Biochemical Pharmacology, Vol. 28, pp. 3063–3068

0006-2952/79/1015-3063 $02.00/0

THE INTERACTION BETWEEN HUMAN PLATELET MONOAMINE OXIDASE, ITS MONOAMINE SUBSTRATES AND OXYGEN

CHRISTOPHER J. FOWLER,* BERTIL EKSTEDT, TORU EGASHIRA,†
HIROYASU KINEMUCHI† and LARS ORELAND
Department of Pharmacology, University of Umeå S-901 87 Umeå, Sweden

(*Received* 23 *February* 1979; *accepted* 30 *April* 1979)

Abstract—The interaction of human platelet MAO-B with three substrates (β-phenethylamine, tryptamine and benzylamine) has been investigated in an attempt to determine whether or not there exists heterogeneity of this enzyme form. Treatment with pargyline, thermal denaturation and 2-butanone affected the enzyme activity to the same degree, regardless of the amine substrate used. Mixed substrate experiments indicated that the substrates inhibited each other in a competitive manner with K_i values close to their K_m values. The activity of MAO-B was increased in an uncompetitive manner as the oxygen concentration was raised. However, the degree of this increase was dependent upon the amine substrate used to assay for activity. These results are consistent for an enzyme with a single binding site for amine substrates and a possible multiplicity of binding sites for oxygen.

In the study of psychiatric disorders, great attention has been drawn to the turnover of monoamine transmitter agents and to the activities of the enzymes involved in their metabolism. Monoamine oxidase (MAO) (EC 1.4.3.4) is an enzyme of major importance for the catabolism of, e.g. noradrenaline, dopamine and 5-hydroxytryptamine [1, 2]. The turnover of 5-hydroxytryptamine in brain as estimated by the levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in autopsy material has recently been found to correlate with the activity of MAO in brain [3]. Furthermore, there are indications that platelet MAO activity is also correlated with serotonin turnover in the brain since a correlation has recently been found between platelet MAO activity and levels of 5-hydroxyindole acetic acid in the cerebrospinal fluid of healthy volunteers [4]. As the human blood platelet is a convenient source of human MAO, a great number of studies on the activity of this enzyme have been performed in a variety of psychiatric disorders [for reviews see 5, 6].

On the basis of the differential sensitivity to inhibition of MAO by the acetylenic inhibitor clorgyline when assayed with different substrates, the activity of MAO has been divided into two forms, MAO-A and B, the A form being inhibited by much lower concentrations of clorgyline [7, for review see 8]. In human blood platelets, only the B form of MAO is present [9]. However, on the basis of mixed substrate experiments and studies on the mechanisms of inhibition by tricyclic antidepressants, it has been suggested that there is more than one catalytic site for the deamination of monoamines by human blood platelet MAO-B, with one preferentially oxidizing benzylamine and tryptamine and the other β-phenethylamine [10, 11].

In this study, an attempt has been made to explore the nature of human blood platelet MAO in order to determine whether or not such multiplicity of catalytic sites exists.

MATERIALS AND METHODS

Preparation of blood platelets. Blood samples (450 ml in a standard buffered citrate solution) were obtained from male volunteers (aged between 24 and 42). The blood was centrifuged at 200 *g* for 10 min to remove the red cells. The cells were washed twice with 0.15 M NaCl and the combined supernatants further centrifuged at 13,000 *g* for 15 min to produce a platelet preparation. The pellet was resuspended in 5.0 ml of 0.01 M potassium phosphate (pH 7.4) and stored at −80°. Before use, the suspension was sonicated in an MSE Ultrasonic Disintegrator at low power for 2 min, to produce a more homogeneous preparation. The properties of rat liver MAO have been shown not to be changed when sonicated in this manner [12].

Lipid depletion of blood platelets was carried out as described previously [13]. Over a period of 5 min, 8 ml of 2-butanone was added to 1 ml of a blood platelet preparation, the suspension being kept at +4° and stirred continuously. This suspension was centrifuged at 20,000 *g* for 5 min, the pellet resuspended in 0.01 M potassium phosphate buffer (pH 7.4), stirred for 5 min at +4°, then recentrifuged and resuspended as before. Aliquots of the pellet and supernatant fractions were assayed for MAO activity.

Monoamine oxidase assay. Monoamine oxidase activity was assayed radiochemically by two different methods, both of which gave essentially similar results. For the mixed substrate and 'oxygen ratio' experiments, the assay described by Callingham and Laverty [14] was used, with two modifications. The buffer solution used in the assay mixture in these experiments was 0.25 M sucrose, buffered with 0.01 M potassium phosphate (pH 7.8), a lower ionic strength buffer than used previously, as the ionic strength of buffer solutions has been shown to influence the activity of MAO [15].

* To whom correspondence should be addressed.
† Present address: Showa University, School of Medicine, Department of Pharmacology, Hatanodai 1-5-8, Shinagawa-ku, Tokyo, Japan.

Secondly, the organic extraction mixture originally used to extract the deaminated metabolites (benzene–ethyl acetate, 1:1 v/v, saturated with water) was replaced by the less toxic mixture of toluene–ethyl acetate, 1:1 v/v, saturated with water. The substitution of the benzene by toluene does not change the efficiency of extraction of the deaminated metabolites into the organic layer [12, 16]. All other experiments reported here were assayed by a standard radiochemical method [17]. Unless otherwise stated, all experiments were carried out under an atmosphere of air and the activity, uncorrected for the efficiency of extraction of the deaminated metabolites into the organic phase, expressed as nmoles (of substrate metabolised) mg protein $^{-1}$ min $^{-1}$. The efficiency of extraction was found not to change for any of the procedures reported here.

Protein was assayed by the method of Lowry *et al.* [18], with human serum albumin as standard.

Materials. The radioactive substrates for MAO, β-phenethylamine-[ethyl-1-^{14}C] hydrochloride and tryptamine-[side chain-2-^{14}C]bisuccinate were obtained from New England Nuclear, Boston, MA, U.S.A. Benzylamine-[methylene-^{14}C]hydrochloride was obtained from ICN Pharmaceuticals Inc., Irvine, CA, U.S.A. Pargyline hydrochloride was a gift from Abbott Laboratories, North Chicago, IL, U.S.A. All other reagents were standard laboratory reagents of analytical grade wherever possible.

RESULTS

Blood platelet preparations were preincubated for 20 min at 37° with tryptamine or β-phenethylamine in the presence of the irreversible inhibitor pargyline. The samples were centrifuged and the pellet then washed to remove any unreacted pargyline before estimation of MAO activity. Table 1 shows a representative experiment. When pargyline alone was present under the conditions indicated, 16 and 21% of the activities of the enzyme towards β-phenethylamine and tryptamine respectively remained. If the blood platelets were preincubated with either of the substrates together with pargyline, substantial protection of the enzyme from inhibition was found, with the same remaining activities towards both substrates.

Upon lipid depletion by 2-butanone, the major part of the activity of MAO was lost, the decrease in activity being similar for all three substrates used (Table 2).

When the thermal stability at 50° of the blood platelets was investigated, it was found that the progressive inhibition of activity with time was the same for all three substrates (Fig. 1).

The activity of MAO was increased in an apparently uncompetitive manner when assayed under an atmosphere of oxygen rather than air (Fig. 2 A–C). The degree of this increase appeared to depend on the amine substrate used to assay for activity. The 'maximum oxygen ratio' (ratio of the V_{max} when assayed in oxygen to the V_{max} in air) was higher for the enzyme when assayed with β-phenethylamine and tryptamine than with benzylamine as substrate (Table 3).

Mixed substrate experiments were performed with binary mixtures of β-phenethylamine, tryptamine and benzylamine, assayed under atmospheres of oxygen and air. In all cases, the inhibition of one substrate by another was essentially competitive in nature. These

Table 1. The effect of preincubation with pargyline in the presence and absence of either β-phenethylamine or tryptamine upon the activity of monoamine oxidase

Preincubation medium containing:			MAO activity remaining (%) towards:	
Pargyline (0.1 μM)	β-Phenethylamine (200 μM)	Tryptamine (800 μM)	β-Phenethylamine (15 μM)	Tryptamine (200 μM)
+	−	−	16	21
+	+	−	61	65
+	−	+	88	91

Blood platelets were incubated in the presence (+) and absence (−) of unlabelled pargyline, β-phenethylamine and tryptamine for 20 min at 37°. After preincubation, the blood platelets were centrifuged at 80,000 *g* for 20 min and the resulting pellets washed, in order to remove any unreacted pargyline, before assay of MAO activity. Results are expressed as the means of the percentage of MAO activity remaining, calculated from duplicate determinations of enzyme activity.

Table 2. Effect of 2-butanone extraction upon the activity of MAO

	MAO activity remaining (%) towards:		
	β-Phenethylamine	Tryptamine	Benzylamine
Before extraction	100	100	100
Pellet after extraction	18.7	17.7	15.3
Supernatant after extraction	0.2	0	0

Blood platelet preparations were treated with 2-butanone as described in Materials and Methods. The values are expressed as percentages of the total activities in each fraction divided by the total activity in the fraction before extraction and are the means of duplicate determinations. Substrate concentrations used: β-phenethylamine, 15 μM; tryptamine, 200 μM; benzylamine, 200 μM.

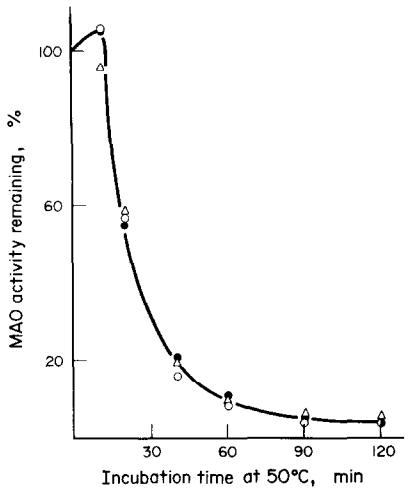

Fig. 1. Thermal inactivation of human blood platelet MAO. One millilitre of blood platelet suspension was preincubated at 50°. After the indicated preincubation time, an aliquot was withdrawn and assayed for MAO activity. Each point represents the mean of duplicate determinations of MAO activity expressed as a percentage of control, plotted against the time in min of preincubation at 50°. Substrates used were: 15 μM β-phenethylamine (○), 200 μM tryptamine (△) and 200 μM benzylamine (●).

data assayed under an atmosphere of air are shown in Fig. 3A–F. Similar results were found when assayed under an atmosphere of oxygen. The K_i values of the monoamines acting as inhibitors were in all cases similar to the K_m values of the same monoamines acting as substrates (Table 4). In all cases, the K_i and K_m values were lower when assayed under an atmosphere of air than when assayed under oxygen (Table 4).

DISCUSSION

Edwards and co-workers [10, 11] have suggested that more than one catalytic site is responsible for the deamination of monoamines by human blood platelet MAO-B. However, the results presented in this study are not consistent with such a hypothesis.

Pargyline is known to cause inhibition by a reversible interaction with the active centre of the enzyme, followed by a 'suicide reaction' with the flavine prosthetic group to form a covalent adduct [19, 20 for review, see 21]. Thus, if the pargyline were to bind to different catalytic sites on the MAO, differences in the sensitivity of the enzyme to inhibition when assayed with different substrates, and differences in the degree of protection of the activity by co-preincubation with different substrates might be expected. However, such differences were not found (Table 1).

Extraction of phospholipids from rat liver MAO by 2-butanone has been shown to inhibit the activity of MAO-A without significant effect on the catalytic properties of MAO-B [22]. However, in the human blood platelet the MAO appears to be sensitive to this form of phospholipid extraction (Table 2), which might suggest that in this tissue, the activity of MAO-B is in some way functionally dependent upon the membrane phospholipid environment. No differences in the degree of inhibition of MAO activity by this procedure were

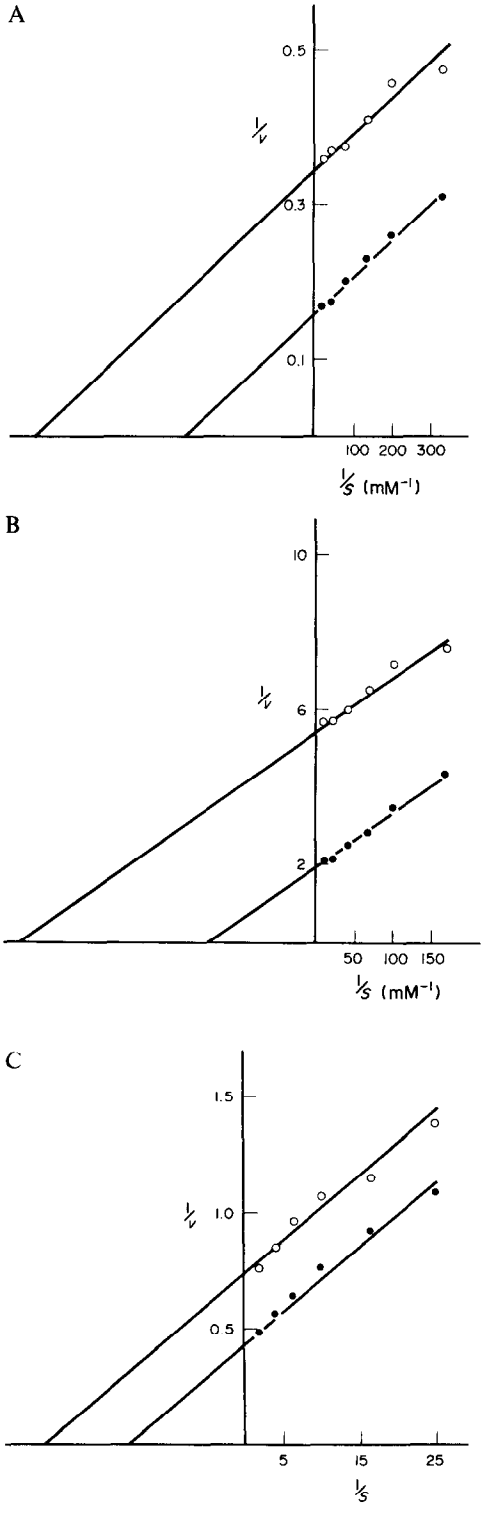

Fig. 2. Double reciprocal plots of the activity of human blood platelet MAO assayed under an atmosphere of oxygen (●) and air (○). Abscissae, 1/(substrate concentration in mM); ordinates, 1/(initial velocity in nmoles. mg protein^{-1} min^{-1}). Substrates used were: A, β-phenethylamine; B, tryptamine; C, benzylamine. Each point represents the mean of duplicate determinations in three blood platelet preparations. At all incubation times used, no deviation from linearity could be detected.

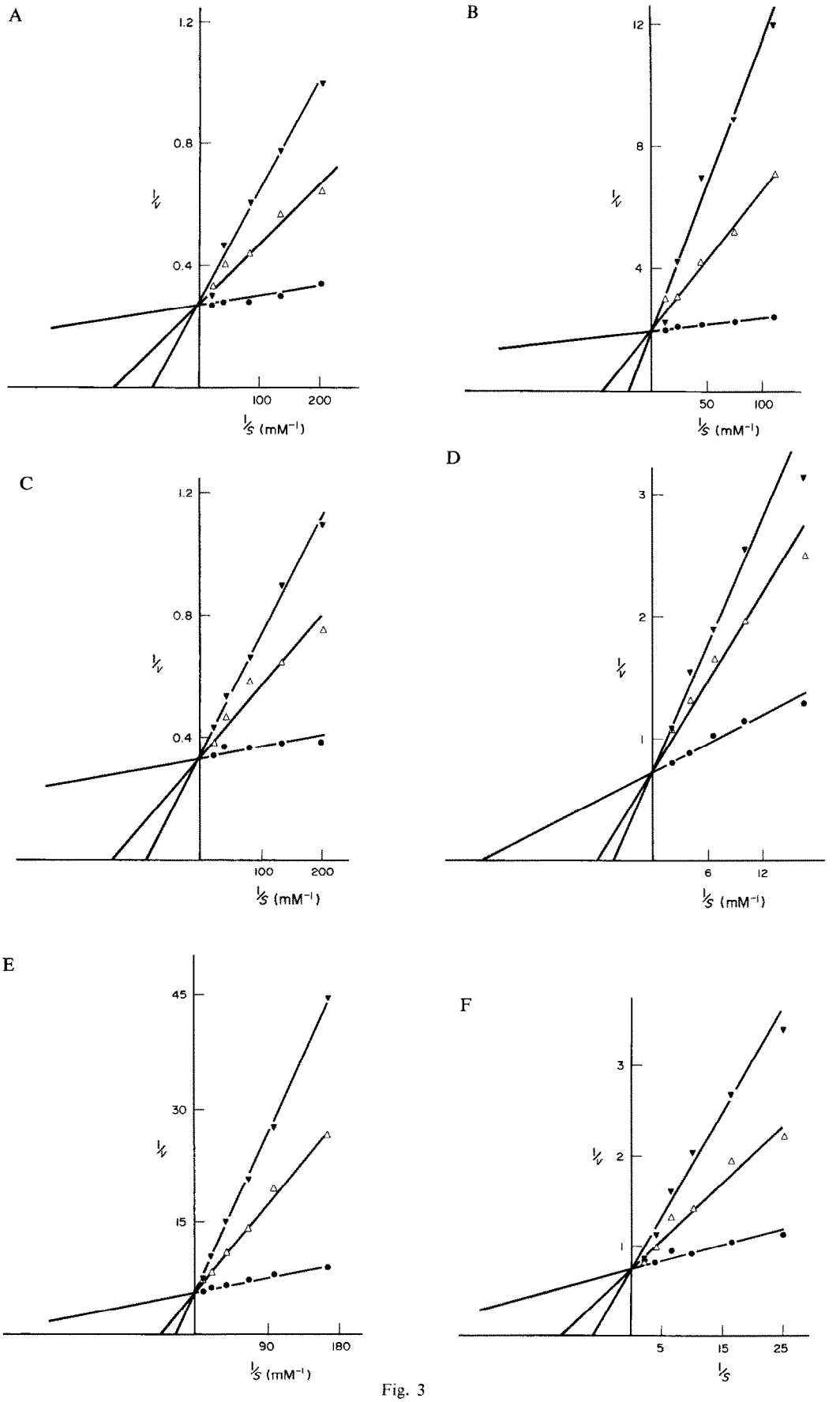
A
1.2
0.8
0.4
1/v
100
200
1/s (mM⁻¹)
B
12
8
4
1/v
50
100
1/s (mM⁻¹)
C
1.2
0.8
0.4
1/v
100
200
1/s (mM⁻¹)
D
3
2
1
1/v
6
12
1/s (mM⁻¹)
E
45
30
15
1/v
90
180
1/s (mM⁻¹)
F
3
2
1
1/v
5
15
25
1/s

Fig. 3

Table 3. Kinetic parameters of human blood platelet MAO assayed under atmospheres of oxygen and air.

Substrate	Oxygen		Air		'Maximum oxygen ratio'
	K_m (μM)	V_{max}	K_m (μM)	V_{max}	
β-Phenethylamine	4.3 ± 1.4	6.56 ± 1.36	1.4 ± 0.5	2.81 ± 0.80	2.45 ± 0.24
Tryptamine	8.5 ± 1.0	0.51 ± 0.14	3.8 ± 1.2	0.18 ± 0.05	2.84 ± 0.17
Benzylamine	81.0 ± 21.0	2.33 ± 0.52	42.0 ± 2.9	1.38 ± 0.36	1.75 ± 0.12

MAO activity was assayed in duplicate with 6 substrate concentrations under atmospheres of either oxygen or air. Results were plotted as $1/v$ against $1/S$ and as S/v against S, and K_m and V_{max} values in each case calculated from linear regression analysis, the values obtained from the two plots being averaged to compensate for bias due to high and low concentrations of substrate. V_{max} values are expressed as nmoles (of substrate metabolised) mg protein^{-1} min^{-1}. 'Maximum oxygen ratios' were calculated as the V_{max} when assayed under an atmosphere of oxygen/V_{max} in air. Results are shown as means ± S.E.M. or S.E.R. as appropriate from determinations in three blood platelet preparations.

Table 4. Inhibition of blood platelet MAO activity towards one substrate by another

Substrate	Oxygen				Air			
	K_m (μM)	K_i (μM) towards:			K_m (μM)	K_i (μM) towards:		
		β- PEA	Trypt	Bz		β-PEA	Trypt	Bz
β-Phenethylamine	4.3 ± 1.4	–	3.9 ± 0.9	6.7 ± 1.3	1.4 ± 0.5	–	1.4 ± 0.4	1.4 ± 0.3
Tryptamine	8.5 ± 1.0	18.3 ± 8.3	–	23.3 ± 1.5	3.8 ± 1.2	5.3 ± 2.0	–	4.1 ± 0.6
Benzylamine	81.0 ± 21.0	84.0 ± 6.7	38.9 ± 4.9	–	42.0 ± 2.9	49.7 ± 11.9	37.0 ± 5.2	–

Two concentrations of unlabelled substrate were used to inhibit the activity of MAO, assayed with 5 concentrations of labelled substrate under an atmosphere of either oxygen or air. All assays were performed in duplicate. K_i values were calculated from data by the method of Dixon [35] and expressed as means ± S.E.M. for determinations in three blood platelet preparations. In all cases, the inhibition of the activity towards one substrate by another was competitive. K_m values shown are the same as in Table 3. Abbreviations: β-PEA. β-phenethylamine; Trypt, tryptamine; Bz, benzylamine.

found with the different substrates (Table 2), again suggesting homogeneity of this enzyme form.

In a variety of tissues it has been shown that the thermal stability of MAO-B is dependent to some extent upon the substrate used to assay for activity, suggesting some kind of heterogeneity of this enzyme form [23, 34]. The human blood platelet, however, appears homogeneous with respect to thermal denaturation, the activity towards all three substrates being equally affected (Fig. 1).

Fig. 3. Double reciprocal plots of the inhibition of activity of human blood platelet MAO towards one substrate by another. Abscissae; 1/(substrate concentration in mM); ordinates, 1/(initial velocity in nmol. mg protein^{-1} min^{-1}). Each point represents the mean of duplicate determinations in three blood platelet preparations, assayed under an atmosphere of air. Substrates used were: A, [^{14}C]β-phenethylamine, assayed in the absence (●) and presence of 20 (△) and 40 μM (▼) unlabelled tryptamine. B, [^{14}C]tryptamine, assayed in the absence (●) and presence of 6.36 (△) and 12.73 μM (▼) unlabelled β-phenethylamine. C, [^{14}C]β-phenethylamine, assayed in the absence (●) and presence of 200 (△) and 400 μM (▼) unlabelled benzylamine. D, [^{14}C]benzylamine, assayed in the absence (●) and presence of 4 (△) and 8 μM (▼) unlabelled β-phenethylamine. E, [^{14}C]tryptamine, assayed in the absence (●) and presence of 200 (△) and 400 μM (▼) unlabelled benzylamine. F, [^{14}C]benzylamine, assayed in the absence (●) and presence of 12.5 (△) and 25 μM (▼) unlabelled tryptamine. At all incubation times used, no deviation from linearity could be detected. Essentially similar results were obtained when assays were conducted under an atmosphere of oxygen rather than air.

The proposed homogeneity of the platelet MAO-B is supported by the mixed substrate experiments shown in Fig. 3 (A–F) and summarised in Table 4. In all cases, the oxidation of one monoamine substrate was competitively inhibited by another when assayed either under an atmosphere of air or oxygen. The K_i of the monoamine as an inhibitor was always similar to the K_m of the enzyme towards the monoamine as a substrate as would be expected for a single enzyme system with alternative substrates.

MAO has been shown to follow a 'ping-pong' or double displacement reaction in a variety of animal tissues [25–28]. The apparent uncompetitive increase in activity of human platelet MAO when assayed in oxygen rather than air would suggest that the human blood platelet also follows this mechanism (Fig. 2A–C). However, the degree of increase appears to depend upon the amine substrate used for the estimation of activity, as found previously for rat heart and liver MAO [12, 29]. In all cases, the increase in activity towards β-phenethylamine and tryptamine were larger than that towards benzylamine. Increases in activity with increases in oxygen tension for human platelets have also been described by Kobayashi and Eiduson [30], although these authors used single amine concentrations. From the 'maximum oxygen ratios' shown in Table 3, approximate values of the apparent Michaelis constant towards oxygen (K_m^0) can be calculated by the method described previously [29], and have values of 642 ± 147 μM for β-phenethylamine as substrate, 947 ± 168 μM for tryptamine as substrate and 255 ± 53 μM for benzylamine as substrate. These dif-

ferences do not appear to be due to substrate inhibition by high concentrations of oxygen, as substrate inhibition in a ping pong system would not be expected to produce parallel regression lines on a Lineweaver–Burk plot [see 31]. In the experiments shown in Fig. 2 no deviation from parallelism could be discerned.

Edwards and Burns [10] have suggested multiplicity of monoamine catalytic centres on human platelet MAO-B on the basis of studies with the tricyclic antidepressant amitriptyline. These authors found that this drug inhibited the oxidation of β-phenethylamine and tryptamine in a mixed fashion, whereas the inhibition of benzylamine oxidation was competitive. Cleland [32] has shown that, for a double displacement reaction, an inhibitor that binds to both oxidised and reduced forms of the enzyme will produce an apparent non-competitive inhibition, which becomes more competitive in nature as the concentration of the second substrate (in this case oxygen) is raised. Roth [33, 34] has shown this to be the case for the inhibition by a variety of tricyclic antidepressants of human brain β-phenethylamine oxidation. However, it can be shown theoretically that a decrease in the apparent Michaelis constant towards the second substrate has the same effect as an increase in the concentration of the second substrate itself [12]. Thus, in the case of MAO, for a fixed oxygen concentration, the inhibition by a tricyclic antidepressant towards an amine substrate with a low K_m^0 value is likely to be more competitive in nature than when a substrate with a high K_m^0 value is used. In the experiments of Edwards and Burns [10], the amine substrate with a low K_m^0 value (benzylamine) is indeed inhibited in a competitive manner, whereas the inhibition of those amine substrates with higher K_m^0 values (β-phenethylamine and tryptamine) is more non-competitive in nature. Thus, these observations can be explained without the need to invoke more than one monoamine binding site.

The results of this study are therefore consistent with a model of amine oxidation by human blood platelet MAO-B with a single binding site for monoamine substrates. The differences in the K_m^0 values for the different amine substrates may be due to the existence of more than one binding site for oxygen in the MAO-B of human blood platelet. However, because of the complicated nature of the enzyme reaction pathway, differences in the K_m^0 values for different amine substrates are not in themselves proof of heterogeneity of oxygen binding sites, and more work is needed to clarify the situation.

Acknowledgements—This work was supported by generous grants from the Swedish Medical Research Council (grant No. 13X-4145) (L.O.) and from the Medical Faculty, University of Umeå (B.E.). C.J.F. would like to thank the Royal Society for a European Science Exchange Programme Fellowship.

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