RESEARCH PAPERS

REVERSAL OF THE EFFECT OF α-METHYLDOPA BY MONOAMINE OXIDASE INHIBITORS

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L-α-Methyldopa, which normally causes sedation, induces a strong central excitation in mice pretreated with a monoamine oxidase inhibitor after a lag of a few hours. It is concluded that this excitation is caused by accumulation of free catecholamines liberated by amines which are slowly formed by decarboxylation of α-methyldopa. The hypotensive and sedative effects of α-methyldopa given alone are attributed to the slow release of catecholamines and subsequent breakdown by monoamine oxidase so that a partial depletion of catecholamines ensues.

DECARBOXYLASE-INHIBITORS represent a new class of pharmacological agents (Sourkes, Murphy and Chavez-Lara, 1962). L-α-Methyl dioxyphenylalanine (α-methyldopa), which is a representative of this class, is widely used as an antihypertensive drug (Bayliss and Harvey-Smith, 1962; Dollery and Harington, 1962; Gillespie, Oates, Crout and Sjoerdsma, 1962; Kirkendall and Wilson, 1962; Oates, Gillespie, Udenfriend and Sjoerdsma, 1960). α-Methyldopa inhibits the enzymatic decarboxylation of L-dioxyphenylalanine (dopa) and of 5-hydroxytryptophan (5-HTP) (Clark, 1959; Sourkes, 1954).

The anti-decarboxylase properties of α-methyldopa have also been shown to exist *in vivo* both in animals (Clark, 1959; Dengler and Reichel, 1958; Hansson and Clark, 1962) and in man (Oates and others, 1961). Serious doubt has been expressed whether inhibition of dopa decarboxylase could be the cause of the antihypertensive properties of α-methyldopa (Gillespie and others, 1962; Hess, Connamacher, Ozaki and Udenfriend, 1961).

From enzymological studies it became evident that α-methyldopa is not only an inhibitor but also a substrate for dopa decarboxylase, having an affinity similar to dopa but a turnover-rate 200 times slower (Lovenberg, Weissbach and Udenfriend, 1962). Also *in vivo* α-methyldopa is slowly converted into the methyl-analogues of the catecholamines (Carlsson and Lindqvist, 1962). Furthermore, from clinical reports it appears that catecholamine-like substances are formed from α-methyldopa during therapy with this drug (Lauwers, Verstraete and Joossens, 1963; Stott, Robinson and Smith, 1963).

The metabolic products (2*S*-α-methyldopamine, 1*R*:2*S*-α-methylnoradrenaline*) formed from α-methyldopa are potent releasers of

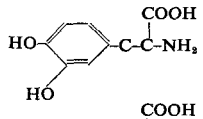
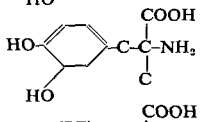
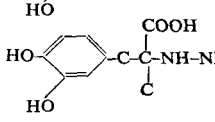
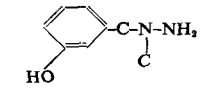
**R* and *S* are notations of absolute configuration according to the sequence rule (Cahn, Ingold and Prelog, 1956).

endogenous catecholamines (Hess and others, 1961; Porter, Totaro and Leiby, 1961). Other far more potent decarboxylase inhibitors (see Table I) than α-methyldopa, themselves unaffected by dopa decarboxylase, have no catecholamine-releasing properties, do not cause depletion of catecholamines and have no antihypertensive effect (Brodie, Kuntzman, Hirsch and Costa, 1962; Drain, Horlington, Lazare and Poulter, 1962). It has recently been shown that these potent and pure decarboxylase inhibitors can completely abolish the catecholamine-depleting and hypotensive effects of α-methyldopa (Davis, Drain, Horlington, Lazare and Urbanska, 1963).

TABLE I

DOPA AND DECARBOXYLASE INHIBITORS

Affinity for the enzyme and relative intrinsic turn-over rate (k_3) obtained from enzymological studies (Lovenberg, Weissenbach, and Udenfriend 1962) and *in vivo* studies (Hansson and Clark, 1962)

Drug	Code	Rel. k_3	Relative affinity	
			Substrate	Inhibitor
	DOPA	100	1·3	—
	α-MeDOPA	0·5	1	1
	MK-485	0	—	45
	NSD-1034	0	-	45

MK-485 is 2-Hydrazino-4-(3,4-dihydroxyphenyl)-2-methylbutyric acid
NSD-1034 is *N*(*m*-hydroxybenzyl)-*N*-methylhydrazine

The conclusion is thus reached that α-methyldopa acts indirectly by being slowly converted into catecholamine analogues which in turn cause release of catecholamines. Since the catecholamines so released are simultaneously metabolised by the enzyme monoamine oxidase a partial depletion ensues.

The antihypertensive action of α-methyldopa thus closely resembles the effect of reserpine. Also α-methyldopa causes some degree of sedation in animals and man (Bayliss and Harvey-Smith, 1962; Oates and others, 1960).

It may be anticipated that when given after pretreatment with a monoamine oxidase inhibitor α-methyldopa will cause an accumulation of free catecholamines and thus hypertension and central excitation.

METHODS

Central effects of α-methyldopa were studied in female mice of the R.Q. strain (an F_1 hybrid of R, Rhodes farm albino, and Q, Extreme dilute). Motor activity was continuously registered with cumulative recorders (Rossum, 1962). Mice of a homogeneous population were

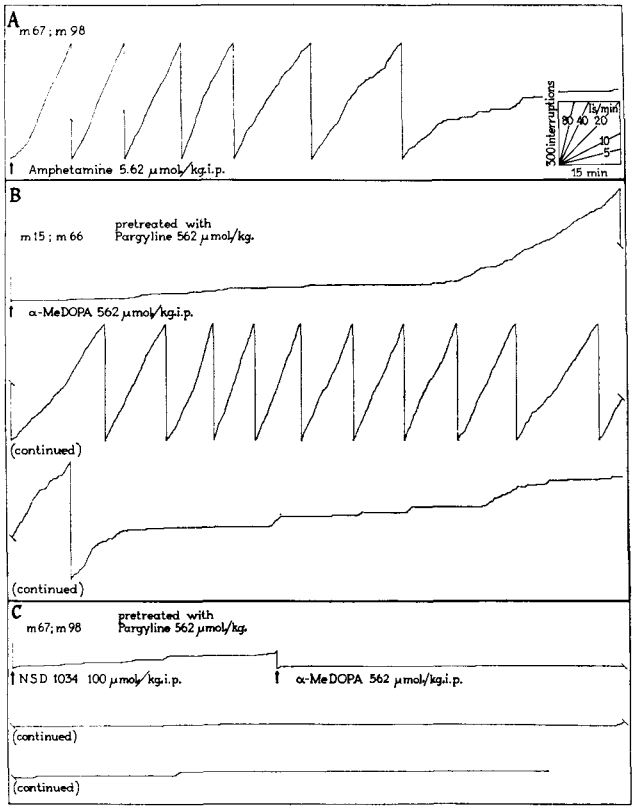


FIG. 1. Cumulative records of the motor activity of two mice under various experimental conditions. In A, mice Nos. 67 and 98 received dexamphetamine (1 mg./kg. of the sulphate) intraperitoneally. The onset of dexamphetamine is almost instantaneous. In B, mice Nos. 15 and 66 which 2 hr. earlier were injected with pargyline (119 mg./kg.) received α-methyldopa (118 mg./kg.). The pargyline as such does not cause an effect. After a lag period of more than 2 hr. a strong increase in locomotor activity occurs which lasts for hours. In C, mice Nos. 67 and 98 which first received pargyline followed 30 min. later by NSD–1034 were injected again 90 min. later with α-methyldopa (118 mg./kg.). The decarboxylase inhibitor completely abolished the central stimulant action of the combination of α-methyldopa and pargyline.

selected for similar sensitivity to dexamphetamine. All mice received a test dose of 5·62 μmol./kg. dexamphetamine (1·0 mg./kg. of the sulphate) one day before the experiment. (See upper row in Fig. 1.) The experiments were conducted in three groups of two mice. A dose of 562 μmol./kg. α-methyldopa (119 mg./kg.) was injected i.p. into (*a*) mice

of the control groups; (*b*) mice which had received 562 μmol./kg. pargyline (*N*-benzyl-*N*-methyl-2-propynylamine hydrochloride) (113 mg./kg. of hydrochloride) 2 hr. previously; and (*c*) in mice that in addition to pargyline also received 100 μmol./kg. *N*-(*m*-hydroxybenzyl)-*N*-methyl-hydrazine (NSD–1034) (25 mg./kg. of H_2PO_4 salt) 30 min. before the α-methyldopa. A typical experiment is presented in Fig. 1. Other groups of two mice were reserpinised by administration of 1 mg./kg.

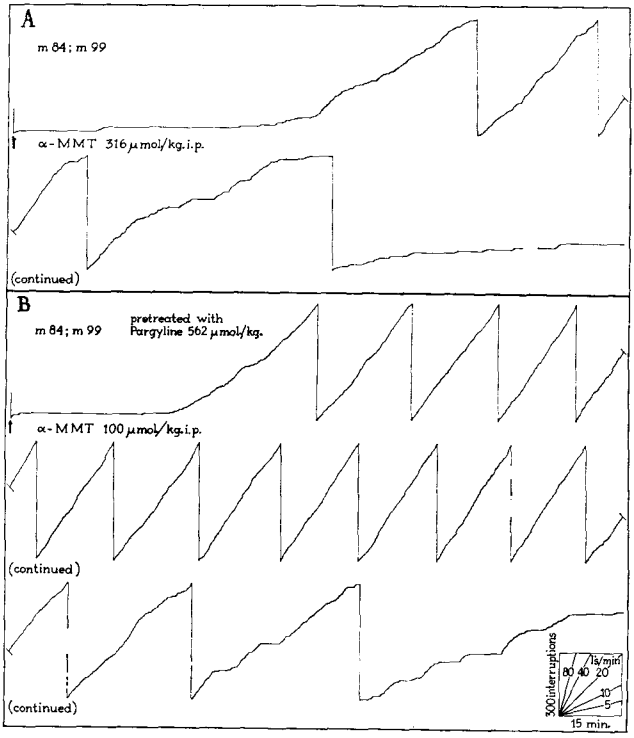


FIG. 2. Cumulative records of motor activity in mice under the influence of α-MMT. In A, mice Nos. 84 and 99 were injected with α-MMT (60 mg./kg.) intraperitoneally. After a lag period of less than 1 hr. an increase of locomotor activity occurs. In B, the same mice one day later were first injected with pargyline (119 mg./kg.) followed 2 hr. later by a threefold smaller dose of α-MMT (19·5 mg./kg.). After a lag time of about 45 min. a strong increase in locomotor activity occurs. The psychomotor stimulant action of α-MMT is potentiated by a monoamine oxidase inhibitor by more than a factor five.

reserpine i.p. over 3 or 4 subsequent days, after which they received 562 μmol./kg. pargyline, followed 2 hr. later by 562 μmol./kg. α-methyldopa. The analogous amino-acid DL-α-methyl-*m*-tyrosine (α-MMT) was also studied in groups of two mice alone in doses of 316 and 562 μmol./kg. and 2 hr. after 562 μmol./kg. pargyline in doses of 31·6 and 100 μmol./kg. A typical experiment is given in Fig. 2.

RESULTS AND DISCUSSION

It was observed that α-methyldopa alone produces a decrease in locomotor activity. In mice pretreated with a monoamine oxidase inhibitor (pargyline) administration of α-methyldopa caused a strong and prolonged increase of motor activity and central excitation resembling an overdose of dexamphetamine. This reversed effect of α-methyldopa occurred after a lag of 2 to 3 hr. (Fig. 1). In contrast, the central stimulant action of dexamphetamine is almost instantaneous. These experiments suggest that the lag in time is caused by a slow conversion in the brain of α-methyldopa to α-methyldopamine or α-methylnoradrenaline or both. This view was further substantiated by experiments with mice which, in addition to a monoamine oxidase inhibitor, also received a strong decarboxylase inhibitor (NSD–1034). The latter drug abolishes the central stimulant action of α-methyldopa in mice pretreated with pargyline. It may be noted here that the catecholamine-depleting and the hypotensive effects of α-methyldopa alone also occur after a lag period of a few hours and that these actions are also completely abolished by the inhibition of dopa decarboxylase (Davis and others, 1963; Drain and others, 1962) suggesting a common mechanism of action.

From the experiments shown in Fig. 1 it seems likely that a release of catecholamines by the amines which are slowly formed from α-methyldopa is the cause of the central stimulant action of the combination of α-methyldopa with the monoamine oxidase. But separately or together it seems unlikely that α-methyldopamine and α-methylnoradrenaline are themselves responsible for the central stimulant action of the combination. In that case it would be expected that α-methyldopa alone would produce a central stimulant action, since α-methyl analogues are resistant to monoamine oxidase. On the contrary, the experiments provide evidence that the α-methyl catecholamines formed from α-methyldopa cause a release of endogenous catecholamines, the oxidation of which is prevented by a monoamine oxidase inhibitor. Further evidence for this supposition is gained by experiments in which the combination of α-methyldopa and pargyline was given to mice previously treated with reserpine. When the catecholamines have been depleted the combination does not exert central excitation. Furthermore, after a large dose of α-methyldopa (1,000 μmol./kg.) alone is given to mice which then receive monoamine oxidase inhibitor the next day, a subsequent dose of α-methyldopa is ineffective as a stimulant. Obviously therefore replenishment of catecholamine stores is essential for the central stimulant action of α-methyldopa when given after a monoamine oxidase inhibitor.

The experiments lend no support to the recent postulation that α-methyldopa could act as a precursor of a false transmitter of noradrenaline (Day and Rand, 1963). Also results from the biochemical work (Gessa, Costa, Kuntzman and Brodie, 1962) pleads against this supposition. α-Methyldopa as well as the analogous amino-acid DL-α-methyl-*m*-tyrosine (α-MMT) cause a depletion of noradrenaline which lasts several days, whereas methyltyramines can be detected only during the first 24 hr. after administration (Carlsson and Lindqvist, 1962).

α-MMT acts similarly by virtue of its decarboxylation products which are potent releasers of catecholamines (Porter and others, 1961; Udenfriend and Zaltman-Nirenberg, 1962). α-MMT causes a central excitation of its own after a lag of about 45 min. (Rossum 1963a). Thus it is a better substrate for dopa decarboxylase than α-methyldopa while the amines formed from it are better releasers of catecholamines. The central stimulant action of α-MMT is strongly potentiated by monoamine oxidase inhibitors (see Fig. 2). Its stimulant action is also dependent on replete catecholamine stores (Rossum, 1963a; Gessa and others, 1962).

The antihypertensive action of α-methyldopa seems unique for this amino-acid, since it is a substrate for dopa decarboxylase with such a low turnover rate that the amines formed from it cause in turn a slow release of endogenous catecholamines. As a result, breakdown by monoamine oxidase keeps up with the release so that depletion of catecholamines occurs without induction of central excitation. Therapeutically α-MMT is inferior because its decarboxylation products cause too fast a release of catecholamines.

A consequence of the mechanism of action of α-methyldopa is its reversal of action by monoamine oxidase inhibition. It might therefore be dangerous to begin therapy with α-methyldopa in patients who have been treated with a monoamine oxidase inhibitor in the two preceding weeks (Rossum, 1963b), whereas administration of monoamine oxidase inhibitors during α-methyldopa therapy is thought to be less dangerous.

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