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THE NATURE OF THE INHIBITION OF RAT LIVER MONOAMINE OXIDASE TYPES A AND B BY THE ACETYLENIC INHIBITORS CLORGYLINE, *l*-DEPRENYL AND PARGYLINE*

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Abstract—The kinetics of inhibition of rat liver mitochondrial monoamine oxidase by clorgyline, *l*-deprenyl and pargyline are consistent with a mechanism whereby a reversible interaction between the inhibitor and the enzyme active site under conditions of thermodynamic equilibrium is followed by a time-dependent formation of the covalently-bound enzyme–inhibitor adduct. The K_i value for the reversible interaction between clorgyline and monoamine oxidase A is about 1000 times lower than that towards the B-form of the enzyme, and this difference is sufficient to account for most, but not all, of the selectivity of the inhibition caused by this compound. The K_i value of the monoamine oxidase B selective inhibitor *l*-deprenyl towards that form of the enzyme is only about 40-fold lower than that towards the A-form. However, in this case, the rate of formation of the irreversible adduct is considerably faster for the B-form than for the A-form and this makes a major contribution to the selectivity of this compound. Pargyline shows a K_i value towards monoamine oxidase B that is only 8 times lower than that towards the A-form and in this case the rates of formation of the enzyme–inhibitor adducts are similar. The significance of these results are discussed in terms of the selective inhibition of monoamine oxidase.

The mitochondrial enzyme monoamine oxidase [amine:oxygen oxidoreductase (deaminating) (flavin-containing), EC 1.4.3.4] (MAO) has been found to exist in two forms, termed MAO-A and MAO-B, in a number of tissues. The A-form of the enzyme is sensitive to inhibition by low (in the nanomolar range) concentrations of clorgyline [*N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy) propylamine], but is not inhibited by *l*-deprenyl (phenyl-isopropyl-methyl-propinylamine) until micromolar concentrations of this inhibitor are used. The reverse is true for the B-form of the enzyme [1, 2]. A great many other compounds have since been shown to be selective towards one or other of the enzyme forms (for review, see Ref. 3), including pargyline (*N*-methyl-*N*-benzyl-propinylamine), a compound structurally related to deprenyl, but which has a less pronounced selectivity towards MAO-B [4]. In the rat liver, 5-hydroxytryptamine (5-HT) is predominantly oxidised by MAO-A whereas β-phenethylamine (PEA) is a substrate for the B-form [5]. At high concentrations of these substrates, however, MAO-B is able to oxidise 5-HT, whilst PEA can be oxidised by MAO-A, albeit to a small extent [6–13].

Clorgyline, *l*-deprenyl and pargyline all belong to the class of acetylenic compounds that act as k_{cat} ('suicide') inhibitors which first interact reversibly with the enzyme to form a non-covalent complex, with subsequent reaction occurring within this complex to form the irreversible, covalently-bound, enzyme–inhibitor adduct (see Refs 3 and 13–16). The reaction between this class of inhibitor and MAO can thus be represented by the mechanism:

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI \xrightarrow{k_2} EI^* \qquad (1)$$

where E and I represent the free enzyme and inhibitor, respectively, EI represents the non-covalently-bound enzyme–inhibitor complex and EI* is the covalent enzyme–inhibitor adduct.

Since the structures of the adducts formed with acetylenic inhibitors are believed to be similar with both forms of monoamine oxidase [17], the selectivity of these compounds must be explicable in terms of kinetic factors alone. Differences in the affinities of the two forms for reversible interaction with an inhibitor, differences in the rates of reaction within the non-covalent complexes to form the irreversibly-inhibited adduct, or a combination of both of these factors could account for the observed selectivities (see Ref. 13). Since the nature of the factors governing selectivity could have particular importance for the design of selective MAO inhibitors, we have studied the kinetics of inhibition of the two forms of MAO by clorgyline, *l*-deprenyl and pargyline in order to elucidate this problem.

MATERIALS AND METHODS

Male Wistar rats of body weight 100–150 g were killed by a blow to the head, and the livers rapidly removed, blotted on filter paper and weighed. All subsequent procedures were performed at 0–4°. The

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livers were homogenised 1 : 5 (w/v) in 0.25 M sucrose, 10 mM potassium phosphate, pH 7.2, in a Dounce homogeniser. The homogenates derived from pairs of rats were pooled and centrifuged at 600 *g* for 10 min to remove nuclei and cell debris. The supernatants were then centrifuged at 15,000 *g* for 10 min to yield mitochondrial pellets, which were washed (by resuspension in the sucrose–phosphate buffer and recentrifugation at 15,000 *g* for 10 min) once, before being resuspended in the sucrose–phosphate buffer to a protein concentration of 5 mg/ml. The mitochondrial fractions were then stored frozen at −20° until used for assay.

MAO activity, determined at 30° and pH 7.2, was determined radiochemically as described elsewhere [18], and the activities, corrected for the efficiencies of extraction of the deaminated metabolites into the organic layer [19] were expressed as nmoles/mg protein/min where appropriate. In experiments designed to assay the activity of a single form of an enzyme, 5-HT at a concentration of 100 μM (for the A-form) and PEA at a concentration of 20 μM (for the B-form) were used. At these substrate concentrations, the substrates can be regarded as being essentially specific for their respective forms in the rat liver [8]. In all cases, care was taken to ensure that product formation was linear with tissue up to the time interval used for the assay so that the values obtained corresponded to the true initial rates and that no time-dependent inhibition of enzyme activity by either the substrate (as has been reported for PEA [20]) or any inhibitor present occurred during the assay.

Protein concentrations were measured by the method of Markwell *et al.* [21], with bovine serum albumin as standard.

[Side-chain-2-^{14}C]5-hydroxytryptamine–creatinine sulphate (5-HT) and [ethyl-1-^{14}C]β-phenethylamine hydrochloride (PEA) were obtained from the Radiochemical Centre (Amersham, U.K.). Clorgyline hydrochloride was a gift from May & Baker Ltd (Dagenham, U.K.). *l*-Deprenyl hydrochloride was a gift from Prof. J. Knoll, Semmelweis University of Medicine (Budapest, Hungary). Pargyline hydrochloride was a gift from Abbott Laboratories Ltd (North Chicago, IL). All other reagents were of analytical grade wherever possible.

RESULTS AND DISCUSSION

Reversible inhibition

The reversible phase of the inhibition of MAO-A and -B (towards 5-HT and PEA as substrates, respectively) by clorgyline, *l*-deprenyl and pargyline was determined in each case by starting the reaction with the addition of enzyme to the substrate–inhibitor mixture. Short incubation times (2 min for PEA, 4–5 min for 5-HT) were used, to ensure that no significant irreversible inhibition of the enzyme by the inhibitors occurred.

The reversible inhibition of MAO activity towards PEA as substrate by clorgyline, *l*-deprenyl and pargyline is shown in Fig. 1A–C. In the case of *l*-deprenyl and pargyline (Fig. 1B and C), the inhibition was competitive, with K_i values of 0.97 and 1.8 μM, respectively. When clorgyline was used as inhibitor,

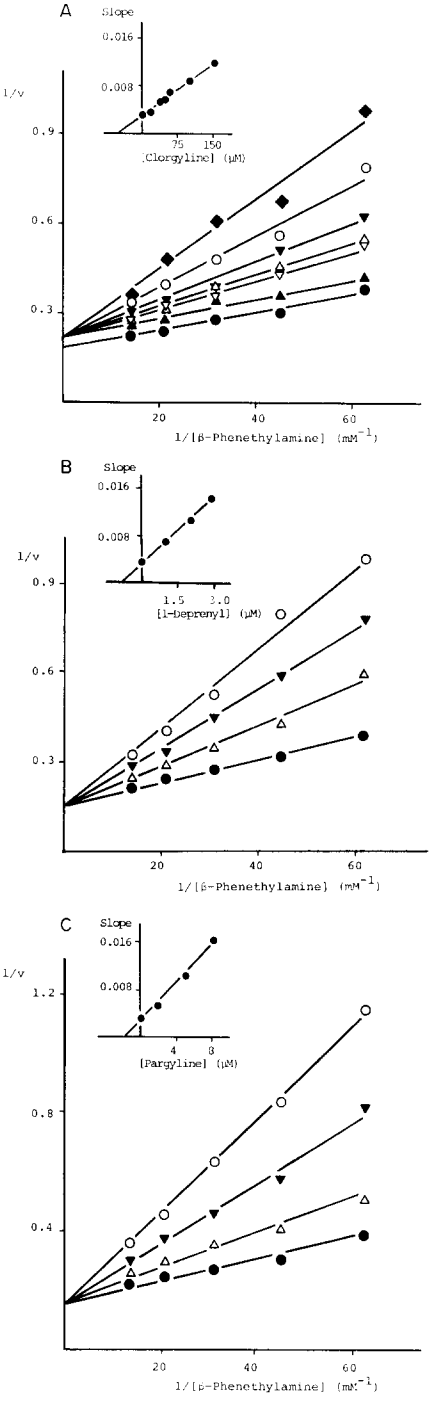

Fig. 1. Double-reciprocal plots of the inhibition of the oxidation of β-phenethylamine by clorgyline, *l*-deprenyl and pargyline. Ordinates: 1/{initial velocity (nmoles/mg protein^{-1}/min^{-1})}; abscissae: 1{β-phenethylamine concentration (mM)}. (A) Samples assayed in the absence (●) and presence of 20 μM (▲), 40 μM (▽), 50 μM (△), 60 μM (▼), 100 μM (○) and 150 μM (◆) clorgyline. (B) Samples assayed in the absence (●) and presence of 1 μM (△), 2 μM (▼) and 3 μM (○) *l*-deprenyl. (C) Samples assayed in the absence (●) and presence of 2 μM (△), 5 μM (▼) and 8 μM (○) pargyline. All values are means of determinations in three mitochondrial fractions, each set to an assay concentration of 0.63 mg/ml. In each case, secondary plots of the slopes (calculated by linear regression analysis) against inhibitor concentration are shown as figure insets.

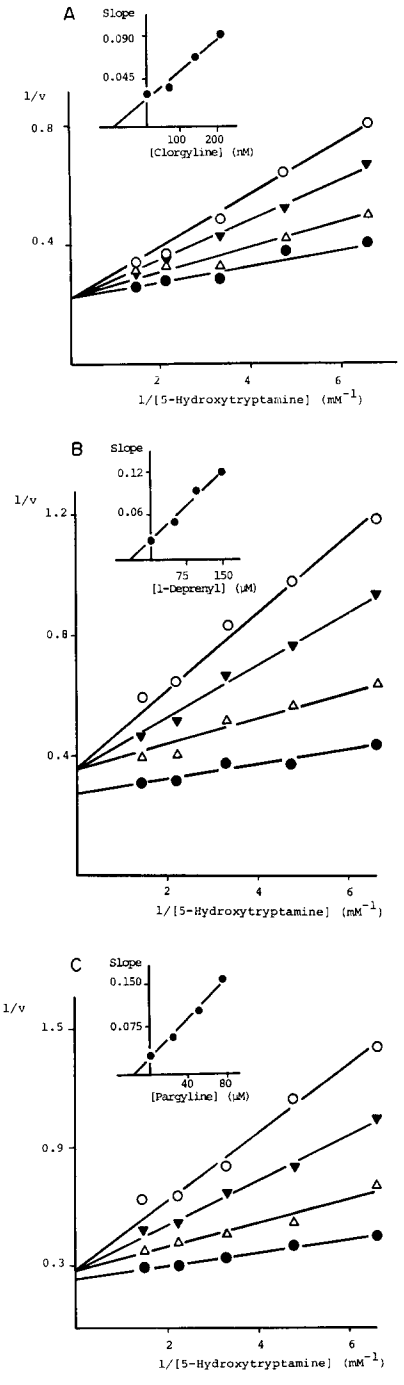

Fig. 2. Double-reciprocal plots of the inhibition of the oxidation of 5-hydroxytryptamine by clorgyline, *l*-deprenyl and pargyline. Ordinates: 1/{initial velocity (nmoles/mg protein/min)}; abscissae: 1/{5-hydroxytryptamine concentration (mM)}. (A) Samples assayed in absence (●) and presence of 65 nM (△), 130 nM (▼) and 195 nM (○) clorgyline. (B) Samples assayed in absence (●) and presence of 50 μM (△), 100 μM (▼) and 150 μM (○) *l*-deprenyl. (C) Samples assayed in absence (●) and presence of 25 μM (△), 50 μM (▼) and 75 μM (○) pargyline. All values are means of determinations in three mitochondrial fractions, each set to an assay concentration of 0.63 mg/ml. In each case, secondary plots of the slopes (calculated by linear regression analysis) against inhibitor concentrations are shown as figure insets.

a competitive interaction was also found for inhibitor concentrations of between 20 and 150 μM, but the line, in the absence of inhibitor, intersected the reciprocal velocity axis at a point lower than the others (Fig. 1A). This result was also found for mitochondrial outer-membrane preparations, but could be eliminated by prior incubation of the membranes at 37° for 240 min with 0.1 μM clorgyline and removal of the excess inhibitor by dilution and centrifugation before assay [13]. Furthermore, a simple competitive interaction was found when benzylamine (which is essentially a substrate for MAO-B alone in the rat liver (see Ref. 22)) was used in place of PEA [13]. The data are thus consistent with the notion that the intercept effect shown in Fig. 1A was due to MAO-A having significant activity towards PEA, but being so sensitive to clorgyline that it was totally inhibited at the lowest concentration of this inhibitor that was used. Assuming this to be the case, it can be calculated from the data in Fig. 1A that the V_{max} of MAO-A towards PEA is 14 ± 2% (mean ± S.E.R., N = 3) of the total V_{max} value. This is in good agreement with the V_{max} value for MAO-A towards PEA of 13% (of total V_{max}) calculated by a different approach [8].

The reversible inhibition of 5-HT activity by clorgyline, *l*-deprenyl and pargyline was also studied, and the data shown in Fig. 2A–C. The inhibition by clorgyline was found to be competitive, with a K_i of 54 nM (Fig. 2A). An intercept effect similar to that observed for the inhibition of activity towards PEA by clorgyline was found for the inhibition of 5-HT by the MAO-B selective inhibitors *l*-deprenyl and pargyline (Fig. 2B and C). Assuming, for the reasons discussed earlier, that this intercept effect was due to the activity of the B-form of the enzyme towards this substrate, the V_{max} of MAO-B towards 5-HT was calculated to be 17 ± 5 and 20 ± 3% (means ± S.E.R., N = 3) of the total V_{max} from the intercepts of the results with *l*-deprenyl and pargyline, respectively (Fig. 2B and C), in line with the value of 11% found previously by an alternative approach [8].

In all cases, secondary plots of the slopes of the graphs against the inhibitor concentrations (shown as insets in Figs 1 and 2) were linear, and the K_i values calculated from these are shown in Table 1. There was a very large difference between the K_i values for clorgyline as inhibitor of the oxidation of 5-HT and PEA, suggesting that the different affinities of the enzyme forms for reversible complex formation with this inhibitor account in large part for the selectivity of this compound. The K_i towards 5-HT is in fact so low that this value may be affected by the nonspecific binding of clorgyline that is found in rat liver [23]. Not surprisingly, therefore, a slightly lower K_i value of 12 nM was found when mitochondrial outer membranes were used [13], since there is a higher ratio of specific : non-specific binding sites in these membranes than in the mitochondrial preparations used in the present study. In any case, these data are in line with K_i values of 66 ± 19 and 200 ± 30 nM (means ± S.E.M., N = 4) for determinations at pH 7.8 and 37° for human brain and rat liver homogenates, respectively, that can be calculated from data given in Refs 24 and 25.

Table 1. Reversible inhibition of rat liver mitochondrial MAO-A and -B by clorgyline, *l*-deprenyl and pargyline

Substrate characteristics	5-HT	PEA
K_m (μM)	110 ± 7	19 ± 0.6
V_{max} (nmoles/mg protein/min)	4.3 ± 0.2	5.9 ± 0.1
K_i (μM) of inhibitors towards the substrates		
Clorgyline	0.054 ± 0.011	58 ± 15
l-Deprenyl	38 ± 2	0.97 ± 0.13
Pargyline	15 ± 3	1.8 ± 0.20

Data as means ± S.E.M. for determinations in three mitochondrial preparations assayed at pH 7.2 and 30°. Protein concentrations at assay were in each case 0.63 mg/ml. Five substrate and three to six inhibitor concentrations were used. In all cases data were plotted as $1/v$ against $1/S$ to calculate K_m, V_{max} and slope values. K_i values were calculated from replots of slope against inhibitor concentration. In all cases, the correlation coefficients of the regression lines used in the calculations were greater than 0.89.

The K_i value of *l*-deprenyl for the B-form of MAO was about 40-fold lower than that towards the A-form (Table 1), but this compound can show a considerably greater selectivity towards the B-form than this after preincubation (e.g. see Ref. 2 for deprenyl inhibition curves). Thus, as discussed later, other factors must play a role in its selectivity. The K_i values for pargyline showed only an eight-fold selectivity for MAO-B (Table 1). However, this compound is not as selective an MAO-B inhibitor as is, for example, *l*-deprenyl [4].

Irreversible inhibition

Irreversible inhibition following the mechanism shown in equation (1) has been analysed by Kitz and Wilson [26]. They showed that if the rate of formation of EI* from EI was slow relative to the dissociation of the EI complex itself, such that the concentration of EI remains in thermodynamic equilibrium with the free inhibitor and enzyme, and the inhibitor concentration is not significantly depleted during the reaction, the rate of irreversible inhibition will be described by the equation:

$$\frac{d[EI^*]}{dt} = k_2[EI] = \frac{k_2([E_t] - [EI^*])}{\frac{K_i}{[I]} + 1} \qquad (2)$$

where I, EI, EI* and k_2 are as defined in equation (1), $[E_t]$ is the total enzyme concentration, and K_i is the dissociation constant of the EI complex (k_{-1}/k_1).

In the present experiments, 350 μl of the mitochondrial fractions at a protein concentration of 2.5 mg/ml^{-1} were incubated with 50 μl of inhibitor at 30°, and 20-μl aliquots were withdrawn at different times and added to 380 μl of assay mixture. Thus, the remaining enzyme activity after a given preincubation time was measured under conditions where the dilution had rendered the degree of reversible inhibition negligible.

Under these conditions, equation (2) may be integrated to give:

$$-k't = \ln\frac{[E_t] - [EI^*]}{[E_t]} = 2.303\{\log_{10}(\%\text{ activity remaining}) - 2\} \qquad (3)$$

where the apparent first-order rate constant for activity loss, k', is given by:

$$k' = \frac{k_2}{\frac{K_i}{[I]} + 1} \qquad (4)$$

Thus k' can be obtained from a graph of $\log_{10}$ (% activity remaining) against preincubation time [equation (3)]. This constant will depend hyperbolically on the inhibitor concentration [equation (4)], and k_2 and K_i may be determined from a plot of $1/k'$ against $1/[I]$. The half-life of enzyme activity at a saturating inhibitor concentration ($t_{\frac{1}{2}}$) may be calculated from the relationship $t_{\frac{1}{2}} = (\ln 2)/k_2 = 0.693/k_2$ (for fuller discussion, see Ref. 27).

When the time-courses of inhibition of MAO-A and -B by pargyline, the inhibition of MAO-A by *l*-deprenyl and the inhibition of MAO-B by clorgyline were determined, the behaviour was found to be in accord with that predicted by these equations, and the kinetic parameters are shown in Table 2. As an example, the data for the inhibition of MAO-A by *l*-deprenyl is shown in Fig. 3A and B. The K_i values calculated in this way are sensibly consistent with those determined by the direct study of the reversible phase of inhibition (Table 1). In addition, the k_2 value for clorgyline is in good agreement with the value obtained in studies with mitochondrial outer-membrane fragments [13], and for an experiment when a high concentration of benzylamine as substrate, rather than dilution, was used to minimise reversible enzyme–inhibitor interactions after the preincubation period [28].

The demonstration of time-dependent inhibition of MAO-A by *l*-deprenyl shown in Fig. 3A contrasts with the results of Egashira *et al.* [29], who reported that *dl*-deprenyl did not cause irreversible inhibition

Table 2. Kinetic constants for the time-dependent inhibition of the activity of MAO-A and -B by clorgyline, *l*-deprenyl and pargyline

Inhibitor and enzyme form	K_i (μM)	k_2 (min^{-1})	$t_{\frac{1}{2}}$ (min)
Clorgyline			
MAO-A	—	>0.76	<0.9
MAO-B	50 ± 10	0.06 ± 0.02	13 ± 4
l-Deprenyl			
MAO-A	25 ± 9	0.14 ± 0.05	7 ± 3
MAO-B	—	>0.99	<0.7
Pargyline			
MAO-A	13 ± 5	0.20 ± 0.06	5 ± 2
MAO-B	0.5 ± 0.08	0.20 ± 0.03	4 ± 0.6

The individual data were plotted as shown in Fig. 3A, and the k' values were calculated by linear regression analysis. $1/k'$ values were plotted against 1/[inhibitor], as shown in Fig. 3B, and the k_2 values (1/intercept on *y*-axis) and K_i values (-1/intercept on *x*-axis) were calculated by linear regression analysis. $t_{\frac{1}{2}}$ values were calculated as 0.693/individual k_2 values. Data are means ± S.E.M. of determinations with three mitochondrial preparations. For inhibition of MAO-A by clorgyline, and MAO-B by *l*-deprenyl, this means of analysis was not applicable (for discussion, see text), and the minimum k_2 values shown are those that could be calculated by use of equation (4) from k' values of 0.60 ± 0.07 and 0.17 ± 0.03 determined with 200 nM clorgyline and 200 nM *l*-deprenyl, respectively. The substrates used to assay for activity of MAO-A and MAO-B were 100 μM 5-HT and 20 μM PEA, respectively.

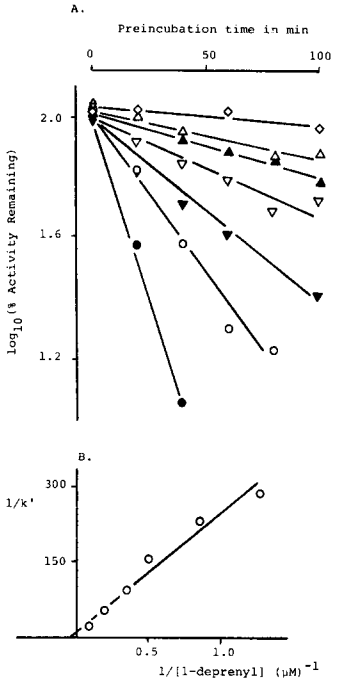

Fig. 3. Time-dependent inhibition of 5-hydroxytryptamine oxidation by *l*-deprenyl. Mitochondrial fractions were preincubated with *l*-deprenyl (protein concentration at preincubation set to 2.2 mg/ml), and after a given time 20-μl aliquots were removed and assayed for activity by addition to 380 μl of an assay mixture containing 100 μM 5-HT. The results represent means of determinations in three mitochondrial preparations. (A) Ordinate, $\log_{10}$ (% activity remaining); abscissa, preincubation time (min). Preincubation concentrations of *l*-deprenyl were 0.3 μM (◇), 0.8 μM (△), 1.2 μM (▲), 2 μM (▽), 3 μM (▼), 5 μM (○) and 10 μM (●). (B) Ordinate, $1/k'$ (calculated from the results shown in panel A by linear regression analysis of the slopes as described in the text); abscissa, 1{*l*-deprenyl concentration (μM)}. The line is that of best fit by regression analysis.

of this form of the enzyme. However, these workers based their conclusions on the behaviour of Ackermann–Potter plots [30], which are unlikely to have the sensitivity to detect small amounts of irreversible inhibition. That the time dependence shown in Fig. 3 was indeed due to irreversible inhibition was confirmed in the present study by the observation that extensive washing of a sample (by centrifugation followed by resuspension) that had been preincubated with *l*-deprenyl failed to give any significant recovery of enzyme activity.

The behaviour of clorgyline and *l*-deprenyl as time-dependent inhibitors of MAO-A and -B, respectively, did not conform to that predicted by equations (3) and (4), in line with a previous observation of the action of *l*-deprenyl on MAO-B [28]. As an example, the inhibition of MAO-A by clorgyline is shown in Fig. 4. At higher concentrations (≥ 25 nM) of clorgyline, the inhibition was apparently first-order with respect to time, but the values of k_2, calculated from each k' value using equation (4), were not constant, but decreased with decreasing inhibitor concentration. For example k_2 values calculated in this way from the individual k' values at 25, 50, 100 and 200 nM clorgyline were 0.15, 0.23, 0.41 and 0.76 min^{-1} (means, N = 3), respectively. A similar situation was found for the inhibition of MAO-B by *l*-deprenyl, although the inhibitor concentration effect was less marked. For example, k_2 values calculated from the individual k' values at 80, 120 and 200 nM *l*-deprenyl were 0.52, 0.68 and 0.99 min^{-1} (means, N = 3), respectively. In addition, with this inhibitor, preliminary experiments suggested that the k' values themselves were dependent upon the enzyme concentration in the assay medium [13].

The most likely explanation for this behaviour is that, at the very low inhibitor concentrations used, there is significant depletion of the free inhibitor as

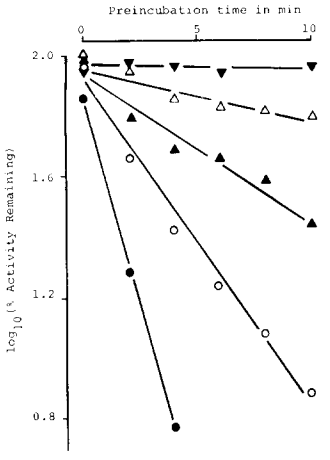

Fig. 4. Time-dependent inhibition of 5-hydroxytryptamine oxidation by clorgyline. The procedure was as described in Fig. 3. Preincubation concentrations of clorgyline were 15 nM (▼), 25 nM (△), 50 nM (▲), 100 nM (○) and 200 nM (●).

a result of non-specific binding to other components in the preparation (see Ref. 23). Inhibitor depletion by enzyme–inhibitor complex formation would lead to a departure from first-order kinetics (see Ref. 28) but it is unlikely that this plays a part in the present study since the inhibitor concentrations used are much higher than the concentration of the enzyme in the membranes (see Ref. 19). However, as a result of the non-specific binding, the value of k_2 calculated from k' at 200 nM clorgyline and *l*-deprenyl must be regarded as a lower limit. Different degrees of non-specific binding could lead to differences between the observed potencies of these inhibitors in crude preparations from different tissues.

Comparison of the values shown in Tables 1 and 2 indicate that, for the inhibition of MAO by clorgyline, the rate of reaction to form the covalent complex occurs at least 10 times more rapidly with the A-form of the enzyme than with the B-form, and this will enhance the already considerable MAO-A selectivity resulting from the greater affinity of that form for the reversible binding of this inhibitor. In the case of *l*-deprenyl, the differences in the affinities of the two forms for non-covalent interactions are not nearly so great and thus the differences (>seven-fold) in the rates of reaction to form the covalent adduct will play a much greater role in determining the selectivity of this compound. The k_2 values for the interaction of the two forms of MAO with pargyline were similar, indicating that the relatively weak selectivity shown by this compound (e.g. see Ref. 4) depends almost completely on the relatively small difference between the affinities of the two forms for non-covalent binding.

The irreversible inhibition of MAO-B by clorgyline (or MAO-A by either *l*-deprenyl or pargyline) is closely analogous to the inhibition of the muscarinic receptor by benzilylcholine mustard [31], in that the irreversible inhibition proceeds via a reversible complex, and that the inhibitor concentration is far greater than the enzyme/receptor concentration. Gill and Rang [31] solved the rate equation for this reaction, so that the combined reversible and irreversible inhibition after any fixed time could be calculated for given inhibitor concentration, k_1, k_{-1} and k_2 values. Since, in the present study, k_2 and K_i (k_{-1}/k_1) values are known, it is possible to use these solutions of Gill and Rang [31] to calculate theoretical inhibition curves using the kinetic parameters in Table 2 and varying the value of k_1 over a wide range whilst making compensatory changes in k_{-1} so that the K_i value is constant. The theoretical inhibition curves calculated in this way for the inhibition of MAO-B by clorgyline (Fig. 5) show that the value of k_1 will indeed affect the response of the system at relatively low values, with the inhibitor sensitivity increasing as k_1 increases. This sensitivity, however, tends to a maximum at k_1 values $> 20{,}000\ M^{-1}\ min^{-1}$, at which point the theoretical curves resemble that found experimentally (Fig. 5). Thus, for the inhibition of the B-form of MAO by clorgyline, $k_1 > 20{,}000\ M^{-1} \cdot min^{-1}$, and the value of k_{-1} must be correspondingly greater than $1\ min^{-1}$ to give a value of K_i of 50 μM. These data indicate that $k_{-1} >> k_2$ which, in agreement with the similarity between the K_i values shown in Tables 1 and 2, validates the equilibrium assumption made in the analysis of equation (1) in terms of equations (3) and (4). Had this assumption not been valid and steady-state conditions applied, similar equations to (3) and (4) might have described the system (see Ref. 32), but the constant K_i would have been more complex and interpretation of its value in terms of the enzyme–inhibitor affinity would not have been appropriate.

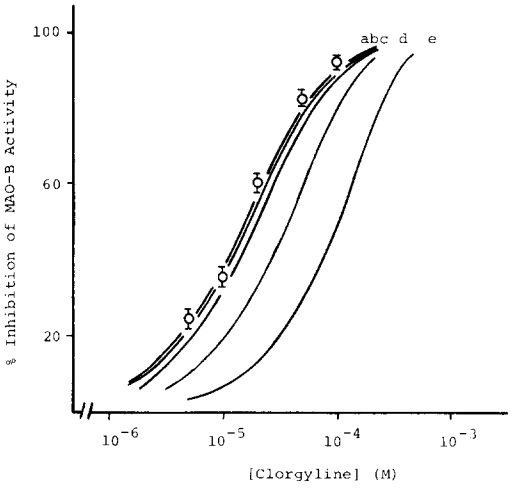

Fig. 5. Theoretical plots of clorgyline inhibition of MAO-B. The curves were calculated by the method of Gill and Rang [31], assuming $K_i = 50\ \mu M$, $k_2 = 0.06\ min^{-1}$ and a preincubation time of 30 min. Curves were drawn for: (a) $k_1 = 2 \times 10^{11}\ M^{-1} \cdot min^{-1}$, $k_{-1} = 10^7\ min^{-1}$; (b) $k_1 = 2 \times 10^4\ M^{-1} \cdot min^{-1}$, $k_{-1} = 1\ min^{-1}$; (c) $k_1 = 5 \times 10^3\ M^{-1} \cdot min^{-1}$, $k_{-1} = 0.25\ min^{-1}$; (d) $k_1 = 10^3\ M^{-1} \cdot min^{-1}$, $k_{-1} = 0.05\ min^{-1}$; (e) $k_1 = 0.25 \times 10^3\ M^{-1} \cdot min^{-1}$, $k_{-1} = 0.0125\ min^{-1}$. In addition, experimental points (○) (as means ± S.E.R. for determinations in three mitochondrial fractions) were determined after 30 min of preincubation, using a low concentration of β-phenethylamine (2 μM) in order to maximise the reversible inhibition present.

The observation that the selectivity of these inhibitors does not reside either simply in differences between the affinities of the two forms for non-covalent binding of the inhibitor or in differences between the rates of reaction within the non-covalent complex but that both these factors may be involved to different extents with individual inhibitors is important for the design of such compounds, since it indicates that modification of a compound known to be a selective reversible inhibitor by incorporating a potentially reactive group will not necessarily result in the selectivity being maintained (see Refs 3 and 33 for discussion).

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