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Evidence that the hypotensive action of methyldopa is mediated by central actions of methylnoradrenaline

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Mean arterial blood pressure was recorded in conscious normotensive rats through indwelling arterial catheters. The effect of L-α-methyldopa (α-MD) (400 mg/kg, i.p.) was studied in animals pretreated with α-methyl-*m*-tyrosine (400 mg/kg i.p.) 27 and 15 h before α-MD, α-methyl-*p*-tyrosine methylester (H 44/68) (250 mg/kg, i.p.) 1 h before α-MD, and DL-α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl) propionic acid (MK 485, 100 mg/kg, i.p.) 30 min before α-MD. This pretreatment, which resulted in a severe depletion of endogenous catecholamines, did not alter the hypotensive effect of α-MD. The effect of α-MD (200 mg/kg, i.p.) was studied 30 min after pretreatment with the dopamine β-hydroxylase inhibitor, bis (4-methyl-1-homopiperazinyl-thiocarbonyl) disulphide (FLA-63) (25 mg/kg, i.p.). The hypotensive response to α-MD was completely abolished in these experiments. The formation of α-methylnoradrenaline from α-MD was prevented after FLA-63 but there was a significant increase in the amounts of α-methyldopamine formed.

A new possibility for the mechanism of action of hypotensive drugs has recently been suggested by the finding that, after inhibition of peripheral decarboxylase, L-dopa exerts a centrally mediated hypotensive effect (Henning & Rubenson, 1970 a, b). There is evidence that this action is brought about through activation of inhibitory mechanisms, possibly of a noradrenergic nature and it is also exerted by certain analogues of L-dopa, e.g. *m*-tyrosine (Rubenson, 1971).

The structurally related amino-acid, L-α-methyl-3,4-dihydroxyphenylalanine (α-methyldopa, α-MD) lowers arterial pressure in animals and man through mechanisms which are not yet completely understood (for review see Henning, 1969b). Current views on the mode of action of α-MD emphasize the importance of its metabolites, in particular α-methylnoradrenaline (α-MNA), which is thought to act as a pseudotransmitter in sympathetic nerves, thereby producing a functional impairment in these nerves and hence a decrease in blood pressure (for review see e.g. Kopin, 1968). There is no doubt that the hypotensive action of α-MD is related to an enzymatic decarboxylation since potent inhibitors of dopa decarboxylase in the central nervous system and peripheral tissues prevent the lowering of blood pressure (Davis, Drain & others, 1963; Henning, 1968, 1969a). However, it has also been demonstrated that selective inhibition of peripheral decarboxylase leaves the hypotensive effect of α-MD unchanged (Henning, 1969a). Taken together, these findings clearly point to the importance of a decarboxylation of α-MD within the central nervous system for the hypotensive action of the drug (for review see Henning, 1969b).

However, these results do not permit any conclusions about which of the decarboxylation products of α-MD is responsible for the central effect, or if their action is mediated by a false transmitter mechanism in the brain. The recent availability of potent

and relatively specific inhibitors of dopamine-β-hydroxylase (Florvall & Corrodi, 1970) seemed to offer an approach to this question. Therefore, the action of α-MD on blood pressure and tissue monoamine levels was studied before and after pretreatment with bis(4-methyl-1-homopiperazinyl-thiocarbonyl)disulphide (FLA-63), an inhibitor of dopamine β-hydroxylase (Svensson & Waldeck, 1969; Florvall & Corrodi, 1970). It was found that the hypotensive action of α-MD in conscious normotensive rats was abolished after pretreatment with FLA-63.

METHODS

Male Sprague-Dawley rats, 200–250 g, were used. Mean arterial pressure was recorded through indwelling arterial catheters connected to Statham P23 Dc pressure transducers writing on a Grass polygraph (Henning 1969b). Using this method blood pressure varies little with time of day or on subsequent days. Therefore, the basal values were obtained by continuous recording for 15–30 min periods and the average of the last 10 min was taken as the level of blood pressure. Similar recordings were made at intervals after administration of saline (0·9%) or drugs. No animal received the same treatment more than once.

The amine contents of isolated organs were measured. The brains, hearts and spleens from two animals were separately pooled. Dopamine and noradrenaline were determined according to Carlsson & Lindqvist (1962) and Bertler, Carlsson & Rosengren (1958). α-Methyldopamine was determined as described by Carlsson & Lindqvist (1962) and α-methylnoradrenaline as described by Waldeck (1968). To avoid interference of noradrenaline in determinations of α MNA, rats were pretreated with reserpine (10 mg/kg) 16h before the experiments.

RESULTS

Blood pressure

The effect of α-MD (400 mg/kg) on blood pressure was studied in animals pretreated with α-MMT (two doses of 400 mg/kg, 27 and 15 h before α-MD), H 44/68 (250 mg/kg, 60 min before α-MD) and MK 485 (100 mg/kg, 30 min before α-MD). The results are shown in Fig. 1. There was a significant decrease in blood pressure 3 h ($P < 0·025$) and 6 h ($P < 0·001$) after α-MD. In another series of experiments 0·9% saline was given instead of α-MD and produced no significant changes in blood pressure.

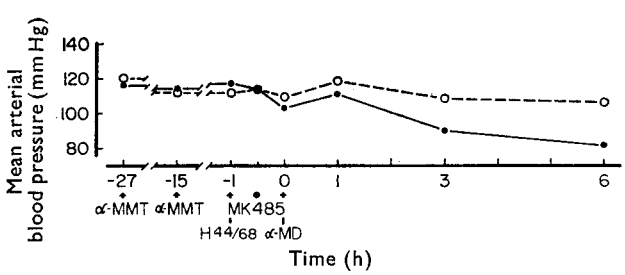

FIG. 1. Changes in mean arterial blood pressure of conscious rats after the following treatments: α-MD, 400 mg/kg, 30 min after MK 485, 100 mg/kg, 60 min after α-methyl-*p*-tyrosine methylester (H 44/68; 250 mg/kg) and 15 h and 27 h after two doses of α-methyl-*m*-tyrosine (α-MMT), 400 mg/kg (solid symbols; error variance 52·3, n = 4); 0·9% saline, 5 ml/kg, 30 min after MK 485, 100 mg/kg, 60 min after H 44/68, 250 mg/kg and 15 h and 27 h after two doses of α-MMT, 400 mg/kg (open symbols; error variance 99·2, n = 4). The values are means in mm Hg; s.e. were calculated by analysis of variance.

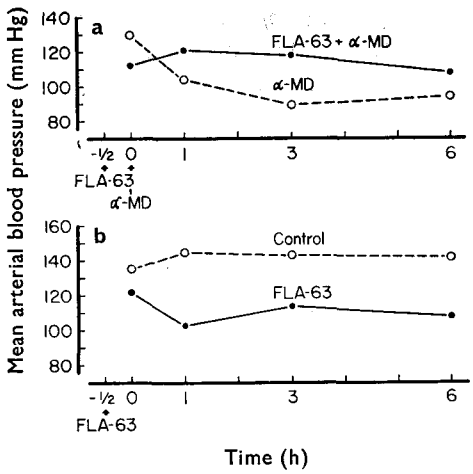

FIG. 2. Changes in mean arterial blood pressure of conscious rats after i.p. injections of drugs as indicated. a. α-MD, 200 mg/kg (open symbols; error variance 100·7, n = 5); α-MD, 200 mg/kg, 30 min after FLA-63, 25 mg/kg (solid symbols; error variance 95·7, n = 5). b. Saline (0·9%) 5 ml/kg, 30 min after FLA-63, 25 mg/kg (solid symbols; s.e. = 3·8, n = 7); two injections of 0·9% saline, 5 ml/kg, 30 min apart (open symbols; s.e. = 4·4, n = 5). The values are means in mm Hg; s.e. were calculated by analysis of variance.

Fig. 2 shows the effect of α-MD alone and after pretreatment with FLA-63. Administration of α-MD (200 mg/kg) lowered mean arterial blood pressure significantly after 1, 3 and 6 h ($P < 0{\cdot}001$). After pretreatment with FLA-63 (25 mg/kg), no significant changes ($P < 0{\cdot}10$) in blood pressure occurred, the hypotensive effect of α-MD (200 mg/kg) being completely abolished. FLA-63 alone (25 mg/kg) caused a slight lowering of blood pressure, particularly 1·5 h after its administration ($P < 0{\cdot}005$) (Fig. 2b). When equivalent volumes of 0·9% saline were injected, no significant changes in blood pressure occurred.

Amine contents of organs

Table 1 shows the levels of dopamine and noradrenaline in brain after pretreatment with αMMT and H 44/68 as described in the blood pressure experiments. There was a marked lowering of both amines. Three h after the injection of α-MD (200 mg/kg) to reserpinized rats, significant amounts of α-methyl-dopamine (α-MDA) and α-MNA had accumulated in all organs examined (Table 2). Pretreatment with FLA-63 (25 mg/kg), significantly prevented the formation of α-MNA

TABLE 1. *Concentrations of dopamine and noradrenaline after administration of two doses of α-methyl-*m*-tyrosine* (α-MMT; 400 mg/kg i.p. 27 *and* 15 h *before death*), *α-methyl-*p*-tyrosine methylester* (H 44/68; 250 mg/kg, i.p. 1 h *before death*) *and* MK 485 (100 mg/kg, i.p. 30 min *before death*). The values are means in μg/g with s.e.

	Brain content of amines (μg/g)			
	No pretreatment		αMMT + H 44/68 + MK 485	
	Dopamine	Noradrenaline	Dopamine	Noradrenaline
Mean	0·640	0·357	0·196	0·028
s.e.	0·011	0·013	0·051	0·005
	(n=2)	(n=2)	(n=3)	(n=3)

Table 2. *Concentration of α-methyldopamine and α-methylnoradrenaline* 3 h *after α-methyldopa* (α-MD) (200 mg/kg, i.p.), *or* FLA-63 (25 mg/kg, i.p.) *plus α-methyldopa* (200 mg/kg, i.p., 30 min *after* FLA-63) in *rats pretreated with reserpine* (10 mg/kg) 16h *previously*, The values are means in μg/g. *P* values are calculated by analysis of variance and *t*-test.

	Brain			Heart			Spleen		
	Control	α-MD	FLA+α-MD	Control	α-MD	FLA+αMD	Control	α-MD	FLA+α-MD
	A	B	C	A_1	B_1	C_1	A^2	B^2	C^2
				α-Methyldopamine					
n =	(1)	(3)	(3)	(1)	(3)	(3)	(1)	(3)	(3)
	0·000	0·252	0·442	0·000	0·045	0·077	0·000	0·066	0·047
ϕ Within group		4			4			4	
Variance within group		0·00567			0·00012			0·00100	
		A–B: $P < 0·001$			A_1–B_1: $P \approx 0·05$			A^2–B^2: $P > 0·1$	
		A–C: $P < 0·001$			A_1–C_1: $P < 0·005$			A^2–C^2: $P > 0·1$	
		B–C: $P < 0·001$			B_1–C_1: $P \approx 0·05$			B^2–C^2: $P > 0·1$	
				α-Methylnoradrenaline					
n =	(4)	(6)	(6)	(3)	(6)	(6)	(4)	(6)	(6)
	0·016	0·175	0·017	0·004	0·176	0·044	0·019	0·200	0·062
		13			12			13	
Variance within group		0·00026			0·00072			0·00443	
		A–B: $P < 0·001$			A_1–B_1: $P < 0·001$			A^2–B^2: $P \approx 0·001$	
		A–C: $P > 0·1$			A_1–C_1: $P > 0·1$			A^2–C^2: $P > 0·1$	
		B–C: $P < 0·001$			B_1–C_1: $P < 0·001$			B^2–C^2: $P < 0·005$	

from α-MD ($P < 0·001$) at the same time. The amounts of α-MDA were significantly increased ($P < 0·001$) in the brain.

DISCUSSION

The results of the present investigation confirm the ability of α-MD to lower mean arterial blood pressure in the conscious normotensive rat (Henning 1967).

Previous work has established that decarboxylation of α-MD in the central nervous system is necessary for its hypotensive action in the rat (Henning, 1968, 1969a). The α-methylated amines could act either directly or indirectly. The present results indicate that an indirect effect would seem less likely since α-MD retained its hypotensive effect after pretreatment with repeated doses of α-MMT in combination with the tyrosine hydroxylase inhibitor H44/68. There is a profound depletion of tissue catecholamines after α-MMT (Hess, Connamacher & others, 1961; Andén, 1964) and the combination used in the present study lowered brain noradrenaline to less than 10% of control values. Further, the lack of effect of α-MMT, in contrast to α-MD, on blood pressure (Henning, 1967) may point to the importance of direct effects of the decarboxylation products of α-MD. There is ample evidence that α-MMT is metabolized to α-methyl-*m*-tyramine and metaraminol in the brain (Carlsson & Lindqvist, 1962; Muscholl, 1966) and it seems likely that these amine products should have indirect actions to a similar extent as those amines formed after α-MD.

The present results show that pretreatment with FLA-63, an inhibitor of dopamine β-hydroxylase (Svensson & Waldeck, 1969; Florvall & Corrodi, 1970), prevented the hypotensive response to α-MD and completely prevented the formation of α-MNA while at the same time there was a significant increase of the amounts of α-MDA found.

Assuming a predominantly direct action of the decarboxylation products of α-MD in mediating the hypotensive action of the drug, the effect of FLA-63 reported here ascribes an important role to α-MNA. The recent observation that FLA-63 may rapidly deplete endogenous noradrenaline stores (Persson & Waldeck, 1970) is

probably less important in the case of α-MD, in view of the results with α-MMT discussed above.

Thus, there is an obvious similarity between the effect of α-MD and that previously observed in similar studies with L-dopa (Henning & Rubenson, 1970b) or *m*-tyrosine (Rubenson, 1971): all three drugs also produce hypotensive effects through central activation of inhibition of sympathetic mechanisms by their decarboxylation products. However, in the case of L-dopa and *m*-tyrosine, indirect mechanisms appear to be of major importance. Although the nature and exact localization of the actions is not known, it is of interest to note that the metabolites of L-dopa (Andén, 1969) as well as α-MD (Andén, Butcher & Engel, 1970) are capable of stimulating noradrenaline receptors in the rat spinal cord, but α-MMT, which lacks hypotensive properties in the rat, had no such effect. Interestingly, the antihypertensive drug clonidine (Catapresan) also stimulates central noradrenaline receptors (Andén, 1969; Andén, Corrodi & others, 1970).

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