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IN VIVO MONOAMINE OXIDASE INHIBITION BY *d*-AMPHETAMINE

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Abstract—*In vitro*, *d*- and *l*-amphetamine (AMPH) are reversible monoamine oxidase (MAO) type A inhibitors, the *d*-form being approximately five times more potent. Experiments were conducted in rats to determine whether MAO inhibition occurs *in vivo*. *d*-AMPH was more effective than *l*-AMPH at decreasing striatal 3,4-dihydroxyphenylacetic acid (DOPAC). However, assays of striatal MAO activity following administration of AMPH *in vivo* failed to show MAO inhibition. In other experiments, rats were treated with *d*-AMPH (zero time) followed by phenelzine (1 hr), an irreversible MAO inhibitor, and were killed at 25 hr. MAO activity was determined *in vitro* for the striatum and the rest of the brain using serotonin (MAO-A) and phenylethylamine (MAO-B) as substrates. *d*-AMPH provided significant protection against MAO-A inhibition by phenelzine, whereas *l*-AMPH and cocaine (used instead of AMPH) were without effect. *d*-AMPH failed to protect against MAO-B inhibition by phenelzine. Thus, *d*-AMPH appears to inhibit reversibly MAO type A *in vivo*. However, using the same 'protection protocol', *d*-AMPH failed to oppose phenelzine-induced lowering of striatal DOPAC. Experiments were undertaken to determine whether the protective effect of *d*-AMPH on MAO type A would influence striatal dopamine depletion by Ro4-1284, a rapidly acting reserpine-like agent. Ro4-1284-induced depletion of dopamine was inhibited by phenelzine. Prior treatment with *d*-AMPH reduced significantly the protective effect of phenelzine, suggesting reversible, intraneuronal MAO inhibition by *d*-AMPH *in vivo*. The possible neuronal mechanisms for these events are discussed.

The *in vivo* effects of amphetamine are commonly considered to result from catecholamine release and reuptake blockade [1]. However, it has been known for many years that amphetamine inhibits monoamine oxidase (MAO; EC 1.4.3.4, monoamine: O_2 oxidoreductase deaminating) *in vitro* [2]. This effect has not been widely accepted as an important action *in vivo* since relatively high concentrations are required for significant inhibition [3]. Recently, however, expanding knowledge in the area of MAO has created renewed interest in the MAO inhibitory property of amphetamine.

Currently, mitochondrial MAO is considered to exist in at least two functionally distinct types (type A and type B), each with particular substrate and inhibitor preferences [4, 5]. Both types of MAO are found extraneuronally but MAO type A is also considered to be the predominant form within peripheral and central monoaminergic neurons [4–7]. Recent *in vitro* studies with *d*-amphetamine have shown preferential inhibition of MAO type A [8–10]. In several rat tissues, *d*-amphetamine was about fifty times more potent in inhibiting MAO type A compared with MAO type B [10]. Additionally, the *d*-form was five times more potent than *l*-amphetamine on the A type, whereas both enantiomers inhibited MAO type B to the same extent [10]. These data led Miller and Clarke [10] to postulate that at least some of the differences in the pharmacologic effects of *d*- and *l*-amphetamine might be related to differential inhibition of MAO type A.

Direct evidence for an MAO inhibitory action of *d*-amphetamine *in vivo* has not been forthcoming, since *d*-amphetamine is a reversible inhibitor. However, Green and El Hait [11] have shown that *d*-amphetamine will protect against irreversible MAO inhibition by phenelzine in the mouse brain. Although possible problems relating to inhibition of phenelzine absorption and neuronal uptake by amphetamine were not addressed by Green and El Hait [11], their data are highly suggestive of an MAO inhibitory action *in vivo*.

In view of the above findings, we have undertaken further studies on the possible MAO inhibitory effect of amphetamine *in vivo*. Our results in rat striatum and the rest of the brain confirm the result described by Green and El Hait [11]. Additionally, the data indicate that inhibition of MAO type A by *d*-amphetamine contributes to its pharmacologic activity.

MATERIALS AND METHODS

Male rats (Sprague–Dawley descendants; Texas Inbred, Houston, TX), weighing between 175 and 275 g, were used. The MAO assays with kynuramine [12] and [^{14}C-]beta-phenylethylamine (40 mCi/mmole) [13] were conducted as described previously. [^{14}C]Serotonin (4.72 mCi/mmole) was assayed using the same procedure as for phenyl-

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ethylamine. The final substrate concentrations used were: kynuramine 100 μM, phenylethylamine 10 μM, and serotonin 400 μM. These concentrations were determined following preliminary studies with the differential MAO inhibitor, clorgyline [14]. In striatal homogenates, serotonin metabolism was found to proceed entirely through MAO type A and phenylethylamine entirely through type B. Kynuramine was a substrate for both types. Additionally, the selected concentrations are two to four times that of the reported K_m values for their respective MAO types [15–18].

Dopamine [19] and 3,4-dihydroxyphenylacetic acid (DOPAC) [20] were measured by established fluorometric techniques. Values were corrected for recovery of authentic dopamine and DOPAC and reported as μg/g tissue.

The following drugs were used: *d*- and *l*-amphetamine sulfate, chlorpromazine HCl, cocaine HCl, clorgyline, haloperidol, phenelzine sulfate and Ro4-1284, (Ro; 2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydrobenzo[*a*]quinolizine).

The significance of differences was determined using Student's *t*-test. P values were expressed as two-tailed.

RESULTS

MAO activity after in vivo *administration of* d-*amphetamine.* *d*-Amphetamine is a reversible MAO inhibitor [8]. Thus, concentrated tissue homogenates (100 mg/ml) were prepared in order to minimize drug dissociation. However, in brains removed from rats treated with *d*-amphetamine (20 mg/kg, s.c., for 1 hr) there was no alteration of MAO activity in the striatum and the rest of the brain, using kynuramine and serotonin as substrates. In view of this result, several different indirect measures were undertaken to test for possible MAO inhibition *in vivo*.

Effects of d- *and* l-*amphetamine on striatal DOPAC levels.* *In vitro*, *d*-amphetamine is five times more potent than *l*-amphetamine at inhibiting MAO type A [10]. Thus, it was of interest to compare the two enantiomers with regard to the ability to lower striatal DOPAC. In some experiments, a dopamine

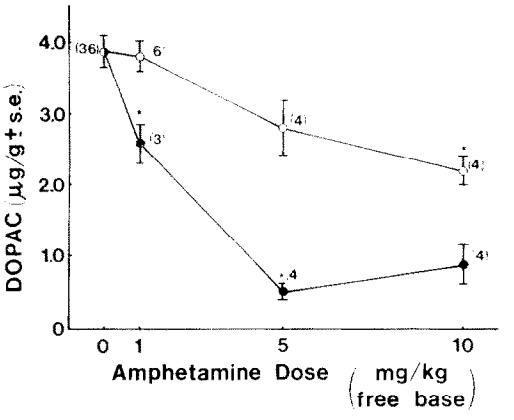

Fig. 1. Effects of *d*-amphetamine (●—●) and *l*-amphetamine (○—○) on the striatal 3,4-dihydroxyphenylacetic acid (DOPAC) level in chlorpromazine (5 mg/kg, free base)-treated rats. The animals were given amphetamine (s.c.) at zero time, chlorpromazine (s.c.) at 30 min and were killed at 2 hr. DOPAC was determined the same day by spectrophotofluorometry (see Materials and Methods) and is expressed as μg DOPAC/g striatum. A single asterisk (*) indicates significance of differences vs control (no amphetamine), $P < 0.05$.

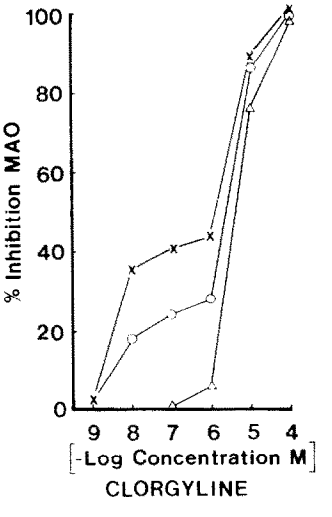

Fig. 2. *In vitro* inhibition of kynuramine (100 μM) deamination in the rat striatum by clorgyline. The animals were treated s.c. for 24 hr as follows: no treatment (×—×), phenelzine (2 mg free base/kg, ○—○) and phenelzine (8 mg free base/kg, △—△). The mean specific activities (nmoles 4-hydroxyquinoline formed/mg fresh tissue/hr) in the absence of clorgyline were 5.74, 3.07 and 1.47 respectively. Each point is the mean of at least two determinations. Variation, expressed as the range of values, falls within the size of the symbols. Note that, as the dose of phenelzine is increased, the type A activity (defined by 10^{-7} M clorgyline) is preferentially diminished in relation to the type B activity (no treatment: 41 per cent A, 59 per cent B; 2 mg/kg phenelzine: 24 per cent A, 76 per cent B; 8 mg/kg phenelzine: 0 per cent A, 100 per cent B). From the specific activities given above it can be calculated that, following 2 mg/kg phenelzine, approximately 68 per cent of the activity defined as type A is inhibited, while approximately 31 per cent of the B activity is inhibited. After 8 mg/kg phenelzine, no type A activity remains, and approximately 57 per cent of the type B activity is inhibited.

Table 1. Effects of *d*- and *l*-amphetamine (AMPH, 5 mg/kg) alone or with haloperidol (HALO, 1 mg/kg) on striatal 3,4-dihydroxyphenylacetic acid (DOPAC) concentration

Drug treatment*	DOPAC (μg/g ± S.E.) (N)
None (control)	1.52 ± 0.09 (39)
d-AMPH	0.77 ± 0.15† (10)
l-AMPH	1.31 ± 0.23‡ (10)
HALO	5.00 ± 0.41 (6)
HALO + *d*-AMPH	1.84 ± 0.22§ (6)
HALO + *l*-AMPH	5.45 ± 0.66‖ (6)

* All drugs were given s.c. at zero time, and the rats were killed at 2 hr.

† Significantly different from control, $P < 0.001$.

‡ Not significantly different from control.

§ Significantly different from HALO, $P < 0.001$.

‖ Not significantly different from HALO.

receptor blocking drug, haloperidol or chlorpromazine, was given to enhance DOPAC production [21, 22]. The results are shown in Table 1 and Fig. 1. Under all experimental conditions, *d*-amphetamine proved to be more effective than the *l*-enantiomer at lowering DOPAC. Figure 1 shows that 1 mg/kg of *d*-amphetamine produced about the same effect as 5–10 mg/kg of the *l*-form.

MAO inhibitory properties of phenelzine and its interaction with d- *and* l-*amphetamine.* Figure 2 characterizes the MAO inhibitory effect of phenelzine (2 and 8 mg/kg) in the striatum of rats 24 hr after subcutaneous injection. Following the convention established by Johnston [14], the control inhibition curve with clorgyline shows an A : B ratio of 41 : 59. The A:B ratio was changed by phenelzine to about 24:76 (2 mg/kg) and 0:100 (8 mg/kg). Thus, phenelzine preferentially inhibited type A MAO. From the specific activities given in the legend to Fig. 2, we calculated that MAO-A was 68 per cent inhibited following 2 mg/kg phenelzine, while MAO-B was inhibited by 31 per cent. After 8 mg/kg phenelzine, MAO-A was inhibited fully, while MAO-B was inhibited by 57 per cent. This differential inhibitory effect of phenelzine in the striatum is further demonstrated in Table 2. Metabolism of the type A substrate serotonin (see Materials and Methods) was inhibited to a greater extent than phenylethylamine, the type B substrate. Similar results were found in the rest of the brain (Table 2). Preferential type A inhibition was also seen after addition of phenelzine to striatal homogenates *in vitro*, but only at a concentration of 1.2×10^{-7} M (Fig. 3).

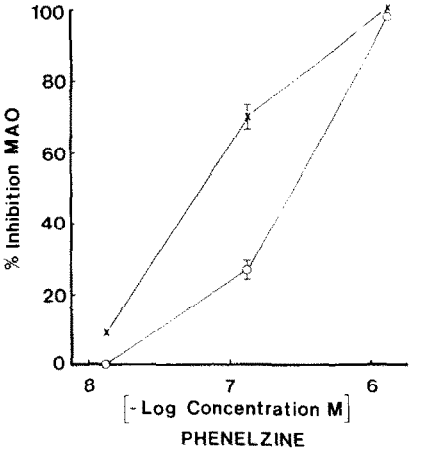

Fig. 3. *In vitro* inhibition of [^{14}C]serotonin (×—×) and [^{14}C]β-phenylethylamine (○—○) deamination in rat striatum by phenelzine. Substrate concentrations were 400 μM and 10 μM respectively. Each point represents at least two determinations. The vertical bars around the central points represent the range of values.

Table 2. Inhibitory effects of phenelzine (2 mg/kg) on MAO activity in the striatum and rest of brain*

Substrate	Mean % inhibition of MAO (N) Striatum	Rest of brain
Phenylethylamine	33 (3)	14 (3)
Kynuramine	44 (6)	ND†
Serotonin	72 (5)	67 (5)

* Phenelzine was given s.c. at zero time, and the rats were killed at 24 hr.
† Not determined.

Experiments were conducted to determine whether the prior administration of amphetamine would protect against phenelzine-induced inhibition of MAO, *in vivo*. Amphetamine (10 mg/kg) was given at zero time, phenelzine (2 mg/kg) at 1 hr and the animals were killed at 25 hr. MAO activity was measured *in vitro* using serotonin (type A) and phenylethylamine (type B) as substrates. Table 3 (*d*-amphetamine) and Table 4 (*l*-amphetamine) show the results obtained in the striatum and the rest of the brain with serotonin. *d*-Amphetamine significantly protected against phenelzine-induced inhibition of MAO, whereas *l*-amphetamine failed to do so. In contrast, no significant protection was found with *d*-amphetamine using phenylethylamine as the substrate (Table 5). With phenylethylamine in the striatum, phenelzine alone, and phenelzine preceded

Table 3. Effects of *d*-amphetamine (*d*-AMPH, 10 mg/kg) on the inhibition of [^{14}C]serotonin deamination by phenelzine (PHEN, 2 mg/kg) in the striatum and rest of brain

Drug treatment*	Specific activity of MAO [nmoles deaminated products/mg/hr ± S.E. (N)] Striatum	% Inhibition	Rest of brain	% Inhibition
None (control)	14.23 ± 1.76 (4)		13.84 ± 1.07 (4)	
PHEN	3.92 ± 0.50† (5)	72	4.54 ± 0.29† (5)	67
d-AMPH	14.30 ± 1.02‡ (4)	0	14.30 ± 0.75‡ (4)	−3
d-AMPH + PHEN	7.17 ± 0.61§ (6)	50	7.13 ± 0.40§ (6)	48

* Amphetamine was given s.c. at zero time, phenelzine s.c. at 1 hr, and all rats were killed at 25 hr. Inhibition of substrate deamination was then measured *in vitro*.
† Significantly different from control, $P < 0.001$.
‡ Not significantly different from control.
§ Significantly different from PHEN, $P < 0.005$.

by *d*-amphetamine, produced 33 per cent inhibition. These data show that the protective effect of *d*-amphetamine relates only to MAO type A. Furthermore, the experiment with phenylethylamine demonstrates that *d*-amphetamine does not inhibit the absorption of phenelzine. Such an effect could have explained the protective action of *d*-amphetamine upon phenelzine-induced inhibition of MAO-A. Table 6 shows that cocaine (15 mg/kg) also failed to alter the extent of MAO inhibition with phenelzine. Thus, it is unlikely that *d*-amphetamine protected against phenelzine by impairing neuronal uptake.

A comparison of Tables 2 and 3 with Tables 4 and 6 reveals a quantitative difference in the MAO inhibitory potency of phenelzine. This variation may be related, at least in part, to the higher specific activities of MAO shown in Table 4. Phenelzine irreversibly titrates MAO, and the per cent inhibition will depend upon the amount of enzyme present. Thus, in order to be certain of the protective action of *d*-amphetamine, the experiment shown in Table 7 was carried out. Of the three drugs used, only *d*-amphetamine produced a significant protective effect.

Interaction of d-*amphetamine with phenelzine on striatal DOPAC.* Experiments were made to determine whether the protective effect of *d*-amphetamine on phenelzine-induced inhibition of MAO would extend to the DOPAC-lowering action of phenelzine. The same experimental protocol was used as described previously for the protection experiments except that haloperidol or chlorpromazine was given 1.5 hr before the animals were killed. The results are given in Table 8. Unlike the acute effect of *d*-amphetamine (Fig. 1 and Table 1), treatment for 25 hr failed to lower DOPAC significantly. Additionally, *d*-amphetamine given before phenelzine failed to show a protective effect.

Interaction of d-*amphetamine with phenelzine on striatal dopamine (DA) depletion by Ro*4-1284 (*Ro*). The experimental protocol was as described above except that Ro (2 mg/kg), a short-acting reserpine-like drug [23], was given 30 min before the animals

Table 4. Effect of *l*-amphetamine (*l*-AMPH, 10 mg/kg) on the inhibition of [^{14}C]serotonin deamination by phenelzine (PHEN, 2 mg/kg) in the striatum and rest of brain

Drug treatment*	Specific activity of MAO [nmoles deaminated products/mg/hr ± S.E. (N)] Striatum	Rest of brain
None (control)	17.29 ± 0.94 (4)	18.44 ± 0.48 (4)
PHEN	7.00 ± 0.75† (4)	7.74 ± 0.75† (4)
l-AMPH	17.65 ± 0.78‡ (4)	17.84 ± 0.09‡ (4)
l-AMPH + PHEN	7.46 ± 0.34§ (6)	7.97 ± 0.37§ (6)

* Amphetamine was given s.c. at zero time, phenelzine s.c. at 1 hr, and all rats were killed at 25 hr. Inhibition of substrate deamination was then measured *in vitro*.
† Significantly different from control, $P < 0.001$.
‡ Not significantly different from control.
§ Not significantly different from PHEN.

Table 5. Effects of *d*-amphetamine (*d*-AMPH, 10 mg/kg) on the inhibition of [^{14}C]phenylethylamine deamination by phenelzine (PHEN, 2 mg/kg) in the striatum and rest of brain

Drug treatment*	Specific activity of MAO [nmoles deaminated products/mg/hr ± S.E. (N)] Striatum	% Inhibition	Rest of brain	% Inhibition
None (control)	2.89 ± 0.09 (3)		4.58 ± 0.13 (3)	
PHEN	1.93 ± 0.10† (3)	33	3.96 ± 0.21‡ (3)	14
d-AMPH	2.81 ± 0.05‡ (3)	3	4.70 ± 0.16‡ (3)	−3
d-AMPH + PHEN	1.94 ± 0.06§ (3)	33	3.77 ± 0.30§ (3)	18

* Amphetamine was given s.c. at zero time, phenelzine s.c. at 1 hr, and all rats were killed at 25 hr. Inhibition of substrate deamination was then measured *in vitro*.
† Significantly different from control, $P < 0.001$.
‡ Not significantly different from control.
§ Not significantly different from PHEN.

Table 6. Effects of cocaine (COC, 15 mg/kg) on the inhibition of [^{14}C]serotonin deamination by phenelzine (PHEN, 2 mg/kg) in the striatum and rest of brain

Drug treatment*	Specific activity of MAO [nmoles deaminated products/mg/hr ± S.E. (N)] Striatum	Rest of brain
None (control)	14.63 ± 0.41 (4)	15.44 ± 0.28 (4)
PHEN	6.35 ± 0.17† (2)	6.60 ± 0.31† (2)
COC	15.06 ± 0.96‡ (3)	15.73 ± 0.50‡ (3)
COC + PHEN	5.87 ± 0.15§ (5)	6.67 ± 0.19§ (5)

* Cocaine was given s.c. at zero time, phenelzine (s.c.) at 1 hr, and all rats were killed at 25 hr. Inhibition of substrate deamination was then measured *in vitro*.
† Significantly different from control, $P < 0.001$.
‡ Not significantly different from control.
§ Not significantly different from PHEN.

Table 7. Comparison of the effects of *d*-amphetamine (*d*-AMPH, 10 mg/kg), *l*-amphetamine (*l*-AMPH, 10 mg/kg) and cocaine (COC, 15 mg/kg) on inhibition of [^{14}C]serotonin deamination by phenelzine (PHEN, 2 mg/kg) in whole brain

Drug treatment*	Specific activity of MAO [nmoles deaminated products/mg/hr ± S.E. (N)]
None (control)	18.08 ± 0.11 (5)
PHEN	7.06 ± 0.27† (4)
d-AMPH + PHEN	9.65 ± 0.92‡ (3)
l-AMPH + PHEN	7.22 ± 0.08§ (5)
COC + PHEN	6.60 ± 0.48§ (3)

* Amphetamine was given s.c. at zero time, phenelzine s.c. at 1 hr, and all rats were killed at 25 hr. Cocaine was given s.c. at zero time, phenelzine s.c. at 1 hr, and all rats were killed at 25 hr. Inhibition of substrate deamination was then measured *in vitro*.
† Significantly different from control, $P < 0.001$.
‡ Significantly different from PHEN, $P < 0.05$.
§ Not significantly different from PHEN.

Table 8. Effects of *d*-amphetamine (*d*-AMPH) on phenelzine (PHEN, 2 mg/kg)-induced lowering of striatal DOPAC measured following haloperidol (HALO, 1 mg/kg) or chlorpromazine (CPZ, 5 mg/kg) administration

Drug treatment*	DOPAC (μg/g ± S.E.) (N)
HALO	5.20 ± 0.42 (4)
d-AMPH (5 mg/kg) + HALO	4.35 ± 0.35† (4)
d-AMPH (10 mg/kg) + HALO	4.65 ± 0.41† (6)
PHEN + HALO	2.10 ± 0.14‡ (4)
d-AMPH (5 mg/kg) + PHEN + HALO	2.12 ± 0.25§ (4)
d-AMPH (10 mg/kg) + PHEN + HALO	2.13 ± 0.35§ (6)
CPZ	4.25 ± 0.38 (13)
PHEN + CPZ	2.37 ± 0.19‖ (8)
d-AMPH (5 mg/kg) + PHEN + CPZ	1.81 ± 0.20¶ (3)
d-AMPH (10 mg/kg) + PHEN + CPZ	2.52 ± 0.33¶ (3)

* *d*-Amphetamine was given at zero time, phenelzine at 1 hr, and either haloperidol or chlorpromazine at 23.5 hr (all drugs s.c.). All rats were killed at 25 hr.
† Not significantly different from HALO.
‡ Significantly different from HALO, $P < 0.001$.
§ Not significantly different from PHEN + HALO.
‖ Significantly different from CPZ, $P < 0.001$.
¶ Not significantly different from PHEN + CPZ.

Table 9. Effects of *d*-amphetamine (*d*-AMPH, 10 mg/kg) on phenelzine (PHEN, 2 mg/kg)-induced inhibition of striatal dopamine depletion by Ro4-1284 (Ro; 2 mg/kg)

Drug treatment*	Dopamine (μg/g ± S.E.) (N)	depletion
None (control)	12.33 ± 0.50 (6)	
Ro	1.56 ± 0.05† (6)	87
d-AMPH	11.32 ± 0.65‡ (6)	
d-AMPH + Ro	1.56 ± 0.10§ (7)	86
PHEN	14.62 ± 0.31† (6)	
PHEN + Ro	8.55 ± 0.35‖ (9)	42
d-AMPH + PHEN	13.51 ± 0.45‡ (5)	
d-AMPH + PHEN + Ro	4.61 ± 0.40¶ (8)	66

* *d*-Amphetamine was given s.c. at zero time, phenelzine s.c. at 1 hr, and Ro4-1284 i.p. at 24.5 hr. All rats were killed at 25 hr.

† Significantly different from control, $P < 0.005$.

‡ Not significantly different from control.

§ Not significantly different from Ro.

‖ Significantly different from Ro, $P < 0.001$.

¶ Significantly different from PHEN + Ro, $P < 0.001$.

were killed. Table 9 shows that Ro alone produced an 87 per cent depletion of dopamine. This effect was not altered by prior administration of *d*-amphetamine. Phenelzine alone produced a significant increase in endogenous dopamine and, when given prior to Ro, markedly impaired dopamine depletion. *d*-Amphetamine significantly inhibited this effect. The drug combination of *d*-AMPH + PHEN + Ro produced a dopamine concentration significantly less than PHEN + Ro, but significantly greater than *d*-AMPH + Ro. Thus *d*-amphetamine is shown to produce a partial protective effect. The per cent protection is very similar to that obtained with direct measurements of MAO (Table 3).

DISCUSSION

Catecholamine release and inhibition of reuptake are considered important properties of *d*-amphetamine *in vivo* [1]. The present data support the view that neuronal MAO inhibition may also be of pharmacologic significance.

Employing serotonin as the substrate, *d*-amphetamine was found to protect against irreversible MAO inhibition of phenelzine. This finding, made in the rat striatum and remaining brain areas, confirms that reported by Green and El Hait [11] in whole mouse brain. *d*-Amphetamine is a reversible inhibitor of MAO *in vitro* [8]. Thus, a reasonable explanation is that *d*-amphetamine impaired irreversible inhibition of MAO by phenelzine by temporary attachment to the enzyme. Evidence was obtained that the protective effect of *d*-amphetamine was not due to impaired absorption, or to decreased neuronal uptake, of phenelzine. *d*-Amphetamine failed to antagonize the inhibitory effect of phenelzine on phenylethylamine deamination, and cocaine did not impair MAO inhibition by phenelzine (Tables 5 and 6).

Table 3 shows that the protective action of *d*-amphetamine amounts to approximately 22 and 19 per cent of the total MAO activity measured in the striatum and rest of the brain respectively. These values are closely similar to those reported by Green and El Hait [11]. The lack of complete protection by *d*-amphetamine probably reflects the much greater potency of phenelzine as an MAO inhibitor. Some displacement of *d*-amphetamine from the enzyme would be expected. A partial differential distribution of the two drugs may also be a contributing factor.

In vitro, *d*-amphetamine is a preferential MAO type A inhibitor [8–10]. Therefore, the failure to observe protection against phenelzine with the type B substrate, phenylethylamine, suggests a similar preference *in vivo*. This contention fits with the ineffectiveness of *l*-amphetamine against phenelzine-induced MAO inhibition (Tables 4 and 7). *In vitro*, *l*-amphetamine is five times less active than the *d*-form on MAO type A, but both enantiomers inhibit type B equally well [10].

This same potency ratio on MAO type A could explain the respective efficacies of the two enantiomers in lowering striatal DOPAC (Fig. 1 and Table 1). Under several different experimental conditions *d*-amphetamine always produced the larger effect, showing evidence of a 5–10-fold greater potency in Fig. 1. Braestrup [24] has shown previously that 50 mg/kg of *l*-amphetamine lowers DOPAC to the same level as 10 mg/kg of the *d*-form. Furthermore, another study in the rat suggests that MAO type A is the primary type governing brain DOPAC formation [25]. Additionally, evidence exists that intraneuronal MAO serves as a primary site for DOPAC production [26, 27]. Thus, the DOPAC lowering action of *d*-amphetamine suggests inhibition of intraneuronal MAO. This suggestion is strengthened by experiments demonstrating the presence of MAO type A within striatal dopaminergic neurons [28, 29].

Since the major evidence for MAO inhibition by *d*-amphetamine *in vivo* derives from 'protection experiments', we utilized this protocol to gain further support for intraneuronal inhibition. The experiment made with Ro (Table 9) offers strong support for such an action. *d*-Amphetamine significantly cur-

tailed the inhibitory effect of phenelzine on Ro-induced dopamine depletion. However, in a comparable experiment, *d*-amphetamine failed to oppose the DOPAC lowering action of phenelzine (Table 8). This discrepancy seems best explained by the degree of MAO protection afforded by *d*-amphetamine, coupled with the intraneuronal concentration of dopamine available for deamination. Accordingly, the amount of MAO protected by *d*-amphetamine is common to both experiments (approximately 22 per cent of the total striatal MAO type A, see Table 3). Thus, the variable factor would seem to be the dopamine concentration. Ro is thought to produce an explosive intraneuronal release of dopamine [29]. Table 9 shows that 87 per cent of striatal dopamine is depleted within 30 min. As with reserpine, the vast majority is deaminated intraneuronally [30], and MAO would tend to be the rate-limiting step governing depletion. Thus, phenelzine alone effectively retards depletion, and *d*-amphetamine prior to phenelzine spares MAO, thereby reducing the effects of phenelzine. Conversely, in the DOPAC experiment, the rate of release of dopamine would seem to be the rate-limiting factor. Neuroleptics are thought to reflexly increase impulse flow in striatal dopaminergic neurons [31], promoting the release of dopamine [21]. Thus, the available concentration of dopamine for intraneuronal deamination is far less than with Ro. Under these conditions, the sparing effect of *d*-amphetamine on MAO would be insufficient to alter measurably the rate of DOPAC production. An alternative possibility is that changes in conjugated DOPAC occurred while free DOPAC remained unaltered. About one-third of the total DOPAC in the rat striatum is conjugated [32, 33] and would not have been measured by the technique of Murphy *et al.* [20] without prior acid hydrolysis [33]. Further experiments are required to resolve this question.

Ro-induced depletion of dopamine provides a measure of intraneuronal dopamine deamination and thus offers strong evidence for MAO inhibition by *d*-amphetamine within striatal dopaminergic neurons. It seems likely that *d*-amphetamine must produce a marked inhibition of MAO at this site, far greater than that revealed in the protection experiments where some displacement of *d*-amphetamine by phenelzine can be expected. Extensive inhibition of MAO type A would be required to explain the acute DOPAC lowering action of *d*-amphetamine if MAO is not the rate-limiting step for DOPAC production. Such an inhibition may well reflect concentration of *d*-amphetamine within dopaminergic neurons, thereby enhancing its effective potency as an MAO inhibitor.

Previous studies have shown that *d*-amphetamine spares [34] or increases [24] homovanillic acid levels while lowering DOPAC [22, 24, 34]. This differential effect has been attributed to blockade of dopamine reuptake subsequent to release [22]. However, more potent uptake inhibitors (amfonelic acid, methylphenidate, nomifensine, cocaine and benztropine) fail to lower DOPAC to the same extent as *d*-amphetamine does [24]. Furthermore, the ability of *d*-amphetamine to block dopamine uptake in the striatum has been questioned [35, 36]. Recently, Fuller and Snoddy [37] have shown that both mazindol and methylphenidate, two of the most active dopamine uptake inhibitors known, failed to prevent the DOPAC lowering action of *d*-amphetamine. Therefore, type A MAO inhibition by *d*-amphetamine at intraneuronal sites offers a viable alternative or additional explanation. Extraneuronal MAO (see beginning of paper) would remain available for homovanillic acid formation since this metabolite is considered to arise primarily from extraneuronal sites [26]. Certain other effects of *d*-amphetamine seen *in vivo* are also compatible with intraneuronal MAO inhibition. In the rat striatum, *d*-amphetamine has been shown to increase endogenous dopamine levels and retard dopamine depletion induced by alpha-methyl-para-tyrosine and haloperidol [38]. Both of these effects are consistent with the actions of an MAO inhibitor. Indeed, phenelzine is shown to enhance the level of striatal dopamine in Table 9. However, the actions of *d*-amphetamine upon dopamine levels and turnover are not always so straightforward. Dopamine turnover has been found to be increased [39] and dopamine levels decreased [40, 41] following certain doses and time schedules. Under such circumstances, it is suggested that the dopamine-releasing action of *d*-amphetamine predominates. A similar suggestion, to explain the 'paradoxical effects' of *d*-amphetamine, has been made recently by Braestrup [24].

Beside the observations made with amphetamine, the present work throws new light on the MAO inhibitory properties of phenelzine. This compound is considered to be a non-specific MAO inhibitor [4]. The present data demonstrate a preferred inhibitory action on MAO type A. The *in vitro* experiment (Fig. 3) shows that this preference is only marginal when compared to compounds such as clorgyline. However, the same preference is seen after administration *in vivo* (Fig. 2 and Table 2). Comparison of Fig. 3 with Table 2 reveals a striking similarity with regard to per cent inhibition. This suggests that the concentration of phenelzine reached in striatal tissue is near 10^{-7} M (after 2 mg/kg, s.c.) and that both type A and type B MAO are equally accessible to phenelzine *in vivo*.

In summary, the present study supports the view that *d*-amphetamine is an MAO type A inhibitor *in vivo* with no demonstrable effect upon type B activity. This effect appears to be exerted within striatal dopaminergic neurons although inhibition of type A MAO at extraneuronal sites is not excluded. A difference in the abilities of *d*- and *l*-amphetamine to lower striatal DOPAC may be related, at least in part, to their relative potencies as MAO type A inhibitors. Whether the MAO inhibitory action of *d*-amphetamine reported here has significance in terms of behavioral effects seen with lower doses of this drug remains to be elucidated.

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