Action on Arterioles[1] (37762)

BURTON M. ALTURA[2]

Departments of Anesthesiology and Physiology, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York 10461

During the late 1950's several important antihypertensive agents were developed—namely, chlorothiazide, guanethidine and α-methyl-DOPA.[3] Although a massive amount of knowledge is available as to the possible antihypertensive mechanisms of action of the thiazides and guanethidine, controversy still exists as to the precise modes of action with respect to these drugs as well as to α-methyl-DOPA [see Ref. (1) for review].

Initially, it was thought that the antihypertensive action of α-methyl-DOPA (Aldomet) was due to the prevention of synthesis, and hence release, of norepinephrine via inhibition of DOPA decarboxylase (2), but other DOPA decarboxylase inhibitors are not necessarily hypotensive (1). Another possibility was that the norepinephrine in the postganglionic sympathetic nerve endings is replaced by α-methylnorepinephrine (3), *i.e., a false neurotransmitter*, via the conversion of α-methyl-DOPA into α-methylnorepinephrine; hypotension would be brought about as a result of the weaker peripheral action of α-methylnorepinephrine (4). But recent evidence suggests that in those animals (and man) in which α-methyl-DOPA is hypotensive, α-methylnorepinephrine is approximately equipotent with norepinephrine on a variety of smooth muscle effector cells (5, 6). These latter types of studies were, however, not done on vascular smooth muscle effector cells of the arterioles, the major sites of peripheral resistance.

More recent work suggests that α-methyl-DOPA may produce hypotension by a direct central action on the nervous system (7, 8). Day, Roach and Whiting (9) contend that the central action is due to formation and release of the false transmitter, *i.e.* α-methylnorepinephrine. Another possibility that must be entertained is that α-methyl-DOPA alters or suppresses peripheral sympathetic activity (10).

Although considerable evidence has been brought forth to counter the latter argument (3, 11–15), only conduction and transmission in the sympathetic nervous system was ascertained in these studies. There is, however, microscopic (16, 17) and indirect (15) evidence to suggest that α-methyl-DOPA may directly produce peripheral vascular vasodilatation. But neither basal sympathetic nerve activity, direct measurement of arteriolar lumen sizes after intra-arterial rather than systemic administration, nor ability of the effector arteriolar smooth muscle cells to respond to the neurotransmitter, norepinephrine (or false neurotransmitter, α-methylnorepinephrine) were ascertained after acute or chronic administration of α-methyl-DOPA. The latter information is especially critical in view of some very recent findings which indicate that periarterial nerve stimulation of isolated rat renal arteries are markedly reduced by pretreatment with α-methyl-DOPA (18). The present experiments, using direct, quantitative *in vivo* microscopy on rat mesenteric arterioles as well as isolated rat aorta, were therefore undertak-

[1] This work was supported by Research Grant HL-12462 from the National Heart and Lung Institute, United States Public Health Service.

[2] This study was performed during the tenure of Research Career Development Award 5-K3-GM-38, 603, U.S. Public Health Service.

[3] DOPA = 3,4-dihydroxyphenylalanine.

en to assess more directly the peripheral vascular actions of α-methyl-DOPA.

Methods. In vivo quantitative microscopic observations were carried out on rat mesenteric arterioles by means of an image-splitting television microscope recording system before and after intra-arterial (branch of ileocolic artery) and ip α-methyl-DOPA. For the latter male rats (Wistar strain, 135 ± 20 g) anesthetized with im pentobarbital (Nembutal, 4 mg/100 g body wt) were utilized. Only male rats were employed, in these *in vivo* as well as in the *in vitro* studies (described below), since estrogenic hormones are known to affect the vascular response to catecholamines (19, 20). The rat mesentery was prepared and kept under physiologic conditions according to procedures described previously (21). Measurements for changes in arteriolar lumen size were made before (controls) and 2, 4 and 6 hr. after intra-arterial administration of α-methyl-DOPA (500 mg/kg/30 min). Simultaneously, these arterioles, as well as those of animals receiving chronic ip α-methyl-DOPA (100 mg/kg/day for 15 days), were examined for their quantitative dose–response relationships to topical application of graded doses (6 to 12 in number) of the catecholamines epinephrine (epinephrine hydrochloride, Parke, Davis and Co.), norepinephrine (norepinephrine bitartrate, Levophed, Winthrop Labs.) and α-methylnorepinephrine HCl (Mann Research Labs.). Synthetic [8-lysine]-vasopressin (270 IU/mg Sandoz Inc.) was also utilized to determine whether the actions of α-methyl-DOPA on reactivity were specific for catecholamines. *In vivo* microscopic observations for discrete drug effects were made at magnifications up to 4000 ×, during temporary interruption of the Ringer gelatin irrigation (21) of the mesentery, using the image-splitting television microscope recording system of Baez (22). These quantitative dose–effect studies were carried out essentially similar to those described previously for catecholamines and vasopressin (23, 24).

For the *in vitro* studies, thoracic aortas were obtained from paired male rats (Wistar strain, 300–400 g), *i.e.*, controls and experimentals (ip α-methyl-DOPA, 100 mg/kg/day for 15 days). These aortas were cut helically into vascular strips and set up isometrically in Krebs–Ringer bicarbonate solution (25) essentially similar to that described previously (24). The Krebs–Ringer solutions were oxygenated continuously with a 95% O_2–5% CO_2 mixture and kept at 37° (pH 7.2-7.5). Complete, cumulative log dose–response curves, similar to those described previously (24, 25) were obtained for the synthetic catecholamines and vasopressin. The results of these experiments are expressed in percentage of the maximal epinephrine response since the latter, along with norepinephrine, yields the greatest maximal response in rat thoracic aorta (26). The α-methyl-DOPA (Aldomet, Merck) was made up fresh daily in normal isotonic saline. Control animals in all experiments received comparable ip or intra-arterial volumes of the normal isotonic saline. It should be noted that the chronic administration of α-methyl-DOPA (*i.e.*, for 15 days) lowered blood pressure, on the average, 10–15 mm Hg when compared to paired untreated controls.

Results and Discussion. If, as a result of the administration of α-methyl-DOPA, norepinephrine (NE) is replaced by α-methylnorepinephrine (MNE) at the postganglionic sympathetic nerve endings on the arteriolar walls (3), and produces hypotension via a weaker action of MNE on the arteriolar effector smooth muscle cells (4), then one might expect MNE to be much less potent than NE *i.e.*, to exhibit a higher ED_{50} and /or to exhibit a lower intrinsic activity (*i.e.*, show a smaller maximal contractile response on arterioles of normal animals). Although Fig. 1 and the ED_{50} values in Table I reveal that MNE is 17 times less potent than epinephrine on rat mesenteric arterioles, it is only two times less potent than the normal transmitter NE. In addition, it should be noted that MNE is equivalent to NE in producing 80% maximal arteriolar constrictions. Figure 2 and Table II reveal that, at least qualitatively, similar dose–response relationships are seen for the three amines on isolated rat aorta; *i.e.*, although MNE is three times less potent than epinephrine it is not very different from NE. In view of these

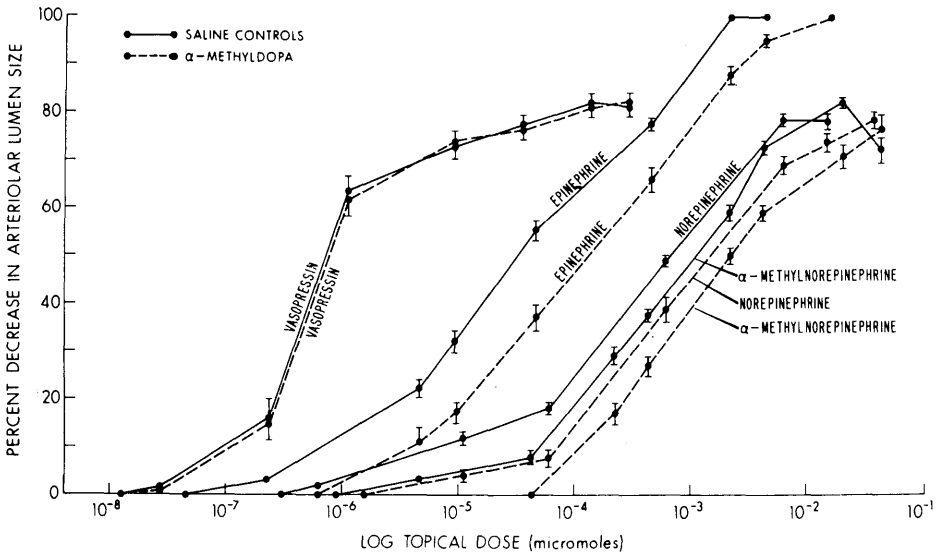

FIG. 1. Influence of α-methyl-DOPA on graded contractile responses of rat mesenteric arterioles to catecholamines, α-methylnorepinephrine and vasopressin. Each point represents the mean value obtained from measurements on vessels from different paired control (● —) and α-methyl-DOPA-treated (● - -) rats ($N = 8$ for all groups). Only one type of vasoactive drug was tested on each rat mesentery. The bars represent 1 SEM. The mean, paired lumen sizes (before vasoactive drug) for the arterioles (μm) were: vasopressin (27.6 ± 2.5; 29.5 ± 2.7), epinephrine (25.6 ± 1.5; 27.8 ± 1.6); norepinephrine (28.0 ± 1.6; 29.4 ± 2.5), and α-methylnorepinephrine (27.1 ± 1.5; 29.2 ± 1.8). Dose-response relationships were ascertained 24 hr after the last dose of α-methyl-DOPA (see text).

direct findings on peripheral blood vessels, it would be very difficult to accept the false neurotransmitter hypothesis of Day and Rand (4), at least in rats.

Another possibility is that α-methyl-DOPA alters or suppresses peripheral sympathetic activity (10). Figure 1, as well as the ED_{50} values in Table II, indicate that systemic administration of α-methyl-DOPA to rats for 15 days, in a dose regimen which is known to both lower blood pressure and induce arteriolar vasodilatation (16, 17), seems rather specifically to depress arteriolar and arterial reactivity to epinephrine, NE and MNE. All three amines, but not vasopressin, exhibit significant losses in affinity for their respective receptors on both the mesenteric arterioles and aorta. The latter suggestion is supported

TABLE I. Influence of Chronic α-Methyl-DOPA Pretreatment on Arteriolar Responsiveness to Catecholamines and α-Methylnorepinephrine.

	Control		After α-methyl-DOPA	
Topical drug	ED_{50}[a] (×10^{-6} moles/liter)	% Maximal[b] response	ED_{50}[a] (×10^{-6} moles/liter)	% Maximal[b] response
Epinephrine	0.35 ± 0.05	100 ± 0.0	1.4 ± 0.07[c]	100.0 ± 0.0
Norepinephrine	2.9 ± 0.2	78.8 ± 2.5	6.7 ± 0.6[c]	78.5 ± 3.2
α-Methylnorepinephrine	5.9 ± 0.6	82.0 ± 2.2	10.0 ± 0.7[c]	76.8 ± 2.6

[a] Mean concentration of agonist (± SEM) required to produce 50% of the maximal contractile response [topical volume (0.1 ml) converted to moles/liter].

[b] % maximal (mean values ± SEM) attainable constrictor response.

[c] Significantly different from untreated control ($P < 0.01$, Student's t test).

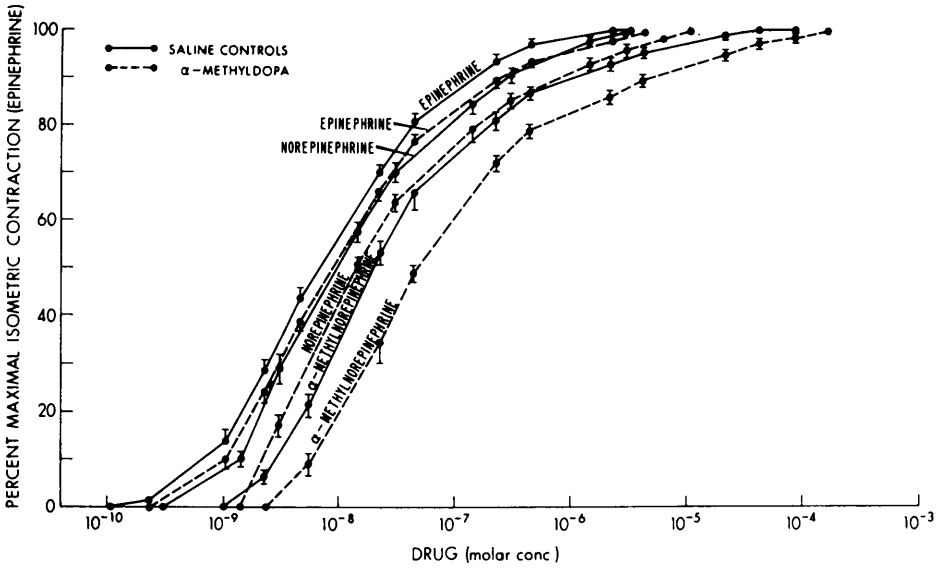

FIG. 2. Influence of chronic treatment with α-methyl-DOPA on cumulative dose–response curves of catecholamines and α-methylnorepinephrine on *in vitro* rat aortic strips mounted isometrically in Krebs–Ringer bicarbonate. Dose–response relationships were ascertained 24 hr after the last dose of α-methyl-DOPA. Maximal contractile response to epinephrine is taken as 100%. All other contractile responses are expressed as a percentage of this value. Values are mean responses ± 1 SEM. $N = 12$ for all groups. 100% response to epinephrine = 1850 ± 110 mg tension. Controls (●—); experimentals (●- -). See text for details of dose regimen.

by the parallel shifts of the log dose–response curves to the right. The maximal contractile responses are, however, not altered. The latter would be anticipated if only receptor sensitivity has been altered. Thus, although the present experiments do not directly indicate that α-methyl-DOPA brings about hypotension by interfering with, or altering, peripheral sympathetic nerve transmission, the findings do suggest that α-methyl-DOPA, at least in the rat, can alter the actions of catecholamines at the effector smooth muscle cells of arterioles and arteries. These direct studies thus support the indirect experiments of previous workers (18, 27, 28) indicating that α-methyl-DOPA may bring about a depression of peripheral sympathetic function.

Such peripheral vascular actions could certainly result in arteriolar vasodilation and hypotension. The data shown in Figs. 3 and 4 lend further support to the latter tenet. Figure 3 indicates that when α-methyl-DOPA is infused intra-arterially, into a branch of the ileocolic artery (supplies rat mesocecal mesentery with blood), there is gradually a development of arteriolar vasodilatation. It is

TABLE II. Influence of Chronic α-Methyl-DOPA Pretreatment on Reactivity of Isolated Rat Aorta to Catecholamines, α-Methylnorepinephrine and Vasopressin.

Drug	Control[a]		After α-methyl-DOPA[a]	
	ED_{50} ($\times 10^{-9}$ moles/liter)	% Maximal response	ED_{50} ($\times 10^{-9}$ moles/liter)	% Maximal response
Epinephrine	6.5 ± 0.5	100.0 ± 0.0	9.0 ± 0.7[b]	100.0 ± 0.0
Norepinephrine	9.6 ± 0.7	100.0 ± 0.0	15.2 ± 0.9[b]	100.0 ± 0.0
α-Methylnorepinephrine	18.0 ± 1.5	100.0 ± 0.0	49.2 ± 2.2[b]	100.0 ± 0.0
[8-Lysine]-vasopressin	2.8 ± 0.3	80.0 ± 5.1	2.9 ± 0.3	80.5 ± 5.0

[a] Mean values ± SEM.

[b] Significantly different from untreated control ($P < 0.01$, Student's t test).

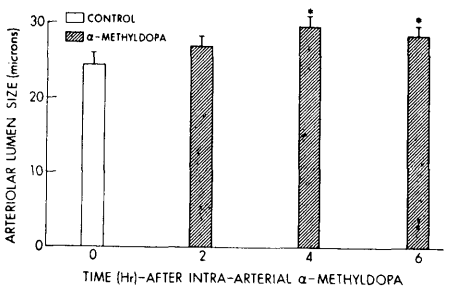

FIG. 3. Effect of intra-arterial administration of α-methyl-DOPA (500 mg/kg) on arteriolar lumen size. Bar represents one SEM. (*) Mean values which are significantly different from control ($P < 0.01$, paired t test). $N = 8$. Although not shown, six paired, control animals receiving intra-arterial injections of saline, comparable in volume to the experimentals, failed to show a change in reactivity over the 6 hr period.

interesting to note that a single intra-arterial dose of 500 mg/kg produces about the same degree of arteriolar dilatation as 100 mg/kg/day for 15 days (compare data in Figs. 3 and 5 in Ref. (17)). Figure 4 demonstrates that such arterioles exhibit depressed constrictor responses to topically applied catecholamines similar to that observed in rats chronically treated with α-methyl-DOPA. Such α-methyl-DOPA-induced vascular effects are most likely due to a peripheral mode of action, possibly direct in nature on the arterioles. In this context, it is of interest to note that acute treatment with α-methyl-DOPA has been reported to also depress cardiac pacemaker activity (29).

Although the experiments presented here suggest that α-methyl-DOPA has important, and possibly direct, peripheral vascular actions which may be entirely or in part, responsible for its antihypertensive action, one must keep in mind that the observed effects are, to my knowledge, limited to (i) a single mammalian species, (ii) normotensive rather than hypertensive animals, and (iii) two types of blood vessels — namely, intact rat mesenteric arterioles and isolated rat aorta.

Summary. The present quantitative *in vivo* and *in vitro* experiments on rat mesenteric arterioles and aortas, respectively, demonstrate that although α-methylnorepinephrine is much less potent than epinephrine on both types of blood vessels, it is only 1.5–2 times less potent than the natural postganglionic neurotransmitter norepinephrine on these blood vessels. Furthermore, α-methylnorepinephrine is equivalent to norepinephrine in its ability to induce maximal contractile responses on rat arterioles and aortas. Systemic administration of α-methyl-DOPA to rats for 15 days shifted the log dose–response curves for all three catecholamines, but not vasopressin, to the right on both intact 20–30 μm arterioles and isolated aorta; the maximal contractile responses to these amines were, however, not affected by chronic treatment with α-methyl-DOPA. In addition, acute, in-

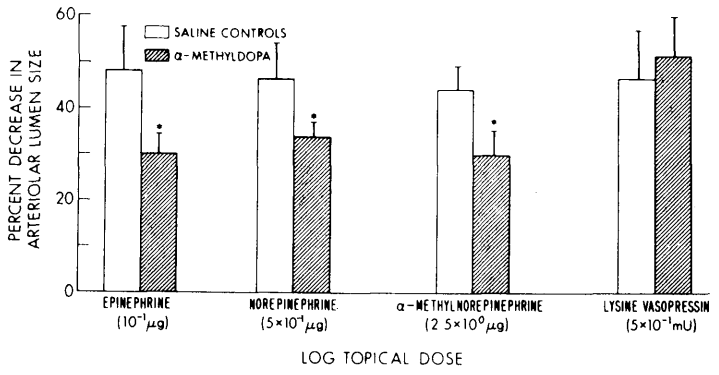

FIG. 4. Influence of intra-arterial adminstration of α-methyl-DOPA (500 mg/kg) on arteriolar responsiveness to catecholamines, α-methylnorepinephrine and vasopressin. $N = 6$ for all groups. * Mean values ± 1 SEM which are significantly different from control ($P < 0.01$, paired t test). Saline controls denotes paired animals receiving ia injections of saline comparable in volume to the experimentals.

tra-arterial administration of 500 mg/kg of α-methyl-DOPA was found to not only gradually induce mesenteric arteriolar vasodilatation, but to depress arteriolar responsiveness to catecholamines. In view of these direct findings on intact and isolated blood vessels it is difficult to accept the hypothesis that α-methyl-DOPA induces hypotension via formation of a false transmitter substance—namely, α-methylnorepinephrine. The present findings rather suggest that α-methyl-DOPA may exert some or all of its antihypertensive action by depressing arteriolar responsiveness to circulating and released catecholamines, at least in the rat.

The author is indebted to Dr. B. Berde (Sandoz Ltd.) for the generous supply of synthetic vasopressin used in this study.

1. Laverty, R., Brit. Med. Bull. **29**, 152 (1973).
2. Sourkes, T. L., Arch. Biochem. Biophys. **51**, 444 (1954).
3. Muscholl, E., and Maitre, L., Experientia **19**, 658 (1963).
4. Day, M. D., and Rand, M. J., J. Pharm. Pharmacol. **15**, 221 (1963).
5. Coupan, I. M., and Turner, P., Brit. J. Pharmacol. **38**, 463P (1970).
6. Trinker, F. R., J. Pharm. Pharmacol. **23**, 306 (1971).
7. Henning, M., Acta Pharmacol. Toxicol. **27**, 135 (1969).
8. Van Zwieten, P. A., J. Pharm. Pharmacol. **25**, 89 (1973).
9. Day, M. D., Roach, A. G., and Whiting, R. L., Brit. J. Pharmacol. **45**, 168P (1972).
10. Dollery, C. T., and Harrington, M., Lancet 1, 759 (1962).
11. Day, M. D., and Rand, M. J., Brit. J. Pharmacol. **22**, 72 (1964).
12. Davies, B. N., Nature (London) **210**, 957 (1966).
13. Haefely, W., Hurlimann, A., and Thoenen, H., Brit. J. Pharmacol. **26**, 172 (1966).
14. Kadzielawa, K., Int. J. Neuropharmacol. **6**, 435 (1967).
15. Mohammed, S., Gaffney, T. E., Yard, A. C., and Gomez, H., J. Pharmacol. Expt. Ther. **160**, 300 (1968).
16. Altura, B. M., Amer. J. Physiol. **212**, 1447 (1967).
17. Altura, B. M., Microvasc. Res. **3**, 361 (1971).
18. Finch, L., and Haeusler, G., Brit. J. Pharmacol. **47**, 217 (1973).
19. Altura, B. M., Eur. J. Pharmacol. **20**, 261 (1972).
20. Altura, B. M., Microvasc. Res. **6**, 117 (1973).
21. Zweifach, B. W., and Metz, D. B., Ergeb. Anat. Entwicklungsgesch. **35**, 176 (1956).
22. Baez, S., J. Appl. Physiol. **211**, 299 (1966).
23. Altura, B. M., Proc. Soc. Exp. Biol. Med. **140**, 1270 (1972).
24. Altura, B. M., Proc. Soc. Exp. Biol. Med. **142**, 1104 (1973).
25. Altura, B. M., and Altura, B. T., Amer. J. Physiol. **219**, 1968 (1970).
26. Altura, B. M., and Altura, B. T., Microvasc. Res. **7**, (1974, in press).
27. Henning, M., and Svensson, L., Acta Pharmacol. Toxicol. **26**, 425 (1968).
28. Salmon, G. K., and Ireson, J. D., Arch. Int. Pharmacol. **183**, 60 (1970).
29. Gillis, R. A., Shister, H. E., and Melville, K. I., Arch. Int. Pharmacol. **159**, 219 (1966).

Received July 13, 1973. P.S.E.B.M., 1974, Vol. 145.