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On the substrate specificities of the two forms of monoamine oxidase

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By use of the selective irreversible inhibitors clorgyline and selegiline [(−)-deprenyl], it is possible to determine the K_m and V_{max} values of the two forms of monoamine oxidase towards a variety of substrates. The validity of this procedure is demonstrated for the deamination of dopamine by rat liver monoamine oxidase -A and -B, and the specificities of the two forms are analysed in terms of their kinetic parameters towards different substrates.

The enzyme monoamine oxidase (MAO, monoamine: oxygen oxidoreductase (deaminating) (flavine containing), EC 1.4.3.4) exists in two forms, termed MAO-A and -B. The activity of MAO-A is inhibited by very low (around nanomolar) concentrations of the acetylenic inhibitor clorgyline, whereas the activity of the B-form of the enzyme is not inhibited until micromolar concentrations of this inhibitor are used (Johnston 1967). The reverse is true for the acetylenic inhibitor selegiline[(−)-deprenyl] (Knoll & Magyar 1972).

By the use of these compounds, it has been possible to study the substrate specificities of the two forms of MAO in a variety of different tissues. Early studies indicated, for example, that while tyramine was a substrate for both forms of MAO in the rat brain, it was metabolized by MAO-A alone in the rat heart and by MAO-B alone in the human platelet (Johnston 1967; Lyles & Callingham 1974: Donnelly & Murphy 1977). In a survey of the literature, it was found that tyramine, generally thought to be a substrate for both forms of MAO, was in fact metabolized by both forms of the enzyme in only 29 out of 57 tissues that had been studied up to that time (Fowler et al 1981).

In 1974, it was shown that the ratio of activities of MAO-A: MAO-B for tryptamine as substrate depended upon the concentration of the substrate used to assay for enzyme activity (Neff et al 1974), suggesting that the K_m of the two forms of this enzyme towards tryptamine were different. Differences in the K_m and V_{max} values of the two forms towards a number of other substrates have since been demonstrated (see Suzuki et al 1981, 1982; Tipton et al 1982), and these may explain, at least partly, the reported variations in the substrate specificities of MAO-A and -B in different tissues.

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In the present study, the technique of selective irreversible inhibition of one or other of the two forms of MAO has been used to determine the kinetic parameters of rat liver MAO-A and -B towards dopamine as substrate. These data have been compared with values for this substrate in other tissues and with those for other substrates in order to provide a new representation of the substrate specificities of the two forms of MAO that may apply to all mammalian tissues.

MATERIALS AND METHODS

Six male Sprague-Dawley rats (AntiCimex AB, Stockholm, Sweden), body weight 150–200 g, were killed by a blow to the head followed by decapitation and the livers were removed and washed in 0.9% NaCl (saline) at 4 °C. Livers were homogenized 1 : 5 (w/v) in 10 mM potassium phosphate buffer, pH 7.4. Homogenates from two livers were in each case pooled and stored frozen until used for assay.

Monoamine oxidase activity was assayed at 37 °C by the method of Otsuka & Kobayashi (1964), with slight modifications as described by Fowler & Strolin Benedetti (1983). In all cases, it was ensured that deamination of substrates was linear with respect to both time and enzyme concentration. In practice, this meant an assay protein concentration of approx. 0.3 mg ml^{-1}, and an incubation time of 12–15 min. Protein concentrations were determined by the method of Markwell et al (1978) with bovine serum albumin as standard.

The radioactive substrates 5-hydroxytryptamine-[side chain-2-^{14}C]-creatinine sulphate, dopamine-[7-^{14}C] hydrochloride and benzylamine-[7-^{14}C] hydrochloride were obtained from Amersham International plc, Amersham, U.K. Non-radioactive substrates (with which the radioactive substrates were mixed to obtain the desired specific activity) were bought as salts, with the exception of benzyl-

amine, which was converted to the hydrochloride by dissolution of the free base in ether followed by addition of ethereal hydrochloric acid. The salt, which precipitated, was then washed with ether and dried. Clorgyline hydrochloride was a gift from May and Baker, Dagenham, U.K. Selegiline [(−)-deprenyl] hydrochloride was a gift from Prof. J. Knoll, Semmelweis University of Medicine, Budapest, Hungary. Amiflamine ((*S*)-(+)-4-dimethylamino-2,α-dimethylphenethylamine-(+)-hydrogen tartrate, FLA 336(+)) was synthesized by Dr L. Florvall, Astra Läkemedel AB, Södertälje, Sweden. All other reagents were standard laboratory reagents of analytical grade wherever possible.

RESULTS AND DISCUSSION

Rat liver homogenates were preincubated at 37 °C and pH 7.4 for 60 min with either clorgyline or selegiline before assay of MAO activity remaining. After this time, it has been shown that further preincubation does not result in any increased inhibition of MAO (see e.g. Fowler et al 1982). The inhibition of the activities towards 5-hydroxytryptamine, benzylamine and dopamine by clorgyline is shown in Fig. 1A. 5-Hydroxytryptamine deamination was inhibited essentially completely at a concentration of 10^{-8} M, a concentration without significant effect on benzylamine deamination. Dopamine, on the other hand, was inhibited in a biphasic manner, with a plateau region at 10^{-8}–10^{-7} M clorgyline, when about 40% of the original activity remained (Fig. 1A). The opposite was obtained for selegiline, with the activity towards benzylamine being inhibited by 10^{-8} M selegiline, that towards 5-hydroxytryptamine being inhibited at higher concentrations, and the activity towards dopamine being inhibited with a plateau where about 60% of the original activity remained (Fig. 1B). These results indicate that 5-hydroxytryptamine is metabolized predominantly by MAO-A, benzylamine by MAO-B and dopamine by both forms of MAO at the concentrations of substrate tested (100 μM), in agreement with previous findings (see e.g. Houslay & Tipton 1974).

Copeland et al (1983) have recently reported that clorgyline increases the binding of spin-labelled amphetamine derivatives to the soluble fraction of rat liver monoamine oxidase. This would suggest that these two compounds interact with different sites on the enzyme and that there is some synergism between them. However, studies with rat liver homogenates and mitochondrial preparations have shown clorgyline to interact with MAO to form an initial non-covalent complex, this interaction being competitive with respect to amine substrates and with a greater affinity for MAO-A (Fowler et al 1982). These studies also showed an initial competitive interaction of selegiline with both forms of

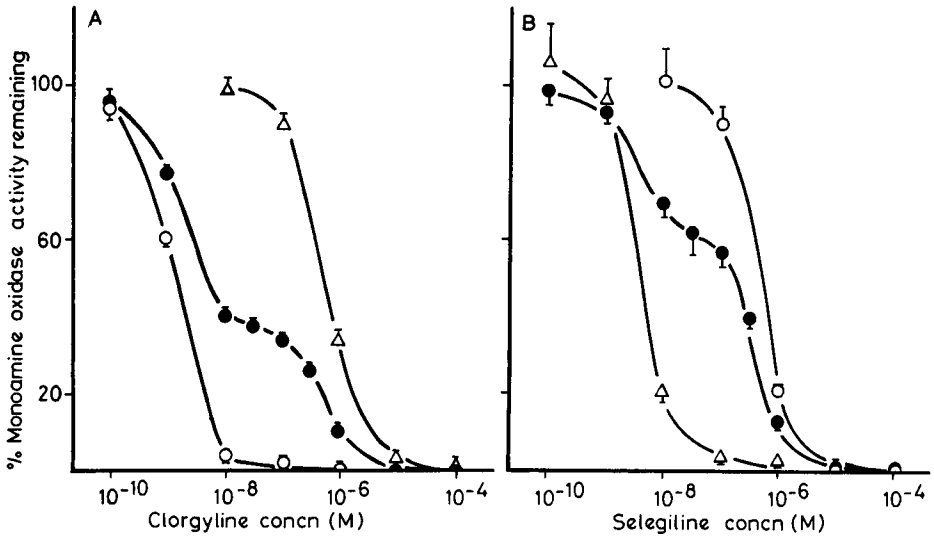

FIG. 1. Inhibition of the deamination of 100 μM 5-hydroxytryptamine (○), 100 μM dopamine (●) and 100 μM benzylamine (△) by: Panel A, clorgyline; Panel B, selegiline. Rat liver homogenates were preincubated for 60 min at 37 °C with the appropriate inhibitor concentration before being assayed for activity. Values are given as means ± s.e.r. (n = 3) of the % activity remaining with respect to samples preincubated for 60 min at 37° with distilled water.

MAO, with a lower K_i value towards MAO-B than towards MAO-A. Amphetamine has been shown to be a competitive inhibitor of MAO with a pronounced selectivity towards the -A form (Mantle et al 1976). In order to investigate any possible effects of clorgyline and selegiline on the potency of inhibition of MAO by a reversible A-selective inhibitor, the effect of pretreatment with these compounds on the inhibition of the enzyme by amiflamine (FLA 336(+)), an α-methyl phenethylamine analogue which has been shown to be a competitive MAO-A selective inhibitor (Ask et al 1982), were studied. Homogenates were preincubated for 60 min at 37 °C with either 3×10^{-8} M clorgyline or 3×10^{-8} M selegiline, concentrations which are on the plateau region of the biphasic dopamine inhibition curves (Fig. 1A and B) and thus can be assumed completely to inhibit the activity of MAO-A or MAO-B, respectively, without significantly affecting the activity of the other form (for further discussion, see e.g. Kinemuchi et al 1980; Tipton et al 1982). After this preincubation period, the inhibitory effects of amiflamine on the MAO activity remaining were studied. The inhibition of benzylamine oxidation by 2×10^{-3} M amiflamine was the same for homogenates pretreated with 3×10^{-8} M clorgyline as for homogenates pretreated with distilled water (Table 1). Similarly, the inhibition of 5-hydroxytryptamine deamination by 10^{-6} M amiflamine was the same for homogenates pretreated with 3×10^{-8} M selegiline as for homogenates pretreated with distilled water (Table 1). Dopamine deamination, divided into its MAO-A and -B components by the selegiline and clorgyline preincubations, respectively, was also inhibited by amiflamine with the same potencies as found with 5-hydroxytryptamine and benzylamine as substrates, respectively, in homogenates pretreated with distilled water (Fig. 2). Thus the rather surprising results of Copeland et al (1983) are not reflected in any changes in the potency of inhibition by this A-selective competitive inhibitor following pretreatment with limiting concentrations of clorgyline or selegiline.

Table 1. Inhibition of 5-hydroxytryptamine and benzylamine oxidation by clorgyline, selegiline and amiflamine. Rat liver homogenates were preincubated for 60 min at 37 °C with either distilled water, 3×10^{-8} M selegiline or 3×10^{-8} M clorgyline. The samples were then further preincubated for 15 min with either distilled water or amiflamine at the concentration given in the table before being assayed for activity with 5-hydroxytryptamine or benzylamine. Values given are means ± s.e.r. (n = 3) of the % activity remaining or % inhibition, as appropriate, with respect to samples treated in the same manner but with distilled water instead of the test compound.

	Substrate: 5-Hydroxytryptamine (100 μM)	Benzylamine (100 μM)
Initial activity (nmol mg protein^{-1} min^{-1})	2·9 ± 0·17	2·3 ± 0·10
% Activity remaining in presence of:		
3×10^{-8} M selegiline	86 ± 5	2 ± 0·2
3×10^{-8} M clorgyline	3 ± 0·5	99 ± 2
% Inhibition by amiflamine		
Amiflamine concentration used:	10^{-6} M	2×10^{-3} M
Homogenates preincubated with:		
Distilled water	48 ± 4	57 ± 2
3×10^{-8} M selegiline	46 ± 0·9	
3×10^{-8} M clorgyline		57 ± 0·6

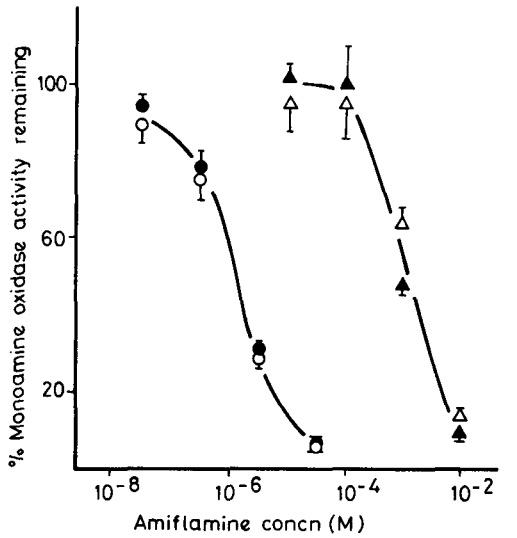

FIG. 2. Inhibition of rat liver MAO-A and -B by amiflamine. Rat liver homogenates were preincubated for 60 min with either distilled water, 3×10^{-8} M selegiline or 3×10^{-8} M clorgyline, then further preincubated for 15 min (to ensure temperature equilibration) with either distilled water or amiflamine before being assayed for activity. Substrates and conditions were: ○, 100 μM 5-hydroxytryptamine as substrate, distilled water-pretreated homogenates; ●, 100 μM dopamine as substrate, 3×10^{-8} M selegiline-pretreated homogenates; △, 100 μM benzylamine as substrate, distilled water-pretreated homogenates; ▲, 100 μM dopamine as substrate, clorgyline-pretreated homogenates. Values are given as means ± s.e.r. (n = 3) of the % activity remaining with respect to homogenates treated with distilled water instead of amiflamine.

After preincubation with either distilled water, 3×10^{-8} M selegiline or 3×10^{-8} M clorgyline, rat liver homogenates were assayed for activity at six different concentrations of dopamine, in order to determine the K_m and V_{max} values of MAO-A and

-B towards this substrate. Double-reciprocal plots of the data are shown in Fig. 3 and the kinetic parameters derived from these plots are given in Table 2. The K_m for MAO-A towards dopamine was lower than that for MAO-B towards this substrate, in agreement with other studies undertaken at pH 7.4 for the rat brain (Schoepp & Azzaro 1981; Fowler & Strolin Benedetti 1983) and for the human cortex at pH 7.4 (Rivett et al 1982) but not at pH 7.2 where the K_m values for the two forms were similar (O'Carroll et al 1983).

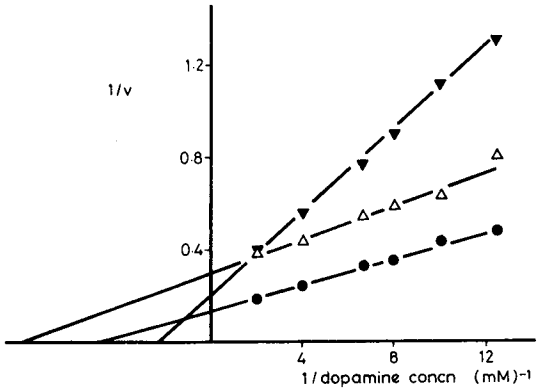

FIG. 3. Double reciprocal plot of dopamine deamination by rat liver MAO-A and -B. Ordinate, 1/(initial velocity in nmol mg protein^{-1} min^{-1}); abscissa, 1/(substrate concentration in mM). Homogenates were preincubated for 60 min with either distilled water (●), 3×10^{-8} M selegiline (△) or 3×10^{-8} M clorgyline (▼) before being assayed for activity. Each point represents the mean activity (n = 3). Regression lines of best fit are given.

Table 2. Kinetic parameters of rat liver MAO-A and -B towards dopamine as substrate.

Pretreatment	MAO form remaining	K_m (μM)	V_{max} (nmol mg protein^{-1} min^{-1})
Distilled water	A + B	220 ± 25	8·0 ± 0·62
3×10^{-8} M 1-Deprenyl	A	140 ± 45	3·6 ± 0·51
3×10^{-8} M Calorgyline	B	430 ± 52	4·9 ± 0·69

Samples were preincubated for 60 min at 37 °C as indicated in the table before being assayed for activity with six different concentrations of dopamine (80, 100, 125, 150, 250, 500 μM). K_m and V_{max} values were calculated by linear regression analysis of the data plotted as 1/v against 1/S. In all cases, the correlation coefficients of the regression lines were greater than r = 0·90. Values are given as means ± s.e.m. for determinations in three homogenates each derived from the livers of two rats.

Studies of the type shown above have indicated that most substrates, even those that have previously been reported to be specific for one or other of the forms of MAO, interact with both forms of the enzyme, but that the K_m value towards one form may be lower (and the V_{max} value higher) than the value for the other form (see e.g. Tipton et al 1982; Suzuki et al 1981). In addition, the relative concentrations of available active centres of the two forms can vary considerably from tissue to tissue, and this can result in differences in substrate specificities of the two forms being found. Because of these effects, many previous studies in which different assay substrate concentrations have been used in different tissues have not given consistent data on the substrate-specificities of MAO-A and -B (see e.g. Fowler et al 1981, for discussion). However, the kinetic parameters of MAO-A and -B towards different substrates can be used to indicate substrate specificity of the two forms of MAO. Ratios of K_m(MAO-B)/K_m(MAO-A) for a variety of substrates reported in the literature and from the present study are shown plotted against their V_{max}(MAO-A)/V_{max}(MAO-B) values in Fig. 4. This plot demonstrates that while no substrates can be considered as being absolutely specific for one or other forms of MAO, certain substrates can be regarded as selective: substrates with high K_m(MAO-B)/K_m(MAO-A) and V_{max}(MAO-A)/V_{max}(MAO-B) values (such as 5-hydroxytryptamine) can be regarded as selective for MAO-A, whereas substrates with low values

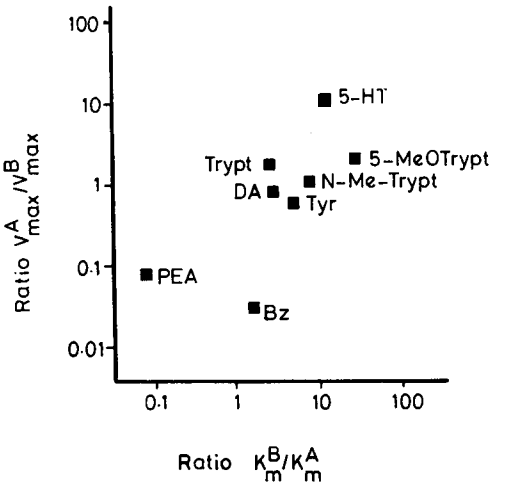

FIG. 4. Substrate specificity of rat liver monoamine oxidase-A and -B. Ordinate, ratio of V_{max}(MAO-A)/V_{max}(MAO-B); abscissa, ratio of K_m(MAO-B)/K_m(MAO-A). Abbreviations and literature sources: DA, dopamine, present study. 5-HT, 5-hydroxytryptamine; Tyr, tyramine; PEA, 2-phenylethylamine; from Tipton et al (1982). 5-MeOTrypt, 5-methoxytryptamine; Trypt, tryptamine; N-Me-Trypt, *N*-methyltryptamine; from Suzuki et al (1981). Bz, benzylamine: estimated value, assuming that 97% of the oxidation is brought about by MAO-B (see Parkinson et al 1980), and that the K_m values for MAO-A and -B towards this substrate are similar to those that can be estimated for the rat heart from the data of Clarke et al (1982).

(such as benzylamine and 2-phenylethylamine) can be regarded as being selective for MAO-B. Fig. 4 also demonstrates that the substrate specificities of the two forms are a continuum, rather than discrete divisions into -A selective, -B selective and 'common' substrates as has been suggested earlier (see e.g. Houslay & Tipton 1974). Fig. 4 can also be adapted to other tissues of the rat, and perhaps also to other species, since previous studies have suggested that for tyramine at least the molecular characteristics of MAO-A and -B towards this substrate are the same in several different tissues (Strolin Benedetti et al 1983). Such an adaptation to other tissues can be made by a parallel shift of the ordinate to higher V_{max}(MAO-A)/V_{max}(MAO-B) values for tissues rich in MAO-A (such as the rat heart) and to lower values for tissues rich in MAO-B.

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