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THE OXIDATION OF TRYPTAMINE BY THE TWO FORMS OF MONOAMINE OXIDASE IN HUMAN TISSUES

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Abstract—The selective monoamine oxidase inhibitors clorgyline and (−)-deprenyl have been used to determine the activities of monoamine oxidase-A and -B towards tryptamine in several human tissues. The results were compared with those obtained with the A-form-selective substrate 5-hydroxytryptamine, the B-form-selective substrate 2-phenethylamine and the common substrate tyramine. Tryptamine was found to be a substrate for both forms of the enzyme in human liver, kidney cortex and medulla and in seven different brain regions. The K_m values of the two forms towards this substrate were similar in all the human tissues examined but the maximum velocities differed. Thus the A-form would contribute approximately 50% of the total monoamine oxidase activity towards this substrate in human cerebral cortex, whereas it would contribute about 60% in kidney cortex and medulla and 75% in liver. These results suggest that both forms of monoamine oxidase would contribute to the metabolism of tryptamine in human tissues and are difficult to reconcile with suggestions that tryptamine excretion may provide a simple index of monoamine oxidase-A inhibition.

In many tissues monoamide oxidase (monoamine: oxygen oxidoreductase (deaminating) (Flavin-containing) EC 1.4.3.4—MAO) is present in two forms, termed the A- and B-forms, that differ in their substrate specificities and inhibitor sensitivities (for reviews see refs 1–5). Inhibitors of the A-form have been shown to be effective antidepressants [6, 7] and this has been attributed to 5-hydroxytryptamine (5-HT) being essentially a specific substrate for that form of the enzyme. The situation with respect to selective inhibitors of MAO-B is less clear. They have been reported not to be antidepressants [8–10] but this has been contested by others [11, 12] and it may be that the type-B inhibitors are only effective in a specific sub-group of depressions [13].

It is possible to monitor the effectiveness of treatment with MAO-B inhibitors in the human by determining their effects on the activity of the enzyme in blood platelets (see ref. 14). However, since the human platelet enzyme is only the B-type [15, 16] this is not suitable for monitoring the effects of the MAO-A inhibitors or for checking whether MAO-B inhibitors retain their selectivity during chronic treatment. Bieck *et al.* [17, 18] have suggested that determination of the urinary excretion of tryptamine may give a measure of the effectiveness of monoamine oxidase-A inhibition. They attributed this to tryptamine being a more specific substrate for MAO-A in human kidney under *in vivo* conditions.

In order to investigate this hypothesis further we have studied the specificities of the two forms of monoamine oxidase towards this amine in human brain, kidney and liver. The activities towards 5-hydroxytryptamine and 2-phenethylamine, preferred substrates for MAO-A and -B respectively, and tyramine, a substrate for both forms (see refs. 1–5), were also determined. A preliminary report of part of this work has been published [19].

MATERIALS AND METHODS

Tissue preparations. Human tissue was obtained within 15 ± 3 hr of death from individuals with no known histories of psychiatric illness or an antidepressant therapy. Brains were dissected into defined regions, homogenised and stored at −20° until use, as previously described [20]. The age range of the individuals (3 male, 2 females) was 25–70 years. Kidney was divided into medulla and cortex which were separately homogenised in 4 vol. of 0.1 M potassium phosphate buffer, pH 7.2, and stored at −20° until use. Liver samples were homogenised and stored in the same way.

Assay. Monoamine oxidase activities were determined by a modification (see ref. 20) of the method of Otsuka and Kobayashi [21]. The assay mixture contained 0.3 ml 0.1 M potassium phosphate buffer, pH 7.2, 0.1 ml tissue preparation and 0.1 ml radioactively-labelled substrate. Reactions were carried out in 25 ml universal tubes equilibrated in a shaking waterbath at 37°. The buffer-tissue preparation mixture was allowed to equilibrate under these conditions for 10 min before the reaction was started by the addition of 0.1 ml of the tissue homogenate. After a fixed time the reaction was stopped by the addition of 0.5 ml 2 M citric acid. Radioactive products were extracted into 10 ml toluene: ethylacetate (1:1 v/v) containing 0.6% (w/v) 2,5-diphenyloxazole (PPO). The aqueous layer was allowed to freeze at −20° and the organic layer was poured off into a vial for scintillation counting. Values for the extraction efficiencies determined by

Fowler *et al.* [22] were used to allow activities to be expressed in absolute terms.

Time-courses were determined in all cases to ensure that the reaction times used were within the range where product formation was linear with time. The initial velocity of the reaction was also shown to be proportional to the concentration of tissue sample over the ranges used in this work.

Protein concentration was determined by the method of Markwell *et al.* [23].

Treatment with inhibitors. For determining the responses to the selective inhibitors clorgyline and (−)-deprenyl (Selegiline), the tissue preparation was incubated with the indicated inhibitor concentration for 60 min at 37° before the activity was determined. Control samples were incubated under the same conditions with the same volume of water. For determining the activities of the A and B forms separately the tissue preparations were preincubated with appropriate concentrations of (−)-deprenyl or clorgyline necessary to cause complete inhibition of the other form. The concentrations of clorgyline that were found to cause complete and selective inhibition of the A-form were 0.3 μM for the brain (see ref. 20) and kidney and 3.0 μM for liver. For inhibition of the B-form without affecting the activity of the A-form the concentrations of (−)-deprenyl were 0.1 μM for brain (see ref. 20), 1.0 μM for kidney and 0.3 μM for liver.

Chemicals. Clorgyline was a gift from May & Baker Ltd (Dagenham, Essex, U.K.) and (−)-deprenyl was given by Professor J. Knoll Semmelweis, University of Medicine, Budapest, Hungary. [^{14}C]-labelled amine substrates were obtained from Amersham International plc.

RESULTS

Human brain

Figures 1a and b show the inhibition by clorgyline and (−)-deprenyl of the monoamine oxidase activities in homogenates of human cerebral cortex towards 100 μM 5-hydroxytryptamine, 20 μM 2-phenethylamine and 100 μM tryptamine. The curves obtained with the MAO-A selective inhibitor clorgyline [24] and the MAO-B selective inhibitor (−)-deprenyl [25] are consistent with 5-HT being a substrate for the former enzyme form and 2-phenethylamine being a substrate for the latter at the concentrations used here (see refs 20, 26–28).

The activities of the two forms in homogenates from seven different brain regions are shown in Table 1. Figure 2 shows the effects of substrate concentration on the oxidation of tryptamine by the two forms in human cerebral cortex, and the K_m values and maximum velocities determined for this substrate, 5-HT, 2-phenethylamine and tyramine are shown in Table 2.

Table 1. Substrate specificities of the two forms of monoamine oxidase in human brain regions

Brain region/ substrate	Total specific activity (pmol · min^{-1} mg^{-1})	% Activity due to: MAO-A	MAO-B
Cerebral cortex			
5-HT	618 ± 60	92 ± 5	3 ± 1
Tryptamine	738 ± 71	55 ± 2	50 ± 2
PEA	666 ± 56	4 ± 1	104 ± 11
Cerebellar cortex			
5-HT	330 ± 42	89 ± 6	8 ± 3.5
Tryptamine	483 ± 62	49 ± 4	44 ± 1
PEA	464 ± 104	4 ± 0.5	96 ± 4
Pons			
5-HT	593 ± 53	91 ± 2.5	1 ± 1
Tryptamine	898 ± 138	44 ± 1.75	62 ± 3.5
PEA	1558 ± 337	6 ± 0.75	94 ± 4
Caudatus			
5-HT	529 ± 66	92 ± 0.25	3 ± 1
Tryptamine	850 ± 86	39 ± 3	62 ± 4.5
PEA	1701 ± 243	7 ± 1	94 ± 6
Substantia Nigra			
5-HT	560 ± 65	99 ± 2	4 ± 2
Tryptamine	831 ± 133	47 ± 3	63 ± 2.5
PEA	1589 ± 287	8 ± 1	101 ± 3
Medulla oblongata			
5-HT	456 ± 54	93 ± 3.5	2 ± 1
Tryptamine	855 ± 107	37 ± 4	74 ± 1
PEA	1523 ± 113	8 ± 1	104 ± 2
Hypothalamus			
5-HT	654 ± 8	92 ± 2	12 ± 6
Tryptamine	879 ± 37	51 ± 4	56 ± 2.5
PEA	1224 ± 57	8 ± 3.5	105 ± 1

Values are means ± S.E. of determinations carried out in three separate tissue samples, each assayed in triplicate.

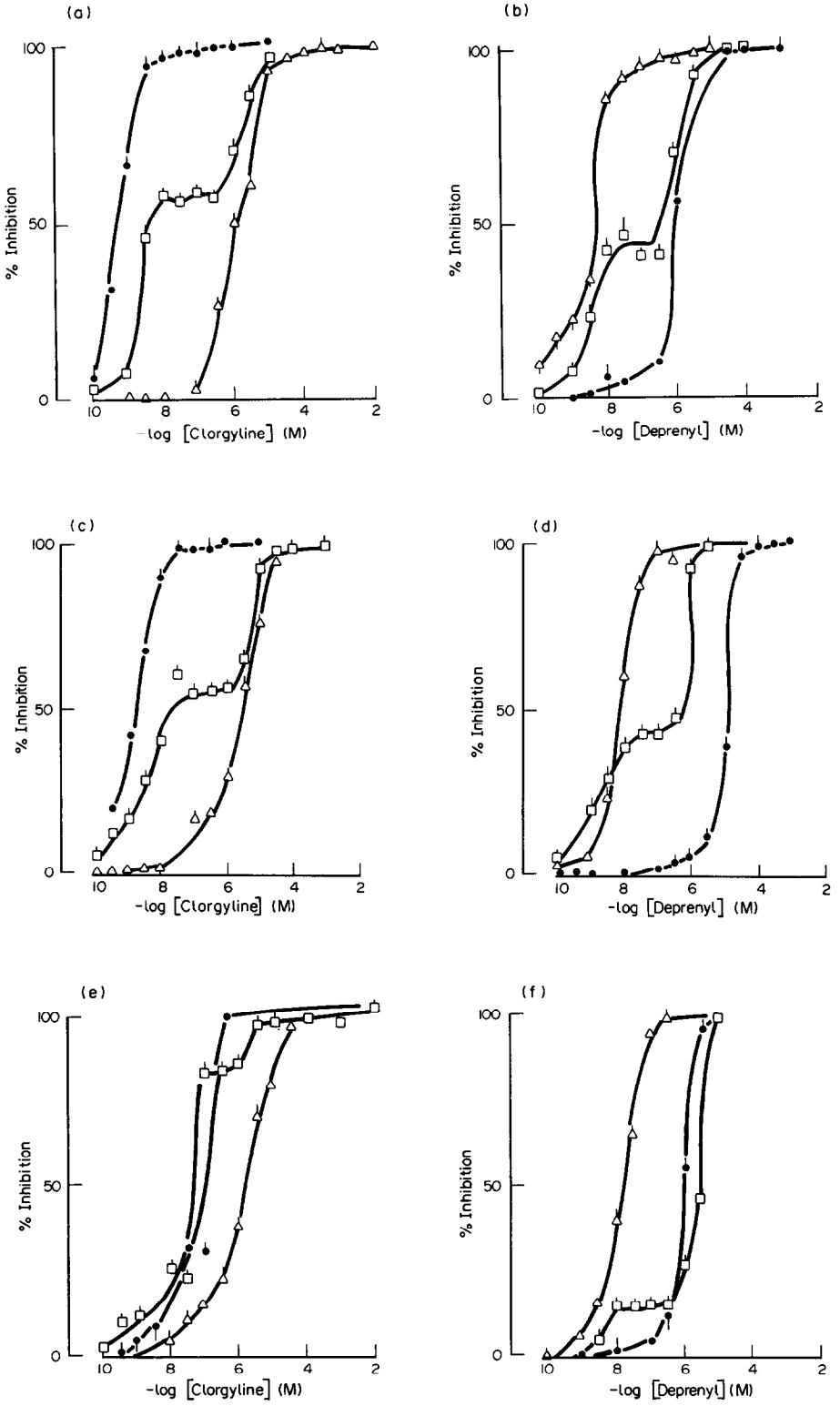

Fig. 1. Inhibition of monoamine oxidase from human tissues by clorgyline (a, c, e) and (−)-deprenyl (b , d, f). Samples of the homogenates were incubated with the indicated inhibitor concentrations for 60 min at 37° before the activities towards 100 μM 5-HT (●), 20 μM 2-phenethylamine (△) and 100 μM tryptamine (□) were determined. Each point represents the mean ± S.E.R. of duplicate determinations made with each of three separate homogenates of the percentage of monoamine oxidase activity remaining with respect to control samples preincubated under the same conditions with a corresponding concentration of water before activity determination. The tissue homogenates were prepared from human cerebral cortex (a, b), kidney cortex (c. d) and liver (e. f).

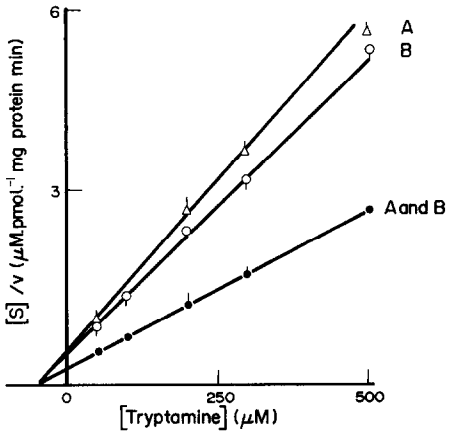

Fig. 2. Half-reciprocal plots of the activities of monoamine oxidase forms from human cerebral cortex towards tryptamine. Samples were preincubated for 60 min at 37° with water for determination of the combined MAO-A plus MAO-B activity, (●) 0.3 μM clorgyline for determination of MAO-B activity (○), or 1.0 μM (−)-deprenyl for determation of MAO-A activity (△) before determination of the activities towards tryptamine. Each point is the mean value ± S.E.R. for determination in three separate homogenates. The lines are the regression lines of best fit.

Human kidney

The inhibition of the activities in homogenates from kidney cortex and medulla towards 5-HT and 2-phenethylamine by clorgyline and (−)-deprenyl (see Fig. 1c and d) confirmed these amines to be substrates for the A- and B-forms of MAO, respectively in these tissues. Tryptamine, at an assay concentration of 100 μM was found to be a substrate for both forms of the enzyme as shown in Table 3 and the kinetic constants, determined from plots similar to those shown in Fig. 2, are shown in Table 2.

Human liver

The inhibition of the activities towards 5-HT, 2-phenethylamine and tryptamine by clorgyline and (−)-deprenyl (Fig. 1e and f) shows tryptamine also to be a suitable substrate for both forms of MAO in this organ but with the A-form making a somewhat larger contribution to the total activity (see Table 3). The kinetic constants determined for these substrates and tyramine are shown in Table 2.

DISCUSSION

Although the oxidation of tryptamine by monoamine oxidase in the rat has been the subject of several different studies, the results have been conflicting in that it has been reported to be a substrate primarily for the A-form [29], for both forms [30] and primarily for the B-form [31]. To some extent this discrepancy may have been due to organ differences since Fuller [29] has shown tryptamine to be a preferred substrate for MAO-A in rat brain and heart but to be a substrate for both forms in rat liver. Differences in the assay concentrations of tryptamine might also have accounted for the reported differences and Suzuki *et al.* [30] have reported rat liver

Table 2. Kinetic parameters for amine oxidation in human organs

Substrate	MAO-form	Cerebral cortex		Kidney cortex		Kidney medulla		Liver	
		K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
5-Hydroxytryptamine	A & B	231 ± 24	3367 ± 167	200 ± 30	6666 ± 208	165 ± 15	4285 ± 34	230 ± 38	8333 ± 104
5-Hydroxytryptamine	A	253 ± 18	3598 ± 104	218 ± 14	5894 ± 382	180 ± 40	4428 ± 82	225 ± 38	7853 ± 89
2-Phenethylamine	A & B	7 ± 1	1964 ± 78	7 ± 1	2187 ± 60	8 ± 1	2245 ± 34	14 ± 1	4405 ± 242
2-Phenethylamine	B	4 ± 2	1727 ± 80	5 ± 2	1864 ± 100	7 ± 2	2086 ± 62	9 ± 2	3921 ± 87
Tyramine	A & B	145 ± 40	4734 ± 326	275 ± 40	21012 ± 323	214 ± 30	10460 ± 426	187 ± 30	9876 ± 128
Tyramine	A	165 ± 30	2985 ± 184	183 ± 25	8098 ± 483	185 ± 45	5285 ± 149	165 ± 15	7500 ± 148
Tyramine	B	135 ± 15	3076 ± 201	360 ± 30	14714 ± 630	250 ± 15	4652 ± 296	180 ± 20	2456 ± 219
Tryptamine	A & B	37 ± 5	1841 ± 57	34 ± 5	3184 ± 84	26 ± 6	2880 ± 60	43 ± 5	5996 ± 151
Tryptamine	A	35 ± 6	955 ± 36	28 ± 7	2262 ± 98	18 ± 4	1200 ± 32	32 ± 5	3856 ± 174
Tryptamine	B	35 ± 8	971 ± 151	41 ± 6	1804 ± 74	24 ± 2	1500 ± 49	27 ± 3	815 ± 20

K_m values are expressed in μM and maximum velocities (V_{max}) in pmol · min^{-1} · mg protein^{-1}. The activities of the A and B forms were determined separately by preincubating the samples for 60 min at 37° with the concentrations of (−)-deprenyl or clorgyline indicated in the text. Values are means ± S.E. of determinations in five separate tissue samples, each performed in triplicate.

Table 3. Substrate specificities of the two forms of monoamine oxidase in human organs

Organ	Substrate	% Activity due to: MAO-A	MAO-B
Liver	5-Hydroxytryptamine	97 ± 3	2 ± 5
	2-Phenethylamine	5 ± 3	91 ± 4
	Tyramine	73 ± 3	22 ± 5
	Tryptamine	76 ± 5	18 ± 8
Kidney cortex	5-Hydroxytryptamine	94 ± 3	7 ± 2
	2-Phenethylamine	7 ± 3	108 ± 10
	Tyramine	58 ± 4	42 ± 1
	Tryptamine	54 ± 6	48 ± 3
Kidney medulla	5-Hydroxytryptamine	91 ± 8	4 ± 2
	2-Phenethylamine	7 ± 2	96 ± 5
	Tyramine	60 ± 5	40 ± 3
	Tryptamine	60 ± 5	37 ± 4
Cerebral cortex	5-Hydroxytryptamine	92 ± 5	3 ± 1
	2-Phenethylamine	4 ± 1	104 ± 11
	Tyramine	46 ± 8	48 ± 3
	Tryptamine	55 ± 2	50 ± 2

Values are means ± S.E. of determinations carried out in three separate tissue samples, each assayed in triplicate.

MAO-A to have a lower K_m value and a higher maximum velocity for this substrate than MAO-B.

The data obtained in the present work indicates that tryptamine, like tyramine, is a substrate for both forms of monoamine oxidase in the human tissues studied. The distribution in human brain regions (Table 1) compares well with those previously reported for dopamine, 5-HT and 2-phenethylamine [20] in showing tryptamine to be oxidised by both forms of the enzyme with equal facility. The similarity between the kinetic parameters for both forms indicate that their relative activities will not depend on the available tryptamine concentration. In the human kidney two forms of monoamine oxidase are also both active towards tryptamine but, whereas they both contribute equally in the cortex, the activity of the A-form is somewhat greater in the medulla (Table 3). A predominance of the activity of the A-form towards this substrate was observed with human liver. In no case did the clorgyline inhibition curves indicate any significant amount of activity to be insensitive to this inhibitor. Thus under these conditions it can be concluded that the so-called clorgyline-resistant amine oxidase activity, which has been equated with a semicarbazide sensitive amine oxidase [32, 33], does not play an important role in the oxidation of tryptamine in these tissues.

This involvement of both forms of monoamine oxidase in the oxidation of tryptamine in human tissue suggests that urinary tryptamine excretion would be sensitive to the inhibition of either form of monoamine oxidase. Thus it might not provide a reliable measure of the inhibition of MAO-A alone.

Iontophoretic [34], receptor binding [35] and other studies (see ref. 36 for review) have indicated that tryptamine may function as a neurotransmitter in brain. In such a case the predominant form of monoamine oxidase involved in its metabolism in that organ might be expected to depend on intraneuronal compartmentalization which may be different for the two forms of the enzyme [37, 38]. It is possible that accessibility factors might also affect the overall mechanism of tryptamine in the periphery but there are no available data to indicate whether this may be the case.

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