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SUBSTRATE- AND INHIBITOR-RELATED CHARACTERISTICS OF HUMAN PLATELET MONOAMINE OXIDASE

CYNTHIA H. DONNELLY

Laboratory of Clinical Science, NIMH, Bethesda, MD 20014 and the Department of Chemistry, University of Maryland, College Park, MD, U.S.A.

and

DENNIS L. MURPHY

Laboratory of Clinical Science, NIMH, Bethesda, MD 20014, U.S.A.

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Abstract—Human platelet monoamine oxidase (MAO) preferentially deaminated benzylamine and phenylethylamine, two substrates relatively specific for type B MAO, in comparison to 5-hydroxytryptamine, a substrate specific for type A MAO. In studies comparing human platelet and rat brain MAO specific activities, benzylamine and 5-hydroxytryptamine deamination by platelets was approximately 90 and 2 per cent, respectively, that of brain, while platelet deamination of dopamine, tryptamine and tyramine was 20 per cent or less than that of brain. Among sixteen drugs studied, platelet MAO activity was selectively inhibited by low concentrations of the MAO-B inhibitors, deprenyl and pargyline, and was relatively insensitive to the MAO-A inhibitors, clorgyline and Lilly 51641. These observations, in addition to the simple sigmoid inhibition curves obtained with increasing concentrations of either clorgyline or deprenyl, suggest that platelet MAO consists of essentially one distinguishable form of MAO which most closely resembles the MAO type B found in other tissues.

Platelet monoamine oxidase (MAO) [monoamine:O_2 oxidoreductase (deaminating) EC 1.4.3.4], like other tissue monoamine oxidases, appears to be primarily located in mitochondria [1]. Human platelet MAO has been purified approximately 12-fold by Collins and Sandler [2], who have estimated its molecular weight to be 235,000. Considering differences in enzyme preparation and experimental conditions, generally similar patterns of substrate specificity have been observed in previous studies of human platelet MAO using tryptamine, tyramine, dopamine, benzylamine and serotonin as substrates [2–4]. Benzylamine is most actively deaminated, while serotonin, the amine present in the highest concentration in platelet, is least actively deaminated [2–4].

Recently two forms of MAO have been described on the basis of substrate specificity and inhibitor sensitivity in brain, liver and other tissues [5–8]. Type A MAO deaminates 5-hydroxytryptamine [5, 6] and is sensitive to inhibition by low concentrations of clorgyline [5–7, 9]. Type B MAO preferentially deaminates benzylamine [6] and phenylethylamine [10] and is sensitive to inhibition by low concentrations of deprenyl and pargyline [7–11]. Other amines such as tryptamine, tyramine and dopamine appear to be deaminated by both forms of MAO [6]. Previous studies of human platelet MAO based on substrate specificities [2–4] and preliminary data on its sensitivity to deprenyl [4, 8] led to the suggestion that this enzyme might primarily represent a type B MAO [4, 8].

In the present study, we have further characterized human platelet MAO with respect to substrate- and inhibitor-related characteristics and have directly compared platelet MAO with that of rat brain, which has previously been described to contain both MAO-A and MAO-B [6, 12, 13], in an attempt to better define the MAO form present in the human platelet.

MATERIALS AND METHODS

Enzyme preparation. Human blood platelet concentrates prepared from venous blood collected in ACD solution (USP formula A, 75 ml/500 ml of whole blood) were obtained from Community Blood and Plasma, Baltimore, MD. The concentrates were centrifuged at 175 *g* for 5 min at 4°. The supernatant was removed and centrifuged at 2000 *g* for 20 min to obtain a platelet pellet. The pellet was washed once with ice-cold saline, resuspended in distilled water and sonicated for 10 sec (Sonifier cell disruptor, Heat Systems-Ultrasonics, Inc., Plainview, L.I., NY). The sonicated preparation was distributed in 50-μl aliquots containing 0.2 to 0.9 mg protein for most studies; the K_m determinations for phenylethylamine used aliquots containing 0.01 to 0.08 mg protein. The samples were stored at either −20° or −70° until assayed.

Wistar and Sprague–Dawley male rats (150–250 g) were obtained from Microbiological Associates, Walkersville, MD, and Taconic Farms, Germantown, NY respectively. Whole brains were homogenized in 10% (w/v) 0.08 M phosphate buffer, pH 7.2. The homogenate was centrifuged at 900 *g* for 10 min at 4°. The supernatant was sonicated for 10 sec, distributed in 50-μl aliquots (0.2 to 0.7 mg protein) and stored at −70° until assayed.

MAO activity studies. MAO activity was determined by a modification of the method used by Robinson *et al.* [3] using the following substrates: [^{14}C]-5-hydroxytryptamine creatinine sulfate (55 mCi/m-mole), [^{14}C]dopamine HCl (57.3 mCi/m-mole), [^{14}C]-1-noradrenaline bitartrate (57 mCi/m-mole) (Amersham/Searle Corp., Arlington Heights, IL); [^{14}C]tyramine HCl (9.2 mCi/m-mole), [^{14}C]tryptamine bisuccinate (47 mCi/m-mole), [^{14}C]-β-phenylethylamine HCl (9.86 mCi/m-mole) (New England Nuclear, Boston, MA); and [^{14}C]benzylamine HCl (3.5 mCi/m-mole) (Mallinkrodt Chemical Works, St. Louis, MO).

The sonicated preparations were assayed in duplicate by incubating 50-μl aliquots in 0.5 ml of 0.08 M phosphate buffer (Na_2HPO_4–KH_2PO_4), pH 7.2, with 25 μl of ^{14}C-labeled substrate (in final concentrations indicated below) for 20 min at 37°. Aliquots heated at 100° for 10 min were assayed simultaneously to establish blank values which were subtracted from those of the active enzyme preparation. When norepinephrine, dopamine or tyramine was used as the substrate, ascorbic acid (10^{-4} M) and EDTA (10^{-4} M) were added to the phosphate buffer to prevent non-enzymatic alteration of the substrate.

After incubation, the samples were placed on ice and then transferred to Pasteur pipettes containing 0.5 × 2.5 cm Amberlite resin (CG-50, 100–200 mesh, Mallinkrodt Chemical Works). The columns were washed twice with 1 ml of distilled water, and the entire 2.5 ml was collected in glass vials containing 17.5 ml Aquasol (New England Nuclear). The radioactivity of the products was determined by liquid scintillation spectrometry. In all of the tissues, monoamine oxidase activity was found to be linear with respect to the previously described protein concentration and time of incubation. Protein was determined by a modification [14] of the method of Lowry *et al.* [15].

K_m and V_{max} determinations. The apparent Michaelis constants and maximum velocities were obtained by fitting the data to the following equation, assuming equal variance for the velocities:

$$v = \frac{VA}{K + A}$$

where v is the reaction velocity for a given substrate concentration, A; K is the apparent Michaelis constant; and V is the apparent maximum velocity of the reaction. All fits were performed by means of the MLAB interactive curve-fitting program [16]. Amine metabolism did not exceed 10 per cent at the lowest concentration of the substrates used with the single exception of phenylethylamine, which did not exceed 15 per cent.

Comparison of platelet and brain MAO activity. MAO specific activities and the inhibitory effects of clorgyline (10^{-7} M) and deprenyl (10^{-6} M) were compared in platelet and brain preparations using the following substrates at the final concentrations and specific activities indicated: 5-hydroxytryptamine (serotonin, 5-HT, 1.67 mCi/m-mole) and tyramine (TYR, 0.5 mCi/m-mole), 10^{-3} M; norepinephrine (NE, 3.3 mCi/m-mole) and dopamine (DA, 3.3 mCi/m-mole), 5×10^{-4} M; benzylamine (BA, 3.5 mCi/m-mole), 2×10^{-4} M; tryptamine (TA, 4.46 mCi/m-mole), 8×10^{-5} M; and phenylethylamine (PEA, 9.86 mCi/m-mole), 2×10^{-5} M. Non-radiolabeled amines [5-hydroxytryptamine creatinine sulfate complex, tyramine monohydrochloride, (−) arterenol bitartrate hydrate (NE), 3-hydroxytyramine HCl (DA), and tryptamine HCl (CalBiochem, San Diego, CA)] were added when required to obtain the desired final concentration. The concentrations chosen were those identified from preliminary studies as (a) approaching saturation according to substrate curves and (b) equal to or greater than the K_m values for the platelet and brain preparations for each substrate. In some instances (e.g. with benzylamine and phenylethylamine), apparent substrate-type inhibition was observed at higher concentrations than those used in this study. In some of these experiments (C. Donnelly and D. L. Murphy, manuscript in preparation), K_m values for rat brain had been found to be generally similar to those found in the present study for the platelet: benzylamine, 1.2×10^{-4} M; dopamine, 2.6×10^{-4} M; tyramine, 1.0×10^{-4} M; phenylethylamine, 5.1×10^{-6} M; and tryptamine, 0.7×10^{-5} M.

MAO inhibition. In cases where inhibitors were used, the samples were preincubated at 25° for 30 min in the presence of inhibitor (final concentration range: 10^{-11} to 10^{-3} M), and then incubated at 37° with 10^{-3} M (final concentration) tyramine as described above. The pI_{50} values (negative logarithms of the inhibitor concentration resulting in 50 per cent inhibition) were determined from plots of per cent MAO inhibition vs concentration of inhibitor (−log M). Inhibitors used in this study were amitriptyline, iproniazid and isocarboxazid (Hoffmann-La Roche Inc., Nutley, NJ), *d*-amphetamine (K & K Labs., Plainview, NY), *l*-amphetamine (Aldrich Labs., Morristown, NJ), clorgyline (May & Baker, Ltd, Essex, England), chlorpromazine and tranylcypromine (Smith, Kline & French Labs., Philadelphia, PA), deprenyl (Prof. J. Knoll, Semmelweis University of Medicine, Budapest, Hungary), desipramine (Geigy Pharmaceuticals, New York, NY), α-ethyltryptamine (Aldrich Chemical Co., Inc., Milwaukee, WI), harmine (Sigma Chemical Co., St. Louis, MO), Lilly 51641 (Eli Lilly & Co., Indianapolis, IN), nialamid (Pfizer Inc., New York, NY), pargyline (Abbott Labs., N. Chicago, IL), and phenelzine (Warner–Lambert Research Institute, Morris Plains, NJ). Semicarbazide (10^{-4} M) final concentration; Fisher Scientific Co., Fair Lawn, NJ) was used only in the study of platelet 5-HT metabolism.

RESULTS

The apparent Michaelis constants and maximum velocities for platelet MAO are listed in Table 1. With the exception of 5-HT, rectangular hyperbolic substrate concentration curves were observed within the indicated concentration ranges for all the substrates studied. In contrast to that of the other amines, the substrate plot obtained with 5-HT (0.1 to 2×10^{-3} M; Fig. 1) did not reflect typical saturation, and attempts to fit the data within reasonable error either to the equation previously described or to an equa-

Table 1. Apparent K_m and V_{max} values for human platelet monoamine oxidase*

Substrate	K_m (M)	V_{max} (nmoles/hr/mg protein)	Concn range studied (M)
Benzylamine	1.3×10^{-4}	49	0.33 to 2×10^{-4}
Dopamine	2.2×10^{-4}	27	0.2 to 10×10^{-4}
Tyramine	1.7×10^{-4}	32	0.1 to 5×10^{-4}
Phenylethylamine	1.5×10^{-6}	15	1.0 to 8×10^{-6}
Tryptamine	3.5×10^{-5}	8	0.1 to 2×10^{-4}

* N = 5.

tion for two enzymes catalyzing the same reaction

$$\left(v = \frac{V_1 A}{Ka_1 + A} + \frac{V_2 A}{Ka_2 + A}\right)$$

[17], were unsuccessful. The metabolism of serotonin was considerably less than that of the other substrates but greater than that of norepinephrine, which was slight and variable and could not be reliably measured in the platelet preparation.

Comparison with rat brain MAO. MAO-specific activities in the platelet and brain preparations were most similar with benzylamine and phenylethylamine as substrates. Platelet specific activities with benzylamine and phenylethylamine were 89 and 40 per cent, respectively, those of brain, while activities with other substrates studied were 20 per cent or less than those of brain (Table 2). The addition of 10^{-6} M deprenyl (Fig. 2) almost completely inhibited MAO activity in the platelet preparations regardless of the substrate used, with the exception of 5-HT metabolism, which was inhibited only about 40 per cent by deprenyl in preparations from seven individuals at 5-HT concentrations 0.1×10^{-3} M (40 per cent ± 10, mean ± S.E.), 0.2×10^{-3} M (38 per cent ± 5), and 1×10^{-3} M (45 per cent ± 6). The addition of semicarbazide (10^{-4} M) to the platelet preparation had no inhibitory effect on 5-HT metabolism.

MAO in rat brain preparations was less sensitive to inhibition by deprenyl. Benzylamine and phenylethylamine deamination in rat brain was inhibited approximately 90 per cent by 10^{-6} M deprenyl and, while activity with other substrates was inhibited almost 40 per cent, activity with 5-HT was inhibited less than 10 per cent (Fig. 2). Human platelet MAO was relatively insensitive to inhibition by 10^{-7} M clorgyline in contrast to rat brain MAO, which was markedly inhibited by this drug for most of the substrates used, especially 5-hydroxytryptamine, whose

Table 2. Specific activity of human platelet and rat brain monoamine oxidase*

Substrate†	Platelet	Brain	Platelet as per cent of brain
Benzylamine	24	27	89
Phenylethylamine	12	30	40
Tyramine	34	174	20
Dopamine	16	84	19
Tryptamine	6	60	10
5-hydroxytryptamine	4	165	2
Norepinephrine		30	

* Specific activity is expressed as nmoles/hr/mg of protein.

† Final concentrations of substrates used were: benzylamine, 2×10^{-4} M; phenylethylamine, 2×10^{-5} M; tyramine, 10^{-3} M; dopamine, 5×10^{-4} M; tryptamine, 8×10^{-5} M; and 5-hydroxytryptamine, 10^{-3} M.

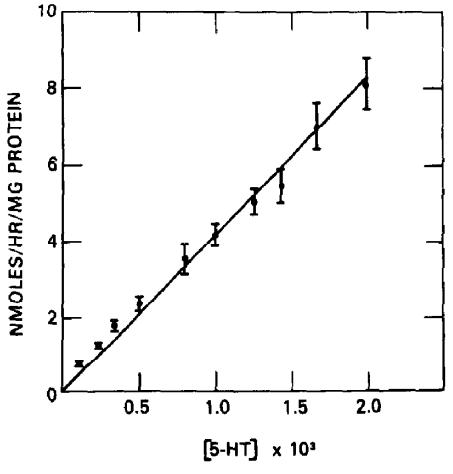

Fig. 1. Effect of 5-hydroxytryptamine concentration on human platelet monoamine oxidase activity. Each point represents the mean (± S. E.) of duplicate determinations on six individuals.

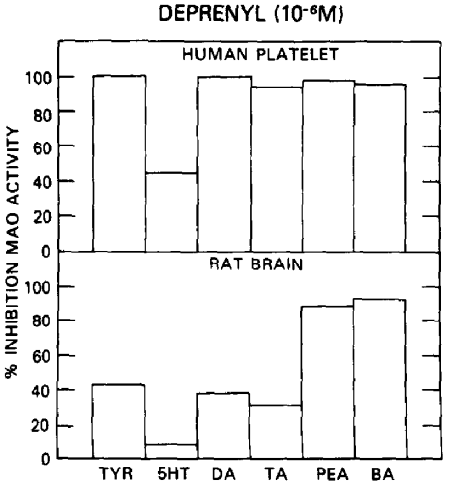

Fig. 2. Deprenyl (10^{-6} M) inhibition of human platelet and rat brain monoamine oxidase activity using 10^{-3} M tyramine (TYR), 10^{-3} M 5-hydroxytryptamine (5-HT), 5×10^{-4} M dopamine (DA), 8×10^{-5} M tryptamine (TA), 2×10^{-5} M phenylethylamine (PEA) and 2×10^{-4} M benzylamine (BA) as substrates.

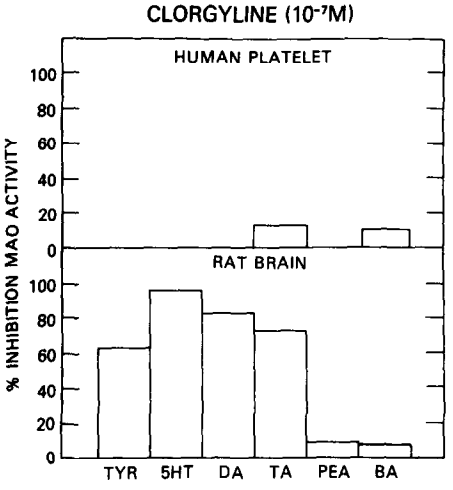

Fig. 3. Clorgyline (10^{-7} M) inhibition of human platelet and rat brain monoamine oxidase activity using 10^{-3} M tyramine (TYR), 10^{-3} M 5-hydroxytryptamine (5-HT), 5×10^{-4} M dopamine (DA), 8×10^{-5} M tryptamine (TA), 2×10^{-5} M phenylethylamine (PEA) and 2×10^{-4} M benzylamine (BA) as substrates.

deamination was inhibited by more than 90 per cent (Fig. 3). Benzylamine and phenylethylamine deamination in brain, however, was inhibited less than 10 per cent by this concentration of clorgyline.

Increasing concentrations of either deprenyl or clorgyline resulted in simple sigmoid inhibition curves for the platelet preparation when 10^{-3} M tyramine was used as the substrate (Figs. 4 and 5). Tyramine deamination by platelet MAO was more than 100-fold more sensitive to inhibition by deprenyl than by clorgyline. Simple sigmoid curves similar to those with tyramine were also obtained for the platelet preparation with tryptamine and benzylamine as substrates. In the rat brain preparations studied with tyramine as substrate, increasing concentrations of these inhibitors resulted in biphasic curves with a distinct plateau region with clorgyline at 58 per cent inhibition and a less distinct plateau with deprenyl, with a midpoint at 44 per cent inhibition. Estimated pI_{50} values from the separate portions of curves presented in Figs. 4 and 5 for rat brain and for the simple sigmoid curves for platelet are listed in Table 3. Of the two pI_{50} values calculated for clorgyline and deprenyl for rat brain MAO, the lower value for deprenyl and higher value for clorgyline closely resembled those estimated for human platelet MAO.

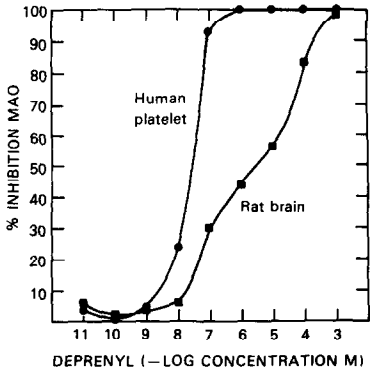

Fig. 4. Inhibition of human platelet (●) and rat brain (■) monoamine oxidase activity by increasing concentrations of deprenyl. Tyramine (10^{-3} M) was used as substrate.

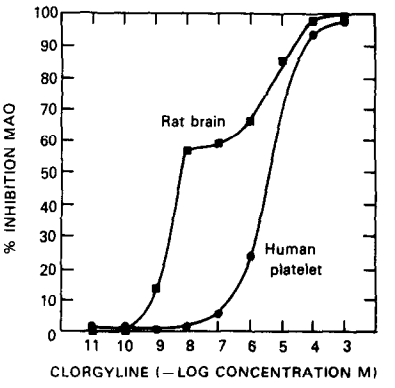

Fig. 5. Inhibition of human platelet (●) and rat brain (■) monoamine oxidase activity by increasing concentrations of clorgyline. Tyramine (10^{-3} M) was used as substrate.

Inhibition by various drugs. Table 4 lists the pI_{50} and ID_{50} values for various drugs (estimated from

Table 3. The pI_{50} values for clorgyline and deprenyl inhibition of tyramine deamination by monoamine oxidase in rat brain and human platelet preparations

Preparation	Clorgyline pI_{50}	Deprenyl pI_{50}
Rat brain	8.6, 5.3*	7.3, 4.3*
Human platelet	5.4	7.6

* Estimated separately for the two phases of the inhibition curves presented in Figs. 4 and 5.

Table 4. Inhibition by various drugs of tyramine (10^{-3} M) deamination by human platelet MAO*

Drug†	pI_{50}	ID_{50} (M)
Deprenyl	7.6	2.5×10^{-8}
Pargyline	7.4	4.0×10^{-8}
Tranylcypromine	6.6	2.5×10^{-7}
Phenelzine	6.0	1.0×10^{-6}
Isocarboxazid	5.6	2.5×10^{-6}
Clorgyline	5.4	4.0×10^{-6}
Lilly 51641	5.2	6.3×10^{-6}
Iproniazid	4.3	5.0×10^{-5}
Nialamide	4.3	5.0×10^{-5}
Amitriptyline	4.2	6.3×10^{-5}
Harmine	4.0	1.0×10^{-4}
Desipramine	3.4	4.0×10^{-4}
d-Amphetamine	3.3	5.0×10^{-4}
Chlorpromazine	3.2	6.3×10^{-4}
l-Amphetamine	3.1	7.9×10^{-4}
α-Ethyltryptamine		$> 10^{-3}$

* N = 5.

† Drug concentrations ranged from 10^{-11} to 10^{-3} M. In each case pI_{50} values were estimated from plots of per cent inhibition of MAO activity vs concentration of the inhibitor (−log M).

curves similar to those presented in Figs. 4 and 5 for human platelet) in the order in which they most effectively inhibit platelet MAO deamination of 10^{-3} M tyramine. Of the various drugs tested, deprenyl and pargyline most effectively inhibited platelet MAO, with clorgyline and Lilly 51641 being 100-fold less effective. The least effective inhibitors were desipramine, clorpromazine, amphetamine and α-ethyltryptamine.

DISCUSSION

Where data involving common substrates are available, a limited comparison of our K_m and V_{max} values for human platelet MAO may be made with those reported in previous studies. The apparent K_m and V_{max} values obtained here for most substrates were similar to those reported by Robinson *et al.* [3] and Edwards and Chang [4], but were generally higher than those reported by Collins and Sandler [2]. Differences in values may be possibly related to the enzyme preparations used. Collins and Sandler [2] determined K_m and V_{max} values using a partially purified MAO preparation. Robinson *et al.* [3] used an unsonicated, freeze-thawed platelet suspension, while Edwards and Chang [4] used outdated sonicated blood bank platelets. It is also possible that individual variations in human platelet MAO may contribute to differences in our values and those previously reported. We found that K_m values for platelet MAO from 21 individuals in our study using tryptamine as substrate ranged from 0.82 to 4.7×10^{-5} M.

In comparison with whole rat brain MAO, the specific activities of human platelet MAO are considerably lower for most of the substrates studied, even though the substrate concentrations chosen were selected to be optimal for the platelet as well as the brain enzyme. Relatively low concentrations of deprenyl (10^{-6} M) almost completely inhibited platelet MAO activity with tyramine, tryptamine, dopamine, benzylamine and phenylethylamine as substrates, in contrast to MAO from brain, where inhibition by deprenyl was most effective with benzylamine and phenylethylamine. Although we were unable to demonstrate complete inhibition of 5-hydroxytryptamine metabolism in our platelet preparation, Edwards and Chang [4], using an ethyl acetate extraction procedure, reported a 98 per cent inhibition of platelet 5-HT deamination by 10^{-7} M deprenyl. Deamination of 5-hydroxytryptamine in two other studies of platelet MAO [3, 18] was apparently not examined for sensitivity to MAO inhibitors. It is possible that in our use of a column procedure, the apparent residual activity we have measured actually reflects binding of 5-HT to the non-boiled platelet preparation. Binding of 5-HT in various brain preparations has been previously reported by several investigators [19, 20]. Other possibilities that might be considered are 5-HT metabolism by other enzymatic processes (e.g. *N*-acetylation [21]), or perhaps deamination by a clorgyline/deprenyl insensitive form of MAO.

Plots of per cent inhibition of MAO activity with increasing concentrations of deprenyl or clorgyline yield simple sigmoid curves with platelet MAO, in contrast to the pair of sigmoid curves joined by a plateau region obtained with rat brain MAO. Studies of clorgyline inhibition of MAO in brain led Johnston [5] to suggest that MAO may exist as a binary system of enzymes, enzyme A and enzyme B, each of which has a detectably different sensitivity to inhibitors; the ratio of MAO-A to MAO-B in rat brain has been reported to be 6/4 using 2.1 mM tyramine as a substrate [13]. In contrast, it would appear that the human platelet contains essentially one detectable tyramine-deaminating form of MAO, which in our study is over 100-fold more sensitive to inhibition by deprenyl than by clorgyline. Edwards and Chang [4] have also recently reported human platelet MAO to be approximately 200-fold more sensitive to inhibition by deprenyl ($ID_{50} = 1.8 \times 10^{-8}$ M) than by clorgyline ($ID_{50} = 4.0 \times 10^{-6}$ M). That platelet MAO consists of only one distinguishable form is also consistent with the single tetrazolium-stained band described by Collins and Sandler [2] which was obtained by polyacrylamide gel electrophoresis, unlike the monoamine oxidases in human brain and liver which exhibit multiple electrophoretic bands [22]. Edwards and Chang [23] recently provided evidence for interacting catalytic sites for human platelet MAO and observed two tetrazolium-stained bands obtained by gel electrophoresis. Each band, however, had the same relative activity for each of the substrates used, and one of the bands of activity localized at the top of the gel appeared to be due to protein aggregation. The characterization of human platelet MAO as type B does not exclude the possibility of the occurrence of subtypes of this form. Species differences in platelet MAO types may also occur, as preliminary evidence suggests that rabbit platelet MAO most closely resembles MAO type A [4]. In previous studies, similar responses to MAO-inhibiting drugs were observed for human whole brain and platelet monoamine oxidases using kynuramine as substrate; both tissues were most sensitive to inhibition by pargyline [2, 24].

Our estimated pI_{50} values for various drugs for human platelet MAO are remarkably similar (with the exception of nialamide and iproniazid) to those reported by Johnston [5] for human brain. These similarities may possibly be related to the specificity and mode of inhibition of the various drugs, or perhaps may reflect the relative proportion of MAO-A to MAO-B in Johnston's particular human brain preparation. Clorgyline was the only drug studied by Johnston for which two separate pI_{50} values (9.2 and 5.3) were reported for human brain; the lower pI_{50} is similar to the value for human platelet MAO (5.4) found in our studies. Deprenyl and pargyline, which have been reported to selectively inhibit MAO type B in other tissues [7, 9–11], were also observed to be the most effective inhibitors of human platelet MAO. Thus, in all characteristics studied, the platelet MAO-B appears similar, if not identical, to the B component of MAO found in other tissues, including brain.

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