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SUBSTRATE AND INHIBITOR SELECTIVITY OF HUMAN HEART MONOAMINE OXIDASE

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Abstract—The subcellular distribution, inhibitor sensitivity, thermostability and pH profiles of monoamine oxidase (MAO) from samples of human heart obtained at post mortem have been investigated with several substrates. A simple subcellular fractionation showed that, with either tyramine or benzylamine as substrate, about 50 per cent of the MAO activity was found in the mitochondrial fraction, with negligible quantities in the high speed supernatant. From the use of clorgyline, it appears that 5-HT is a substrate for MAO-A, benzylamine and β-phenethylamine are substrates for MAO-B, while tyramine and dopamine are substrates for both forms of the enzyme. *d*-Amphetamine was shown to be a selective competitive inhibitor of MAO-A, of similar potency to that observed with MAO from rat liver. No significant difference between the thermostability at 50° of the MAO activity towards 5-HT and benzylamine was observed. Preliminary results for the effect of pH on human heart MAO are presented. The results are discussed with respect to similar data obtained for MAO from other human and animal tissues.

Nearly all animal tissues contain monoamine oxidase (EC 1.4.3.4; MAO). Although some of the enzyme activity can be found in monoamine-containing neurons, where it is involved in the control of the neuronal content of transmitter, most is located in extraneuronal cells, where it appears to act as a detoxifying enzyme [1]. Data obtained from studies of the thermostability, pH optimum, substrate and inhibitor sensitivities of the enzyme activity, have suggested that MAO may exist in at least two catalytically separable forms [2], which Johnston [3] termed "MAO enzyme A" and "enzyme B". Use of the irreversible inhibitor, clorgyline, together with several monoamines as substrates, has shown that the nature and proportions of the two forms may vary, not only between species, but also between the different organs of the same animal [4]. As a result, extrapolation of conclusions obtained in one species to another must be done with caution. This is of particular importance when the second species is man.

Heart tissue is a relatively rich source of MAO in most animal species so far studied. However, little information is available concerning the nature of this enzyme in the human heart. Since previous work has shown that MAO is relatively stable after death, at least in the brain and in the rat heart [6], a study has been made of the enzyme activity in the left ventricle of human hearts obtained at post-mortem dissection, to determine whether or not it exhibits more than one form of MAO activity. Preliminary results of this work were presented to the British Pharmacological Society [7].

MATERIALS AND METHODS

Samples of human left ventricle were obtained within 48 hr of sudden death and within 1 hr of post-mortem dissection from six subjects, three male and three female (aged 25–89 years). The tissue was freed of fat, coarsely chopped and then homogenised in 10 vol. ice-cold 0.25 M sucrose–10 mM potassium phosphate buffer, pH 7.8, with a Polytron homogeniser (PT-10-35, setting 5, 30 sec). Nuclei and cell debris were sedimented by centrifugation at 600 *g* for 15 min. The supernatant was taken as the crude homogenate. Aliquots of this homogenate were then centrifuged as described in Table 1 to provide the sub-cellular fractions. Washed mitochondrial fractions were prepared by sedimenting the crude mitochondrial fraction twice from sucrose–phosphate buffer by centrifugation at

Table 1. Subcellular distribution of MAO in human heart left ventricle

Fraction	Procedure: RCF (*g*) × time (min)	Pellet(P) or supernatant(S)	Tyramine: Activity (%)	Tyramine: Purification	Benzylamine: Activity (%)	Benzylamine: Purification
Crude suspension	600 × 10	S	100		100	
High speed supernatant	100,000 × 60	S	0		0.5 ± 0.4	
"Light" microsomes	12,500 × 40	S	15.4 ± 1.3		16.6 ± 1.5	
"Heavy" microsomes	7000 × 20	S	13.3 ± 3.9		14.3 ± 0.9	
Mitochondria	7000 × 20	P	48.9 ± 7.0	3.16 ± 0.31	52.7 ± 2.2	3.51 ± 0.44

Mean values (from 4 individuals) ± S.E.R.
Activity expressed as a per cent of the crude suspension
Purification expressed as the increase in enzyme activity related to protein concentration.

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7000 *g* for 20 min. The pellet from the second washing was resuspended in 10 mM potassium phosphate buffer, pH 7.8 and stored at −20° in aliquots for later use. These washed mitochondrial fractions were used in all experiments, other than those described in Table 1.

MAO assay. MAO activities were determined radiochemically by the method of McCaman *et al.* [8] as modified by Callingham and Laverty [9]. Briefly, aliquots of enzyme were incubated with inhibitors as necessary (i.e. clorgyline, 30 min; *d*-amphetamine, 10 min), cooled on ice, and radioactive substrate (1–3 mCi/mM) added in buffer (to a final strength of 100 mM potassium phosphate, pH 7.8, in 100 μl). After incubation at 37° under an atmosphere of oxygen, the reaction was stopped by cooling the tubes on ice and acidifying with 10 μl of 3 N HCl. Deaminated products were extracted in 500 μl of toluene:ethylacetate (1:1, saturated with water) and a 400 μl aliquot taken for liquid scintillation counting with quench correction. When the pH profile of the enzyme was investigated the total amine concentration was 0.5 mM in the final incubation volume with potassium phosphate buffer of the appropriate pH.

Initial velocities were determined by computer program with data from progress curves.

Protein assay. Protein was determined by the method of Lowry *et al.* [10], with bovine serum albumin as standard.

Chemicals. Clorgyline (M&B 9302) was a gift from May & Baker Ltd., Dagenham, Essex.

The radioactive substrates for MAO, [G-^{3}H]-tyramine hydrochloride and [ethyl-1-^{14}C]-β-phenethylamine hydrochloride were obtained from New England Nuclear GMBH, Dreieichenhain, Germany; and [G-^{3}H]-5-hydroxytryptamine creatinine sulphate, [ring-G-^{3}H]dopamine hydrochloride and [3-^{3}H]benzylamine hydrochloride (custom preparation) from The Radiochemical Centre, Amersham, U.K. All other chemicals were standard laboratory reagents of analytical grade where possible.

RESULTS

Simple subcellular fractionation of homogenates of human heart left ventricular tissue from four individuals, showed that about 50 per cent of the MAO activity against either tyramine or benzylamine was found in that fraction which sedimented at 7000 *g* for 20 min (Table 1). With both substrates, a significant amount of activity could be separated in the crude microsomal fraction. The high speed supernatant fraction contained very little deaminating activity which could only be detected with benzylamine as substrate; neither tyramine nor 5-HT were metabolised by this fraction. This activity was totally inhibited by 10^{-3} M clorgyline, but not by 10^{-3} M semicarbazide.

Clorgyline was a more potent inhibitor of the MAO activity towards 5-hydroxytryptamine (5-HT) than of the activity towards benzylamine and β-phenethylamine. For example, preincubation of the enzyme with 10^{-9} M inhibitor for 30 min at 37° prior to the addition of substrate, reduced the activity towards 5-HT by about 50 per cent, while 10^{-5} M was required to produce the same degree of inhibition when either benzylamine or β-phenethylamine was used (Fig. 1).

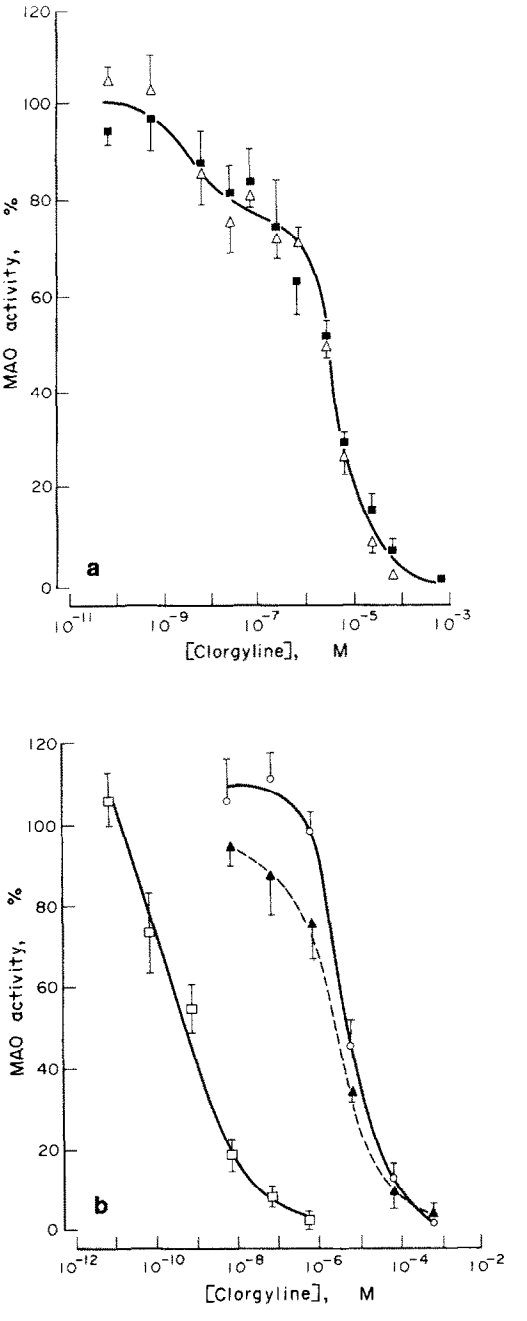

Fig. 1. The effect of clorgyline on the *in vitro* activity of MAO in mitochondrial fractions of human heart left ventricle. Each point represents the mean MAO activity (± S.E.R., n = 6) assayed in triplicate at each inhibitor concentration. Enzyme activities are expressed as percentages of the activity of MAO in the absence of inhibitor. (a) tyramine (■), or dopamine (△) as substrate. (b) 5-HT (□), benzylamine (○), or β-phenethylamine (▲) as substrate. The final concentration of substrate was 0.5 mM in all cases.

When tyramine and dopamine were used as substrates, the profiles of plots of the enzyme activities against log clorgyline concentrations appeared to be biphasic, with plateau regions at about 80 per cent of control activity (Fig. 1). These contrast with the simple sigmoid shapes of the curves obtained with 5-HT, benzylamine and β-phenethylamine as substrates.

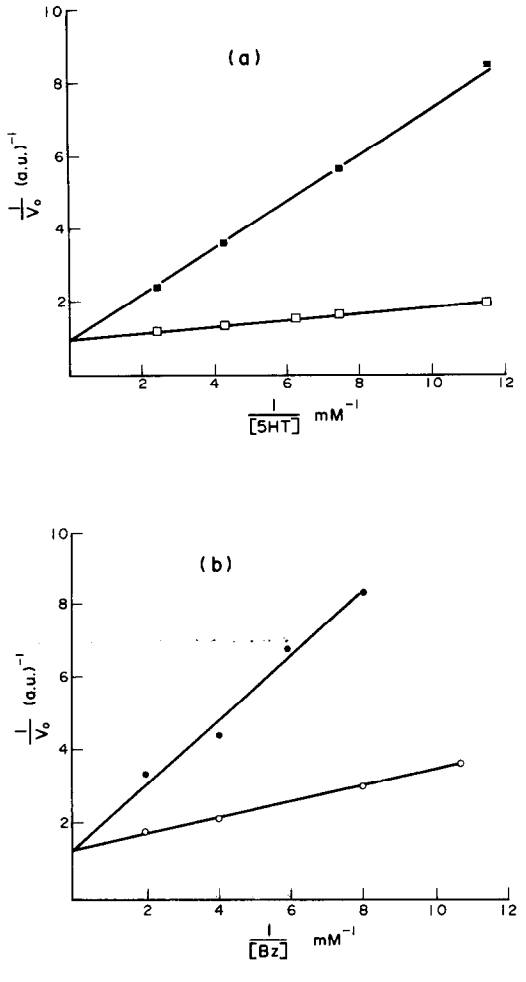

Fig. 2. The inhibition of human heart mitochondrial MAO activity by *d*-amphetamine. Representative double reciprocal plots. Initial velocities in arbitrary units (a.u.) at each concentration were calculated from progress curves by computer program. (a) 5-HT as substrate, with (■) or without (□) 3×10^{-5}M *d*-amphetamine. The K_i value from this determination was 5.4×10^{-6}M. (b) benzylamine as substrate, with (●) or without (○) 1×10^{-3}M *d*-amphetamine. The K_i value from this determination was 3.3×10^{-4}M.

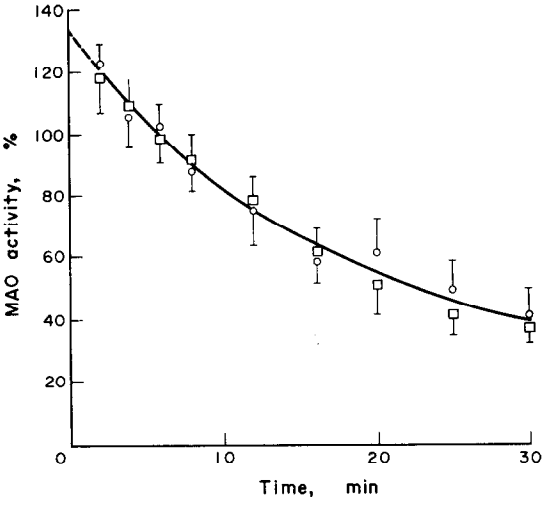

Fig. 3. The effect of prior incubation at 50° on the *in vitro* activity of MAO in human heart mitochondrial fractions. The activities are expressed as mean percentages (± S.E.) of the activity of the enzyme without prior incubation. Assays were performed in triplicate for each exposure time on mitochondrial fractions from 6 individuals. 5-HT (□), or benzylamine (○) as substrate at 0.5 mM final concentration.

The K_m values, calculated from Lineweaver–Burk plots, for the substrates, 5-HT, benzylamine and β-phenethylamine, were closely similar to each other (Table 2). However, *d*-amphetamine was found to be a competitive inhibitor of both 5-HT and of benzylamine deamination in mitochondrial fractions, while pH-independent K_i values (K_i') for *d*-amphetamine with 5-HT and benzylamine as substrates were found to be $4.82 \pm 0.71 \times 10^{-8}$ M and $1.24 \pm 0.61 \times 10^{-6}$ M respectively, when calculated by the method of McEwen *et al.* [11] (assuming a pK_a of 9.93 for amphetamine [12]). Table 2 also compares these results with those obtained by Mantle, Tipton and Garrett [13] for MAO from rat liver.

The sensitivity of the MAO activity to inhibition by heat was examined by incubating mitochondrial fractions at 50° for various time intervals before the addition of either 5-HT or benzylamine (Fig. 3). No differ-

Table 2. Substrate and inhibitor constants for human heart mitochondrial MAO

		d-Amphetamine			
		Human heart		Rat liver	
Substrate	K_m(M)	K_i(M)	K_i'(M)	K_i(M)*	K_i'(M)
5-HT	1.32×10^{-4} (6) ± 0.02	8.25×10^{-6} ± 1.21 (6)	4.82×10^{-8} ± 0.71	2.0×10^{-5}	3.68×10^{-8}
Benzylamine	1.51×10^{-4} (4) ± 0.40	2.12×10^{-4} ± 1.05 (3)	1.24×10^{-6} ± 0.61	7.7×10^{-4}	1.43×10^{-6}
β-phenethylamine	1.97×10^{-4} (3) ± 0.46				

Mean values (± S.E.M.) with number of individual values in parentheses obtained from reciprocal plots derived from triplicate determinations of 5 substrate concentrations. K_i' is the pH independent K_i value (for explanation see text).

* Data of Mantle *et al.* [13] determined at pH 7.2.

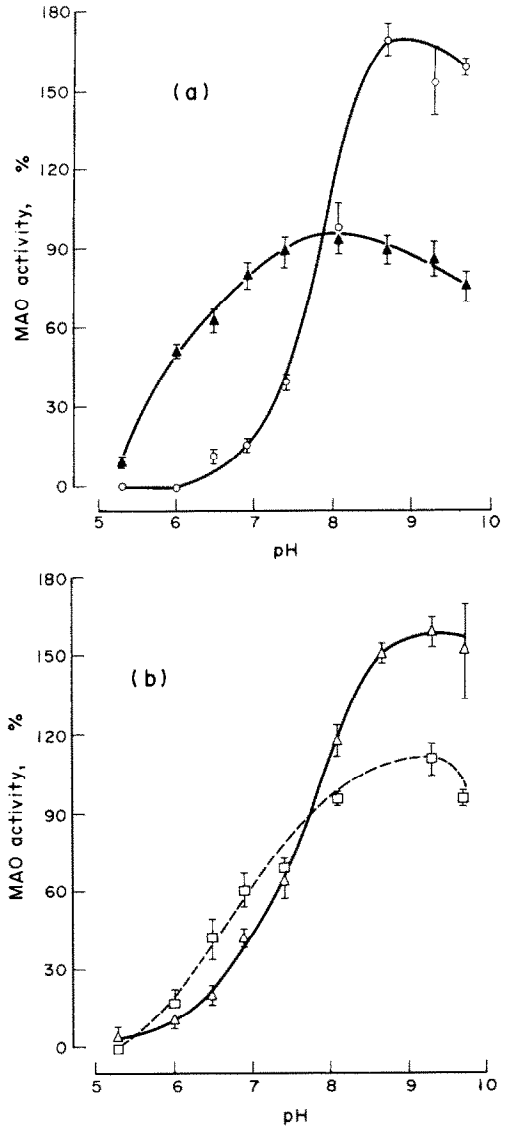

Fig. 4. The effect of change of pH of the incubation medium (100 mM potassium phosphate) on the activity of MAO in mitochondrial fractions of human hearts. The activities are expressed as mean percentages (± S.E.R.) of the activity of the enzyme at pH 7.8 for data from 6 individuals. (a) Benzylamine (○), or β-phenethylamine (▲) as substrate. (b) 5-HT (□), or tyramine (△) as substrate. The total concentration of substrate was 0.5 mM in all cases.

ence could be detected in the sensitivity of the enzyme activity to heat inactivation between the two substrates. An apparent increase in MAO activity was seen following short periods of exposure at 50°.

The pH "optimum" of the activity of the MAO towards tyramine, 5-HT, benzylamine and β-phenethylamine was determined with 5 min incubation periods at 37° in an atmosphere of oxygen over a pH range of 5.3–9.7. The data, presented in Fig. 4, are expressed as percentages of the enzyme activity at pH 7.8 for each substrate and are uncorrected for the ionisation of the substrate. Maximum activity towards 5-HT, tyramine and benzylamine was observed at the higher pH values, greater than 8.5. However with β-phenethylamine as substrate maximum activity was seen at pH 7.8.

DISCUSSION

From these results, it appears that the oxidative deamination of monoamines in mitochondrial fractions of human heart left ventricular tissue is almost entirely brought about by MAO. However, the use of the selective inhibitor, clorgyline and the convention introduced by Johnston [3], which designates that form of the enzyme that is more sensitive to the inhibitor, as MAO-A and the less sensitive form as MAO-B, enables classification of the substrate specificities to be made. 5-HT is a substrate for MAO-A, benzylamine and 2-phenethylamine are substrates for MAO-B while tyramine and dopamine are deaminated by both forms of the enzyme. No clorgyline resistant activity such as that found in rat heart [14] was seen in the human heart tissue. Although a significant amount of MAO activity was found in microsomal fractions, it is probable that a large proportion of this activity was due to the presence of mitochondrial fragments produced during the homogenization of this very tough tissue. The small amount of clorgyline-sensitive activity towards the MAO-B substrate, benzylamine, could also originate from the mitochondrial membrane due to sonication produced in the homogenization procedure. Other tissues that are homogenized with difficulty such as the rat heart show a similarly high proportion of MAO activity in microsomal fractions [14–16].

The clorgyline-determined substrate specificities for MAO from the human left ventricle are similar to those found for human liver [17] and human brain [17, 18]. Although Glover *et al.* [19] have reported that dopamine is a substrate for MAO-B in human brain, White and Glassman [20] found that dopamine is metabolised by both types of MAO in human brain mitochondria.

These results could not have been predicted by extrapolation from results obtained with the rat heart, where tyramine, 5-HT and β-phenethylamine are all substrates of MAO-A, and only benzylamine deamination is inhibited in a biphasic manner characteristic of both enzyme forms [14]. The substrate specificity of both rat brain and liver more closely resembles that reported here for the human heart, although the K_m value for rat liver MAO towards 2-phenethylamine is lower (about 2×10^{-5}M, [20]).

Mantle, Tipton and Garrett [13] reported that *d*-amphetamine was a competitive inhibitor of the deamination, by MAO, of both 5-HT and benzylamine but showed a 35-fold selectivity towards the inhibition of 5-HT metabolism to that of benzylamine with K_i values of 2×10^{-5}M and 7.7×10^{-4}M respectively. We have shown a similar substrate selective inhibition of human heart MAO by *d*-amphetamine with K_i values of 8×10^{-6}M and 2×10^{-4}M for MAO-A and MAO-B respectively. Mantle *et al.* [13] determined the inhibitor constants at pH 7.2, while the values we have reported here were determined at pH 7.8. The apparent differences between K_i values determined here and those determined by Mantle *et al.* [13] can be resolved if it is assumed that only the non-protonated form of the inhibitor can associate with the enzyme [11]. Calculation of the pH-independent inhibitor constant (K_i') for *d*-amphetamine, produces similar values for rat liver and human heart (Table 2).

The use of simple optima curves to suggest enzyme multiplicity for MAO has been criticised by Houslay,

Tipton and Youdim [2]. However our results do suggest that at least with tyramine, 5-HT and benzylamine, it is the non-protonated form of the amine which is susceptible to deamination since preliminary results show that the enzyme is relatively stable over the pH range 6.5–9. The results obtained with β-phenethylamine as substrate are more difficult to interpret, in particular the significant amount of activity observed at pH values below 7, and the apparent maximum at pH 7.8. While interpretation of this data must wait upon a detailed kinetic analysis of the effect of pH on MAO, it is of interest that the deamination of two amines, benzylamine and β-phenethylamine, with similar pK_a values (9.35 and 9.83 respectively, [22]) should have such different pH profiles.

Finally, observations of the relative stability of MAO activity at high temperatures towards different substrates has been used to support the hypothesis of multiplicity (see [2]). There does not appear to be any difference between the thermostability at 50° of MAO activity towards 5-HT or benzylamine of human heart mitochondria.

A common problem encountered in studies such as this, where human material obtained after death is used, concerns the uncertainty that the results represent the state of affairs in life. There have been several reports that the MAO activity in the brain is relatively stable after death [5], and we have previously found that there is little change in the specific activity of the MAO towards several substrates in rat brain, liver and heart, after storage at 4° for periods up to 24 hr after death [6]. In addition there were no significant changes in the profiles of the biphasic inhibition curves produced with clorgyline towards the appropriate substrate (tyramine in liver and brain and benzylamine in heart). It seems unlikely, therefore, that the results presented here will be greatly different from those that would be obtained immediately after death.

In conclusion, it appears that the mitochondrial MAO activity of the human heart in its substrate specificity and inhibitor sensitivity more closely resembles the MAO in the rat liver than in the rat heart.

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