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EFFECTS OF MONOAMINE OXIDASE INHIBITION BY CLORGYLINE, DEPRENIL OR TRANYLCYPROMINE ON 5-HYDROXYTRYPTAMINE CONCENTRATIONS IN RAT BRAIN AND HYPERACTIVITY FOLLOWING SUBSEQUENT TRYPTOPHAN ADMINISTRATION

A.R. GREEN & M.B.H. YOUDIM

MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford OX2 6HE

1 The effect of various doses of tranylcypromine on the degree of inhibition of rat brain monoamine oxidase (MAO) using 5-hydroxytryptamine (5-HT), dopamine and phenylethylamine as substrates has been examined 120 min after injection of the inhibitor. The concentration of brain 5-HT was also examined both after tranylcypromine alone and also when L-tryptophan (100 mg/kg) had been given 30 min after the tranylcypromine.

2 All doses of tranylcypromine greater than 2.5 mg/kg totally inhibited MAO oxidation of 5-HT, phenylethylamine and dopamine as measured *in vitro* and produced a similar rise of brain 5-HT *in vivo*. When tryptophan was also given, there was a further rise of brain 5-HT, which was comparable after all doses of tranylcypromine above 2.5 mg/kg and the characteristic syndrome of hyperactivity made its appearance.

3 Clorgyline (a 'Type A' MAO inhibitor), in doses up to 10 mg/kg, did not totally inhibit MAO activity towards phenylethylamine although it did inhibit 5-HT oxidation by 100%. Deprenil (a 'Type B' MAO inhibitor) at doses up to 10 mg/kg did not fully inhibit 5-HT oxidation although phenylethylamine oxidation was inhibited almost completely. Administration of either compound alone did not produce as great an accumulation of brain 5-HT as that seen after tranylcypromine (2.5 mg/kg) and subsequent administration of tryptophan did not cause hyperactivity or the rise of brain 5-HT seen after tranylcypromine (2.5 mg/kg) plus tryptophan.

4 Administration of clorgyline plus deprenil (2.5 mg/kg of each) almost totally inhibited oxidation of both 5-HT and phenylethylamine; subsequent tryptophan administration resulted in a rise of brain 5-HT nearly as great as that seen following tranylcypromine (2.5 mg/kg) plus tryptophan and the animals became hyperactive.

5 No evidence was found pointing to the formation of any other 5-substituted indole in the brain following tranylcypromine plus L-tryptophan administration as suggested by others.

6 It is concluded that while 5-HT may normally be metabolized in the brain by 'Type A' MAO *in vivo*, when this form is inhibited, 5-HT can still be metabolized by 'Type B' enzyme. It is only when both forms are almost totally inhibited that the largest rise of brain 5-HT is seen and subsequent tryptophan administration produces the hyperactivity syndrome.

Introduction

Administration of tryptophan to rats pretreated with the monoamine oxidase (MAO) inhibitor, tranylcypromine, results in a characteristic hyperactivity syndrome (Grahame-Smith, 1971). The evidence suggests that the hyperactivity is the result of increased 5-hydroxytryptamine (5-HT) synthesis with spill over on to post-synaptic receptors, and inhibition of MAO appears to be an essential factor for the production of the syndrome. Different forms of MAO have been demonstrated both *in vitro* and *in vivo* (see Sandler & Youdim, 1972 for review). Using clorgyline, Johnston (1968) distinguished between two enzyme systems, 'Type A' and 'Type B'. Type A is sensitive to inhibition by clorgyline and oxidatively deaminates 5-hydroxytryptamine, noradrenaline and dopamine. Type B is more resistant to clorgyline but is inhibited by deprenil and oxidizes phenylethylamine and dopamine but not 5-HT (Sandler & Youdim, 1974; Houslay & Tipton, 1974). It thus seemed reasonable to predict that if the hyperactivity syndrome were

due to 5-HT alone, then hyperactivity would appear after tryptophan administration to rats pretreated with clorgyline but not deprenil. However, Squires & Buus Lassen (1975) reported that tryptophan after clorgyline did not produce hyperactivity whereas it was seen if tryptophan followed clorgyline plus deprenil.

We have now investigated the reasons for the absence of hyperactivity following clorgyline alone by studying brain 5-HT and tryptophan concentrations and brain MAO activity following tranylcypromine, clorgyline and deprenil administration and correlated these changes with the appearance of the behavioural changes.

A preliminary report has been given of some of these findings (Green & Youdim, 1975).

Methods

Male Sprague-Dawley rats (160-200 g) (Anglia Laboratory Animals, Alconbury, Huntingdon) were used in all experiments. Tranylcypromine (Smith, Kline & French, Ltd.), clorgyline (May & Baker, Ltd.) or deprenil were dissolved in 0.9% w/v NaCl solution (saline) and injected intraperitoneally in various doses to groups of 6 rats. After 30 min, L-tryptophan (100 mg/kg i.p.) was given to 3 rats and saline to the other three. After a further 90 min, the rats were killed, the brains removed and divided along the mid-line. One half was homogenized in acidified butanol (Chang, 1964); 5-HT was measured by the method of Curzon & Green (1970) and tryptophan by that of Denckla & Dewey (1967). The other half was homogenized in 0.32 M sucrose and MAO activity towards the substrates [1-^{14}C]-dopamine, [1-^{14}C]-5-hydroxytryptamine (both from the Radiochemical Centre, Amersham) and [1-^{14}C]-phenylethylamine (NEN Chemicals, GmbH) was measured by the method of Southgate & Collins (1969). Protein was estimated by the procedure of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as standard. All enzyme activities were calculated as nmol deaminated product formed/mg protein per 30 min incubation and results are expressed as the mean ± s.e.mean of the percentage inhibition compared to saline-injected controls. For analysis of variance of these groups, arcsin transformation of the percentage inhibition values was performed (Snedecor & Cochran, 1967) before analysis.

Activity was measured on both groups of 3 animals for 120 min after MAO inhibition using Animex activity meters (sensitivity and tuning settings: 30 μA) as described previously (Grahame-Smith, 1971; Green & Grahame-Smith, 1974).

Results

Effect of L-tryptophan on rat brain 5-hydroxytryptamine, tryptophan and monoamine oxidase activity

Initial experiments were performed to see whether L-tryptophan administration altered MAO activity. Rats were injected with saline and 30 min later given either saline or L-tryptophan (100 mg/kg). After a further 90 min they were killed and brain 5-HT, tryptophan and MAO activity measured. After L-tryptophan, there was a large increase in brain tryptophan concentrations and an increase in 5-HT (Table 1). Tryptophan did not inhibit MAO activity towards any of the substrates examined and no increased behavioural activity was observed.

Effect of various doses of tranylcypromine followed by L-tryptophan on hyperactivity and brain 5-hydroxytryptamine, tryptophan and MAO activity

Rats were injected with 1.0, 2.5, 5.0 and 10 mg/kg tranylcypromine and the experiment continued as described in the methods section. None of the dose of tranylcypromine alone produced hyper-

Table 1 Effect of L-tryptophan (100 mg/kg) on brain tryptophan and 5-hydroxytryptamine (5-HT) concentrations 90 min later

Injected	*Brain tryptophan (μg tryptophan/g (wet wt))*	*Brain 5-hydroxytryptamine (μg 5-HT/g brain (wet wt))*
Saline	4.71 ± 0.38 (3)	0.49 ± 0.01 (10)
L-tryptophan (100 mg/kg)	35.0 ± 0.59 (3)	0.81 ± 0.05 (3)

Results of brain 5-HT and tryptophan concentrations expressed as mean ± s.e. mean with number of determinations in brackets.

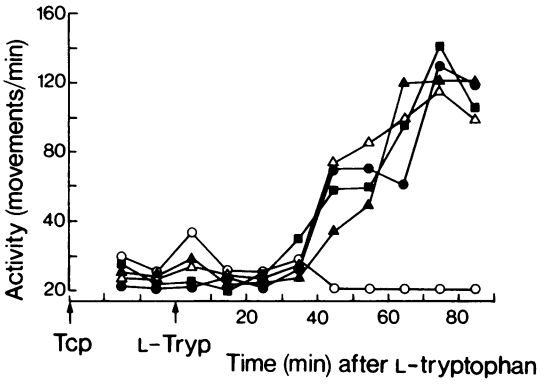

Figure 1 Effect of L-tryptophan (100 mg/kg) injection following various doses of tranylcypromine on hyperactivity. Rats were injected with tranylcypromine and with L-tryptophan (100 mg/kg) 30 min later. Activity measured as movements/minute. Tranylcypromine doses: 1.0 mg/kg (○), 2.5 mg/kg (●), 5.0 mg/kg (△), 10.0 mg/kg (▲), 20.0 mg/kg (■).

activity nor was there any change when tryptophan (100 mg/kg) was given after the 1 mg/kg dose of tranylcypromine. However, tryptophan plus tranylcypromine at a dose of 2.5 mg/kg or more resulted in the appearance of the hyperactivity syndrome (Grahame-Smith, 1971). The degree of hyperactivity was then independent of the dose of tranylcypromine (Figure 1).

Biochemical measurements showed that there was only a small increase in brain 5-HT concentration following tranylcypromine (1 mg/kg). When L-tryptophan was given after this dose of tranylcypromine, brain 5-HT concentration was no higher 90 min later than that seen when L-tryptophan alone had been given (Table 2). With this dose of tranylcypromine MAO activity was inhibited by 86% and 85% with 5-HT and phenylethylamine respectively as substrates. At the higher doses of tranylcypromine, both forms of the enzyme are totally inhibited (Table 2).

The dose of 20 mg/kg tranylcypromine used in previous investigations of hyperactivity (for review see Green & Grahame-Smith, 1975) only produces a similar degree of hyperactivity to that seen after lower doses (Figure 1). A study of the time course of alterations in brain 5-HT and tryptophan concentrations was made. Brain 5-HT increases linearly with time following tranylcypromine (20 mg/kg) with a much steeper rate of increase after L-tryptophan (100 mg/kg). Brain tryptophan concentrations following L-tryptophan (100 mg/kg) increased rapidly to reach a maximum 60 min later, followed by a fairly rapid decline in the next 30 min (Figure 2).

Effect of various doses of clorgyline or deprenil followed by L-tryptophan on hyperactivity and brain 5-hydroxytryptamine and MAO activity

Rats were injected with 1.0, 2.5, 5.0 and 10.0 mg/kg clorgyline or deprenil and the experiment continued as described in the methods section. None of the doses of clorgyline resulted in the hyperactivity syndrome, even after further administration of tryptophan (Figure 3). Essentially complete inhibition of 5-HT oxidation by MAO was produced by 2.5 mg/kg or more of clorgyline whereas phenylethylamine oxidation was not strongly inhibited even by 10 mg/kg clorgyline (Table 3).

After deprenil, hyperactivity was not seen at doses up to 5.0 mg/kg, even when tryptophan was given (Figure 4). However, following the 10 mg/kg

Table 2 Effect of various doses of tranylcypromine with or without L-tryptophan injection (100 mg/kg) 30 min later on brain 5-hydroxytryptamine (5-HT) concentrations and monoamine oxidase (MAO) activity towards 5-HT, dopamine and phenylethylamine 120 min after initial injection

Dose of tranylcypromine (mg/kg)	*Brain 5-hydroxytryptamine (μg 5-HT/g brain (wet wt))*		*% Inhibition of MAO activity towards substrates*		
	Saline	*L-tryptophan (100 mg/kg)*	*5-HT*	*Dopamine*	*Phenylethylamine*
1.0	0.60 ± 0.03 (3)	0.75 ± 0.14 (3)	86 ± 5 (6)	86 ± 1 (6)	85 ± 1 (3)
2.5	0.78 ± 0.02 (3)	1.12 ± 0.14 (3)	100 (6)	100 (6)	100 (6)
5.0	0.77 ± 0.02 (3)	1.16 ± 0.06 (3)	100 (6)	100 (6)	100 (6)
10.0	0.79 ± 0.05 (3)	1.18 ± 0.07 (3)	100 (6)	100 (6)	N.D.

Results of brain 5-HT shown 120 min after injection of inhibitor when saline or L-tryptophan (100 mg/kg) had also been given 30 min after tranylcypromine. % Inhibition of MAO activity also shown 120 min after injection of tranylcypromine. Results expressed as mean ± s.e. mean with number of determinations in brackets N.D.: Not determined.

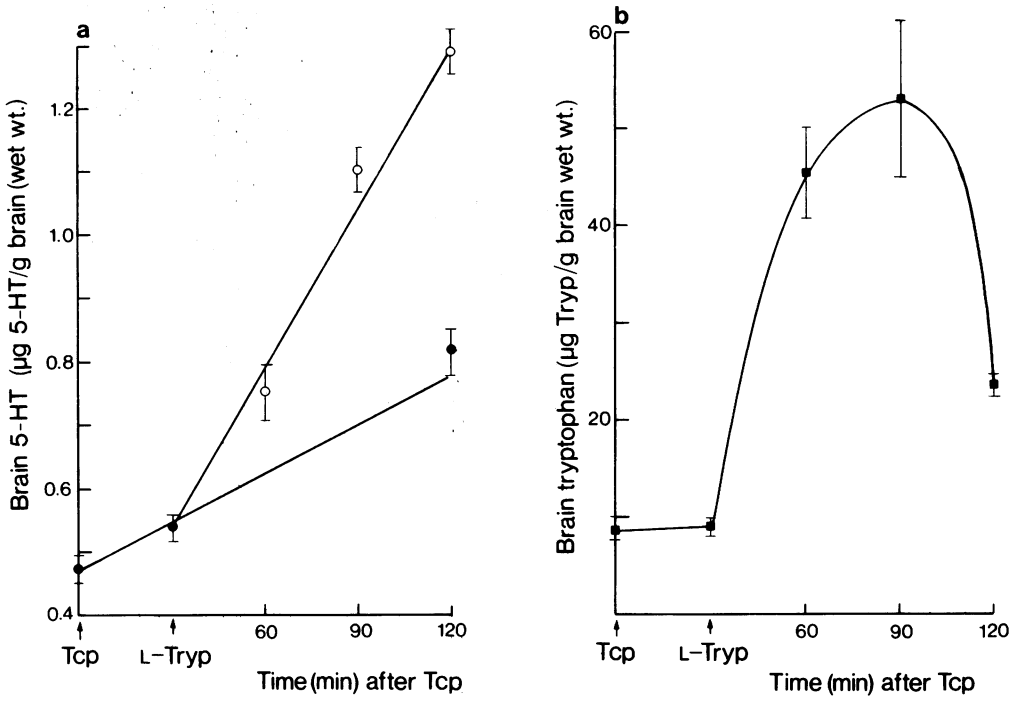

Figure 2 Effect of L-tryptophan (L-Tryp) (100 mg/kg) following tranylcypromine (Tcp) (20.0 mg/kg) on rat brain 5-hydroxytryptamine (5-HT) and tryptophan concentrations. (a) Effect on brain 5-HT concentrations. Brain 5-HT concentrations following tranylcypromine 20 mg/kg (●) and following L-tryptophan 30 min after tranylcypromine (○). (b) Effect of tranylcypromine 20 mg/kg with L-tryptophan 100 mg/kg 30 min later on brain tryptophan (■).

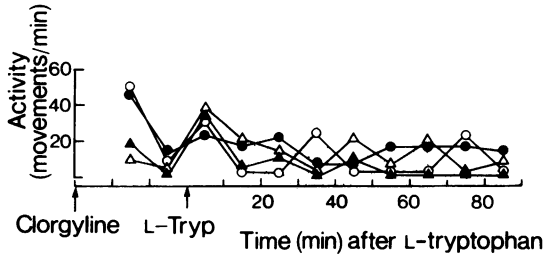

Figure 3 Effect of L-tryptophan (L-Tryp) (100 mg/kg) injection following various doses of clorgyline on hyperactivity. Rats were injected with L-tryptophan (100 mg/kg) 30 min later. Activity measured as movements/minute. Clorgyline doses 1.0 mg/kg (○), 2.5 mg/kg (●), 5.0 mg/kg (△), 10.0 mg/kg (▲).

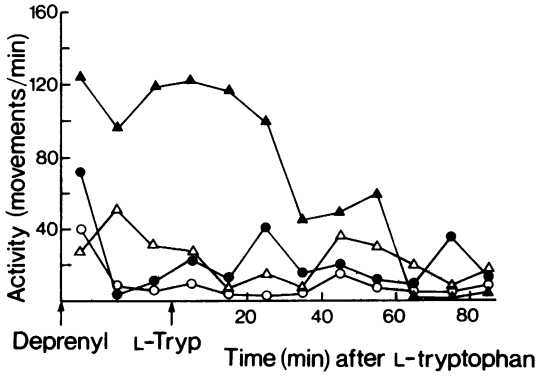

Figure 4 Effect of L-tryptophan (100 mg/kg) injection following various doses of deprenil on hyperactivity. Rats were injected with deprenil and with L-tryptophan (100 mg/kg) 30 min later. Activity was measured as movements/minute. Deprenil doses 1.0 mg/kg (○), 2.5 mg/kg (●), 5.0 mg/kg (△), 10.0 mg/kg (▲).

dose, much spontaneous locomotor activity was measured on the meters, which was not accompanied by any of the other behavioural changes associated with the hyperactivity syndrome (for details of these changes, see Grahame-Smith, 1971) and not altered by L-tryptophan administration. None of the doses of deprenil when given with tryptophan resulted in a rise in brain 5-HT concentration greater than that seen in animals not given deprenil. Nor did deprenil at any dosage schedule inhibit MAO activity towards 5-HT by more than 50% although phenylethylamine oxidation was almost totally inhibited by 5.0 mg/kg deprenil (Table 3).

Effect of various doses of deprenil plus clorgyline followed by L-tryptophan on hyperactivity, brain tryptophan, 5-hydroxytryptamine and MAO activity

An attempt was made to confirm the finding of Squires & Buus Lassen (1975) that when deprenil and clorgyline are given in combination, hyperactivity follows the subsequent administration of tryptophan. Experimental procedures were as before except that the two inhibitors were given together. While no hyperactivity was observed when 1 mg/kg of each inhibitor was given 30 min before L-tryptophan (100 mg/kg), the hyperactivity syndrome appeared when the dose of each inhibitor was increased to 2.5 mg/kg (Figure 5). 5-HT and phenylethylamine oxidation were only inhibited by more than 90% at the higher dose, whilst 5-HT concentrations only rose to about

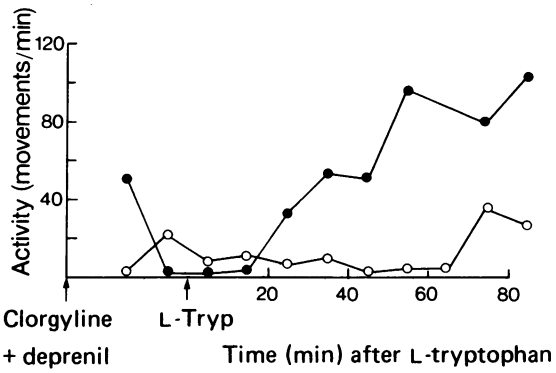

Figure 5 Effect of L-tryptophan (L-Tryp) (100 mg/kg) injection following various doses of clorgyline plus deprenil on hyperactivity. Rats were injected with clorgyline plus deprenil and with L-tryptophan (100 mg/kg) 30 min later. Activity measured as movements/minute. Clorgyline (1.0 mg/kg) + deprenil (1.0 mg/kg) (○), clorgyline (2.5 mg/kg) + deprenil (2.5 mg/kg) (●).

those observed after tranylcypromine (2.5 mg/kg) at the same dosage combination (Table 4).

Measurement of brain 5-HT by two methods after tranylcypromine and L-tryptophan

Squires (1975) suggested that hyperactivity is seen only when clorgyline and deprenil are given together because it stems from the action of a

Table 3 Effect of various doses of clorgyline or deprenil with or without L-tryptophan injection (100 mg/kg) 30 min later on brain 5-hydroxytryptamine (5-HT) concentrations and monoamine oxidase (MAO) activity towards 5-HT, dopamine and phenylethylamine 120 min after initial injection

		Brain 5-hydroxytryptamine (μg 5-HT/g brain (wet wt))		*% Inhibition of MAO activity towards substrates*		
Injected	*Dose of deprenil or clorgyline (mg/kg)*	*Saline*	*L-tryptophan (100 mg/kg)*	*5-HT*	*Dopamine*	*Phenylethylamine*
Clorgyline	1.0	0.60 ± 0.06 (9)	0.90 ± 0.10 (6)	61 ± 7 (11)	51 ± 6 (12)	19 ± 7 (3)
	2.5	0.67 ± 0.06 (9)	0.96 ± 0.11 (6)	88 ± 6 (12)	71 ± 10 (12)	24 ± 9 (3)
	5.0	0.65 ± 0.04 (9)	0.91 ± 0.10 (6)	98 ± 1 (12)	87 ± 5 (12)	29 ± 10 (3)
	10.0	0.69 ± 0.03 (6)	0.90 ± 0.09 (6)	99 ± 1 (9)	96 ± 18 (9)	67 ± 8 (3)
Deprenil	1.0	0.51 ± 0.01 (9)	0.73 ± 0.09 (6)	23 ± 7 (12)	28 ± 5 (9)	58 ± 5 (3)
	2.5	0.58 ± 0.03 (9)	0.72 ± 0.02 (6)	29 ± 6 (12)	32 ± 6 (12)	79 ± 1 (3)
	5.0	0.56 ± 0.03 (9)	0.77 ± 0.07 (6)	31 ± 4 (12)	43 ± 4 (12)	91 ± 4 (3)
	10.0	0.57 ± 0.02 (6)	0.78 ± 0.12 (6)	44 ± 8 (9)	57 ± 5 (9)	N.D.

Results of brain 5-HT shown 120 min after injection of inhibitor when saline or L-tryptophan (100 mg/kg) had also been given 30 min after inhibitor. % Inhibition of MAO activity also shown 120 min after injection of inhibitor. Results expressed as mean ± s.e. mean with number of determinations in brackets. N.D.: Not determined.

5-HT metabolite metabolized by Type B MAO which is being measured as 5-HT in the brain. Certainly the method of measuring 5-HT concentration by an *o*-phthalaldehyde (OPT) reaction used in this study (Curzon & Green, 1970) and that of Squires & Buus Lassen (1975) is somewhat non-specific, for it will measure other 5-substituted indoles (Maickel & Miller, 1966). Since 5-methoxytryptamine is present in the hypothalamus (Green, Koslow & Costa, 1973), produces a strong fluorescent reading with OPT and gives rise to behavioural changes similar but not identical to those seen following increased 5-HT synthesis (Green, Hughes & Tordoff, 1975), it was important to ascertain that the apparent rise of 5-HT seen after tranylcypromine and tryptophan does not derive spuriously from this or another 5-substituted pharmacologically active indoleamine.

Rats were injected with tranylcypromine (20 mg/kg) followed by L-tryptophan (100 mg/kg) 30 min later. After a further 60 min, the rats were killed, the brains divided down the mid-line and 5-HT measured in one half by the OPT method (Curzon & Green, 1970) and in the other by the ninhydrin method of Snyder, Axelrod & Zweig (1965) which at the appropriate wave length appears to measure only 5-HT (Snyder *et al.*, 1965). As they were so similar, the results suggested that the OPT method was measuring only 5-HT (Table 5) and that no other 5-substituted indoles were present.

Discussion

In agreement with Squires & Buus Lassen (1975), we found that tryptophan administration to rats pretreated with either clorgyline or deprenil alone does not result in the hyperactivity syndrome, which was, however, observed after the administration of both inhibitors at low doses. Squires (1975) has suggested that this hyperactivity derives from the formation of an *N*-substituted derivative of 5-HT which is deaminated by Type B MAO to 5-hydroxyindoleacetic acid. However, our results lead us to quite different conclusions.

Pretreatment of rats with tranylcypromine at a dose of 2.5 mg/kg or more, totally inhibited phenylethylamine and 5-HT metabolism by MAO as measured *in vitro*. All doses of this magnitude or greater caused a similar 5-HT rise, measured as a

Table 4 Effect of various doses of clorgyline plus deprenil with or without L-tryptophan injection (100 mg/kg) 30 min later on brain 5-hydroxytryptamine (5-HT) concentrations and monoamine oxidase (MAO) activity towards 5-HT, dopamine and phenylethylamine 120 min after initial injection

Dose of clorgyline plus deprenil (mg/kg) of each)	*Brain 5-hydroxytryptamine (μg 5-HT/g brain (wet wt))*		*% Inhibition of MAO activity towards substrates*		
	Saline	*L-tryptophan (100 mg/kg)*	*5-HT*	*Dopamine*	*Phenylethylamine*
1.0 + 1.0	0.68 ± 0.03 (9)	0.80 ± 0.08 (3)	84 ± 7 (10)	83 ± 4 (12)	76 ± 20 (3)
2.5 + 2.5	0.76 ± 0.05 (8)	1.04 ± 0.14 (3)	97 ± 14 (12)	99 ± 1 (12)	87 ± 13 (3)

Results of brain 5-HT shown 120 min after injection of inhibitor when saline or L-tryptophan (100 mg/kg) had also been given 30 min after the inhibitor. % Inhibition of MAO activity also shown 120 min after injection of the inhibitor. Results expressed as mean ± s.e. mean with number of determinations in brackets.

Table 5 Concentration of brain 5-hydroxytryptamine (5-HT) measured by two methods following tranylcypromine and L-tryptophan administration

	Brain 5-hydroxytryptamine (μg 5-HT/g brain (wet wt))	
	Ninhydrin method	*OPT method*
Saline	0.49 ± 0.01 (4)	0.49 ± 0.02 (4)
Tranylcypromine (20 mg/kg) + L-tryptophan (100 mg/kg)*	1.32 ± 0.06 (6)	1.24 ± 0.10 (6)

* Tranylcypromine given 30 min before L-tryptophan with measurement of brain 5-HT 60 min later.

rate of 5-HT synthesis or 5-HT accumulation when tryptophan was also administered. In contrast, clorgyline at doses above 2.5 mg/kg, while totally inhibiting MAO activity to 5-HT, did not produce a greater than 70% inhibition of phenylethylamine oxidation (Table 3). Pretreatment with deprenil did not inhibit 5-HT oxidation by more than 50% even at a dose of 10 mg/kg. None of the dosage levels of either clorgyline or deprenil resulted in a rise of 5-HT concentration comparable to that observed after tranylcypromine (2.5 mg/kg) nor was 5-HT accumulation after subsequent tryptophan administration as great as that seen after tranylcypromine (2.5 mg/kg) plus tryptophan.

In view of the limited and specific distribution pattern of the clorgyline-insensitive 5-HT oxidizing form of MAO recently reported (Gascoigne, Williams & Williams, 1975), it seems unlikely that this enzyme is responsible for the continuing metabolism of 5-HT following clorgyline injection which is seen in our experiments.

Deprenil and clorgyline (1 mg/kg of each), when injected together, also failed to inhibit totally both forms of the enzyme. However 2.5 mg/kg of each almost totally inhibited both forms of the enzyme. Brain 5-HT also rose to the concentration seen after administration of tranylcypromine (2.5 mg/kg), with a further similar increase after tryptophan.

These results suggest that while 5-HT may normally be predominantly metabolized *in vivo* by Type A monoamine oxidase, when it is inhibited (e.g. by clorgyline), Type B MAO continues to act on the amine. Thus 5-HT concentrations rise more slowly after clorgyline than after the non-specific inhibitor tranylcypromine. When deprenil is given, of course, Type A MAO is only partially inhibited. These findings strengthen the view (Youdim, 1973) that MAO acts *in vivo* as an integrated enzyme system with properties which differ from the individual enzyme forms studied *in vitro*.

The results relating to hyperactivity demonstrate that it is only when both forms of MAO are almost totally inhibited, as when clorgyline and deprenil are given together, that the phenomenon is seen. Only then, presumably, is 5-HT not being oxidized in the presynaptic compartment by intraneuronal MAO and is thus able to 'spill-over' into functional activity. Tranylcypromine (1 mg/kg) inhibited 5-HT oxidation by 86% and phenylethylamine oxidation by 85% but even so it did not cause either hyperactivity or the rise of 5-HT concentration seen after the larger doses which totally inhibited the enzyme. This finding suggests that amounts of MAO are present in the brain grossly in excess of normal requirements. If true, this may be one reason that successful therapy of depressive states by monoamine oxidase inhibitors is difficult to achieve. Previous studies on human brain have indicated that MAO activity towards various substrates is not normally inhibited much above 70% by therapeutic doses of inhibitors (Youdim, Collins, Sandler, Bevan-Jones, Pare & Nicholson, 1972).

Our results pertaining to the inhibition of dopamine oxidation after either clorgyline or deprenil indicate that dopamine is metabolized both by Type A and Type B MAO in agreement with the report by Yang & Neff (1974).

Analysis of 5-HT in rats treated with tranylcypromine (20 mg/kg) and L-tryptophan using the *o*-phthalaldehyde and ninhydrin methods gave essentially similar results (Table 5). Squires (1975) suggested that an N-methylated derivative might be formed following MAO inhibition and L-tryptophan administration, although it would be indistinguishable from 5-HT using the OPT method. Thus results with the OPT method would have been higher than those with ninhydrin, as the former measures any 5-substituted indole (Maickel & Miller, 1966). However, the similarity between the two sets of results reported here argues against involvement of any other 5-substituted indole, as does the observation that the brain 5-HT rise is smaller after clorgyline (even at a dose of 10 mg/kg) than after tranylcypromine (2.5 mg/kg), pointing to incomplete inhibition of 5-HT degradation. Nor does the rise of 5-HT concentrations seen after injection of either clorgyline plus deprenil (2.5 mg/kg of each) or tranylcypromine (2.5 mg/kg) appear to be due to formation of a 5-HT derivative, as the value obtained when translated into a rate of 5-HT synthesis agrees well with those calculated by other methods not requiring the use of a MAO inhibitor (Neff & Tozer, 1968).

The rapid decrease in brain typtophan observed 90 min after tranylcypromine injection is probably due to the induction of hepatic tryptophan pyrrolase activity by its substrate, leading to rapid peripheral metabolism of tryptophan. This in turn would presumably decrease brain tryptophan. Recent evidence suggests that variations in activity of this enzyme can alter brain tryptophan concentrations (Green, Sourkes & Young, 1975; Green, Woods, Knott & Curzon, 1975).

Thus while the involvement of any other indole cannot totally be excluded, we nevertheless feel that the most plausible explanation for the results obtained by Squires & Buus Laasen (1975) and ourselves is also the simplest, that unless both Type A and Type B MAO are almost totally inhibited, hyperactivity will not occur; while 5-HT may normally be metabolized by Type A enzyme *in vivo*, when this form is blocked, oxidation can be continued by Type B enzyme. If this hypo-

thesis is correct, it suggests that increasing the functional activity of 5-HT by using selective inhibitors may be difficult to achieve.

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