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A comparison of the pharmacological and biochemical properties of substrate-selective monoamine oxidase inhibitors

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Summary

1. M&B 9302, E-250, NSD 2023, and Lilly 51641, substrate-selective inhibitors of monoamine oxidase (MAO), and two non-selective inhibitors of MAO (tranylcypromine and phenelzine) have been compared in the rat for activity in (i) inhibiting rat brain monoamine oxidase *in vitro* and *in vivo* using tyramine, 5-hydroxytryptamine (5-HT) and benzylamine as substrates; (ii) increasing brain levels of noradrenaline (NA) and 5-HT and (iii) antagonizing tetrabenazine-induced sedation.

2. Concentrations of M&B 9302 and Lilly 51641 required to produce 50% inhibition of 5-HT oxidation by brain mitochondrial MAO were $1{\cdot}4 \times 10^{-8}$M and $2{\cdot}5 \times 10^{-7}$M respectively. Higher concentrations were required to inhibit tyramine oxidation whilst benzylamine oxidation was inhibited only at concentrations above 10^{-5}M.

3. E-250 showed the reverse substrate-selectivity in inhibiting the oxidation of benzylamine at concentrations below that required to inhibit the oxidation of 5-HT. NSD 2023 showed little substrate selectivity *in vitro*.

4. Qualitatively similar results were obtained *in vivo*, except that NSD 2023 showed more marked substrate-selectivity.

5. All the inhibitors except E-250 produced a dose-related rise in brain 5-HT levels. Only phenelzine and Lilly 51641 showed a linear relationship between NA levels and dose.

6. All the drugs antagonized, in dose-related fashion, the effects of tetrabenazine in reducing locomotor activity. E-250 and NSD 2023 failed to restore locomotor activity to control levels whilst in high doses the other inhibitors, when given before tetrabenazine, produced a considerable increase in locomotor activity.

7. Antagonism of tetrabenazine sedation appears to be correlated with (a) inhibition of the enzyme species that oxidize 5-HT and NA but not with inhibition of the enzyme species that oxidize benzylamine; (b) the rise in brain 5-HT levels rather than NA levels.

Introduction

Monoamine oxidase (EC 1.4.3.4) (MAO), present both in central and peripheral nervous tissues, can act on a number of amines including noradrenaline (NA), 5-hydroxytryptamine (5-HT) and dopamine. Inhibitors of monoamine oxidase

(MAOIs) are able to elevate mood in some patients with depressive illness and can antagonize the central depressant properties of the reserpine-like drugs and potentiate the actions of substrates for MAO in experimental animals. It is not yet clear whether these actions are due to the inhibition of oxidation of biogenic amines, or result from some other property.

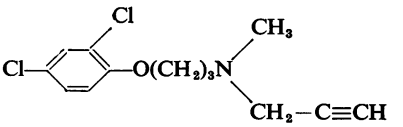
Recently a number of drugs have been described which show a selective action in that they inhibit the oxidation of some amines by MAO at concentrations which do not inhibit the oxidation of other substrates. In contrast, the earlier MAOIs show little or no substrate selectivity.

This paper reports data on the pharmacological action of four substrate-selective MAOIs, and compares their activity in inhibiting the oxidation of a number of biogenic amines by rat brain mitochondrial MAO *in vitro* and *in vivo*. Furthermore, by the use of these substrate-selective inhibitors, an attempt has been made to determine which biogenic amine(s) might be involved in the reversal, produced by MAOIs, of tetrabenazine-induced sedation in the rat.

Throughout this paper the term 'substrate-selective MAOI' is used to denote an inhibitor which shows a selective action in inhibiting the oxidation of some substrates in preference to others.

The four substrate-selective MAOIs used in these investigations were:

(i) M&B 9302 (clorgyline) (Johnston, 1968)



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(ii) Lilly 51641 (Fuller, 1968)

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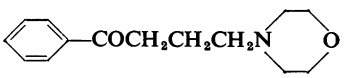
(iii) NSD 2023 (Squires & Lassen, 1968)



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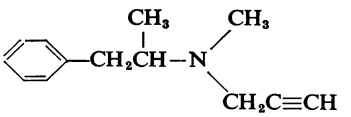
(iv) E-250 (Knoll, Ecseri, Kelemen, Nievel & Knoll, 1965)



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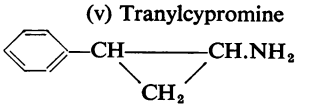
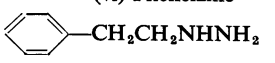
These were compared with two MAOIs that are not substrate-selective:

(v) Tranylcypromine



(vi) Phenelzine



A preliminary account of some of this work was given to a meeting of the British Pharmacological Society (Christmas, Hall, Hayward & Maxwell, 1970).

Methods

Male albino rats of the May & Baker, Sprague-Dawley derived, breeding colony weighing 120 to 200 g were used throughout.

Inhibition of monoamine oxidase activity in vitro

Mitochondrial preparation

Whole brains from rats were homogenized in 0·1 M tris-(hydroxymethyl)aminomethane chloride buffer at pH 7·5 (3 ml buffer per 1 g wet weight of tissue). The homogenates were spun at 1,000 *g* for 10 min at 4° C. The pellets were discarded and the supernatants were re-spun at 33,500 *g* for 20 min at 4° C. The pellet obtained was resuspended in buffer (1 ml buffer per g of wet weight of tissue). The final suspensions contained approximately 5 mg of protein/ml (Lowry, Rosebrough, Farr & Randall, 1951).

MAO assay–radioactive substrate

Aliquots of mitochondrial suspension (containing 5 mg of protein) were incubated with 25 μl of substrate (10^{-3}M, 62·5 nCi) in 0·1 M tris chloride buffer at pH 7·5. Graded concentrations of the test compounds were preincubated for 15 min with mitochondrial suspensions before substrate was added. After a further 15 min incubation (1 h for NA, dopamine) with substrate at 37° C, the reaction was terminated by the addition of: (a) 0·3 ml of 2 N HCl for 5-HT, benzylamine and tyramine assays, (b) 1 ml of 1 N perchloric acid for NA and dopamine estimation. For NA and dopamine assays, the deaminated metabolites were separated from unreacted substrate by the column chromatography procedure of Izumi, Oka, Yoshida & Imiazumi (1969) and an aliquot taken for liquid scintillation counting. The products of 5-HT, benzylamine and tyramine reaction, however, were extracted with 7 ml toluene, 4 ml of which was taken for scintillation counting. This was carried out in a Packard Scintillation Counter using as scintillator a mixture of toluene/Triton-X-100 (2:1 by volume) containing 5 g/l. of 2,5-diphenyloxazole. After boiled enzyme blank values had been subtracted, the MAO activity of mitochondrial suspensions incubated with test compounds was compared with the enzyme activity of untreated mitochondria.

MAO assay-kynuramine oxidation

This followed the method of Weissbach, Smith, Daly, Witkop & Udenfriend (1960) with 10^{-4}M substrate. Conditions of pH, temperature etc. were similar to those employed in the radiochemical assay.

Inhibition of monoamine oxidase in vivo

Rats (at least five per dose level) were treated with graded oral doses of the MAOI. The animals were killed 18 h later, or in the case of NSD-2023, 1 h later because of its short duration of action (Squires & Lassen, 1968); the brain was removed and the mitochondrial preparation of MAO prepared as described above.

The MAO content of the brain mitochondrial preparation was determined *in vitro* with various substrates by the method described above.

In the majority of experiments the rats used were those in which the antagonism of tetrabenazine-induced sedation had been observed. Preliminary experiments confirmed that the presence of tetrabenazine would not interfere with the assay of MAO. Furthermore, the degree of inhibition of MAO produced in groups of rats receiving M&B 9302 only did not differ significantly ($P<0{\cdot}05$) from groups of rats treated with M&B 9302 and tetrabenazine.

Determination of brain levels of 5-hydroxytryptamine and noradrenaline

Rats were dosed orally with the drugs under test and 18 h later (1 h in the case of NSD-2023) the animals were killed and the brains removed.

Amines were extracted from brain tissue by the method of Maickel, Cox, Saillant & Miller (1968) as modified by Curzon & Green (1970). Aliquots of the extract were examined for 5-HT by the method of Maickel *et al.* (1968) and for NA by the method of Laverty & Taylor (1968).

Brain amine levels were determined in animals that were not used for the determination of tetrabenazine sedation. At least eight animals per dose level were used, and the brain level determined individually in duplicate samples.

Prevention of tetrabenazine-induced sedation

Groups of rats (5 per dose level) were treated with graded doses of MAOI 18 h before receiving tetrabenazine 4 mg/kg subcutaneously. NSD 2023, however, was administered 1 h before tetrabenazine. One half-hour after tetrabenazine, the animals were placed individually in an enclosure based on the open field described by Hall (1934). This consisted of a circular enclosure 110 cm in diameter and 48 cm high, painted flat white inside and resting on a rubber mat which was divided into roughly equal sectors by two concentric circles and lines radiating from the centre to the periphery. An even illumination of about 650 lux was produced by the room lights.

Locomotor activity was measured by recording the number of sectors entered by an animal with all four feet over a period of 3 minutes. In each experiment a group of undosed animals, and a group of animals that had received tetrabenazine only, were included as controls. Mean ambulation scores for each group were expressed as a percentage of the mean undosed control score. Treatment with tetrabenazine alone reduced ambulation to near zero.

Immediately after the test the animals were killed by stunning, and the brains were removed and placed on ice before determination of MAO activity.

Computer analysis

Product moment correlation coefficients (r) were determined by computer analysis. The programme derived regression equations from 'r' if a correlation reached significance at $P<0{\cdot}05$.

Drugs

^{14}C-5-Hydroxytryptamine creatinine sulphate (56 mCi/mM); ^{14}C-(±)-noradrena-

line bitartrate (57 mCi/mM); ^{14}C-tyramine hydrochloride (44 mCi/mM) and ^{14}C-dopamine hydrochloride (55 mCi/mM) were obtained from the Radiochemical Centre, Amersham, and ^{14}C-benzylamine hydrochloride (2 mCi/mM) from Tracerlab. Kynuramine hydrobromide and benzylamine hydrochloride (Koch-Light) were recrystallized once before use. Other drugs used in this study were Lilly 51641 (Dr. Fuller, Eli Lilly); NSD 2023 (Dr. Squires, Ferrosan); E-250 (Deprenaline; Dr. Knoll, Institute of Pharmacology, University Medical School, Budapest); M&B 9302 (clorgyline); tranylcypromine sulphate (Smith, Kline & French); phenelzine sulphate (Warner) and tetrabenazine (Roche). All drugs were dissolved in distilled water immediately after use. Concentrations and dosages are expressed in terms of the salt.

Results

Inhibition of monoamine oxidase in vitro

In previous work from these laboratories (Hall, Logan & Parsons, 1969) it was shown that the apparent degree of inhibition of MAO produced by M&B 9302 depended on the substrate used, i.e. it exhibits substrate-selectivity. These findings have been extended to include NA, dopamine and kynuramine as substrates *in vitro*.

When the activity of rat brain mitochondrial MAO was determined in the presence of various concentrations of M&B 9302 with NA as substrate, the inhibition of the enzyme could be represented by a single sigmoid-shaped curve which resembled the curve obtained with 5-HT as substrate. When kynuramine or benzylamine was used as the substrate MAO was found to be resistant to the actions of M&B 9302. With dopamine as substrate a curve was obtained with a plateau similar in position and shape to that noted with tyramine. The curves for 5-HT, tyramine and benzylamine are illustrated in Fig. 1.

Lilly 51641 like M&B 9302 inhibited the oxidation of 5-HT to a greater extent than that of benzylamine (Fig. 1). E-250, on the other hand, strongly inhibited the oxidation of benzylamine at concentrations which had little effect on the oxidation of 5-HT. Tyramine oxidation was inhibited to a similar extent by these three drugs, and occupied a position intermediate between 5-HT and benzylamine oxidation. In contrast, NSD 2023, and phenelzine and tranylcypromine (two MAOIs in clinical use) showed little significant substrate-selectivity.

If the degree of substrate-selectivity of a drug is considered in terms of the ratio of the concentrations required to produce 50% inhibition of the oxidation of various substances, M&B 9302 showed the greatest degree of substrate-selectivity *in vitro* (Table 1). The concentration of M&B 9302 required to inhibit the oxidation of 5-HT *in vitro* by 50% was $1{\cdot}4 \times 10^{-8}$M, whilst a concentration of 1×10^{-4}M gave 50% inhibition of benzylamine oxidation (ratio of inhibitory concentrations ca. 7×10^{3}). Lilly 51641 was next in order of selectivity for 5-HT giving 50% inhibition of 5-HT oxidation at $2{\cdot}5 \times 10^{-7}$M and 50% inhibition of benzylamine oxidation at 3×10^{-5}M (ratio ca. 120). However, the specificity of Lilly 51641 would be negligible at 100% inhibition. In inhibiting the oxidation of tyramine M&B 9302 showed a clear-cut 'plateau region' over which increases in concentration of the inhibitor had little effect. Lilly 51641, E-250, NSD 2023, phenelzine and tranylcypromine did not show a 'plateau region'.

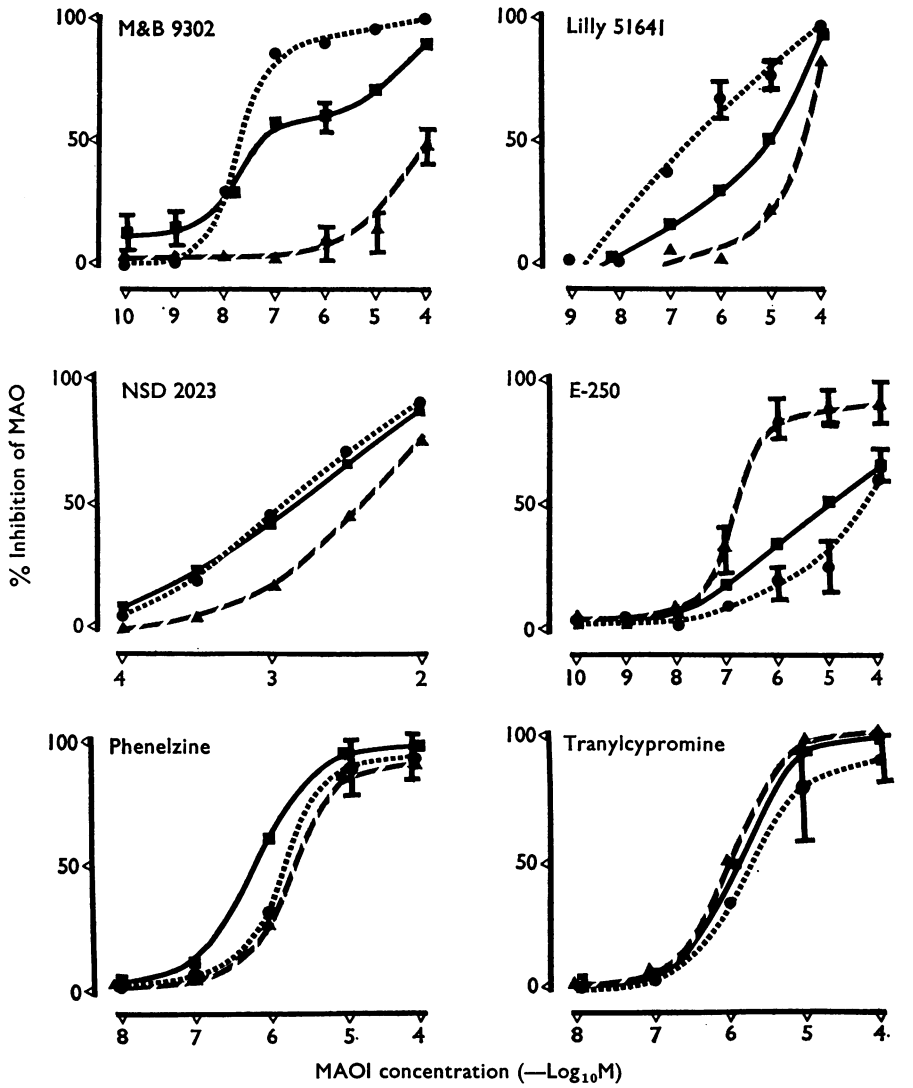


FIG. 1. Effect of MAOIs on rat brain MAO activity *in vitro* with 5-hydroxytryptamine (●); tyramine (■) or benzylamine (▲) as substrate. Aliquots of brain mitochondrial suspension were incubated with various concentrations of inhibitor for 15 min before addition of the substrate (10^{-3}M). Abscissae: concentration of MAOI in medium ($-\text{Log}_{10}$M). Ordinates: percentage inhibition of MAO. Each point represents the mean of at least four determinations. For clarity, vertical lines indicating the S.E.M. have only been inserted where the value exceeds 5% of the mean value. This practice has also been followed in Fig. 2.

TABLE 1. *Substrate-selectivity of monoamine oxidase inhibitors* in vitro

Inhibitor	Molar concentration producing 50% (I50) inhibition of oxidation of			Ratio $\left(\frac{\text{I50 benzylamine}}{\text{I50-5HT}}\right)$
	5-HT	Tyramine	Benzylamine	
M&B 9302	$1{\cdot}4\times10^{-8}$	3×10^{-8}	1×10^{-4}	7150
Lilly 51641	$2{\cdot}5\times10^{-7}$	9×10^{-6}	3×10^{-5}	120
NSD 2023	$1{\cdot}2\times10^{-3}$	$1{\cdot}2\times10^{-3}$	$3{\cdot}8\times10^{-3}$	3·16
E-250	3×10^{-5}	$5{\cdot}6\times10^{-6}$	$1{\cdot}7\times10^{-7}$	0·006
Phenelzine	1×10^{-6}	7×10^{-7}	$1{\cdot}5\times10^{-6}$	1·5
Tranylcypromine	2×10^{-6}	1×10^{-6}	1×10^{-6}	0·5

Concentrations of drug producing 50% inhibition of the oxidation of the substrate by rat brain mitochondrial MAO (I50) were obtained from the curves of drug concentration against % inhibition.

Inhibition of monoamine oxidase in vivo

M&B 9302, E-250 and Lilly 51641 retained their substrate-selectivity when administered orally. However, whereas M&B 9302 showed the highest degree of substrate-selectivity *in vitro*, it had less substrate-selectivity than Lilly 51641 *in vivo* (Fig. 2). In contrast, NSD 2023 showed a greater degree of substrate-selectivity *in vivo* than *in vitro* in that *in vivo* it had little inhibitory effect on benzylamine oxidation. E-250 showed a high degree of substrate-selectivity *in vivo*, by inhibiting the oxidation of benzylamine to a greater extent than that of 5-HT or tyramine. The four substrate-selective MAOIs produced 50% inhibition of the oxidation of tyramine at concentrations intermediate between those required to produce 50%

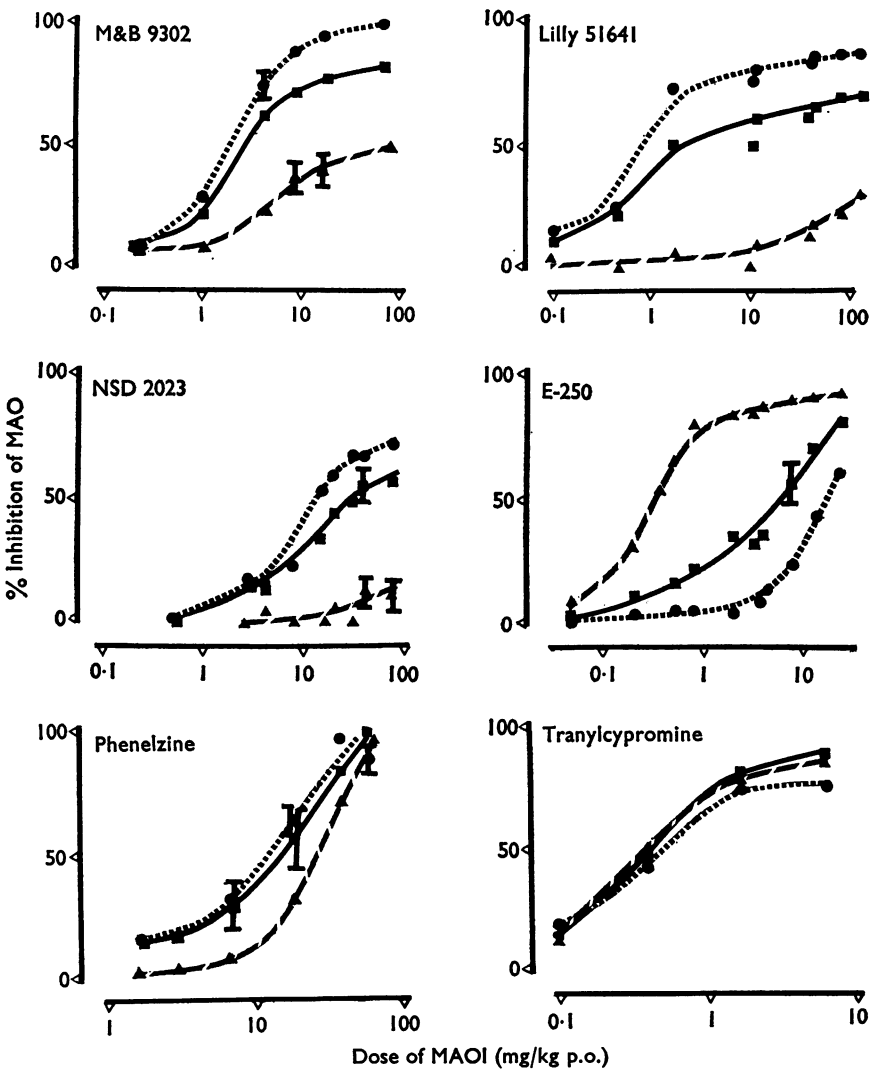


FIG. 2. Effect of MAOIs on rat brain MAO activity *in vivo* with 5-hydroxytryptamine (●); tyramine (■) or benzylamine (▲) as substrate. The drugs were administered orally to rats 18 h (or in the case of NSD 2023, 1 h) before killing. Abscissae: oral dose of MAOI (mg/kg). Ordinates: percentage inhibition of MAO. Points refer to the mean of three experiments.

inhibition of the oxidation of 5-HT and of benzylamine. The 'plateau region' seen with M&B 9302 in the *in vitro* tests was not seen when the drug was used *in vivo*. Whereas the potency of phenelzine and tranylcypromine was similar *in vitro*, tranylcypromine was about 40 times more active than phenelzine *in vivo*.

Effects on brain levels of noradrenaline and 5-hydroxytryptamine

E-250 differed from the other three substrate-selective inhibitors in not producing any significant increase in brain levels of NA (Fig. 3). Somewhat surprisingly, even low doses of E-250 produced small but significant increases in brain levels of 5-HT. These increases in 5-HT levels did not appear to be dose-related and were produced by doses of E-250 which gave little inhibition of the oxidation of 5-HT by MAO (Fig. 2). Higher doses of E-250, which produced significant increases in inhibition of 5-HT oxidation, did not result in any further increase in levels of 5-HT.

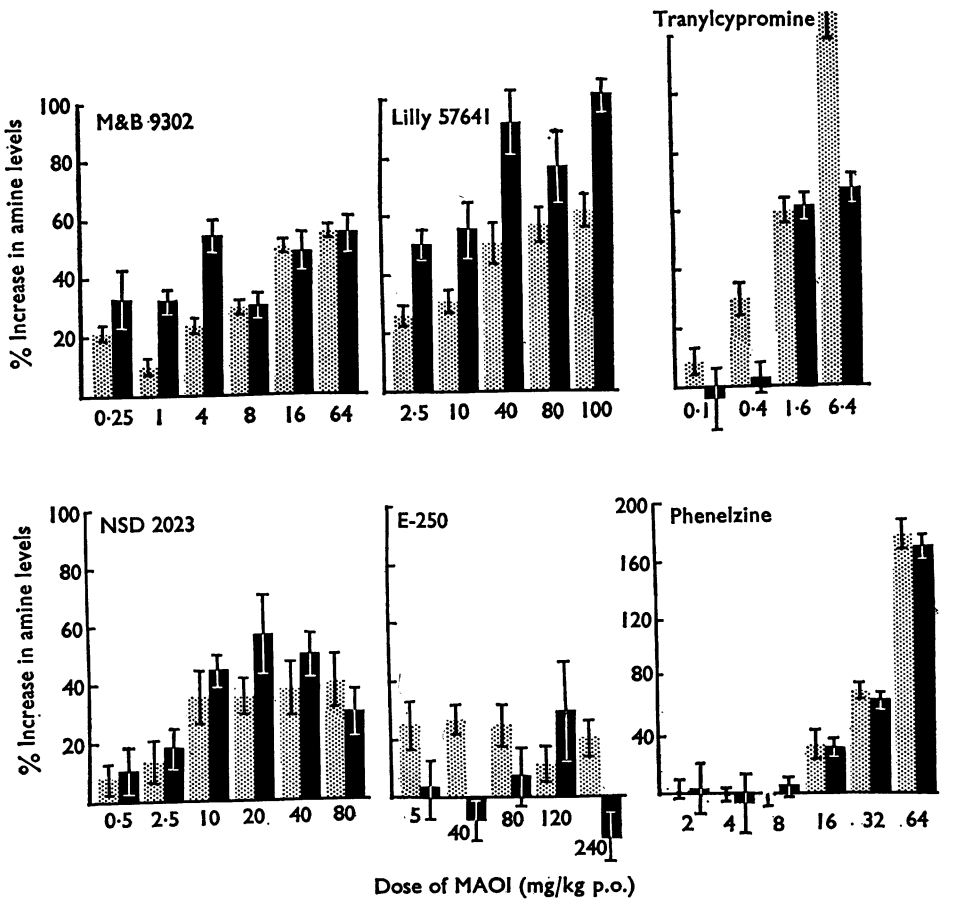


FIG. 3. Effect of MAOIs on the concentrations of 5-hydroxytryptamine (5-HT) (speckled blocks) and noradrenaline (NA) (solid blocks) in rat brain. Animals were killed 18 h (or in the case of NSD 2023, 1 h) after oral administration of MAOIs. Abscissae: dose of MAOI (mg/kg). Ordinates: percentage increase in amine levels from control values which were 0·63±S.E.M. 0·01 μg 5-HT/g and 0·34±S.E.M. 0·01 μg NA/g. Bars represent S.E.M. obtained from 6 to 16 animals.

Although M&B 9302 and Lilly 51641 showed, in general, similar spectra of inhibition of MAO, Lilly 51641 showed a significantly greater ($P<0{\cdot}01$) percentage increase in brain levels of NA than of 5-HT, at all dose levels except at 80 mg/kg. In contrast, M&B 9302 produced similar increases in the brain levels of both amines. NSD 2023 showed a biphasic effect on brain levels of NA, an oral dose of 80 mg/kg producing a smaller increase than 20 mg/kg. Phenelzine and tranylcypromine both yielded large increases in brain amine levels. The curve for phenelzine showed a very steep rise in the levels of both amines.

Antagonism of tetrabenazine-induced sedation

As a measure of the pharmacological actions of these substrate-selective monoamine oxidase inhibitors, their relative potency in antagonizing the sedative properties of tetrabenazine was determined. Tetrabenazine was used rather than reserpine because of its preferential effects on central stores of catechol- and indolamines, and because of its more rapid onset of action (Quinn, Shore & Brodie, 1959).

All six MAOIs produced a dose-dependent antagonism of tetrabenazine-induced

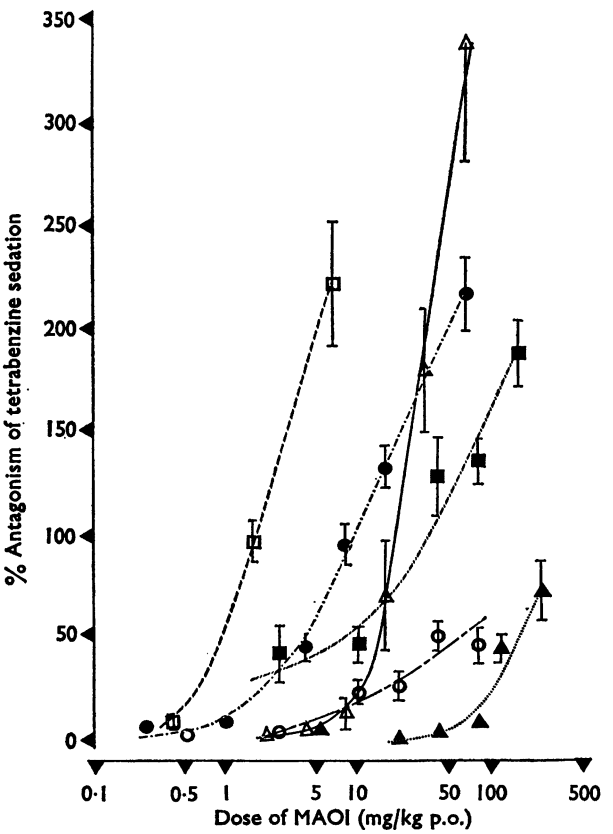


FIG. 4. Effect of MAOIs on tetrabenazine-induced sedation. Rats were pretreated with varying doses of M&B 9302 (●); Lilly 51641 (■); NSD 2023 (○); E-250 (▲); phenelzine (△); or tranylcypromine (□); 18 h later (1 h later after NSD 2023), tetrabenazine was administered (4 mg/kg s.c.) and locomotor activity determined in the open field test. Results are expressed as percentage of activity exhibited by undosed control animals. Figures refer to the mean from at least five animals, vertical lines to S.E.M.

sedation (Fig. 4). Tranylcypromine was the most potent of these, dose for dose. At doses of the MAOIs that produced less than 100% inhibition of tetrabenazine-induced sedation, the behavioural effects produced by the combination of MAOI and tetrabenazine were similar to those produced by a low dose of tetrabenazine alone. As the dose of MAOI was increased, tetrabenazine-induced sedation was progressively reduced. Eventually the combination of high doses of some MAOIs and tetrabenazine produced symptoms of central stimulation (tetrabenazine antagonism >100%), characterized by an increase in co-ordinated locomotor activity and exophthalmos. This central stimulation was produced by the combination with tetrabenazine of either M&B 9302, Lilly 51641, tranylcypromine or phenelzine. It was, however, not observed with NSD 2023 plus tetrabenazine at the doses studied. This difference may be quantitative rather than qualitative, in that higher doses of NSD 2023 could not be given because of its toxicity.

The behavioural effects produced by E-250, and by the combination of E-250 and tetrabenazine, were different from those produced by the other drugs and tetrabenazine. In high doses (120–240 mg/kg orally), E-250 produced behavioural symptoms similar to those produced by lower doses of dexamphetamine (4–16 mg/kg orally) (Christmas, unpublished results). These were characterized by the presence of stereotyped motor behaviour, increased locomotor activity, sniffing, and exophthalmos. With the exception of E-250 and NSD 2023 the MAOIs produced a greater stimulant effect following tetrabenazine injection as the dose of MAOI was increased.

Phenelzine exhibited a dose response curve with a much steeper slope than those obtained with the other MAOIs. It is of interest that the rise in amine levels produced by phenelzine also increased more markedly with increasing dose than did the rise in brain amine levels produced by the other MAOIs.

TABLE 2. *Relation between the ability of MAOIs to antagonize tetrabenazine-induced reduction in locomotor activity and their ability to inhibit brain MAO activity with 5-hydroxytryptamine, tyramine or benzylamine as substrates*

Compound	% Antagonism tetrabenazine	% Inhibition of MAO activity		
		5-HT	Tyramine	Benzylamine
M&B 9302	25	75	38	0
	50	82	45	2
	75	85	53	6
Lilly 51641	25	74	46	5
	50	84	63	11
	75	87	74	15
NSD 2023	25	63	49	7
	50	73	65	11
	75	77	82	16
E-250	25	36	66	89
	50	47	76	92
	75	57	82	95
Phenelzine	25	40	40	17
	50	52	57	30
	75	60	57	38
Tranylcypromine	25	56	55	52
	50	67	65	61
	75	74	72	68

The dose of MAOI required to produce 25, 50 and 75% inhibition of tetrabenazine-induced sedation was obtained by extrapolation from dose-response curves (Fig. 4). The percentage inhibition of MAO produced by the compounds at these dose levels were then read off from % inhibition-dose curves (Fig. 2).

Correlation between biochemical and pharmacological effects

Only Lilly 51641 ($r=0.959$; $P<0.01$) and phenelzine ($r=0.917$; $P<0.01$) showed a significant correlation between increase in brain NA levels and antagonism of tetrabenazine. The reversal of tetrabenazine-induced sedation correlated to a significant degree with the increases in the brain level of 5-HT produced by M&B 9302 ($r=0.940$; $P<0.01$), Lilly 51641 ($r=0.997$; $P<0.01$), NSD 2023 ($r=0.910$; $P<0.05$), phenelzine ($r=0.991$; $P<0.01$), and tranylcypromine ($r=0.958$; $P<0.05$).

From the data in Figs. 2 and 4, the degree of inhibition of brain MAO corresponding to a given anti-tetrabenazine effect has been determined (Table 2). With the exception of E-250, 50% reversal of tetrabenazine effect corresponds to 52–84% inhibition of 5-HT oxidation and to 45–65% inhibition of tyramine oxidation. The steep dose-response curve already noted for phenelzine may account for the fact that the figure for phenelzine inhibition of 5-HT oxidation (52%) is somewhat below the range for the other four drugs (67–84%).

Discussion

The main aim of this study was to examine the biochemical and pharmacological properties of substrate-selective MAOIs, and to attempt to determine which biogenic amine(s) are important in the pharmacological action studied.

Inhibition of monoamine oxidase

Four MAOIs reported to have some degree of substrate-selectivity have been examined. Johnston (1968) and Hall *et al.* (1969) reported on the substrate-selectivity of M&B 9302 in inhibiting MAO of various mammalian tissues. Tipton (1971) and Goridis & Neff (1971) have confirmed and extended these findings. Our study has shown that Lilly 51641, which has previously been shown to inhibit the oxidation of various substrates to different degrees (Fuller, 1968), and NSD 2023 have substrate-selectivities *in vivo* similar to M&B 9302. NSD 2023 may form a metabolite with substrate-selectivity *in vivo* since only a low degree of substrate-selectivity was noted *in vitro*. E-250 was included in the study because of its contrasting pattern of inhibition with respect to M&B 9302 and Lilly 51641. Low concentrations of the latter compounds inhibit 5-HT oxidation and higher ones, benzylamine oxidation. E-250, in sharp contrast, inhibits benzylamine oxidation at low concentrations whereas 5-HT oxidation is only inhibited at high concentrations.

It is important to consider whether the substrate-selectivity of these inhibitors is possibly related to the existence of multiple forms of MAO. Johnston (1968) and Hall *et al.* (1969) found that substrates for MAO could be placed into three groups according to the sensitivity of their oxidation to inhibition by M&B 9302. 5-HT oxidation was most sensitive to inhibition by M&B 9302, whilst benzylamine was least affected by M&B 9302. Tyramine, tryptamine and dopamine fell into an intermediate position. From the present data NA as a substrate resembles 5-HT with respect to inhibition by M&B 9302. To explain these findings, Johnston (1968) suggested that MAO existed as two enzyme species (enzymes A and B). Enzyme A was responsible for the oxidation of 5-HT while benzylamine was preferentially oxidized by enzyme B. He further postulated that tyramine could be oxidized by both forms of MAO. Support for this proposal has been pre-

sented by Jarrott (1971) who has proposed that at least two forms of MAO exist in the rat vas deferens. Sympathectomy selectively reduces the activity of the enzyme species that are sensitive to low concentrations of M&B 9302. Similar findings have been presented by Goridis & Neff (1971) who have shown that in the pineal gland enzyme A, the NA-metabolizing enzyme, is associated with sympathetic nerve endings while enzyme B occurs mainly in the pineal cells. The classification of MAO into enzymes A and B as a consequence of M&B 9302 inhibition may correspond to a physiological distinction between the enzyme species.

The results in the present paper are consistent with the above interpretation. Although E-250 shows a different substrate-selectivity, the MAO inhibition curves produced by this drug can be explained in terms of two MAO enzymes (A and B), E-250 preferentially inhibiting enzyme B. In contrast, low concentrations of M&B 9302, Lilly 51641 and to some extent NSD 2023 *in vivo* inhibit enzyme A whilst higher concentrations are required to affect enzyme B.

Using gel electrophoresis, Youdim, Collins & Sandler (1969) have separated rat brain MAO into four enzyme species (MAO_{1-4}) each showing different degrees of substrate selectivity. There is no indication from our work how enzymes A and B may be subdivided into MAO_{1-4}. Shih & Eiduson (1971) have also reported the electrophoretic fractionation of rat brain MAO into four isoenzymes. They found that all four isoenzymes could oxidize tryptamine but only two could oxidize 5-HT, which suggests that these two isoenzymes are equivalent to enzyme A. Benzylamine was oxidized only by the remaining two isoenzymes.

Effect on brain levels of noradrenaline and 5-hydroxytryptamine

M&B 9302 and Lilly 51641 showed similar spectra of activity in inhibiting MAO both *in vivo* and *in vitro*. However, the effects of these two compounds on the concentrations of 5-HT and NA in rat brain differed in that Lilly 51641 produced a more pronounced increase in the level of NA. This suggests that Lilly 51641 or M&B 9302, or indeed both, may affect the metabolism of brain amines in a way additional to inhibition of MAO. We can offer no sound explanation as to why NSD 2023 produces a lower increase in brain NA at a high rather than at an intermediate dose, or why E-250 produces an increase in brain 5-HT at dose levels which do not significantly inhibit MAO. Further work is required to clarify this.

Antagonism of tetrabenazine sedation

The behavioural effects produced by E-250 when administered alone to rats were qualitatively different from those produced by the other MAOIs studied, since increased motility together with stereotyped movements were observed. These effects were similar to those produced by dexamphetamine, and were in agreement with the finding of Knoll *et al.* (1965). As the behavioural responses to E-250 resemble those to amphetamine, it is possible that E-250 like amphetamine (Fuxe & Ungerstedt, 1970), releases dopamine from dopaminergic neurones. An alternative explanation is that E-250 increases receptor stimulation by inhibiting reuptake of dopamine by nerve terminals.

Relationship between the biochemical effect and pharmacological action

One might expect that if the biochemical effects (inhibition of MAO) being

measured caused the antagonism of tetrabenazine, there should be a correlation between inhibition of MAO and antagonism of tetrabenazine irrespective of MAOI used. From a consideration of the data set out in Table 2, the following tentative conclusions may be drawn:

(i) It seems improbable that the enzyme species mediating benzylamine oxidation (enzyme B) is in any way related to the antagonism of tetrabenazine.

The degree of inhibition of benzylamine oxidation by M&B 9302, Lilly 51641 or NSD 2023 is small compared with that seen with E-250. If, therefore, the inhibition of the benzylamine-oxidizing enzyme were important, one would expect marked pharmacological effects with E-250. This is not observed.

(ii) It is therefore unlikely that the observed anti-tetrabenazine effect of E-250 is due to MAO inhibition.

This conclusion agrees with the finding that E-250 appears to have an amphetamine-like action, and produces a much smaller increase in brain levels of 5-HT or NA than the other MAOIs.

(iii) With the exception of E-250, pharmacologically equiactive doses of the MAOIs produce similar degrees of inhibition of the oxidation of 5-HT. A similar effect is noted for tyramine.

Since tyramine is a substrate for enzyme A as well as for enzyme B (Goridis & Neff, 1971), inhibition of 5-HT oxidation will inevitably be associated with inhibition of tyramine oxidation. Our results are therefore consistent with the hypothesis that the pharmacological effects observed are related to the inhibition of the enzyme species responsible for 5-HT oxidation.

Our study has shown that there is a significant correlation between the anti-tetrabenazine effects of all the drugs used, except E-250, and the increases in brain 5-HT levels produced by these MAOIs. Only Lilly 51641 and phenelzine showed a significant correlation between increase in NA levels and anti-tetrabenazine sedation. Since the metabolism of NA, unlike that of 5-HT, is affected by enzymes other than MAO this lack of correlation is not so unexpected. As the locomotor effects of the MAOI/tetrabenazine combination differ from those of amphetamine, we suggest that dopamine plays a minor role, if any, in this test although it is known that dopamine may be important in other tetrabenazine-induced behavioural effects (Butcher & Andén, 1969 ; Butcher, Butcher & Larsson, 1969).

Conclusions as to which amine is important in locomotor activity differ because different tests have been employed to examine locomotion, and different means have been used to alter amine levels so that differing functional pools have been affected. Present evidence suggests that amines are present in at least two compartments in nerve terminals, a large non-functional entity which is very sensitive to reserpine-like drugs, and a smaller functional pool which can be depleted by inhibitors of amine synthesis. It is the concentration of amines in this reserpine-resistant pool, and not the overall level, that is important in controlling locomotion (Chan & Webster, 1971 ; Grahame-Smith, 1971). In our study, the amines that are released by tetrabenazine from the non-functional pool cannot be metabolized by MAO and, therefore, the levels of NA and 5-HT that are available for receptor stimulation approach the overall concentrations of the amines in the nerve terminals. Under such conditions, we have found a closer correlation between antagonism of tetrabenazine-induced sedation and elevated 5-HT levels, as produced by these substrate-selective MAOIs, than with elevation in NA levels.

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