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The deamination of n-pentylamine by monoamine oxidase and a semicarbazide-sensitive amine oxidase of rat heart

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n-Pentylamine is deaminated by homogenates of rat heart. Clorgyline inhibition curves at 10 and 100 μM n-pentylamine indicated that this substrate was deaminated by MAO-A, -B and a clorgyline-resistant amine oxidase sensitive to inhibition by semicarbazide. These results have been compared with two other commonly used monoamine substrates, β-phenethylamine and benzylamine.

The deamination of monoamines is usually brought about by the flavine-containing enzyme monoamine oxidase (MAO; monoamine: oxygen oxidoreductase (deaminating) (flavine containing), EC 1.4.3.4). This enzyme is divided into two catalytically distinguishable forms, MAO-A and MAO-B, where the -A form is sensitive to inhibition by very low (ca nanomolar) concentrations of clorgyline and the -B form sensitive to the inhibitor selegiline((−)-deprenyl) (Johnston 1968; Knoll & Magyar 1972).

In tissues such as the rat liver and brain where the concentrations of MAO-A and -B are similar, 5-hydroxytryptamine (5-HT) is metabolized preferentially by MAO-A, although a small amount of oxidation by MAO-B is observable at high concentrations of substrate, whereas the reverse is true for β-phenethylamine (see e.g. Dial & Clarke 1979; Kinemuchi et al 1980; Suzuki et al 1981; Fowler & Tipton 1982). Tyramine is a substrate for both forms of the enzyme, but the K_m of MAO-A towards this substrate is lower than that for MAO-B (Fowler & Tipton 1981). Benzylamine is deaminated essentially by MAO-B alone, although a very small (about 3%) deamination by MAO-A has been found in selegiline-treated liver mitochondria (Parkinson et al 1980).

In the rat heart, the concentration of MAO-A has been found to be 17 times higher than that of MAO-B (Parkinson et al 1980), and as a consequence 5-HT, tyramine and β-phenethylamine are metabolized mainly by MAO-A in this tissue (Dial & Clarke 1979; Lyles & Callingham 1979; Fowler & Callingham 1979). Benzylamine is metabolized by MAO-A, -B and a clorgyline-resistant semicarbazide-sensitive enzyme (Lyles & Callingham 1975; Clarke et al 1982). This latter enzyme has a much lower K_m for benzylamine than does MAO (Lyles & Callingham 1975; Andree & Clarke 1981; Barrand et al 1981; Clarke et al 1982) and is found in a variety of tissues (see e.g. Lyles & Callingham 1975; Fowler & Callingham 1977; Lewinsohn et al 1978, 1980; Ryder et al 1979; Andree & Clarke 1981; Barrand et al 1981; Norqvist et al 1981; Kobayashi et al 1981; Clarke et al 1982). Recently, it was reported that n-pentylamine is a substrate for MAO-B in the rat brain and lung (Strolin Benedetti et al 1981). In those experiments, no apparent deamination by MAO-A was found. A simple way to test whether or not this amine is specific for MAO-B is to determine the nature of its deamination in a tissue where the -A form predominates, such as the rat heart. The results of such a study are reported here.

MATERIALS AND METHODS

The radioactive substrate 5-hydroxytryptamine-[side chain-2-^{14}C] creatinine sulphate, tyramine-[7-^{14}C] hydrochloride and benzylamine-[7-^{14}C] hydrochloride were obtained from the Radiochemical Centre, Amersham, U.K.; β-phenethylamine-[ethyl-1-^{14}C] hydrochloride was obtained from New England Nuclear, Boston, Mass., U.S.A.; and pentylamine-[1-^{14}C] (1 μCi μmol^{-1} at assay) was a special synthesis by C.E.A., Gif sur Yvette, France. Clorgyline hydrochloride was synthesized by the Department of Organic Chemistry, Centre de Recherche Delalande, Rueil-Malmaison, France. All other reagents were standard laboratory reagents of analytical grade wherever possible.

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Male Wistar-Kyoto rats (Iffa Credo, L'Arbresle, France), aged between 14–15 weeks (290–320 g) were killed by a blow to the head; the hearts, after removal, blotting on filter paper and excision of the large blood vessels, were homogenized (1 : 20, w/v) in 0·1 M sodium phosphate buffer, pH 7·8. Freshly made homogenates were always used. Monoamine oxidase activity was assayed under an atmosphere of air at 37 °C and at pH 7·8 by the method of Otsuka & Kobayashi (1964) as modified by Fowler & Strolin Benedetti (1982). It was ensured that initial velocities were always measured. Activities are given as nmoles (of substrate metabolized) (mg protein)$^{-1}$ min^{-1}, and have been corrected for the efficiencies of extraction of the deaminated metabolites into the organic phase of the assay mixture (Fowler & Oreland 1980; Strolin Benedetti et al 1981). With n-pentylamine, [^{14}C]pentanoic acid may be further metabolized to $^{14}CO_2$. However, in preliminary experiments where the volatile radioactivity produced by the action of MAO was determined (for methodology, see Strolin Benedetti et al 1981) it was found that release of $^{14}CO_2$ was at all times $\leq$10% of the total recovered radioactivity, in agreement with results in the rat brain (Strolin Benedetti et al 1981). Thus, for n-pentylamine as substrate, the activity was determined as the amount of [^{14}C]pentanoic acid extracted/unit time. Protein contents of the homogenates were assayed by the method of Lowry et al (1951).

RESULTS AND DISCUSSION

The kinetic parameters of the deamination of five monoamines by rat heart homogenates are given in Table 1 and, as an example, the data from which the values for n-pentylamine were obtained are given in Fig. 1. Although it should be stressed that these are the kinetic parameters of the total deamination rather than for any particular form of MAO, it is possible to draw certain conclusions from these data. In the rat heart, 5-HT is the substrate most readily metabolized, followed by tyramine, β-phenethylamine, n-pentylamine and benzylamine. Thus, the substrates most readily metabolized are those most favourable towards MAO-A. On the other hand, platelet monoamine oxidase, which is only of the -B type (Donnelly & Murphy 1977), has the highest V_{max} values (at pH 7·8) towards β-phenethylamine and benzylamine, followed by tyramine and 5-HT (Fowler & Oreland 1982). Indeed, in the human platelet, 5-hydroxytryptamine is oxidized at a rate of only 12% of that of tyramine (Donnelly & Murphy 1977) whereas in the rat heart the value is 151% (Table 1). No value is available for the activity of n-pentylamine in the human platelet, but the V_{max} of MAO-B towards this substrate in the rat brain is approximately 2 nmol (mg protein)$^{-1}$ min^{-1} (Strolin Benedetti et al 1981), which is approximately 80% of the V_{max} for tyramine, whereas the V_{max} value for rat heart pentylamine is only about 7% of that for tyramine. These data are consistent, therefore, with the notion that MAO-A predominates in the rat heart.

Such a conclusion is confirmed by the clorgyline inhibition studies on three 'MAO-B' substrates shown in Fig. 2A–C. The deamination of 10 μM β-phenethylamine was brought about mainly by MAO-A although a small (approximately 15%) component of MAO-B activity was visible.

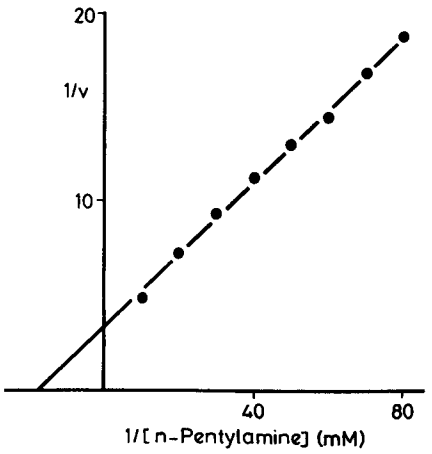

FIG. 1. Double reciprocal plot of the deamination of n-pentylamine assayed at different substrate concentrations. Ordinate: 1/[initial velocity in nmol (mg protein)$^{-1}$ min^{-1}]; abscissa: 1/(substrate concentration in mM). Each point represents the mean for three rat heart homogenates. The regression line of best fit (r = 0·998) is drawn through the points.

Table 1. Kinetic parameters of the deamination of five substrates by rat heart homogenates. K_m and V_{max} values were calculated from initial velocities at 6–8 substrate concentrations. The data was in each case plotted as 1/v against 1/S and the K_m and V_{max} values determined by linear regression analysis. In all cases, the correlation coefficients of the regression lines were greater than r = 0·97. Values are means ± s.e.m. for determinations in three rat heart homogenates (n = 5 in the case of benzylamine).

	K_m (μM)	V_{max} nmol (mg prot)$^{-1}$ min^{-1}
5-HT	75 ± 4	6·2 ± 0·2
Tyramine	33 ± 0·7	4·1 ± 0·4
β-Phenethylamine	54 ± 2	1·0 ± 0·05
n-Pentylamine	56 ± 3	0·30 ± 0·03
Benzylamine	4·9 ± 0·6	0·13 ± 0·006

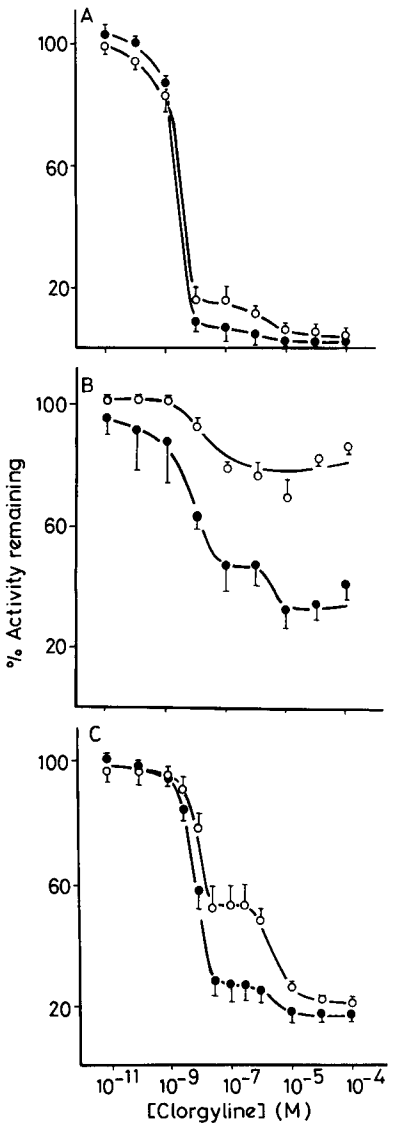

FIG. 2. The effect of clorgyline upon the in-vitro deamination of: A. 10 μM (○) and 100 μM (●) β-phenethylamine; B. 10 μM (○) and 100 μM (●) benzylamine; C. 10 μM (○) and 100 μM (●) n-pentylamine. Homogenates were preincubated for 20 min with either distilled water or clorgyline before assay for activity remaining. Preincubation protein concentrations were: for β-phenethylamine as substrate, 0·7 mg ml^{-1}; for benzylamine and n-pentylamine as substrates, 1·7 mg ml^{-1}. Each point represents the mean ± s.e.r. of determinations in three rat heart homogenates.

However, this component was not visible at 100 μM β-phenethylamine (Fig. 2A). These results are consistent with earlier results (Dial & Clarke 1979) and indicate that, as in other tissues, the K_m of MAO-B towards β-phenethylamine is lower than that of MAO-A towards this substrate (Dial & Clarke 1979; Kinemuchi et al 1980). At low concentrations of benzylamine, the deamination of this substrate was brought about in the brain by an enzyme activity resistant to inhibition by clorgyline (Fig. 2B), in agreement with previous studies (Clarke et al 1982). This clorgyline-resistant activity was inhibited by the carbonyl-reagent semicarbazide (Table 2). At high concentrations of benzylamine, the deamination is brought about by MAO-A, -B and a much smaller proportion by the clorgyline-resistant, semicarbazide-sensitive enzyme (Fig. 2B, Table 2), suggesting that the K_m of the semicarbazide-sensitive enzyme is much lower that that of MAO towards this substrate. From the data of Fig. 2B, given the initial velocities at each benzylamine concentration, it is possible to estimate the relative activities of MAO and the semicarbazide-sensitive enzyme at 10 and 100 μM substrate concentrations. The mean activities for the semicarbazide-sensitive enzyme are [in nmol (mg protein)$^{-1}$ min^{-1}]: 10 μM benzylamine, 0·048 and at 100 μM benzylamine, 0·045, indicating that the enzyme is saturated at 10 μM benzylamine. For MAO the values are 0·012 and 0·084 nmol (mg protein)$^{-1}$ min^{-1} for 10 and 100 μM benzylamine, respectively, from which K_m and V_{max} values of 200 μM and 0·25 nmol (mg protein)$^{-1}$ min^{-1}, respectively, can be estimated. Although the values must be considered approximations, they are in good agreement with the K_m values of 2·9 and 169 μM for the semicarbazide-sensitive enzyme and MAO

Table 2. Effect of clorgyline, semicarbazide and both drugs upon the deamination of β-phenethylamine, n-pentylamine and benzylamine in the rat heart. Values represent means ± s.e.r. of the inhibition of the deamination of the substrates (at the concentrations shown) in three rat heart homogenates after preincubation with the inhibitors for 20 min, with respect to samples preincubated for the same length of time with distilled water. The protein concentrations at preincubation were: for β-phenethylamine as substrate, 0·7 mg ml^{-1}; for n-pentylamine and benzylamine as substrates, 1·7 mg ml^{-1}.

	% inhibition of deamination		
	10^{-3} M clorgyline	10^{-3} M semicarbazide	10^{-3} M clorgyline + 10^{-3} M semicarbazide
β-Phenethylamine			
10 μM	96 ± 2	19 ± 5	100 ± 3
100 μM	98 ± 3	9 ± 6	98 ± 6
n-Pentylamine			
10 μM	81 ± 2	21 ± 2	100 ± 1
100 μM	81 ± 4	23 ± 2	100 ± 1
Benzylamine			
10 μM	12 ± 3	76 ± 3	100 ± 2
100 μM	55 ± 8	37 ± 5	100 ± 7

reported previously for this substrate for the isolated perfused rat brain (Andree & Clarke 1981).

Approximately 50% of the deamination of n-pentylamine at a concentration of 10 μM was brought about by MAO-A, while MAO-B accounted for approximately 30% of the activity (Fig. 2C). The remaining 20% was brought about by the clorgyline-resistant, semicarbazide-sensitive enzyme form (Table 2). At a concentration of 100 μM, MAO-A was the predominant enzyme form (Fig. 2C). From these data, together with the initial velocities at 10 and 100 μM pentylamine concentrations, the following kinetic parameters could be estimated for this substrate:

MAO-A, K_m = 170 μM, V_{max} = 0·36 nmol (mg protein)$^{-1}$ min^{-1}

MAO-B, K_m = 4 μM, V_{max} = 0·02 nmol (mg protein)$^{-1}$ min^{-1}

Clorgyline-resistant, semicarbazide-sensitive enzyme, K_m = 40 μM, V_{max} = 0·04 nmol (mg protein)$^{-1}$ min^{-1}.

Although these values must be considered estimates, the K_m of MAO-B is in good agreement with the K_m of rat brain MAO-B (measured at pH 7·4) towards n-pentylamine of 29 μM (Strolin Benedetti et al 1981), when it is taken into account that the K_m value decreases as the pH value increases for substrates of MAO (for discussion, see McEwen et al 1968; Williams 1974). The K_m of human platelet MAO-B towards tyramine, for example, is 220 μM at pH 7·4 and 120 μM at pH 7·8 (Fowler & Oreland 1982). The large difference in the K_m values would suggest that a double reciprocal plot of pentylamine deamination over an extended concentration range (i.e. larger than that shown in Fig. 1) would not be linear.

From the data of Fig. 2C, the IC50 value of the inhibition of MAO-A towards pentylamine by clorgyline can be estimated to be approximately 7 nM. Since, after the preincubation time of 20 min used in this experiment, clorgyline effectively titrates the concentration of MAO-A active centres (Fowler & Callingham 1979; see also Fowler et al 1981), a rough MAO-A concentration of 8 pmol mg^{-1} protein can be estimated. More detailed experiments performed with 0, 1, 2, 3, 4, 5, 6 and 7 nM clorgyline, 60 min preincubation and a preincubation protein concentration of 0·6 mg ml^{-1} gave IC50 values of 4·9 nM for both 100 μM β-phenethylamine and 100 μM 5-HT as assay substrates, corresponding to an MAO-A concentration of 17 pmol (mg protein)$^{-1}$. This is rather higher than previously reported by titration methods (Fowler & Callingham 1979), but is in good agreement with the value of 9 pmol (mg protein)$^{-1}$ found by a [^{3}H]pargyline binding assay for rat heart mitochondria (Parkinson et al 1980). The molecular turnover number of MAO-A towards a 5-HT (100 μM) was 400 mol. (mol MAO-A)$^{-1}$ min^{-1}, rather lower than previously reported (Fowler & Callingham 1979), but in line with values for human brain and rat liver MAO-A towards this substrate (for review, see Fowler et al 1981).

The data in the study would support the contention made earlier (Parkinson et al 1980) that no substrate can be considered an absolute substrate for MAO-B: even benzylamine, n-pentylamine and isoamylamine that appear to be classical MAO-B substrates (Strolin Benedetti et al 1981; Hall et al 1969; Peers et al 1980) have MAO-A activities that can be observed either at high substrate concentration (in the case of isoamylamine, Peers et al 1980), when MAO-B activity is inhibited (in the case of benzylamine, Parkinson et al 1980), or when a tissue rich in MAO-A, such as the rat heart, is used as the enzyme source.

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