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THE DEAMINATION OF MONOAMINES BY PIG DENTAL PULP

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Abstract—The deamination of monoamines by porcine dental pulp has been investigated. Both 'soluble' and membrane-bound monoamine oxidising activities were found. The 'soluble' activity, however, had an inhibitor specificity similar to pig plasma benzylamine oxidase, and could be due to contamination by this enzyme, since there is a large blood flow to the dental pulp of piglets. The membrane-bound activity was found to be due to both monoamine oxidase and a clorgyline-resistant, but semicarbazide-sensitive enzyme. Both enzymes were active towards all substrates tested (benzylamine, tryptamine, tyramine, β-phenethylamine and 5-hydroxytryptamine). Some kinetic properties of the monoamine oxidase and the clorgyline-resistant enzyme are presented.

The deamination of monoamines in most mammalian tissues is brought about primarily by monoamine oxidase (MAO; monoamine O_2: oxidoreductase, flavine-containing; E.C. 1.4.3.4). This enzyme appears to exist as two catalytically active forms, termed MAO-A and MAO-B, on the basis of the differential inhibition by substrate-selective inhibitors, such as clorgyline and l-deprenil (for review, see [1]). MAO-A is sensitive to inhibition by clorgyline but relatively insensitive to inhibition by l-deprenil, whereas the reverse is true for MAO-B [2, 3]. In the rat liver, for example, 5-hydroxytryptamine (5-HT) is metabolised by MAO-A alone, benzylamine by MAO-B alone, and tyramine by both forms of the enzyme [4]. However, the substrate specificities of the two forms vary considerably from tissue to tissue (see [1]).

The deamination of benzylamine is also brought about, in a variety of tissues, by a clorgyline and l-deprenil-resistant, but semi-carbazide-sensitive amine oxidase [5–9]. The function of this enzyme is at present unknown, but may be associated with the connective tissue (see [10]). In addition, a benzylamine oxidase has also been found in both pig and human plasma [11, 12].

Dental pulp is a tissue which contains both specialised connective tissue and undifferentiated cells, and in consequence might be expected to be a source of both the clorgyline-resistant enzyme and MAO. In ox dental pulp, benzylamine and kynuramine are deaminated by a pyridoxal phosphate-containing enzyme distinct from lysyl oxidase [13, 14]. However, nothing is known about the deamination of other monoamines by dental pulp.

In the present communication, the deamination of five monoamines by pig dental pulp has been investigated in an attempt to determine whether or not both MAO and a clorgyline-resistant amine oxidase are present in this tissue and to study some of their properties.

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MATERIALS AND METHODS

The radioactive substrates for MAO, 5-hydroxytryptamine-[side chain-2-^{14}C]-binoxalate (5-HT), tyramine-[ethyl-1-^{14}C]-hydrochloride, tryptamine-[side chain-2-^{14}C]-bisuccinate and β-phenethylamine-[ethyl-1-^{14}C]-hydrochloride were obtained from New England Nuclear, Boston, Mass., USA. Benzylamine-[methylene-^{14}C]-hydrochloride was obtained from ICN Pharmaceuticals Inc., Irvine, CA, USA. All non-radioactive substrates, with the exception of benzylamine, were bought as salts, as the purity of the salts over a long period could be guaranteed better than the free bases, and substrate contamination has been shown to produce inaccuracies in kinetic experiments (see [15]). Benzylamine was converted into the hydrochloride by dissolution of the free base in ethyl acetate and addition of concentrated hydrochloric acid, the insoluble hydrochloride being collected and recrystallised in ethyl acetate/ethanol.

Clorgyline hydrochloride was a gift from May and Baker Ltd., Dagenham, U.K. 1-Deprenil hydrochloride was a gift from Prof. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary. Semicarbazide hydrochloride and phenanthroline hydrochloride were obtained from Merck, Darmstadt, West Germany. β-Aminopropionitrile fumarate was obtained from Sigma Chemical Company, St. Louis, Mo., USA; harmaline hydrochloride and cuprizone from Fluka, Buchs, Switzerland. All other reagents were standard laboratory reagents of analytical grade whereever possible. Pig lower jaws and blood were obtained from Norrlands Slakteriförening, Umeå, Sweden.

Preparation of subcellular fractions from tooth embryos. Lower jaws from 3–4 month old piglets were obtained after slaughter. For each preparation, tooth embryos (used because of their greater size and ease of extraction compared with the corresponding fully developed teeth) from 40 piglets were dissected, pooled (total weight approx 250 g) and

homogenised 1:4 (w/v) in 0.05 M potassium phosphate, pH 7.8, in an Ultra-Turrax homogeniser (type 18/2) at 0°. The homogenates were centrifuged at 13,000 *g* for 15 min, the pellets rehomogenised (1:4 w/v, in the same buffer) and recentrifuged, and the supernatant fractions combined. The combined supernatants were then centrifuged at 73,000 *g* for 60 min to give high-speed supernatants ('soluble fractions') and pellets ('membrane fractions'). The membrane fractions were set to 10 mg · ml^{-1} protein before assay. All fractions were stored frozen until used to assay for activity. Approximately 50 per cent of the total benzylamine oxidising activity was found in the soluble fraction, the remaining activity being associated with the membrane fractions. The properties of the 13,000 *g* × 15 min pellets and the 73,000 *g* × 60 min pellets with respect to their substrate specificities and sensitivities to inhibition by clorgyline and semicarbazide were very similar. However, there is a great deal of contaminating collagen in the 13,000 *g* × 15 min pellets, which makes for a very low specific activity. Since the degree of contaminating collagen is much lower in the 79,000 *g* × 60 min pellets, it was felt wiser to use these pellets in the investigation. Attempts to release the enzyme activity from the 13,000 *g* × 15 min pellets by either sonication, treatment with Triton X-100 (0.1% v/v) or urea (up to 4 M) were unsuccessful.

Partial purification of the soluble enzyme. The soluble fractions were purified two-fold with respect to their specific activity towards benzylamine by the first three steps of the method described by Yamada and Yasunobu [16]. Pig plasma, obtained by centrifugation of blood at 13,000 *g* for 15 min, was also treated in the same manner, the purification of the benzylamine oxidase obtained being four-fold.

Assays. Monoamine oxidase activity was determined radiochemically by the method of Callingham and Laverty [17] as modified by Fowler *et al.* [18] with ^{14}C-5-HT, ^{14}C[tyramine], ^{14}C[tryptamine], ^{14}C-β-[phenethylamine] and ^{14}C-[benzylamine] as substrates. Unless otherwise stated, all reactions were performed under an atmosphere of air. In all cases, incubation times were chosen so that the deamination of monoamines was linear with respect to both time and enzyme concentration. When clorgyline, 1-deprenil and semicarbazide were used to inhibit the oxidising activity, the fractions were preincubated with the inhibitors for 30 min, unless otherwise indicated in the text, before the addition of substrate. Specific activities, expressed as pmoles (of substrate metabolised) · (mg protein)$^{-1}$ · min^{-1}, were in all cases corrected for the efficiencies of extraction of the deaminated metabolites into the organic layer used in the assay medium [19]. Protein concentrations of the preparations were determined by the method of Lowry *et al.* [20], with human serum albumin as standard.

RESULTS

The substrate specificities of the soluble and membrane-bound deaminating activities from pig dental pulp towards the five substrates used in this study are shown in Table 1. Both fractions deaminate tryptamine and benzylamine with similar substrate specificities, but the soluble fractions deaminate 5-HT and β-phenethylamine very poorly.

The sensitivity of benzylamine oxidation to inhibition by a variety of compounds was determined in both soluble and membrane-bound preparations and in pig plasma. The inhibitor sensitivities of the soluble fraction enzyme and the pig plasma benzylamine oxidase were very similar, but differed greatly from the inhibitor sensitivities of the membrane-bound deaminating activity (Table 2). In the membrane-bound fractions, the oxidation of benzylamine was only partially inhibited by clorgyline, 1-deprenil and harmaline, and also only partially by classical amine oxidase inhibitors such as semicarbazide and cuprizone (Table 2), which would suggest the presence of both monoamine oxidase and a clorgyline-resistant amine oxidising activity. With all five substrates used in the study, the inhibition of activity in the membrane-bound fractions by clorgyline and semicarbazide was additive (Table 3), although the relative activities of the clorgyline-sensitive and semi-

Table 1. Activities of the enzymes responsible for the deamination of monoamines by membrane and soluble fractions of porcine dental pulp

Substrate	Enzyme activities (pmoles · mg protein^{-1} · min^{-1}) Membrane fractions	Soluble fractions
Benzylamine	23.9 ± 2.5 (100%)	15.0 ± 1.1 (100%)
Tryptamine	98.4 ± 24.8 (412%)	62.1 ± 9.3 (414%)
Tyramine	40.4 ± 5.1 (169%)	9.4 ± 1.2 (63%)
β-Phenethylamine	88.8 ± 13.5 (372%)	1.0 (7%)
5-HT	71.6 ± 4.0 (300%)	0.9 (6%)

Enzyme activities are given as the V_{max} values assayed under an atmosphere of air, calculated by linear regression analysis of duplicate determinations of activity at 6 substrate concentrations, plotted as S/v against S. Each value represents the mean (± S.E.M.) of determinations in three preparations. Because of the extremely low activity of the soluble fractions towards β-phenethylamine and 5-HT, it was not possible to estimate these V_{max} values. In consequence, for these two substrates, the values for the soluble activities are the means of duplicate determinations of activity at 100 μM substrate concentrations. Values in brackets represent the mean activities with respect to the value for benzylamine, expressed as a percentage.

Table 2. Inhibitor specificities of the enzymes responsible for the deamination of benzylamine in pig dental pulp and plasma

	% Inhibition of benzylamine oxidation in: Pig dental pulp		
	Membrane fractions	Soluble fractions	Pig plasma
Clorgyline			
10^{-4} M	64 ± 2	15	6
10^{-3} M	59 ± 4	16	19
10^{-2} M	66 ± 3	76	75
1-Deprenil			
10^{-4} M	66 ± 2	0	0
10^{-3} M	63 ± 3	0	3
10^{-2} M	70 ± 2	34	35
Harmaline			
10^{-4} M	24 ± 8	21	2
10^{-3} M	34 ± 11	49	44
10^{-2} M	49 ± 8	77	73
Phenanthroline			
10^{-4} M	53 ± 3	26	18
10^{-3} M	48 ± 2	67	66
10^{-2} M	52 ± 4	96	96
Semicarbazide			
10^{-4} M	15 ± 10	96	95
10^{-3} M	40 ± 12	98	97
10^{-2} M	75 ± 2	100	99
Cuprizone			
10^{-4} M	19 ± 9	74	75
10^{-3} M	25 ± 8	94	93
10^{-2} M	41 ± 4	97	95
β-Aminopropionitrile			
10^{-4} M	56 ± 3	0	0
10^{-3} M	64 ± 2	0	0
10^{-2} M	89 ± 1	40	25

Values represent the means (± S.E.R. where appropriate) of the per cent inhibition of benzylamine oxidation in three membrane fractions, two soluble fractions (partially purified) and two pig plasma fractions (partially purified). When cuprizone was used to inhibit the activity, it was dissolved in 50% ethanol, and compared with control preparations treated with the same concentration of ethanol. The concentration of benzylamine used to assay for activity was 100 μM. Assays were performed in duplicate. None of the drugs were preincubated with the enzyme preparations before addition of substrate.

Table 3. The effect of clorgyline, semicarbazide, and both drugs upon the deamination of monoamines by porcine dental pulp membranes

	% Inhibition of deamination by:		
	Clorgyline (0.33 mM)	Semicarbazide (0.33 mM)	Clorgyline (0.33 mM) +Semicarbazide (0.33 mM)
Benzylamine	47 ± 6	42 ± 4	99 ± 0.4
Tryptamine	84 ± 3	14 ± 8	99 ± 1
Tyramine	53 ± 1	34 ± 2	99 ± 1
β-Phenethylamine	65 ± 9	42 ± 15	99 ± 0.2
5-HT	27 ± 14	78 ± 6	100 ± 0

All assays were performed in duplicate in three membrane preparations, and the results expressed as means (± S.E.R.) of the percentage inhibition of the deamination produced by each drug. Substrate concentrations used were: 50 μM (benzylamine, tryptamine); 150 μM (tyramine, 5-HT) and 38 μM (β-phenethylamine).

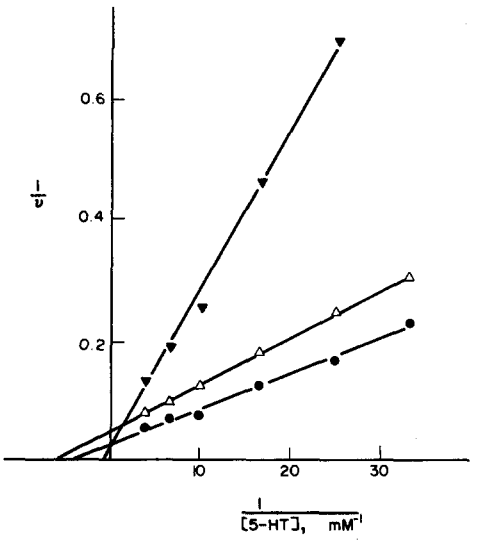

Fig. 1. Double reciprocal plots of the activity of pig dental pulp membrane fractions preincubated for 30 min at 37° with distilled water (●), 0.33 mM clorgyline (△), and 0.33 mM semicarbazide (▼). 5-HT was used as the substrate. Each point represents the mean of duplicate determinations of activity in three membrane preparations. Abscissa: 1/(substrate concentration in mM); ordinate: 1/(initial velocity in pmoles·mg protein^{-1}·min^{-1}). There was no difference found in the kinetic properties of membranes in either the absence or presence of 30 min of preincubation at 37° with distilled water.

carbazide-sensitive enzymes differed from substrate to substrate.

The additive nature of the inhibition by clorgyline and semicarbazide was also demonstrated in kinetic experiments. For benzylamine as substrate, V_{max} values (calculated as described in Tables 1 and 4) were determined as follows: membrane fractions, V_{max} = 43.2 ± 2.7; membrane fractions preincubated for 30 min with distilled water, V_{max} = 43.5 ± 3.1; membrane fractions preincubated for 30 min with 0.33 mM clorgyline, V_{max} = 20.1 ± 3.1; membrane fractions preincubated for 30 min with 0.33 mM semicarbazide, V_{max} = 33.0 ± 3.6 (values, given as pmoles·mg protein^{-1}·min^{-1}, are means ± S.E.M. for determinations in three membrane preparations). There were no significant differences in the K_m values of the different groups. For tryptamine as substrate, the V_{max} values were: 131.8 ± 4.4, 136.0 ± 3.4, 48.7 ± 4.3 and 118.4 ± 10.9 pmoles·mg protein^{-1}·min^{-1}, for the four groups, respectively, assayed under an atmosphere of air. The K_m values for the four groups were of the same order of magnitude. When 5-HT was used as a substrate, however, the K_m value of the MAO was very much higher than that of the clorgyline-resistant enzyme (Fig. 1).

The oxidation of benzylamine and tryptamine by the membrane-bound preparations appeared to be increased in an uncompetitive manner when the activities were determined under an atmosphere of oxygen rather than air (Fig. 2A and B). From these data, values of the Michaelis constants towards

Table 4. The effect of oxygen upon the activities of the clorgyline-resistant enzyme and MAO-B towards tryptamine and benzylamine

	K_m (μM)	V_{max} (pmoles·mg protein^{-1}·min^{-1})	Maximum oxygen ratio	Estimated K_0 (μM)
Benzylamine				
Clorgyline-resistant enzyme				
Oxygen	36 ± 21	52.9 ± 17.8	1.34 ± 0.03	101 ± 10
Air	28 ± 15	39.9 ± 13.6		
Monoamine oxidase				
Oxygen	44 ± 20	47.1 ± 14.5	1.28 ± 0.02	82 ± 7
Air	38 ± 17	37.1 ± 11.7		
Tryptamine				
Clorgyline-resistant enzyme				
Oxygen	315 ± 5	74.8 ± 2.9	1.23 ± 0.05	67 ± 14
Air	279 ± 21	68.8 ± 7.2		
Monoamine oxidase				
Oxygen	106 ± 9*	237.6 ± 15.8*	1.93 ± 0.06*	330 ± 28*
Air	60 ± 5*	122.9 ± 5.7*		

K_m and V_{max} values were calculated by linear regression analysis of duplicate determinations of activity at 6 substrate concentrations (benzylamine range 5–200 μM; tryptamine range 50–400 μM), plotted as S/v against S. In all cases, the correlation coefficients for the regression lines were greater than $r = 0.94$. Maximum oxygen ratios were calculated from the individual data as V_{max} assayed under an atmosphere of oxygen divided by V_{max} assayed under an atmosphere of air. The estimated K_0 values were calculated from the individual maximum oxygen ratio values as described elsewhere [21]. Values represent the means (± S.E.M. or S.E.R. as appropriate) of determinations in three membrane preparations. Clorgyline-resistant amine oxidase and monoamine oxidase activities were determined after prior preincubation at 37° for 30 min of porcine dental pulp membrane preparations with 0.33 mM clorgyline and 0.33 mM semicarbazide, respectively.

* Significantly different for the corresponding value for the clorgyline-resistant enzyme ($P < 0.05$, two-tailed paired t-test).

oxygen were estimated as described previously [21], and were 140 and 240 μM for benzylamine and tryptamine as amine substrates, respectively. Since both tryptamine and benzylamine oxidations are composed of both monoamine oxidase and a clorgyline-resistant amine oxidase, the effect of changed oxygen tension upon the activity of the membrane preparations towards these two substrates was determined after prior preincubation with 0.33 mM clorgyline and semicarbazide (Table 4). In all cases, the interaction between the enzymes and oxygen appeared to be uncompetitive in nature. The kinetic parameters are given in Table 4. The activities of both MAO and the clorgyline-resistant enzyme were affected only to a small degree with changing oxygen tension when benzylamine was used as substrate, as was the clorgyline-resistant enzyme when tryptamine was used as substrate. However, with tryptamine as substrate, the maximum oxygen ratio (calculated as V_{max} (oxygen)/V_{max} (air)), and hence the estimated Michaelis constant towards oxygen, was significantly higher for MAO than for either the value of the clorgyline-resistant enzyme with tryptamine as substrate or the MAO with benzylamine as substrate ($P < 0.05$, two-tailed paired t-test) (Table 4).

The inhibition of the deamination of the 5 substrates used in this study by increasing concentrations of clorgyline, in the presence of 0.33 mM semicarbazide, is shown in Fig. 3A and B. From this figure, and the data shown in Table 3, it would appear that the oxidation of benzylamine, tryptamine, tyramine and β-phenethylamine is brought about by MAO-B and by the clorgyline-resistant enzyme, but not by MAO-A. The oxidation of 5-HT appears to be brought about by the clorgyline-resistant enzyme and an MAO that is more sensitive to inhibition by clorgyline, presumably corresponding to MAO-A.

DISCUSSION

Dental pulp is a highly specialised soft connective tissue, containing not only fibroblasts and undifferentiated mesenchymal cells, but also specialised types of cells such as histiocytes and odontoblasts. For this study, piglets were chosen as the source of teeth, because of their morphological similarities to human teeth (see [22]). Although more is known about the deamination of benzylamine by bovine dental pulp [14], this species was not chosen here as ox teeth grow continuously, whereas pig and human teeth do not.

Both 'soluble' and membrane-bound fractions derived from porcine dental pulp were found to be able to deaminate tryptamine, benzylamine and tyramine. However, the membrane-bound fractions appeared to be responsible for the bulk of the deamination of 5-HT and β-phenethylamine. The inhibitor specificity of the 'soluble' activity was very similar to that for pig plasma benzylamine oxidase (Table 2), which would suggest strongly that the 'soluble' activity is due to contamination by the plasma enzyme, especially since there is a relatively large blood supply to the dental pulp of the young piglet [22]. In consequence, the work discussed below was done on the membrane-bound oxidising activity.

The oxidation of benzylamine by the membrane fractions was partially inhibited by both classical MAO inhibitors such as clorgyline, 1-deprenil and harmaline as well as by inhibitors of pyridoxal–phosphate and copper containing amine oxidases (semicarbazide and cuprizone). The lysyl oxidase inhibitor β-propionitrile was also found to inhibit the activity, although, in view of the high concentrations used, this could be a non-specific effect unrelated to its ability to inhibit lysyl oxidase. Complete inhibition by either the MAO inhibitors or by the amine oxidase inhibitors was not found, even at concentrations as high as 10^{-2} M (Table 2), which would suggest that the oxidation of benzylamine by porcine dental pulp membranes is brought about not only by MAO but also by a clorgyline-resistant amine oxidase. This conclusion is supported by the data shown in Table 3, which indicates that, with all five substrates used, the inhibitions produced by clorgyline and semicarbazide are additive. Clorgyline-resistant, but semicarbazide-sensitive enzyme activities have been reported for a variety of rat and human tissues [5, 8, 9, 23], and also for the chick heart, where it has been shown to be localised to the mitochondrion [6]. However, the pig dental pulp appears to be the only tissue so far reported where a 5-HT metabolising, clorgyline-resistant enzyme has been found

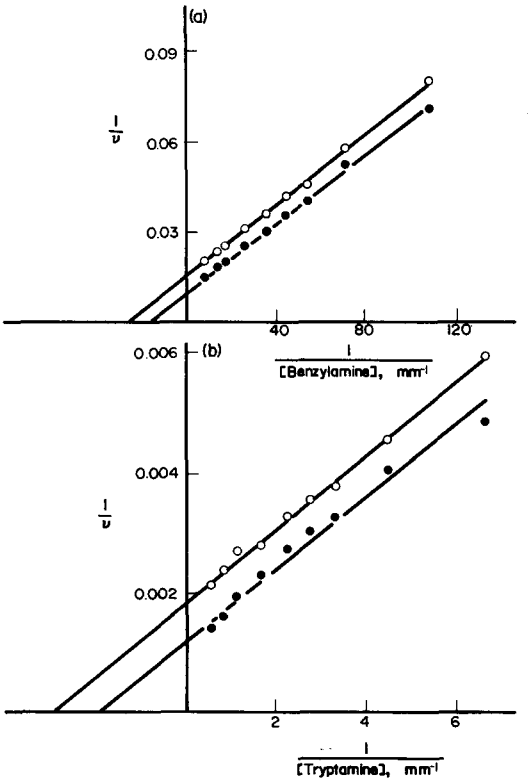

Fig. 2. Double reciprocal plots of the activity of pig dental pulp membrane fractions towards: A, benzylamine; B, tryptamine, assayed under an atmosphere of oxygen (●) and air (○). Abscissae: 1/(substrate concentration in mM); ordinates: 1/(initial velocity in pmoles·mg protein^{-1}·min^{-1}). Each point represents the mean of duplicate determinations in a preparation of membranes pooled from tooth embryos from the lower jaws of 40 piglets.

(Table 3). In a large number of tissues of the rat, for example, no such activity was found [24]. The possibility that this dental pulp clorgyline-resistant 5-HT oxidising activity could be due to tightly bound plasma amine oxidase is unlikely, since the soluble enzyme appears not to be able to deaminate 5-HT to any significant degree (Table 1).

The nature of the monoamine oxidase component in the dental pulp was investigated by clorgyline inhibition studies in the presence of semicarbazide (Fig. 3A and B). Benzylamine, tryptamine, tyramine and β-phenethylamine all appear to be substrates for MAO-B but not MAO-A, whereas the oxidation of 5-HT is more sensitive to inhibition by clorgyline. Most pig tissues are thought to contain only the -B form of MAO, (see e.g. [25, 26]), although 5-HT has been found to be a substrate for both forms of the enzyme in pig brain and liver [27].

Monoamine oxidase is thought to follow a ping-pong, or double displacement reaction in a variety of tissues [28–31]. A similar mechanism is also thought to occur for diamine oxidase [32]. As a consequence of such a mechanism, the enzyme activity is increased in an uncompetitive manner as the concentration of the second substrate, oxygen, is raised (see [21, 33]). Such an uncompetitive increase in activity was found for the oxidation of benzylamine and tryptamine by pig liver membranes (Fig. 2). When the component parts of the oxidation were studied by preincubation of the membranes with clorgyline and semicarbazide, both the clorgyline-resistant enzyme and the monoamine oxidase-B were found to be affected in an uncompetitive manner by increasing concentrations of oxygen since the ratio of V_{max} (oxygen)/V_{max} (air) is approximately equal to the ratio of K_m (oxygen)/K_m (air) (data shown in Table 4). From the maximum oxygen ratios shown in Table 4, the values of the approximate Michaelis constants of the enzymes towards oxygen could be estimated (Table 4), although it should be stressed that these values must be considered as approximate since they are determined from two concentrations of oxygen. However, the K_0 values obtained are in good agreement with those found for monoamine oxidase in a variety of tissues [7, 18, 21, 28–31, 33–35]. The data shown in Table 4 would also suggest that the value of the Michaelis constant of the MAO-B towards oxygen is dependent upon the amine substrate used to assay for activity, a result also found for rat liver, human brain and human platelet [18, 21, 34, 35]. This dependency of the Michaelis constant towards oxygen upon the amine substrate is not due to enzyme heterogeneity, but is due to the nature of the enzyme reaction pathway [see 34, 35].

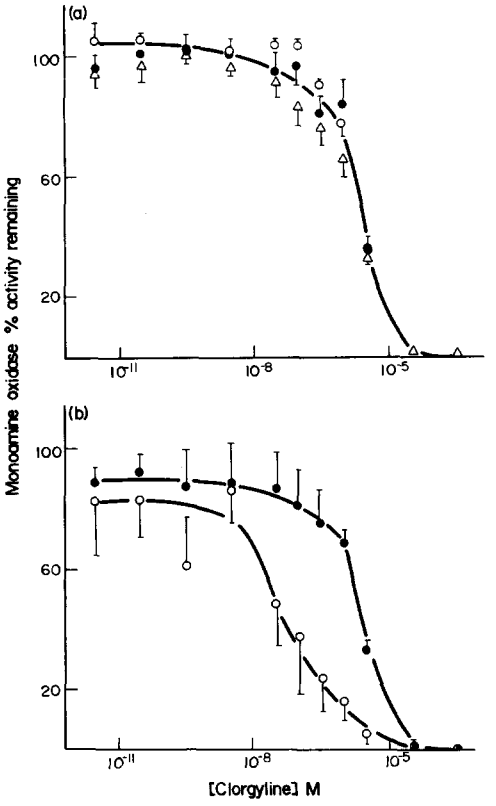

Fig. 3. The effect of clorgyline, in the presence of 0.33 mM semicarbazide (in order to inhibit the clorgyline-resistant enzyme), on the *in vitro* deamination of: A, benzylamine (○), tryptamine (●) and tyramine (△); B, β-phenethylamine (●) and 5-HT (○) in membrane preparations of pig dental pulp. Each point represents the mean MAO activity (± S.E.R.), assayed in duplicate in three groups of membrane preparations, expressed as a percentage of its uninhibited control, plotted against the molar concentration of clorgyline. Preincubation time: 30 min. Substrate concentrations were: 50 μM (benzylamine, tryptamine), 150 μM (tyramine, 5-HT), 38 μM (β-phenethylamine).

The K_m and V_{max} values of both MAO-B and the clorgyline-resistant enzyme towards benzylamine as substrate were similar (Table 4), indicating that under physiological conditions, each enzyme is responsible for the oxidation of about 50 per cent of the benzylamine. Tryptamine appears preferentially to be metabolised by MAO-B, since this enzyme metabolises tryptamine with a higher V_{max} and a lower K_m than the clorgyline-resistant enzyme. On the other hand, the K_m value of the MAO towards 5-HT was found to be much higher than the K_m of the clorgyline-resistant enzyme towards this substrate (Fig. 1), which would suggest that, at physiological concentrations of 5-HT, the bulk of this substrate will be metabolised by the clorgyline-resistant enzyme alone.

The physiological role of the clorgyline-resistant enzyme is not elucidated as yet. In the chick heart, both the MAO and clorgyline-resistant enzyme appear to be under similar hormonal control, as they are affected to the same extent by age and thyroid hormones [6], which might indicate some sort of functional interdependence of the two enzymes one upon the other, although in the rat heart, the two enzymes appear to have a different subcellular localisation in the cell, and are affected differently by age and thyroid hormones [5, 36, 37]. The clorgyline-resistant enzyme may also be associated with lysyl oxidase, an enzyme involved in the formation of collagen and elastic fibres [see 10]. However, until more is known about the nature of the clorgyline-resistant enzyme, it is impossible to speculate upon its functional significance.

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REFERENCES

1. C. J. Fowler, B. A. Callingham, T. J. Mantle and K. F. Tipton, *Biochem. Pharmac.* **27**, 97 (1978).
2. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
3. J. Knoll and K. Magyar, *Adv. Biochem. Psychopharmac.* **5**, 393 (1972).
4. D. W. R. Hall, B. W. Logan and G. H. Parsons, *Biochem. Pharmac.* **18**, 1447 (1969).
5. G. A. Lyles and B. A. Callingham, *J. Pharm. Pharmac.* **27**, 682 (1975).
6. C. J. Fowler and B. A. Callingham, *J. Pharm. Pharmac.* **29**, 593 (1977).
7. C. J. Fowler, PhD Thesis, University of Cambridge (1978).
8. R. Lewinsohn, K.-H. Böhm, V. Glover and M. Sandler, *Biochem. Pharmac.* **27**, 1857 (1978).
9. T. A. Ryder, M. L. MacKensie, J. Pryse-Davies, V. Glover and M. Sandler, *Histochemistry* **62**, 93 (1979).
10. H. Blanschko, *Rev. Physiol. Biochem. Pharmac.* **70**, 83 (1974).
11. F. Buffoni, L. Della Corte and D. B. Hope, *Proc. R. Soc. B.* **195**, 417 (1977).
12. R. Lewinsohn, *Clin. Chim. Acta* **81**, 247 (1977).
13. G. Nakano, M. Harada and T. Nagatsu, *Biochim. biophys. Acta* **341**, 366 (1974).
14. K. Shimozato, T. Kawai, M. Hino, H. Kuzuya and T. Nagatsu, *Archs. oral Biol.* **21**, 495 (1976).
15. K. F. Tipton, T. J. Mantle and M. D. Houslay, *Biochem. Pharmac.* **26**, 1525 (1977).
16. H. Yamada and K. T. Yasunobu, *J. biol. Chem.* **237**, 1511 (1962).
17. B. A. Callingham and R. Laverty, *J. Pharm. Pharmac.* **25**, 940 (1973).
18. C. J. Fowler, B. Ekstedt, T. Egashira, H. Kinemuchi and L. Oreland, *Biochem. Pharmac.* **28**, 3063 (1979).
19. C. J. Fowler, J. Marcusson, L. Oreland and B. Winblad, *N.S. Arch. Pharmac.* **311**, 263 (1980).
20. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
21. C. J. Fowler and B. A. Callingham, *Biochem. Pharmac.* **27**, 1995 (1978).
22. E. B. Jump and M. E. Weaver, in *Swine in Biomedical Research* (Eds L. K. Bustad, R. D. McClellan and M. P. Burns) pp. 543–557. Richland, Washington (1965).
23. J. A. Fuentes and N. H. Neff, *Biochem. Pharmac.* **26**, 2107 (1977).
24. K.-H. Böhm, V. Glover, M. Sandler, M. Petty and J. L. Reid, *J. Neurochem.* **33**, 607 (1979).
25. G. A. Lyles and J. W. Greenawalt, *Biochem. Pharmac.* **27**, 923 (1978).
26. H. C. Stanton, R. A. Cornejo, H. J. Mersmann, L. J. Brown and R. L. Mueller, *Archs Int. Pharmacodyn. Ther.* **213**, 128 (1975).
27. B. Ekstedt and L. Oreland, *Archs Int. Pharmacodyn. Ther.* **222**, 157 (1976).
28. K. F. Tipton, *Eur. J. Biochem.* **5**, 316 (1968).
29. A. G. Fischer, A. R. Schulz and L. Oliner, *Biochim. biophys. Acta* **159**, 460 (1968).
30. S. Oi, K. Shimada, M. Inamasu and K. T. Yasunobu, *Archs Biochem. Biophys.* **139**, 28 (1970).
31. M. D. Houslay and K. F. Tipton, *Biochem. J.* **135**, 735 (1973).
32. A. Finazziagro, G. Rotilio, M. T. Costa and B. Mondovi, *FEBS Lett.* **4**, 31 (1969).
33. K. F. Tipton, *Adv. Biochem. Psychopharmac.* **5**, 11 (1972).
34. C. J. Fowler and L. Oreland, *Biochem. Pharmac.* **29**, 2225 (1980).
35. J. A. Roth, in *Monoamine Oxidase; Structure, Function and Altered Functions* (Eds T. P. Singer, R. W. Von Korff and D. L. Murphy) pp. 153–168. Academic Press, New York (1979).
36. G. A. Lyles and B. A. Callingham, *J. Pharm. Pharmac.* **26**, 921 (1974).
37. B. A. Callingham and G. A. Lyles, *Br. J. Pharmac.* **53**, 458 P (1975).