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CUMULATIVE EFFECTS OF IRREVERSIBLE MAO INHIBITORS *IN VIVO*

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Abstract—The effects of repeated treatment with clorgyline, pargyline, deprenyl and tranylcypromine on MAO activity in rat brain and liver were investigated. MAO was measured with the substrates serotonin (5-HT), phenethylamine (PEA) and, in some cases, tyramine (TYR). The rates of recovery of MAO activity in brain tissue after single and repeated administrations of 10 mg/kg s.c. clorgyline or deprenyl were also compared. Single doses of clorgyline (1 and 10 mg/kg s.c.) completely blocked the deamination of 5-HT. PEA deamination gradually decreased during the 14-day treatment. Pargyline in a dose of 0.3 mg/kg s.c. reduced both 5-HT and PEA deamination progressively over the same period. In the course of repeated treatment the effects of clorgyline and deprenyl on 5-HT and PEA deamination increased in intensity, by a factor of about 10 in the brain and about 3 in the liver. The potentiation of the effect of tranylcypromine was less marked (brain: × 4; liver: × 2). The rates of recovery of MAO activity were not greater after repeated than after single administrations of high doses of clorgyline and deprenyl, suggesting that the withdrawal of the drugs is not followed by a rebound phenomenon. Our results indicate that repeated treatment with suitable doses of clorgyline or deprenyl leads to specific reduction of either MAO A or B activity in brain, without producing any appreciable effect in the liver.

It has been shown in recent years that in mammals monoamine oxidase (monoamine: O_2 oxidoreductase, MAO; EC 1.4.3.4) exists in at least two forms with different substrate specificities. Of the two identified so far, one, termed A-type MAO, preferentially deaminates serotonin and noradrenaline and is inhibited relatively selectively by clorgyline, harmaline, and Lilly 51641; the other, B-type MAO, preferentially deaminates phenethylamine and benzylamine and is inhibited by pargyline and deprenyl [1].

However, evidence is accumulating that substrate specificities and susceptibility to inhibition by specific inhibitors can vary greatly, depending on the sources of the enzymatic preparations, which suggests that the two forms represent two different classes rather than single entities [2]. There is also some evidence that the lipid environment of the active site might determine substrate specificity and susceptibility to different inhibitors, which would explain the large variations in these parameters observed with enzymatic preparations obtained from different sources [3, 4]. It nevertheless seems permissible for the time being to retain the broad classification into A and B forms based on specificity for the substrates serotonin, tyramine, benzylamine and phenethylamine and sensitivity to clorgyline [2]. These substrates appear to be deaminated by the same forms of MAO in rat and human brain and liver [2], so that studies of the effects of MAO inhibitors on enzymatic activity in the brain and liver of rats might be of some relevance to the use of these drugs in man. The MAO inhibition produced by drugs of the classical hydrazine and propargyl types is irreversible† and long-lasting *in vivo* [5, 6] and this also holds good for the newer drugs, deprenyl and clorgyline [7, 8], which have been shown to inactivate specifically B- and A-type enzymes, respectively [8–10].

The fact that these drugs inhibit MAO irreversibly suggests that the degree of inhibition *in vivo* might increase with repeated treatment. Moreover, the selective effect of some MAO inhibitors like clorgyline or deprenyl on A- or B-type MAO observed after the administration of single doses might be gradually lost after repeated treatment. An indication that this may in fact happen was observed by Long *et al.* [11] in their investigations of irreversible inhibitors of a cyclopropylamino-substituted oxadiazole type.

In this laboratory, we have recently obtained evidence that substrate selectivity is indeed gradually lost after repeated administration of relatively high doses of deprenyl to rats [12].

We have now extended this study and compared the effects of single and repetitive administration of various doses of clorgyline, deprenyl and tranylcypromine (a non-selective drug causing only partially irreversible MAO inhibition [13] in rat brain and liver). We have also determined the rates of recovery of MAO activity after single and repeated administration of the two former drugs, to see whether the synthesis of the enzyme is accelerated as a consequence of continued treatment with an inhibitor.

MATERIALS AND METHODS

Clorgyline HCl was synthesized for us by Dr. E. Schmid, deprenyl by Dr. A. Storni and pargyline HCl by Dr. W. Bencze in our Chemical Department. Tranylcypromine was kindly provided by Smith, Kline and French Labs., Philadelphia, PA.

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† The result of the interaction of "irreversible inhibitors" with MAO is an inactivation of the enzyme and in a strict sense it is not correct to use the term "inhibition". However, as that latter term is commonly used by pharmacologists, we will also do so in this article.

MAO activity was determined by radioassay essentially as described by Wurtman and Axelrod [14]. Groups of 4 female Tif: RAIf (SPF) rats (Tierfarm Sisseln, Switzerland) weighing 160–220 g at the beginning of the treatment were used. The animals were killed by decapitation 2 hr after the last dose and the brains and livers homogenized in 3 vol. 0.1 M phosphate buffer of pH 7.9. Further dilutions of the homogenates were made in the same phosphate buffer.

2-[^{14}C]-5-Hydroxytryptamine binoxalate (5-HT; 44 mCi/m-mole, New England Nuclear, Boston, MA), β-ethyl-1-[^{14}C]phenethylamine HCl (PEA; 51 mCi/m-mole, New England Nuclear, Boston, MA) and 1-[^{14}C]tyramine HCL (TYR: 55 mCi/m-mole, Radiochemical Centre, Amersham, England) served as substrates at a concentration of 20.8μM (10 nCi per sample) at pH 7.9. The final volume of the incubation mixture was 0.3 ml and the reaction was carried out with 2.5 mg and 0.5 mg of brain and liver tissue, resp. After incubation at 37° for 20 min, the labelled deaminated metabolites were extracted into 6 ml ethyl acetate. Aliquots of 4 ml were counted after addition of 1 ml ethanol and 10 ml scintillator (Butyl-PBD Ciba–Geigy, 0.6% in toluene). The assay was linear with homogenates from brain and liver with the above substrates under our conditions.

RESULTS

Effect of treatment of varying duration with clorgyline on MAO activity in whole brain

Rats were treated with 1 or 10 mg/kg s.c. clorgyline for 1, 2, 4, 7 or 14 days and decapitated 2 hr after the last injection. Inhibition of 5-HT deamination was already complete after acute treatment with both doses of the MAO inhibitor (Fig. 1). After 7 days' treatment with the lower dose of 1 mg/kg s.c. (but not earlier), the extent of the inhibition of the deamination of PEA and TYR was only very slightly increased. At the higher dose of 10 mg/kg, however, the increase was more clear-cut. After 4 days' treatment with this dose, PEA deamination was inhibited by about 55 per cent, as compared to 25 per cent after acute treatment. The extent of inhibition then increased progressively, but at a slower rate, until the last day of treatment, by which time it had reached about 75 per cent. Inhibition of TYR deamination increased from about 70 per cent after a single dose of 10 mg/kg s.c. clorgyline to about 90 per cent after 14 days' treatment (Fig. 1). Very similar results were obtained in the rat liver (results not shown).

Effect of repeated treatment with pargyline on cerebral MAO activity

After repeated treatment with 0.3 mg/kg s.c. pargyline, similar results were obtained. Inhibition of PEA deamination amounted to about 45 per cent after one dose, increased to about 70 per cent after the second injection, and from then onwards rose slowly to reach about 85 per cent after 14 days' treatment. In contrast, deamination of 5-HT, which was not affected by a single injection of this dose of pargyline, was only slightly inhibited (10–15 per cent) after 7 days' treatment, but then diminished to about 55 per cent of controls after 14 days (Fig. 2). Again, very similar results were obtained in the rat liver (results not shown).

Comparison of the dose–response curves for inhibition of the deamination of PEA and 5-HT by some MAO inhibitors in brain and liver after acute and repeated treatment

Clorgyline. The acute threshold dose for inhibition of the deamination of 5-HT in the rat brain (Fig. 3) was between 0.01 and 0.03 mg/kg s.c. The ED_{50} was approximately 0.1 mg/kg, and almost complete inhibition was produced by 1 m/kg s.c. (Fig. 3). In contrast,

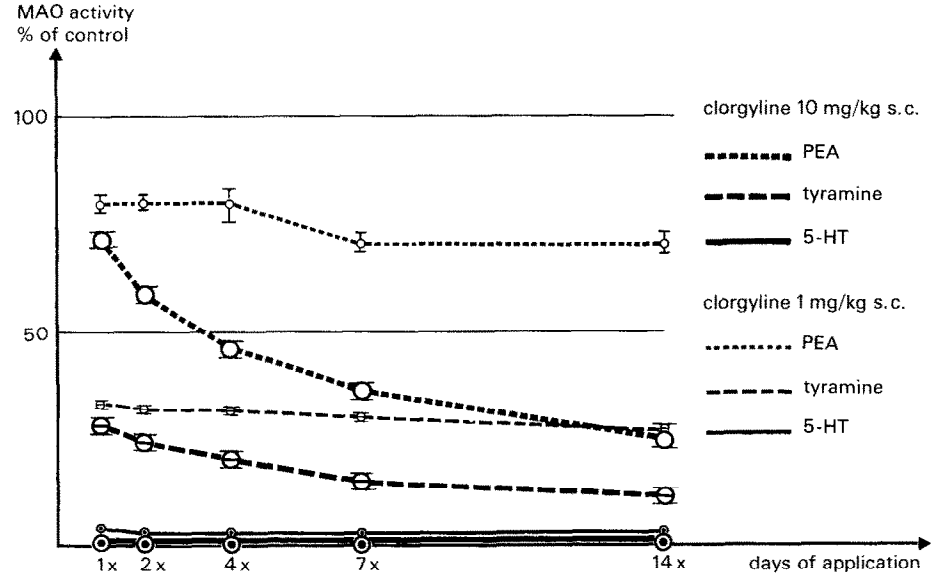

Fig. 1. Inhibition of the deamination of phenethylamine (PEA), tyramine and serotonin (5-HT) in rat brain after acute and repeated treatment of varying duration with clorgyline. Points and bars represent means ± S.E.M. of the values obtained from four rats each.

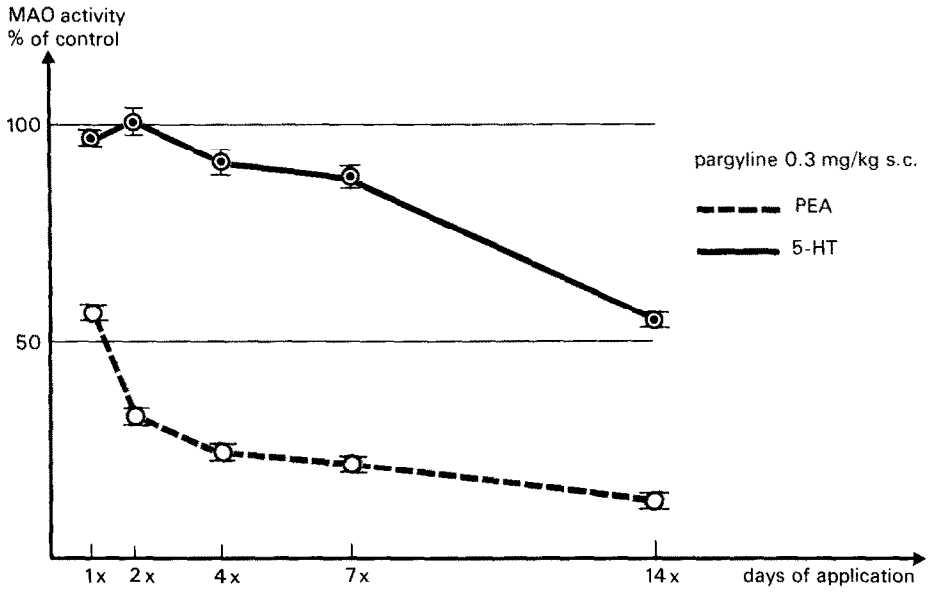

Fig. 2. Inhibition of the deamination of phenethylamine(PEA) and serotonin (5-HT) in rat brain after acute and repeated treatment of varying duration with pargyline. Points and bars represent means ± S.E.M. ($n = 4$).

after 14 daily injections of the very low dose of 0.003 mg/kg, 15 per cent inhibition of 5-HT deamination was already noted: the ED_{50} under these conditions was about 0.01 mg/kg, and 0.1 mg/kg caused almost complete inhibition. This corresponds to a shift to the left of the dose–response curve by a factor of about 10.

A similar shift was observed with respect to PEA deamination. After acute administration, the dose–response curve was very flat: the threshold dose was between 0.03 and 0.1 mg/kg, and at 10 mg/kg only 30 per cent inhibition was reached. After 14 daily injections, the threshold dose was not clearly changed, but the curve was appreciably steeper, the ED_{50} being 2.5 mg/kg (Fig. 3).

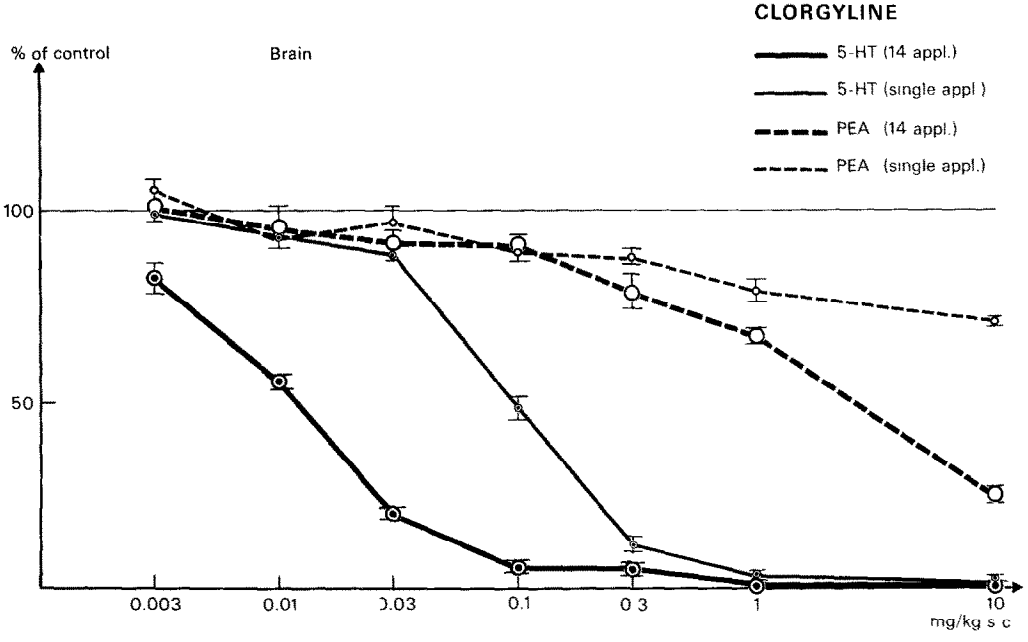

Fig. 3. Comparison of the dose–response curves of the effect of clorgyline on the deamination of phenethylamine (PEA) and serotonin (5-HT) in rat brain after acute and repeated (14 days') treatment. Points and bars represent means ± S.E.M. ($n = 4$).

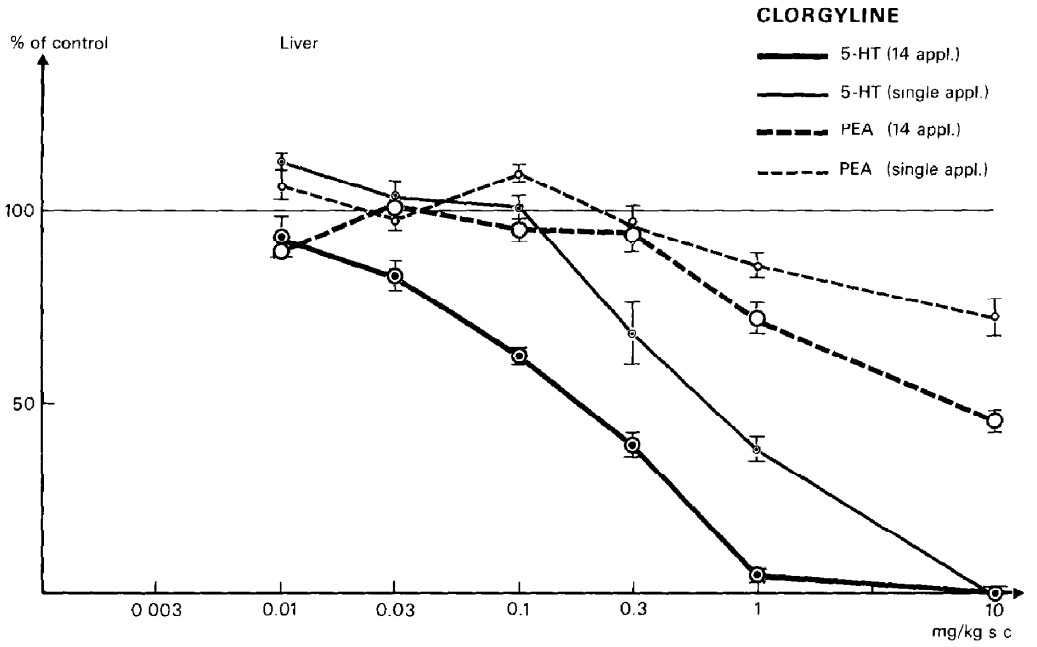

Fig. 4. Comparison of the dose–response curves of the effect of clorgyline on the deamination of phenethylamine (PEA) and serotonin (5-HT) in rat liver after acute and repeated (14 days') treatment. Points and bars represent means ± S.E.M. ($n = 4$).

In the rat liver (Fig. 4), a corresponding, though less marked shift was also observed. The threshold dose for the inhibition of 5-HT deamination after acute administration of clorgyline was about 0.1 mg/kg. The ED_{50} was about 0.6 mg/kg, and almost complete inhibition was reached with 10 mg/kg. After 14 days' administration, the threshold was about 0.01 mg/kg, and the ED_{50} 0.2 mg/kg, while almost complete inhibition was produced by 1 mg/kg. This corresponds to a shift of the dose–response curve by a factor of about 3. Threshold inhibition of PEA deamination in the rat liver was observed after a single dose of 0.3 mg/kg. At 10 mg/kg, deamination was inhibited by 30 per cent. After 14 days' treatment, the threshold dose was not changed,

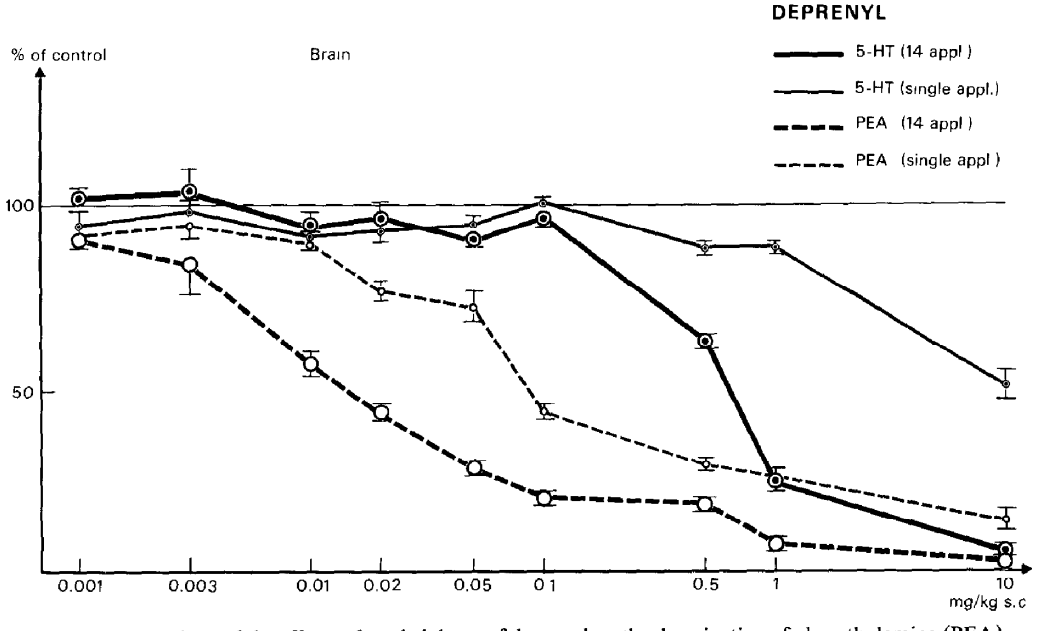

Fig. 5. Comparison of the effects of graded doses of deprenyl on the deamination of phenethylamine (PEA) and serotonin (5-HT) in rat brain after acute or repeated (14 days') treatment. Points and bars represent means ± S.E.M. of four independent determinations.

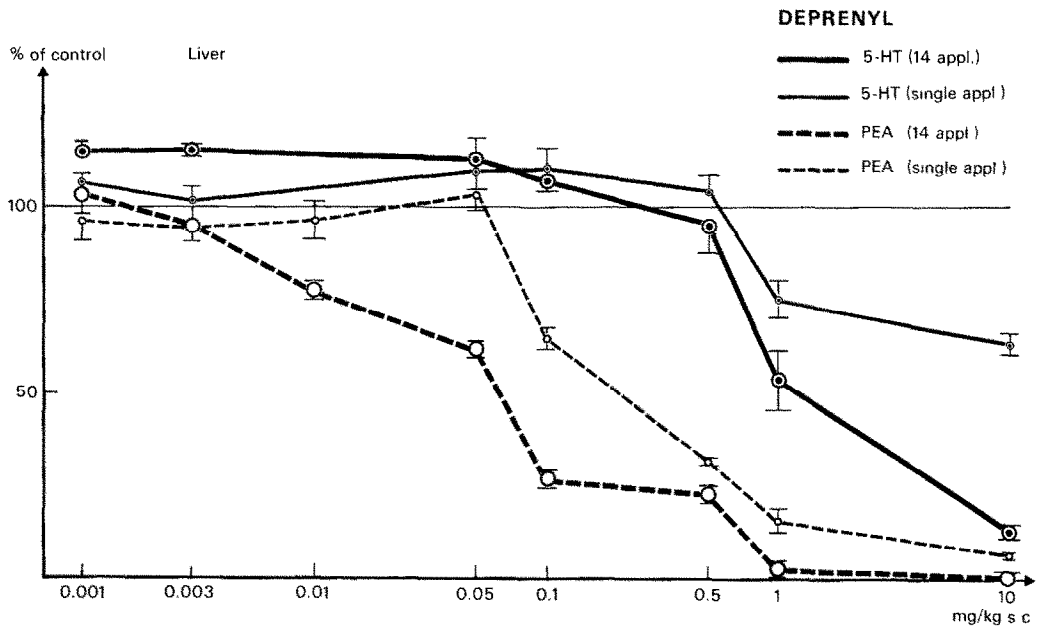

Fig. 6. Comparison of the effects of graded doses of deprenyl on the deamination of phenethylamine (PEA) and serotonin (5-HT) in rat liver after acute and repeated (14 days') treatment. Points and bars represent means ± S.E.M. ($n = 4$).

but the curve grew steeper, 50 per cent inhibition occurring at a dose of 10 mg/kg.

Deprenyl. Analogous results were obtained with the B-type inhibitor deprenyl. In rat brain (Fig. 5), the threshold dose for PEA deamination after acute administration of deprenyl was about 0.01 mg/kg. The ED_{50} was about 0.1 mg/kg, and the curve flattened out thereafter: inhibition amounted to about 85 per cent at 10 mg/kg s.c. After 14 daily injections, the threshold dose was about 0.001 mg/kg, and the ED_{50} about 0.015 mg/kg, while almost complete inhibition was reached at about 1 mg/kg s.c. This corresponds to a shift to the left of the dose–response curve by a factor of 6–8.

The threshold-dose for the inhibition of 5-HT deamination after acute treatment, was found to be about 0.1 mg/kg and the ED_{50} about 10 mg/kg s.c. After 14 daily injections, the threshold dose was not markedly changed, but the ED_{50} was approximately 0.6 mg/kg s.c., and almost complete inhibition was reached at

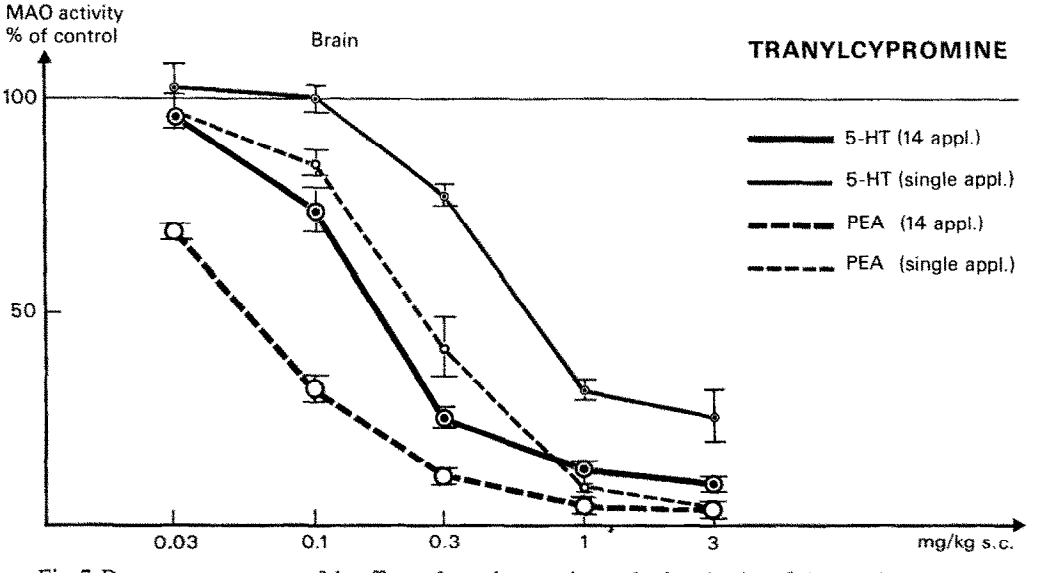

Fig. 7. Dose–response curves of the effects of tranylcypromine on the deamination of phenethylamine (PEA) and serotonin (5-HT) in rat brain after acute or repeated (14 days') treatment. Points and bars represent means ± S.E.M. ($n = 4$).

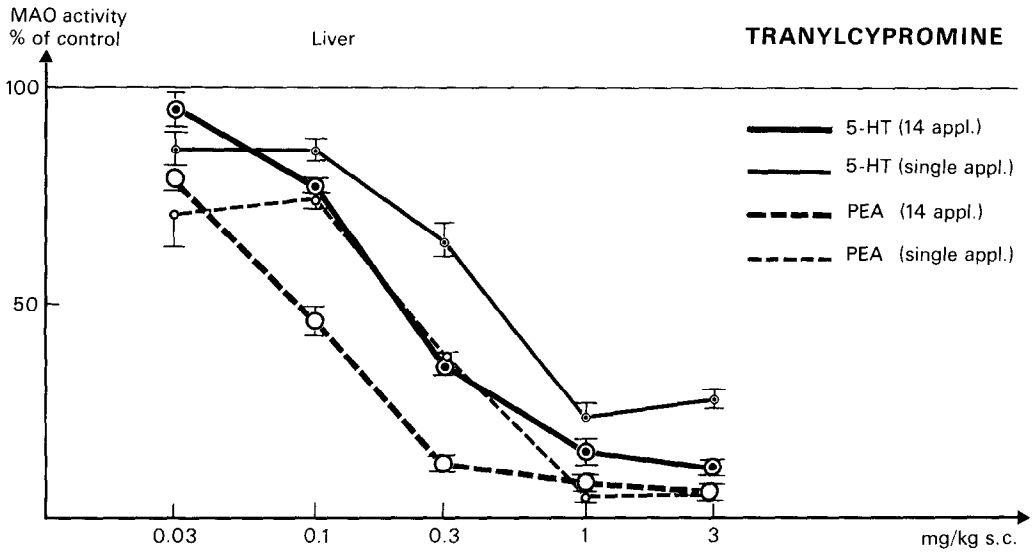

Fig. 8. Dose–response curves of the effects of tranylcypromine on the deamination of phenethylamine (PEA) and serotonin (5-HT) in rat liver after acute or repeated treatment. Points and bars represent means ± S.E.M. ($n = 4$).

10 mg/kg s.c. The shift to the left of the dose–response curve was therefore even somewhat larger than with PEA, corresponding to about a factor of 10–15.

In the rat liver (Fig. 6), deprenyl was also more active after repeated than after acute administration. However, as with clorgyline, the shift of the dose–response curves for the inhibition of both PEA and 5-HT deamination was smaller in magnitude than in the brain. The threshold dose for inhibition of PEA deamination after acute treatment proved to be 0.05 mg/kg and the ED_{50} 0.2 mg/kg, almost complete inhibition being observed at 10 mg/kg. After repeated treatment, the threshold dose was 0.003 mg/kg, and the ED_{50} 0.06 mg/kg, and total inhibition was produced by 1 mg/kg s.c. The dose–response curve was thus shifted to the left by a factor of 3 after repeated treatment. The threshold dose for inhibition of 5-HT deamination was 0.5 mg/kg after acute administration, and 10 mg/kg produced 35 per cent inhibition. No change in the threshold dose was noted after repeated administration, but the ED_{50} was about 1 mg/kg, and inhibition was nearly complete after 10 mg/kg s.c. Hence the dose–response curve appears to have shifted to the left by a factor of between 3 and 10.

Tranylcypromine. This compound showed only slight preference for the inhibition of PEA deamination over that of 5-HT deamination in both rat brain (Fig. 7) and liver (Fig. 8). The dose–response curves for inhibition of both PEA and 5-HT deamination were shifted to the left by factors of about 4 in the brain and 2 in the liver.

The ED_{50}'s of the three MAO inhibitors for inhibition of PEA and 5-HT deamination after acute and repeated treatment are shown in Table 1.

Comparison of the rates of recovery of MAO activity after acute and repeated treatment with clorgyline and deprenyl

To determine whether continued blockade of MAO stimulates the synthesis of the enzyme, the rates of recovery of MAO activity after acute treatment and after 14 days' treatment with high doses of clorgyline and deprenyl (10 mg/kg s.c.) were compared, using 3 different substrates: 5-HT, TYR and PEA. After irreversible inhibition of MAO, the reappearance of enzyme activity was shown to reflect the *de novo* synthesis of enzyme protein [13].

Table 1. ED_{50}'s for inhibition of PEA and 5-HT deamination after acute administration and 14 days' treatment with clorgyline, deprenyl and tranylcypromine

		5-HT		PEA	
Drug	Administration	Brain mg/kg s.c.	Liver mg/kg s.c.	Brain mg/kg s.c.	Liver mg/kg s.c.
Clorgyline	1 ×	0.10	0.60	> 10	> 10
	14 ×	0.012	0.17	2.5	6
Deprenyl	1 ×	10	> 10	0.09	0.2
	14 ×	0.6	1.2	0.015	0.06
Tranylcypromine	1 ×	0.60	0.45	0.25	0.20
	14 ×	0.17	0.20	0.06	0.09

ED_{50} values obtained by interpolation from the curves shown in Figs. 3–8.

Table 2. Comparison of the recovery rates of MAO activity in rat brain with different substrates after acute and repeated treatment

Drug	Administration	5-HT $t_{\frac{1}{2}}$ days	5-HT % Inhibition at time zero	5-HT r	TYR $t_{\frac{1}{2}}$ days	TYR % Inhibition at time zero	TYR r	PEA $t_{\frac{1}{2}}$ days	PEA % Inhibition at time zero	PEA r
Clorgyline	1 ×	12.0 b, c	105.0	0.970	9.7 b, c	85.3	0.955	14.3 b	19.3	0.606
10 mg/kg s.c.	14 ×	12.0	101.6	0.978	10.4 a	94.4	0.925	7.9	70.2	0.871
Deprenyl	1 ×	14.3 b	41.3	0.810	6.5 b	63.5	0.823	7.9	101.2	0.956
10 mg/kg s.c.	14 ×	12.0 a	84.9	0.952	9.7	91.2	0.986	11.6	88.9	0.966

a: Regression line deviates slightly from linearity. b: regression lines parallel ($P < 0.05$). c: regression lines identical ($P < 0.05$).

Rats were treated once or once daily on 14 consecutive days with 10 mg/kg s.c. of either clorgyline or deprenyl. Groups of 4 animals were decapitated 1, 2, 7, 10, 14, 17 and 21 days after the single or the last injection and MAO activity determined with 5-HT, TYR, and PEA as substrates. (An additional group of animals treated with deprenyl was decapitated 4 days after the end of treatment.) The percentage of MAO inhibition for each individual sample was plotted against the time since decapitation. Linear regression was calculated and the regression lines tested for linearity. The half-lives ($t_{1/2}$) for the normalization of MAO activity were determined and given together with the intersections on the abscissae (= per cent inhibition at time zero) as representative parameters for the regression lines. r is the correlation coefficient. The regressions obtained with each substrate after acute and chronic application were also tested for parallelism, and, if found to be parallel, for identity. The statistical methods used were taken from *Scientific Tables* (Eds K. Diem and C. Lentner) J.R. Geigy AG, Basle, Switzerland (1968).

The results are summarized in Table 2. The half-lives and the percentages of inhibition at time zero were taken as representative parameters of the recovery rates and are shown together with the correlation coefficient. It is also indicated whether the calculated regression lines are parallel, and if so, whether they can be considered identical. The latter point refers to what was shown earlier in this paper, i.e., that MAO inhibition can be increased by repeated treatment.

With all 3 substrates, the rates of recovery of MAO activity were the same after acute and repeated treatment with clorgyline ($P < 0.05$). The nearly two-fold difference in the half-lives found when PEA was used as substrate can be ascribed to the relatively low degree of inhibition resulting from acute treatment: the slope is consequently not at all certain. This is reflected in the low correlation coefficient (Table 2) and the fact that, in spite of the large difference in the calculated half-lives, the slopes of the regression lines are not significantly different.

The regression lines obtained after acute and repeated treatment with deprenyl were parallel, but not identical, when MAO activity was determined with 5-HT and TYR as substrates. With PEA as substrate, the half-life was significantly longer after repeated than after acute treatment (Table 2).

DISCUSSION

The shifts to the left in the dose–response curves for MAO inhibition by clorgyline, deprenyl, and tranylcypromine in the rat liver and brain demonstrated by the foregoing results indicate that the inhibitory effects of these drugs are cumulative. These shifts were similar in extent with both the MAO-B substrate phenethylamine (PEA) and the MAO-A substrate serotonin (5-HT). With all three MAO inhibitors they were more marked in the brain than in the liver. After 14 days' administration of the totally irreversible inhibitors clorgyline and deprenyl, the dose–response curves for the inhibition of 5-HT and PEA deamination in brain tissue were shifted leftwards by a factor of about 10, as compared with acute treatment. With the partly reversible inhibitor tranylcypromine the factor was about 4.

By contrast, liver tissue showed less marked shifts than in the brain, the factors being 3 for clorgyline and deprenyl, and 2 for tranylcypromine.

This difference is probably related to the fact that the half-life of MAO synthesis is about 2–3 times longer in rat brain (about 10 days) [13, 15, 16] than in rat liver [13].

Owing to the faster turnover of the enzyme in the liver, probably less accumulation of the irreversible inhibitors occurs in this tissue. However, it cannot be excluded that other factors like differential accumulation or metabolism of the drugs in liver and brain might also play a role in the observed differences. The more pronounced cumulative effect of irreversible MAO inhibitors in the brain, is evident upon comparison of the dose–response curves (Figs. 3–8) and even more clearly so from the ED_{50}'s shown in Table 1. Clorgyline, for instance, was about six times more potent in inhibiting 5-HT deamination in brain than in liver when given acutely [8], and 15 times more potent after repeated administration.

The inhibitory effect of deprenyl on PEA deamination was twice as potent in brain as in the liver after acute and 4 times more potent after repeated administration. In contrast, no increase in the potency of the shorter-acting inhibitor tranylcypromine in brain as compared to liver was observed after repeated treatment.

From the data shown in Fig. 1 and similar data on deprenyl reported earlier [12] it seems that a "steady

state" of MAO inhibition is reached earlier after large than after small doses. The increase in potency, especially of small doses, may therefore not be terminated after 14 days. Hence, treatment with small doses of clorgyline, for example, over periods of much longer than two weeks might well lead to a fairly selective inhibition of MAO A in rat brain with negligible inhibition of MAO B in the same organ and of both forms in the liver. If comparable conditions exist in man, which probably largely depends on the relative half-lives of MAO in human brain and peripheral organs similar effects might be obtained by long-term treatment with suitably small doses of clorgyline-like MAO inhibitors.

A second set of experiments was performed to ascertain whether enzyme synthesis in the brain is accelerated by continued blockade. The recovery rates after acute and repeated treatment with high doses of clorgyline or deprenyl (10 mg/kg s.c.) were compared by determining MAO activity in rat brain tissue with the three substrates 5-HT, TYR and PEA at different times after the last injection of the MAO inhibitor, as reported in the literature [13, 15, 16].

From the results summarized in Table 2 it can be concluded that there is no appreciable acceleration of MAO synthesis in rat brain after continuous blockade of the enzyme for more than two weeks. If these results can be extrapolated to man, this finding is reassuring as regards the treatment of depressed patients with MAO inhibitors: it might mean that there is no "rebound" of MAO activity to be expected after the discontinuation of treatment, which might result in a relapse into depression.

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