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SOME PROPERTIES OF MONOAMINE OXIDASE AND A SEMICARBAZIDE SENSITIVE AMINE OXIDASE CAPABLE OF THE DEAMINATION OF 5-HYDROXYTRYPTAMINE FROM PORCINE DENTAL PULP

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Abstract—The deamination of 5-hydroxytryptamine, tryptamine and benzylamine by porcine dental pulp membrane preparations is brought about not only by monoamine oxidase, but also by a clorgyline (and deprenyl) resistant, semicarbazide sensitive enzyme. The semicarbazide sensitive enzyme was also inhibited by aminoguanidine, hydroxylamine and phenylhydrazine, but was not affected to any significant extent by incubation at 50° for up to 100 min. There was, on the other hand, considerable inhibition of monoamine oxidase activity after incubation at this temperature. The semicarbazide sensitive enzyme neither metabolised, nor was inhibited by putrescine or cadaverine. Mixed substrate experiments indicated that 5-hydroxytryptamine and tryptamine interacted at the same catalytic centre on the semicarbazide sensitive enzyme.

In most tissues, the enzyme monoamine oxidase [MAO, monoamine O_2: oxidoreductase (flavine containing), EC 1.4.3.4] is responsible for the deamination of a large variety of monoamines such as the transmitter amines (noradrenaline, adrenaline, dopamine and 5-hydroxytryptamine) as well as amines found in foodstuffs (e.g. tyramine and β-phenethylamine) and a variety of amines not found in the body, such as benzylamine and kynuramine (for reviews see [1, 2]). The enzyme exists in two forms, termed MAO-A and MAO-B, where the A form is sensitive to inhibition by low concentrations of the acetylenic inhibitor clorgyline and the B form sensitive to inhibition by l-deprenyl [3, 4].

In addition to MAO, several tissues contain an enzyme capable of the deamination of benzylamine (and in some cases also tyramine and β-phenethylamine) that is not inhibited by either clorgyline or l-deprenyl, but is sensitive to inhibition by the carbonyl reagent semicarbazide [5–11]. The function of this enzyme is at present unknown.

In a recent study, it was found that pig dental pulp contained both monoamine oxidase (of which the B form was predominant) and a semicarbazide-sensitive enzyme activity that was capable of the deamination of not only benzylamine, tryptamine, tyramine and β-phenethylamine, but also 5-hydroxytryptamine, a feature apparently unique to this tissue in mammals [12]. The presence of both MAO and the semicarbazide-sensitive enzyme within a single tissue makes the pig dental pulp a useful tissue in which to compare and contrast the properties of the two enzymes. In the present study, the thermostability, kinetic properties and some inhibitor sensitivities of the two enzymes have been investigated.

MATERIALS AND METHODS

The radioactive substrates 5-hydroxytryptamine-[side chain-2-^{14}C]binoxalate (5-HT) and tryptamine-[side chain-2-^{14}C]bisuccinate were obtained from New England Nuclear (Boston, MA). Benzylamine-[methylene-^{14}C]hydrochloride was obtained from ICN Pharmaceuticals Inc. (Irvine, CA). Clorgyline hydrochloride was a gift from May & Baker (Dagenham, U.K.). FLA 336(+) (4-dimethylamino-α,2-dimethylphenethylamine) was a gift from Astra Läkemedel AB (Södertälje, Sweden). Semicarbazide hydrochloride was obtained from Merck (Darmstadt, West Germany). Aminoguanidine bicarbonate, hydroxylamine hydrochloride and phenylhydrazine hydrochloride were obtained from Sigma London (Poole, U.K.). All other reagents were standard laboratory reagents of analytical grade wherever possible. Pig lower jaws were obtained from Norrlands Slakteriförening (Umeå, Sweden).

Lower jaws from 3–4 month old piglets were obtained after slaughter. For each preparation, tooth embryos from 40 piglets were dissected, pooled (giving a total weight of about 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 4°. The homogenates were centrifuged at 13,000 *g* for 15 min, the pellets rehomogenised (1:4 w/v, in the same buffer), recentrifuged, and the supernatant fractions combined and centrifuged at 73,000 *g* for 60 min. The pellets ('membrane fractions') were set to 10 mg/ml protein before assay. Only about 10% of activities of MAO:semicarbazide-sensitive amine activity was found in the 'membrane fractions', the remainder being associated with the 13,000 *g* 15 min pellet. For all substrates tested, however, the ratios of activities of MAO: semicarbazide-sensitive amine oxidase were the same in the membrane fractions

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as in the 13,000 *g* 15 min fractions. The specific activities of the two enzymes were considerably higher in the membrane fractions than in the 13,000 *g* 15 min fractions. Furthermore, the 13,000 *g* 15 min fractions were difficult to use in microassays due to the large amount of collagen present. Attempts to release the enzyme activities from the 13,000 *g* 15 min fractions by either sonication, treatment with Triton X-100 or urea were unsuccessful. In consequence, the 'membrane fractions' were used in all the experiments reported here.

MAO and the semicarbazide sensitive enzyme activities were determined radiochemically as described elsewhere [13], with [^{14}C]5-HT, [^{14}C]tryptamine and [^{14}C]benzylamine as substrates. All incubation times, which were performed under an atmosphere of air, were chosen so that the deamination of monoamines was linear with respect to both time and enzyme concentration. When clorgyline and semicarbazide were used as inhibitors of the two enzymes, the fractions were preincubated with the inhibitors at a concentration of 1 mM for 30 min at 37° before addition of substrate to assay for remaining enzyme activity. Specific activities, expressed as pmoles (of substrate metabolised)/mg protein/min, were in all cases corrected for the efficiencies of extraction of the deaminated metabolites into the organic layer used in the assay medium [14]. Protein concentrations of the preparations were determined by the method of Markwell *et al.* [15], with human serum albumin as standard.

RESULTS AND DISCUSSION

In agreement with the results from the previous study [12], the deamination of 5-HT, tryptamine and benzylamine were sensitive to partial inhibition by the MAO inhibitor clorgyline and the carbonyl reagent semicarbazide, the inhibitors acting in an additive manner. Thus, for example, at the concentration of tryptamine tested (200 μM), 38% of the total activity was brought about by the semicarbazide sensitive enzyme whereas about 59% was brought about by MAO (Table 1). 5-HT, at a concentration of 400 μM, was deaminated preferentially by the semicarbazide sensitive enzyme (Table 1), a feature unique to this tissue in mammals, since no other mammalian tissue so far investigated has been found to contain a semicarbazide sensitive enzyme capable of the deamination of 5-HT [9].

The semicarbazide sensitive enzyme activity was further studied in preparations preincubated for 30 min with 1 mM clorgyline to inhibit the MAO activity. The activity of this semicarbazide sensitive enzyme, with benzylamine as substrate, was inhibited not only by semicarbazide (IC_{50} 130 μM), but also by the classical inhibitors of the EC 1.4.3.6 class: aminoguanidine (IC_{50} 60 μM), hydroxylamine (IC_{50} 5 μM) and phenylhydrazine (IC_{50} 10 μM) (Fig. 1). Hydroxylamine appears to be almost as potent an inhibitor of the semicarbazide sensitive enzyme as benserazide, which has an IC_{50} of about 1 μM. dependent upon the tissue and the preincubation time used [16].

Neither cadaverine nor putrescine, even at concentrations of 10 mM, inhibited the oxidation of benzylamine or tryptamine by either MAO or the semicarbazide sensitive enzyme to any great extent (Table 2). Histamine, at a concentration of 10 mM, however, was inhibitory towards MAO (Table 2), in agreement with a previous result [17]. By use of a photometric assay for tissue deaminating activity [18], it was found that there was no detectable deamination of putrescine, cadaverine or histamine by the dental pulp membranes. This absence of detectable activity was not due to methodological errors, since the assay was checked with a pig kidney preparation, and, with putrescine as substrate, gave significant deaminating activity with a K_m value of 150 μM. These experiments would therefore suggest that the semicarbazide sensitive enzyme is distinct from the classical diamine oxidase (DAO, EC 1.4.3.6), since the enzyme neither metabolises nor is inhibited by putrescine, cadaverine or histamine to any significant extent.

Pig dental pulp MAO and the semicarbazide sensitive enzyme differ considerably in their sensitivities to thermal inactivation. After incubation of the membrane fractions at 50° for varying times followed by selective inhibition of the enzymes with 1 mM clorgyline or semicarbazide as appropriate, large differences in the % activity remaining for the two enzymes were found (Table 3). As an example, the data for benzylamine as substrate is shown in Fig. 2. The semicarbazide sensitive enzyme was extremely thermostable, with little or no inhibition of enzyme activity being found even after 100 min of preincubation at 50°. This result was found regardless of the amine substrate used to assay for activity (Table 3). The monoamine oxidase component was,

Table 1. The effect of clorgyline and semicarbazide upon the deamination of monoamines by pig dental pulp

	% Activity remaining in the presence of:	
Substrate	1 mM Clorgyline	1 mM Semicarbazide
5-HT (400 μM)	80 ± 6	23 ± 5
Tryptamine (200 μM)	38 ± 8	59 ± 4
Benzylamine (200 μM)	65 ± 21	46 ± 14

Results are expressed as means ± S.E.R. of duplicate determinations in 3 membrane fractions of the activity remaining in the presence of either clorgyline (1 mM) or semicarbazide (1 mM) after preincubation for 30 min at 37° with respect to samples preincubated for the same length of time with distilled water. The substrate concentrations used to assay for activity are given in the table.

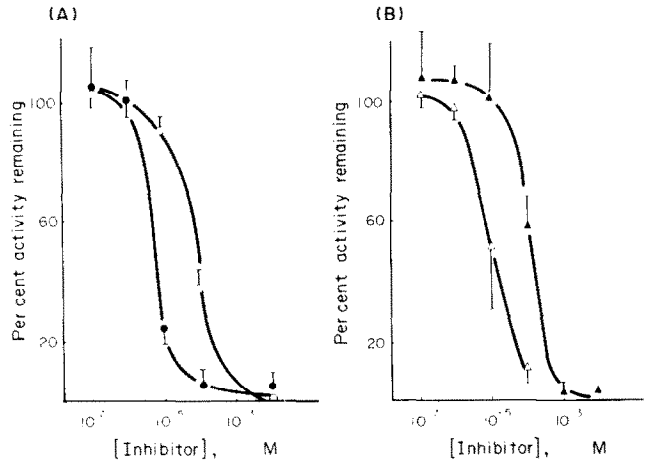

Fig. 1. Inhibition of the semicarbazide-sensitive amine oxidising activity by (A) aminoguanidine (○) and hydroxylamine (●); and (B) phenylhydrazine (△) and semicarbazide (▲). Membrane preparations were preincubated for 30 min at 37° with 1 mM clorgyline in order to inhibit the MAO activity before assay for activity with 50 μM benzylamine as substrate in the absence or presence of the inhibitors. Each point represents the mean ± S.E.R. of duplicate determinations in three membrane preparations.

Table 2. The effect of cadaverine, putrescine and histamine upon the deamination of tryptamine (50 μM) and benzylamine (50 μM) by MAO and the semicarbazide-sensitive enzyme from pig dental pulp

		% Inhibition of enzyme activity			
		Monoamine oxidase		Semicarbazide-sensitive amine oxidase	
Substrate		Tryptamine	Benzylamine	Tryptamine	Benzylamine
Cadaverine,	10^{-3} M	1 ± 1	0.3 ± 0.3	6 ± 4	1 ± 0.7
	10^{-2} M	10 ± 2	9 ± 1	7 ± 1	8 ± 0.2
Putrescine,	10^{-3} M	0 ± 0	0 ± 0	2 ± 1	3 ± 2
	10^{-2} M	22 ± 2	16 ± 1	6 ± 3	4 ± 0.3
Histamine,	10^{-4} M	5 ± 3	3 ± 1	5 ± 3	0 ± 0
	10^{-3} M	14 ± 3	9 ± 3	15 ± 1	0.3 ± 0.1
	10^{-2} M	30 ± 5	51 ± 1	20 ± 2	12 ± 4

MAO and semicarbazide-sensitive enzyme activities were assayed with prior preincubation for 30 min at 37° with 1 mM semicarbazide and 1 mM clorgyline respectively. Results are given as means ± S.E.R. of determinations in 3 dental pulp membrane preparations.

Table 3. Thermostabilities at 50° of the MAO and clorgyline-resistant enzyme towards three substrates

Time at 50° (min)	Substrate	% Activity remaining		
		No inhibitor present	+ 1 mM Clorgyline	+ 1 mM Semicarbazide
10	5-HT	91 ± 9	107 ± 6	94 ± 6
	Tryptamine	83 ± 5	115 ± 10	74 ± 3
	Benzylamine	83 ± 4	89 ± 4	70 ± 7
40	5-HT	89 ± 2	104 ± 6	71 ± 3
	Tryptamine	68 ± 10	115 ± 5	61 ± 4
	Benzylamine	59 ± 9	76 ± 10	33 ± 10
80	5-HT	93 ± 9	104 ± 3	67 ± 7
	Tryptamine	69 ± 8	117 ± 11	51 ± 7
	Benzylamine	54 ± 8	84 ± 2	28 ± 6

Aliquots of porcine dental pulp membrane preparations were preincubated at 50° for the times given in the text, followed by a further preincubation at 37° for 30 min with either distilled water, 1 mM clorgyline or 1 mM semicarbazide as appropriate, before addition of substrate to assay for activity. 5-HT (400 μM), tryptamine (200 μM) and benzylamine (200 μM) were used as substrates, and the results expressed as the activity as a percentage of the activity in the absence of incubation at 50°, and as means ± S.E.R. of duplicate determinations in 3 membrane preparations.

however, considerably more thermosensitive (Table 3, Fig. 2), the degree of thermal inhibition depending to some extent upon the substrate used to assay for enzyme activity. For example, the activity of MAO towards benzylamine was more thermosensitive than the activity towards tryptamine (Table 3), despite the fact that both substrates are metabolised by the same form of MAO [12]. A similar dependence of the thermal inhibition of pig heart MAO-B on the substrate used to assay for activity has also been reported by Lyles and Greenawalt [19]. However, such differences in the thermal inactivation pattern of an enzyme with different substrates does not constitute definite proof of enzyme heterogeneity, since the thermal denaturation is likely to involve changes in the enzyme reaction mechanism as well as involving straightforward inactivation of the enzyme.

In order to assess further the nature of the interaction of the two enzymes with their monoamine substrates, mixed substrate experiments were undertaken for both clorgyline and semicarbazide inhibited preparations. In all cases tested, the inhibition of the oxidation of one substrate by another was competitive. As examples, the inhibition of benzylamine oxidation by tryptamine in both clorgyline and semicarbazide inhibited preparations is shown in Figs. 3A and 3B. For the semicarbazide inhibited preparations, i.e. where the activity of MAO is being assayed, the K_i values of tryptamine towards the oxidation of either 5-HT or benzylamine were similar to the K_m value of MAO-B towards this substrate (Table 4). The K_i values for 5-HT as an inhibitor of the oxidation of benzylamine and tryptamine were, however, much higher than the K_m value of 5-HT as a substrate (Table 4). In the previous study [12], it was shown that although the deamination of benzylamine, tyramine, tryptamine and β-phenethylamine were brought about by MAO-B alone, a possible contribution of MAO-A as well as MAO-B was found for 5-HT. In the present study, it was found that approximately 50% of the MAO activity towards 5-HT (at an assay concentration of 150 μM) was inhibited by 5–25 μM FLA 336(+) (4-dimethyl-

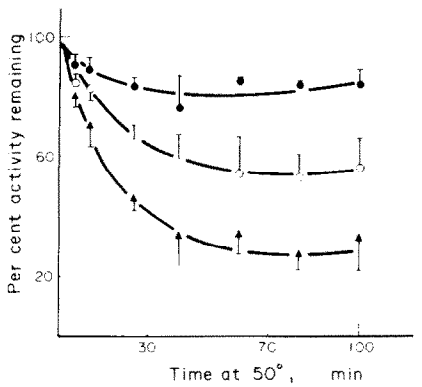

Fig. 2. Thermal denaturation of MAO and the semicarbazide-sensitive amine oxidising activity of pig dental pulp. The procedure employed is described in the legend to Table 2. Each point represents the mean ± S.E.R. of determinations in three membrane fractions, with 50 μM benzylamine as substrate. ○, uninhibited preparation; ▲, monoamine oxidase; ●, semicarbazide-sensitive amine oxidase.

Table 4. Inhibition of the activity of dental pulp MAO and semicarbazide-sensitive amine oxidase towards one substrate by another

Substrate	K_m (μM)	V_{max}	K_i (μM) of monoamine oxidase towards oxidation of: 5-HT	Tryptamine	Benzylamine
Monoamine oxidase					
5-HT	340 ± 140	71 ± 18	—	1,360 ± 140	1,430 ± 200
Tryptamine	60 ± 15	107 ± 14	60 ± 20	—	30 ± 20
Benzylamine	60 ± 10	54 ± 2	210 ± 70	250 ± 30	—
Semicarbazide-sensitive amine oxidase					
5-HT	120 ± 40	46 ± 6	—	370 ± 90	210 ± 20
Tryptamine	130 ± 20	47 ± 9	310 ± 30	—	100 ± 30
Benzylamine	2 ± 0.3	11 ± 0.3	830 ± 200	400 ± 90	—

MAO and semicarbazide-sensitive amine oxidase activities were assayed in duplicate after preincubation for 30 min at 37° with 1 mM semicarbazide and 1 mM clorgyline, respectively. K_m and V_{max} (in μM and pmoles/mg protein/min, respectively) were calculated by linear regression analysis of determinations at 5–6 substrate concentrations, plotted as $1/v$ against $1/S$. K_i values were calculated from secondary replots of slope against inhibitor concentration of the data plotted as $1/v$ against $1/S$ with 1–3 concentrations of non-radioactive substrate used in each case. Values are means ± S.E.M. of determinations in three membrane fractions. In all cases, the inhibition of enzyme activity towards one substrate by another was competitive.

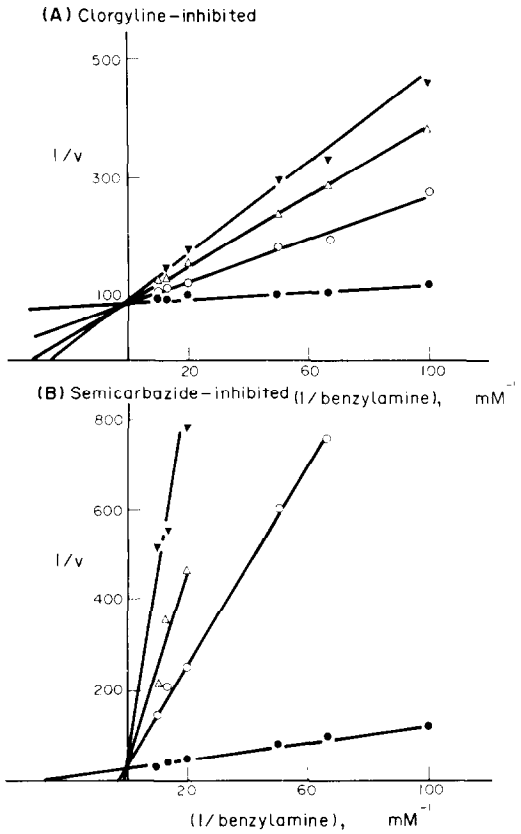

Fig. 3. Inhibition of the oxidation of benzylamine by tryptamine for (A) semicarbazide-sensitive amine oxidase; (B) monoamine oxidase. Concentrations of non-radioactive tryptamine were: ●, none; ○, 400 μM; △, 800 μM; ▼, 1200 μM. Each data point represents the mean of duplicate determinations in three membrane fractions. Data plotted as: ordinates, 1/(initial velocity in nmoles/mg protein/min); abscissae, 1/(benzylamine concentration in mM).

amino-α,2-dimethylphenethylamine), whereas only about 10% of the MAO activity towards benzylamine (at an assay concentration of 50 μM) was inhibited by this compound, even at a concentration of 200 μM. Since FLA 336(+) has been shown to be a competitive reversible MAO-A selective inhibitor [20, 21], these results would suggest that at least half of the deamination of 5-HT by MAO is brought about by the A form of the enzyme. In the pig liver, rat liver and rat brain, the K_m value of MAO-A towards 5-HT is about ten times lower than the K_m value of MAO-B towards this substrate [22–24]. If this is also the case for pig dental pulp, the K_i of 5-HT as an inhibitor of the oxidation of substrates for MAO-B alone, such as benzylamine and tryptamine, would be expected to be higher than the K_m value of 5-HT towards MAO-A and MAO-B assayed together. Thus, the mixed substrate experiments are consistent with the presence in pig dental pulp of an MAO-A enzyme form with activity towards 5-HT, and an MAO-B enzyme form where the metabolism of 5-HT, tryptamine and benzylamine all take place at the same catalytic centre.

For the semicarbazide sensitive enzyme, the K_i values of both 5-HT and tryptamine are similar to their K_m values, consistent with the notion that these monoamines are deaminated at the same catalytic site. However, the K_m value of benzylamine is considerably lower than the K_i values of benzylamine as an inhibitor of the oxidation of 5-HT and tryptamine (Table 4). This result raises the possibility that the semicarbazide sensitive deaminating activity may be composed of more than one enzyme species with one form active towards benzylamine and the other form active towards tryptamine and 5-HT. Further experiments are clearly necessary to explore this possibility.

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Note added in proof: Recently Kobayashi, Takahara and Kamijo [*Comp. Biochem. Physiol.* **69**, 179 (1981)] have reported a clorgyline-resistant, semicarbazide-sensitive 5-HT-metabolising activity in the frog liver.