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EVIDENCE FOR DOPAMINE DEAMINATION BY BOTH TYPE A AND TYPE B MONOAMINE OXIDASE IN RAT BRAIN *in vivo* AND FOR THE DEGREE OF INHIBITION OF ENZYME NECESSARY FOR INCREASED FUNCTIONAL ACTIVITY OF DOPAMINE AND 5-HYDROXYTRYPTAMINE

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1 Tranylcypromine (20 mg/kg) administration to rats totally inhibited brain monoamine oxidase (MAO) oxidation of 5-hydroxytryptamine (5-HT), phenylethylamine and dopamine as measured *in vitro*. When L-3,4-dihydroxyphenylalanine (L-DOPA) (50 mg/kg) was given 30 min after the tranylcypromine, brain dopamine and noradrenaline concentrations rose markedly and the rats displayed characteristic behavioural changes and locomotor activity.

2 Clorgyline (5 mg/kg) administration inhibited 5-HT oxidation by almost 100% but phenylethylamine by only 29% while (–)-deprenil (5 mg/kg) injection almost totally inhibited phenylethylamine oxidation and inhibited 5-HT metabolism by only 31%. Administration of L-DOPA after pretreatment with either drug did not alter brain dopamine or noradrenaline concentrations and the animals did not display any behavioural changes.

3 Administration of clorgyline plus (–)-deprenil (5 mg/kg of each) almost totally inhibited oxidation of both phenylethylamine and 5-HT; there was a large rise of brain dopamine and noradrenaline concentrations and the animals displayed the behavioural changes observed when tranylcypromine and L-DOPA had been given.

4 The effects of tranylcypromine (20 mg/kg) on brain 5-HT, dopamine and noradrenaline concentrations up to 48 h after injection were recorded. Brain 5-HT concentrations were considerably elevated for 18 h after injection and then fell steadily. In contrast, brain dopamine concentrations rose slightly and remained at this level for 48 h while noradrenaline levels doubled and also remained at this level for 48 hours.

5 When L-tryptophan (50 mg/kg) was given at various times after tranylcypromine the characteristic hyperactivity syndrome appeared at 12 h but not 18 h after tranylcypromine and a further rise in brain 5-HT was only observed at 12 hours. When L-DOPA (50 mg/kg) was given at various times after tranylcypromine a further large rise in brain dopamine and noradrenaline occurred at 12 h but not at 18 h and all the behavioural changes were observed only at 12 hours.

6 Measurement of MAO activity at the above times after tranylcypromine showed that the half-life of recovery of the enzyme activity with 5-HT and dopamine as substrates was 4.5 days and 8.5 days with phenylethylamine as substrate. Inhibition of MAO oxidation of dopamine and 5-HT was approximately 85%, 18 h after tranylcypromine injection.

7 It is concluded from both the studies with clorgyline and deprenil and the recovery of MAO activity after tranylcypromine, that dopamine is metabolized by both Type A and Type B MAO *in vivo* and that it is only when both forms are almost totally inhibited that there is an increase in dopamine and 5-HT functional activity, as judged by the appearance of the hyperactivity syndromes.

Introduction

In a previous paper we demonstrated that while *in vitro* it could be shown that 5-hydroxytryptamine (5-HT) was metabolized by the Type A form of monoamine oxidase (MAO-A), nevertheless when this form was inhibited *in vivo* by clorgyline, Type B monoamine oxidase (MAO-B) continued to metabolize the amine (Green & Youdim, 1975). Recently Ekstedt (1976) has shown that *in vitro* 5-HT is metabolized by MAO-B in liver mitochondria when MAO-A is inhibited by clorgyline.

Previous *in vitro* experiments have suggested that dopamine is metabolized by both forms of the enzyme (see Houslay, Tipton & Youdim, 1976) and we have now investigated whether this is true *in vivo* in the brain. To do this, both biochemical measurements and a behavioural model have been used in an analagous way to the previous study on 5-HT. The behaviour studied was that of the locomotor activity which follows administration of an MAO inhibitor and L-3,4-dihydroxyphenylalanine (L-DOPA) and which has been shown to be the result of increasing dopamine concentrations in the brain (Everett, Wiegland & Rinaldi, 1963; Everett, 1966; Green & Kelly, 1976).

Results indicate that both MAO-A and MAO-B must be inhibited by about 85% to increase dopamine functional activity. This observation led to the second part of this study, since our previous investigation (Green & Youdim, 1975) also indicated that this degree of inhibition of both enzyme types was necessary in order to increase 5-HT functional activity, following subsequent tryptophan administration.

We have now therefore also examined the length of time after administration to rats of tranylcypromine that an increase in functional activity of 5-HT or dopamine (as indicated by the appearance of the behavioural changes following respectively L-tryptophan and L-DOPA) can be demonstrated. The rise in brain 5-HT and dopamine at this time has also been measured. Results have been compared to the degree of inhibition of MAO seen at various times after tranylcypromine injection. Results again indicate the importance of inhibiting MAO by about 85% in order to increase amine functional activity as judged by the hyperactivity syndrome.

Methods

Male Sprague-Dawley rats (150–200 g) (Anglia Laboratory Animals, Alconbury, Huntingdon) were used in all experiments.

Deprenil and clorgyline 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 to two groups of 3 rats. After 30 min L-DOPA (50 mg/kg) suspended in saline containing 1% carboxymethylcellulose was given to one group and saline to the other group. After a further 90 min the rats were killed, the brains removed and divided along the mid-line. One half was homogenized in 0.32 M sucrose and MAO activity towards the substrates [1-^{14}C]-dopamine, [1-^{14}C]-5-hydroxytryptamine (both Radiochemical Centre, Amersham) and [1-^{14}C]-phenylethylamine (NEN Chemicals GmbH) measured by the method of Tipton & Youdim (1976). Protein was measured by the procedure of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as standard. Enzyme activity was calculated as nmol of deaminated product formed/mg protein per 30 min incubation and results expressed as the mean ± s.e. mean of the percentage inhibition compared to saline-injected controls. The other half of the brain was homogenized in acidified butanol, and noradrenaline and dopamine measured as described by Chang (1964).

Activity was measured on both groups of 3 animals for 120 min after administration of the MAO inhibitor using Animex activity meters (sensitivity and tuning 30 μA) as described elsewhere (Grahame-Smith, 1971; Green & Grahame-Smith, 1974). Behavioural changes following the L-DOPA administration were exactly as described by Green & Kelly (1976).

Time course after tranylcypromine experiments

Tranylcypromine was dissolved in saline and injected intraperitoneally. After various times the animals were injected with either L-DOPA (50 mg/kg) suspended in saline containing 1% carboxymethylcellulose or L-tryptophan (50 mg/kg) dissolved in saline. After 60 min the rats were killed, the brains removed and divided along the mid-line. After L-DOPA injection, dopamine and noradrenaline were measured as described above and after L-tryptophan injection, 5-HT was measured by the method of Curzon & Green (1970). The other half of the brain was homogenized in 0.32 M sucrose and MAO activity measured as described above.

Activity was measured in groups of 3 animals for 60 min after amino acid administration by means of Animex activity meters as outlined above. Behavioural changes are described in the text.

Results

Effect of L-DOPA on rat brain dopamine and noradrenaline concentrations and monoamine oxidase activity

Rats were injected with saline and 30 min later given L-DOPA (50 mg/kg). After a further 90 min they were killed and dopamine, noradrenaline and MAO activity measured. L-DOPA did not alter MAO activity and caused only a small rise in noradrenaline and dopamine (Table 1).

Effect of tranylcypromine, clorgyline or (−)-deprenil administration on the locomotor activity following subsequent L-DOPA administration

Tranylcypromine (20 mg/kg) administration 30 min before L-DOPA (50 mg/kg) resulted in the characteristic behavioural changes and locomotor

activity previously described (Everett *et al.*, 1963; Green & Kelly, 1976; Figure 1). When clorgyline (5 mg/kg) or (−)-deprenil (5 mg/kg) was given in place of the tranylcypromine no significant increase in locomotor activity was observed (Figure 1). Even a dose of 10 mg/kg of either of these inhibitors before L-DOPA did not result in increased locomotor activity. However, administration of both inhibitors (clorgyline, 5 mg/kg plus (−)-deprenil, 5 mg/kg) did result in the behavioural changes when L-DOPA was given 30 min later (Figure 1). Without the second L-DOPA injection there was no hyperactivity following any of the inhibitors in agreement with previous observations (Green & Youdim, 1975).

Effect of tranylcypromine, clorgyline or (−)-deprenil on brain dopamine and noradrenaline concentrations and monoamine oxidase activity

When tranylcypromine (20 mg/kg) was given before the L-DOPA there was a large rise in brain dopamine and noradrenaline concentrations and MAO activity was totally inhibited with all substrates tested, 5-HT being used as an *in vitro* index of MAO-A activity and phenylethylamine for MAO-B (Table 1). In contrast neither clorgyline nor (−)-deprenil alone (at a dose of 5 mg/kg) produced as large a rise of dopamine and noradrenaline on subsequent L-DOPA injection, as that seen when tranylcypromine was given. When both clorgyline and deprenil were given together (5 mg/kg of each) brain dopamine and noradrenaline rose to the values seen when tranylcypromine (20 mg/kg) had been given. Almost total inhibition of

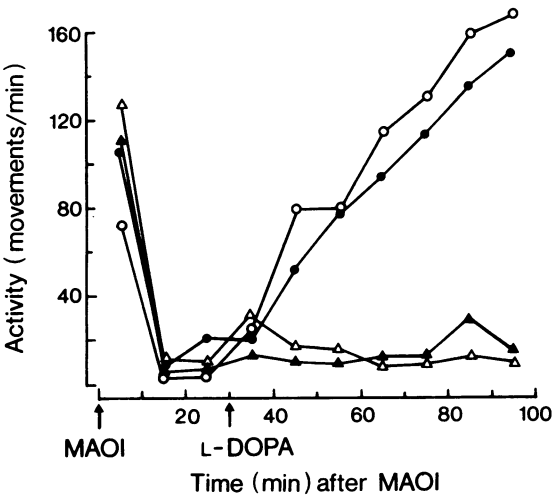

Figure 1 Effect of L-DOPA administration to rats pretreated with clorgyline, deprenil or tranylcypromine. Groups of rats were injected with a monoamine oxidase inhibitor (MAOI), either clorgyline 5 mg/kg (▲), (−)-deprenil 5 mg/kg (△), clorgyline (5 mg/kg) plus (−)-deprenil (5 mg/kg) (●) or tranylcypromine (○). Thirty min later all groups were given L-DOPA (50 mg/kg). Figure shows the result of a typical experiment showing the change in locomotor activity (measured in movements/min) in the different groups of rats. Rats injected with either L-DOPA alone or tranylcypromine, clorgyline or (−)-deprenil (without subsequent L-DOPA injection) do not display increased locomotor activity.

Table 1 Effect of monoamine oxidase inhibitors with or without L-DOPA (50 mg/kg) injection 30 min later on brain noradrenaline and dopamine concentrations and monoamine oxidase (MAO) activity towards 5-hydroxytryptamine (5-HT), dopamine and phenylethylamine 90 min after initial injection

Injected		*Brain catecholamine concentration (μg catecholamine/g brain (wet wt.))*		*% Inhibition of MAO activity towards substrates*		
0 min	*30 min*	*Noradrenaline*	*Dopamine*	*5-HT*	*Dopamine*	*Phenylethylamine*
Saline	Saline	0.23 ± 0.02 (7)	1.65 ± 0.10 (7)	—†	—†	—†
Saline	L-DOPA	0.23 ± 0.02 (6)	1.82 ± 0.12 (6)	0	0	0
Clorgyline (5 mg/kg)	L-DOPA	0.33 ± 0.05 (6)	1.66 ± 0.28 (6)	98 ± 1 (12)	87 ± 5 (12)	29 ± 10 (3)
Deprenil (5 mg/kg)	L-DOPA	0.26 ± 0.03 (6)	1.72 ± 0.05 (6)	31 ± 4 (12)	43 ± 4 (12)	91 ± 4 (3)
Clorgyline + deprenil (5 mg/kg of each)	L-DOPA	0.44 ± 0.02 (4)	6.05 ± 0.04 (4)	92 ± 1 (7)	89 ± 1 (7)	99 ± 3 (7)
Tranylcypromine (20 mg/kg)	L-DOPA	0.47 ± 0.02 (6)	8.80 ± 0.85 (6)	100	100	100

Brain noradrenaline and dopamine concentrations shown 90 min after MAO inhibitor injection, when L-DOPA had been given 30 min later. Brain MAO activity shown at same time. Results expressed as mean ± s.e. mean with number of determinations in brackets. Absolute MAO activities (in nmol deaminated product formed per mg protein/30 min incubation); dopamine: 59.2 ± 2.5; 5-HT: 75.6 ± 5.1; phenylethylamine: 51.5 ± 1.3 (all 6 determinations). Some MAO inhibition results taken from Green & Youdim (1975).

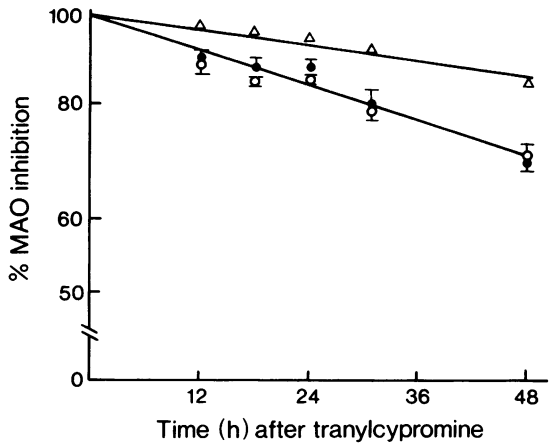

Figure 2 Recovery of monoamine oxidase (MAO) activity, following injection of tranylcypromine. Rats were injected with tranylcypromine (20 mg/kg) and after various times they were killed and MAO activity measured with phenylethylamine (△), dopamine (○) and 5-hydroxytryptamine (●) as substrates. The half-life of reappearance of MAO activity was calculated and is reported in the text. Points show mean and bars ± s.e. mean of determinations in 6 rats. Where bars are not shown the s.e. mean was smaller than the point.

both forms of the enzyme was seen only when both inhibitors had been given (Table 1).

Return of monoamine oxidase activity following tranylcypromine administration

Rats were injected intraperitoneally with tranylcypromine (20 mg/kg). At various times after the injection they were killed and MAO activity to 5-HT, dopamine and phenylethylamine measured. Results shown in Figure 2 demonstrate that the recovery of MAO activity towards 5-HT and dopamine was the same, with a half-life of 4.5 days, whereas the half-life with the substrate phenylethylamine was 8.5 days.

Brain 5-hydroxytryptamine, dopamine and noradrenaline at various times after tranylcypromine injection

Following tranylcypromine (20 mg/kg) injection, brain 5-HT increased considerably being around 1 μg 5-HT/g (wet wt.) at both 12 and 18 h; after this time it started to decrease (Table 1). Brain dopamine concentration on the other hand showed a relatively small increase which was seen up to 48 h after injection. Brain noradrenaline concentration increased and also remained elevated for 48 h (Table 2).

Behavioural changes and brain 5-hydroxytryptamine concentrations following L-tryptophan administration to rats previously given tranylcypromine

Administration of L-tryptophan (50 mg/kg) to rats given tranylcypromine (20 mg/kg) 30 min previously results in a complex series of behavioural changes including hyperactivity (Grahame-Smith, 1971). These changes were seen in this study (Figure 3) and also when the L-tryptophan was given 12 h after the tranylcypromine, although at this time the hyperactivity syndrome was seen to occur immediately after tryptophan administration (Figure 3). The activity was quantitatively similar to that seen in the rats given tranylcypromine 0.5 h before the tryptophan, although it could not be seen in these animals until about 40 min after tryptophan.

Table 2 Changes in brain 5-hydroxytryptamine (5-HT), dopamine and noradrenaline at various times after injection of tranylcypromine (20 mg/kg) and the concentration of these amines 60 min after L-tryptophan or L-DOPA administration

Time (h) after tranylcypromine (20 mg/kg) Injected	*Brain amine concentrations in μg amine/g brain (wet wt.)*					
	5-hydroxytryptamine		*Dopamine*		*Noradrenaline*	
	Saline	*L-tryptophan*	*Saline*	*L-DOPA*	*Saline*	*L-DOPA*
0.5	0.53 ± 0.04 (6)	0.93 ± 0.07 (6)	1.65 ± 0.08 (6)	8.80 ± 0.85 (6)	0.23 ± 0.02 (7)	0.47 ± 0.02 (6)
12	1.01 ± 0.04 (8)	1.43 ± 0.03 (4)	2.64 ± 0.22 (3)	6.93 ± 1.44 (3)	0.46 ± 0.01 (3)	0.67 ± 0.10 (3)
18	0.98 ± 0.05 (4)	0.91 ± 0.08 (6)	3.32 ± 0.36 (4)	3.37 ± 0.51 (3)	0.42 ± 0.06 (4)	0.48 ± 0.08 (3)
24	0.73 ± 0.01 (4)	0.93 ± 0.08 (6)	2.66 ± 0.22 (4)	4.30 ± 0.87 (9)	0.59 ± 0.02 (4)	0.56 ± 0.05 (6)
48	0.57 ± 0.02 (4)	0.62 ± 0.04 (3)	2.48 ± 0.31 (4)	3.36 ± 0.39 (6)	0.43 ± 0.06 (3)	0.42 ± 0.03 (6)
Saline	0.44 ± 0.03 (6)	0.66 ± 0.03 (4)	1.72 ± 0.09 (11)	1.94 ± 0.03 (3)	0.23 ± 0.01 (9)	0.24 ± 0.03 (3)

Rats were injected with tranylcypromine (20 mg/kg). At various times after this injection they were killed and brain 5-HT, dopamine and noradrenaline measured while other groups were injected with either L-tryptophan (50 mg/kg), when brain 5-HT was measured 60 min later, or L-DOPA (50 mg/kg), when brain dopamine and noradrenaline were measured 60 min later.

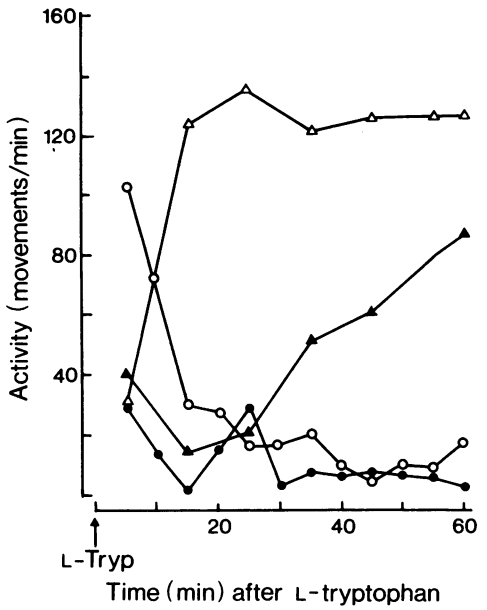

Figure 3 Hyperactivity response of rats following L-tryptophan injection at various times after administration of tranylcypromine. Rats were injected with tranylcypromine (20 mg/kg). After 0.5 h (▲), 12 h (△), 18 h (●) and 24 h (○) they were given L-tryptophan (50 mg/kg) and activity measured over the next 60 min at which time they were killed and brain 5-hydroxytryptamine concentrations measured (Table 2). The 48 h result is not shown but was essentially the same as the 24 h result shown. No hyperactivity was observed in any group until after the L-tryptophan injection.

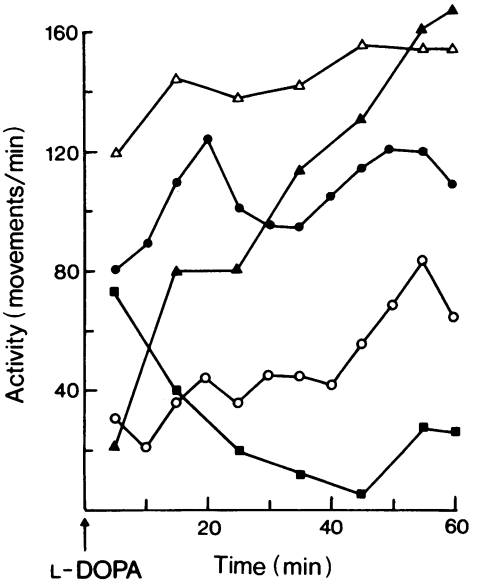

Figure 4 Locomotor response of rats following L-DOPA injection at various times after administration of tranylcypromine. Rats were injected with tranylcypromine (20 mg/kg). After 0.5 h (▲), 12 h (△), 18 h (●), 24 h (○) and 48 h (■) they were given L-DOPA (50 mg/kg) and activity measured over the next 60 min at which time they were killed and brain dopamine and noradrenaline concentrations were measured (Table 2). No hyperactivity was observed in any group until after the L-DOPA injection.

The animals given tryptophan either 0.5 h or 12 h after tranylcypromine both showed a further rise in 5-HT above that produced by the MAO inhibitor.

In contrast, those animals given tryptophan 18 h, 24 h and 48 h after tranylcypromine showed neither a large 5-HT rise 60 min after L-tryptophan administration nor hyperactivity (Table 2 and Figure 3).

The hyperactivity syndrome was not seen in any group of animals until after the L-tryptophan administration.

Behavioural changes and brain dopamine and noradrenaline concentrations following L-DOPA administration to rats previously given tranylcypromine

Administration of L-DOPA (50 mg/kg) to rats pretreated with tranylcypromine (20 mg/kg) either 0.5 h or 12 h earlier, results in a large brain dopamine rise (Table 2) and the appearance of all behavioural changes described by Green & Kelly (1976). These include irritability, squeaking, aggression, Straub tail, forepaw padding, headweaving, salivation, piloerection, rearing to other animals and locomotor activity. Eighteen and 24 h after tranylcypromine, L-DOPA administration resulted in a smaller dopamine rise than that seen after 12 hours. Furthermore, the behaviour changed. Locomotor activity occurred (although the effect was less by 24 h than at 18 h) but was not accompanied by many other of the behavioural features. There was little rearing, squeaking or aggression and headweaving, piloerection and forepaw padding were absent. The animals now looked much more as if they had been given methamphetamine at a low dose (1 mg/kg). At 18 h and 24 h only some of the animals displayed hyperactivity and this is probably due to the large variation at this time in brain dopamine levels from near normal to very elevated. No locomotor activity was observed when the rats were given L-DOPA 48 h after tranylcypromine and there was an even smaller brain dopamine rise, nor was it seen in any group until after the L-DOPA injection.

It was found that L-DOPA administration failed to alter significantly brain noradrenaline concentrations (Table 2). This is in accord with previous studies on the influence of L-DOPA administration on central noradrenaline concentrations (Everett & Borcherding, 1970; Green & Grahame-Smith, 1974).

Discussion

The first part of this study clearly demonstrates that in the brain, dopamine is metabolized by both Type A and Type B MAO. Unless both forms of the enzyme are inhibited, L-DOPA administration neither produces the rise of brain dopamine nor the behavioural changes seen after administration of a non-specific inhibitor such as tranylcypromine. Similar observations have also been made by Squires (personal communication).

Previously Yang & Neff (1974) suggested that dopamine is metabolized by both Type A and Type B MAO on the basis of *in vitro* studies. While our *in vivo* work on hyperactivity and the rise in brain dopamine agrees with this observation, the estimation of MAO activity *in vitro* after rats have been treated *in vivo* with selective MAO inhibitors suggests that inhibition of dopamine metabolism is influenced much more by inhibition of Type A than Type B MAO (Table 1). This is also supported by the observation that the recovery of dopamine metabolizing activity *in vitro* following total inhibition by tranylcypromine injection exactly parallels the recovery of MAO activity towards 5-HT (a Type A substrate, Figure 2). Therefore, we suggest that dopamine may normally be metabolized by Type A MAO but that Type B enzyme can continue to metabolize the amine when the Type A enzyme is inhibited. This is exactly analagous to the situation with 5-HT in the brain (Green & Youdim, 1975).

Our figures on the half-life of the return of MAO activity following total inhibition agree with some of those obtained by Maître, Delini-Stula & Waldmeier (1976). For example they also found a half-life of the disappearance of inhibition of MAO of 8.5 days using phenylethylamine as a substrate, after giving either iproniazid or deprenil but not after tranylcypromine. When 5-HT was used as a substrate however they obtained a half-life of about 10 days which is considerably longer than that found here and again tranylcypromine was reported to show a different pattern from other inhibitors tested. However, like us, Maître *et al.* (1976) found a similar time course to 5-HT when dopamine was used as a substrate.

The results with clorgyline and (−)-deprenil showed that the large rise in brain dopamine and the behavioural changes occurred only when both forms of the enzyme were almost completely inhibited. This observation was taken one stage further by studying the recovery of MAO activity towards various substrates after total inhibition of the brain enzyme. It was found that when MAO inhibition dropped below about 85% (this occurs around 18 h after injection) L-tryptophan injection did not produce a large 5-HT rise nor did the animals display the hyperactivity syndrome. When L-DOPA was given at 18 h there was a smaller brain dopamine rise than that seen 12 h after tranylcypromine and some behavioural changes still occurred. However, the behavioural change was much less marked than that seen 12 h after injection and many of the behavioural components of the change were absent. These findings complement our previous suggestion on the basis of studies on 5-HT (Green & Youdim, 1975), that total MAO activity is present in the brain in large amounts and that it is only when both forms of the enzyme are inhibited by about 85% that there is an increase in the functional activity of 5-HT or dopamine.

This observation has implications in the use of MAO inhibitors in the treatment of psychiatric illness. First, because this degree of inhibition does not normally appear to be reached during administration of MAO inhibitors (Youdim, Collins, Sandler, Bevan-Jones, Pare & Nicholson, 1972) and second, because of the research being carried out on the feasibility of using selective monoamine oxidase inhibitors to increase preferentially the concentration of specified neurotransmitters.

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