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INTESTINAL METABOLISM OF TYRAMINE BY BOTH FORMS OF MONOAMINE OXIDASE IN THE RAT

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Abstract—The two forms of monoamine oxidase (MAO) in rat intestine and brain homogenates were found to have different K_m and V_{max} values towards tyramine. The K_m values for the A-form of the enzyme towards this substrate were around 120 μM in both cases, whereas the values for the B-form were about 240 μM. As a consequence, the ratio of activities (MAO-A : MAO-B) towards tyramine are dependent upon the substrate concentration. The MAO-A-selective inhibitors, toloxatone and cimoxatone, were found to be competitive inhibitors of the oxidation of tyramine by the A-form of this enzyme in the rat intestine, with K_i values of 3.4 μM and 3.7 nM respectively. The significance of these results in relation to the "cheese effect", a pressor response to tyramine after monoamine oxidase inhibition, are discussed.

Monoamine oxidase (MAO) (amine : oxygen oxidoreductase (deaminating) (flavin-containing), EC 1.4.3.4) exists as two forms, termed MAO-A and MAO-B, where the A-form of the enzyme is sensitive to inhibition by very low (around nanomolar) concentrations of the acetylenic inhibitor clorgyline but the B-form of the enzyme is not inhibited until micromolar concentrations of this inhibitor are used [1]. The reverse is true for the MAO-B-selective inhibitor *l*-deprenyl [2]. A great many other compounds have also been shown to inhibit one or other of the forms of MAO selectively (for review see Ref. 3).

Intestinal MAO plays a very important role in the breakdown of orally ingested tyramine that is present in large quantities in foodstuffs such as cheese (for reviews see Refs. 4–6). Little, however, is known about the properties of MAO from the intestine. In consequence, in the present study, the kinetic parameters of rat intestinal MAO-A and MAO-B towards tyramine have been investigated. As a comparison, data of rat brain MAO-A and MAO-B are also included. In addition, the nature of the inhibition of intestinal MAO-A by two reversible selective MAO-A inhibitors, toloxatone [5-(hydroxymethyl)-3-(3-methylphenyl)-2-oxazolidinone] and cimoxatone [3-(4-(3-cyanophenyl-methoxy)phenyl)-5-(methoxymethyl)-2-oxazolidinone; MD780515], have been investigated.

MATERIALS AND METHODS

Male Sprague–Dawley rats of body weight 200–250 g were killed by a blow to the head followed by decapitation, and the brains and intestines rapidly removed and blotted on filter paper. The intestines were dissected into 15-cm lengths, and, unless otherwise stated, the portion of the intestine proximal to the stomach was used. The samples were homogenised 1 : 16 (w/v) in 100 mM sodium phosphate buffer, pH 7.4, in an Ultra-Turrax homogeniser.

MAO activity was determined radiochemically at 37° as described elsewhere [7, 8] with [7-^{14}C]tyramine hydrochloride (Amersham International, Amersham, U.K.) as substrate. At all times it was ensured that initial velocities were measured. MAO activities are given as nmoles (of tyramine deaminated) · (mg protein)$^{-1}$ · min^{-1}. Protein concentrations of the homogenates were determined by the method of Lowry *et al.* [9], with bovine serum albumin as standard. In experiments where the acetylenic inhibitors clorgyline and *l*-deprenyl were used, they were preincubated with the homogenates at 37° before addition of substrate to assay for remaining enzyme activity. Preliminary time courses of the inhibition of MAO-A by clorgyline and MAO-B with *l*-deprenyl indicated that, for both brain and intestinal homogenates, the reaction between enzyme form and inhibitor had reached completion by 20 min, in agreement with a recent kinetic study in the rat liver [10]. In consequence, in the present study, a 20-min preincubation period was used for these inhibitors. There was no loss of enzyme activity for samples preincubated with distilled water over this time period.

RESULTS AND DISCUSSION

Initial experiments indicated that tyramine oxidation by rat intestine MAO was inhibited in a biphasic manner by clorgyline (Fig. 1), consistent with the notion that both forms of MAO are active towards this substrate in this tissue. From the plateau height shown in Fig. 1, approximately 75% of the intestinal tyramine oxidation (at a tyramine concentration of 285 μM) is brought about by MAO-A and 25% by MAO-B. A similar ratio of activities was found with *l*-deprenyl as inhibitor (data not shown).

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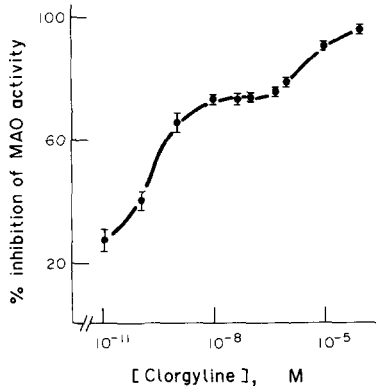

Fig. 1. Inhibition of rat intestinal MAO by clorgyline. Samples were preincubated with the indicated concentrations of clorgyline for 20 min at 37° before addition of tyramine at an assay concentration of 285 μM. Points represent mean ± S.E.R. of triplicate determinations of the % inhibition of MAO activity with respect to samples preincubated for the same length of time with distilled water.

Such a ratio of activities has also been found for rat intestinal MAO towards this substrate by Tipton *et al.* [11] and for kynuramine by Squires [12]. In order to determine whether the MAO-A and MAO-B activities towards this substrate varied along the intestine, five separate sections of intestine were studied. There was a very small variation in activity from section to section, but the ratio of activities (MAO-A : MAO-B) towards tyramine were constant across the intestine (Table 1). In consequence, in all subsequent experiments, the 15-cm section of the intestine proximal to the stomach was used.

From the data in Fig. 1, it can be seen that a clorgyline concentration of 5×10^{-7} M is on the plateau region of the biphasic inhibition curve of tyramine oxidation, and could thus be assumed to inhibit completely the activity of intestinal MAO-A without an effect on the activity of MAO-B. Similar consideration with *l*-deprenyl indicated that a concentration of 10^{-7} M gave a selective inhibition of intestinal MAO-B. In the rat brain, it was found that a clorgyline concentration of 2.5×10^{-7} M and a *l*-deprenyl concentration of 2.5×10^{-8} M inhibited selectively MAO-A and MAO-B, respectively. By use of these inhibitors, it was possible to study the kinetic parameters of the two enzyme forms separately towards tyramine as substrate. The kinetic parameters of rat brain and intestine MAO-A and MAO-B towards tyramine are given in Table 2, and examples of the data shown in Fig. 2A and B. As expected, the sum of the V_{max} values of the MAO-A and MAO-B activities determined separately were equal for both the brain and intestine to the V_{max} values of the uninhibited enzyme activities. However, for both brain and intestinal MAO, the K_m value of the MAO-A form towards tyramine was lower than that for the B-form towards this substrate

Table 1. Tyramine deaminating activity of different regions of the rat intestine*

Intestine section	MAO activity [nmoles·(mg protein)$^{-1}$·min^{-1}]	% inhibition by clorgyline 5×10^{-8} M	5×10^{-7} M
First	1.4 ± 0.2	74 ± 2	78 ± 1
Second	1.2 ± 0.1	75 ± 1	77 ± 1
Third	1.2 ± 0.1	75 ± 0.5	76 ± 2
Fourth	1.2 ± 0.1	75 ± 0.5	77 ± 0.5
Fifth	1.3 ± 0.03	76 ± 3	77 ± 2

* The intestine was divided into five sections, the first section referring to the 15 cm proximal to the stomach followed by successive 20-cm lengths (termed second–fifth sections). Values are given as means ± S.E.M. or S.E.R. as appropriate for determination in four separate homogenates, assayed with 285 μM tyramine as substrate.

Table 2. Kinetic parameters of rat brain and intestinal MAO-A and MAO-B towards tyramine*

Inhibitor	Form remaining	K_m (μM)	V_{max} [nmoles·(mg protein)$^{-1}$·min^{-1}]
		Rat brain	
None	A and B	145 ± 20	2.55 ± 0.07
l-Deprenyl	A	120 ± 10	1.74 ± 0.05
Clorgyline	B	300 ± 50	0.94 ± 0.13
		Rat intestine	
None	A and B	130 ± 20	1.63 ± 0.32
l-Deprenyl	A	120 ± 20	1.23 ± 0.19
Clorgyline	B	240 ± 20	0.51 ± 0.09

* The homogenates were preincubated for 20 min at 37° with either distilled water, clorgyline or *l*-deprenyl (concentration used given in the text) before addition of tyramine to assay for enzyme activity. Eight substrate concentrations, in the range 50–1500 μM were used and the results plotted as $1/v$ against $1/S$. K_m and V_{max} values were calculated from each plot by linear regression analysis. In all cases, the correlation coefficient of the regression line was greater than 0.96. All values represent means ± S.E.M. in three preparations.

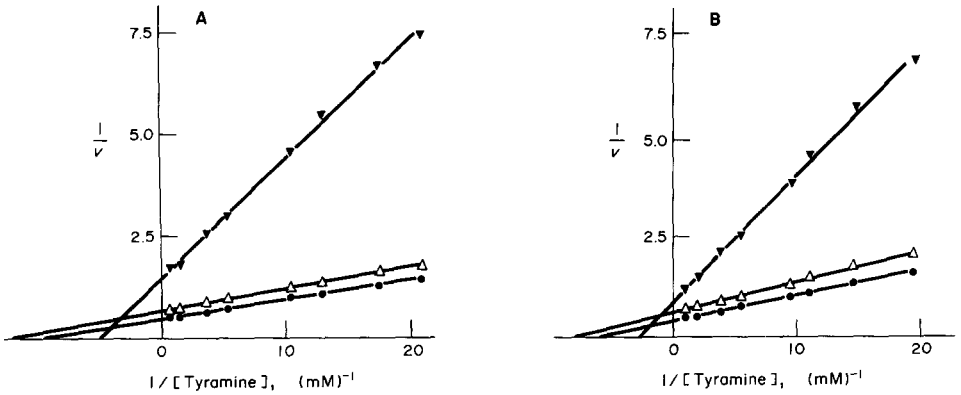

Fig. 2. Double-reciprocal plots of the activity of MAO-A and MAO-B towards tyramine. Before assay, samples were preincubated with distilled water, clorgyline or *l*-deprenyl for 20 min. Points represent means of triplicate determinations of MAO activity. Tissues and inhibitor concentrations used were: (A) rat intestine, incubated with distilled water (●), 10^{-7} M *l*-deprenyl (△) and 5×10^{-7} M clorgyline (▼); and (B) rat brain, incubated with distilled water (●), 2.5×10^{-8} M *l*-deprenyl (△) and 2.5×10^{-7} M clorgyline (▼).

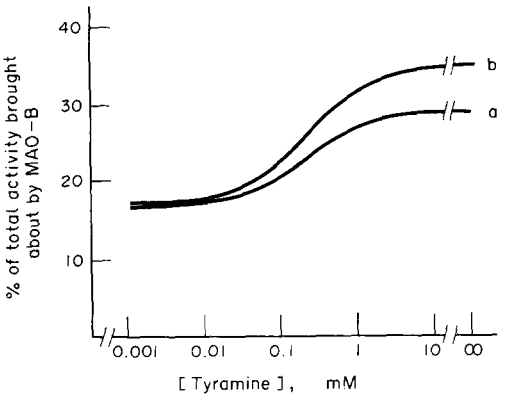

Fig. 3. The percentage of the total MAO activity due to the B-form of the enzyme towards tyramine as substrate in: (a) rat intestine and (b) rat brain. Activities of MAO-A and MAO-B were calculated separately for different substrate concentrations from the K_m and V_{max} values given in Table 2, and the % of the total activity due to MAO-B determined as: [MAO-B activity/(MAO-A activity + MAO-B activity)] × 100.

(Table 2). A similar result has been reported for rat liver MAO [13].

From the data given in Table 2, it is possible to calculate the contribution that each of the two forms of MAO make to the deamination of tyramine at different concentrations of this substrate. Thus, in the intestine, the data from Table 2 would predict that at a tyramine concentration of 285 μM, 24 ± 1% (mean ± S.E.R.) of the total deamination is brought about by the B-form of the enzyme. This value corresponds well with the value obtained experimentally from clorgyline inhibition curves (Fig. 1, Table 1). Since the K_m values of the two forms of MAO towards tyramine are different (Table 2), the relative contribution of MAO-B to the total deaminating activity is dependent upon the concentration of substrate used to assay for activity. Such a dependence can be calculated from the data of Table 2, and is shown in Fig. 3. At low tyramine concentrations, for both the brain and intestine, the bulk (about 83%) of the tyramine deamination is brought about by MAO-A. With increasing tyramine

Table 3. Estimated pH-independent K_m values ($K_{\bar{m}}$) of MAO-A and MAO-B towards tyramine in four rat tissues, assayed under an atmosphere of air and at 37°*

Rat tissue	Study pH	$K_{\bar{m}}$ (μM) towards MAO-A	MAO-B	Reference
Brain	7.4	0.22	0.56	This study
Heart	7.8	0.18	—	[21]
Liver	7.2	0.13	0.68	[13]
	7.4	0.19	0.54	†
Intestine	7.4	0.22	0.45	This study

* $K_{\bar{m}}$ were calculated from the Henderson–Hasselbalch equation assuming that the non-ionized form of the substrate is preferentially metabolized (see Ref. 17). The pK_a values for the amino and phenolic groups of tyramine were taken as 10.13 and 9.59, respectively [17]. No value for rat heart MAO-B has been reported due to the very low concentration of this enzyme form in this tissue [22].

† T. Boucher and M. Strolin Benedetti, unpublished results.

concentrations, however, the contribution by MAO-B increases up to a maximum of 29% (intestine) and 35% (brain) of the total deaminating activity (Fig. 3). This concentration dependence in the brain will be even more marked in old rats than in young rats, since there is in any case an increased ratio of activities (MAO-B : MAO-A) with age in the rat brain [14, 15], due to the neurone loss associated with age (see Ref. 16).

In order to compare the K_m values for the two forms of MAO towards tyramine in the intestine and brain with values of other rat tissues that were determined at different assay pH values, the pH-independent K_m values ($K_{\bar{m}}$) for the two forms were calculated as described by McEwen *et al.* [17], assuming that for both enzyme forms it is the non-ionized form of the substrate that is metabolised preferentially. Such an assumption has been shown to be justified in the human liver [17, 18], pig brain [19] and human platelet [20]. The $K_{\bar{m}}$ values for the two forms of MAO towards tyramine from data for four tissues are given in Table 3. In each case, the $K_{\bar{m}}$ for MAO-A is in the range 0.13–0.22 μM and is lower than that for MAO-B (range 0.45–0.68 μM) (Table 3). Consistent with these values are the findings that human platelet MAO-B has a $K_{\bar{m}}$ towards tyramine in the range 0.4–0.9 μM [20], and human liver MAO-A + MAO-B a value of 0.38 μM [17]. The similarities of the $K_{\bar{m}}$ values for the respective forms of MAO towards tyramine in the different tissues of the rat (Table 3) raise the possibility that the different substrate specificities of MAO-A and MAO-B reported in the literature for different tissues (see Ref. 23) are due to the presence of different concentrations of the enzyme forms assayed at different substrate concentrations rather than due to a variation in enzyme molecular structure from tissue to tissue (for further discussion see Refs 24 and 25).

Since little is known about the inhibitor sensitivity of rat intestinal MAO-A, it was decided to investigate the inhibition of this enzyme form with toloxatone and cimoxatone, two inhibitors that have been shown to be reversible and selective for MAO-A in the rat brain [26, 27]. Such selectivity was also demonstrated in preliminary experiments in the rat intestine in the present study: the oxidation of 200 μM 5-hydroxytryptamine (an MAO-A substrate) was inhibited by toloxatone with an IC_{50} of 5 μM whereas that of 20 μM β-phenethylamine (an MAO-B substrate) was inhibited with an IC_{50} of 180 μM.

For cimoxatone, the IC_{50} values were 9 and 300 nM for 5-hydroxytryptamine and β-phenethylamine, respectively. The compounds were tested for inhibition of tyramine oxidation in intestinal homogenates preincubated for 20 min with 10^{-7} M *l*-deprenyl (to inhibit selectively the MAO-B activity). The inhibition was in both cases competitive in nature, with mean K_i values of 3.4 μM and 3.7 nM being found for toloxatone and cimoxatone, respectively (Fig. 4A and B). These values are in line with the potencies of these compounds found in other tissues of the rat (e.g. Refs 26 and 27). Toloxatone is rapidly absorbed after oral administration of the drug to volunteers, and peak plasma levels are reached within 30 min [28]. Since toloxatone is an effective antidepressant at daily doses of 600–1400 mg [29, 30] such doses would result in a peak plasma toloxatone concentration in the range of 4–15 μM, assuming a regime of four oral doses per day (calculated from Ref. 28). Thus, it is likely that at therapeutic doses of toloxatone there will be considerable inhibition of intestinal MAO-A activity.

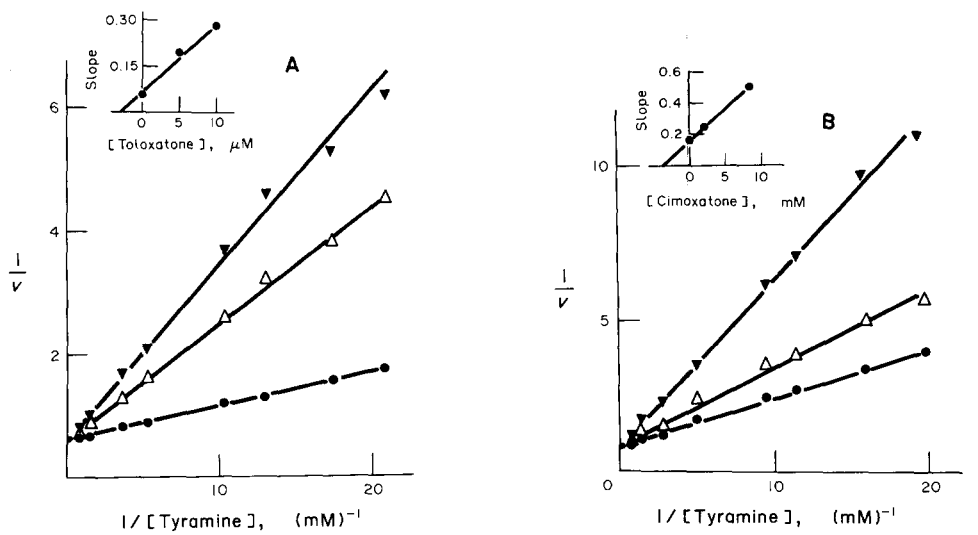

Fig. 4. Double-reciprocal plots of the inhibition of MAO-A activity by toloxatone and cimoxatone. Intestinal homogenates were preincubated with 10^{-7} M *l*-deprenyl for 20 min at 37°, then further incubated with either distilled water, toloxatone or cimoxatone for 5 min at 37° before addition of tyramine to assay for activity. Inhibitor concentrations used were: (A) distilled water (●), 5 μM (△) and 10 μM (▼) toloxatone; and (B) distilled water (●), 2.5 nM (△) and 10 nM (▼) cimoxatone. K_i values were calculated from secondary replots of the slopes against the inhibitor concentration (shown as insets in the graphs).

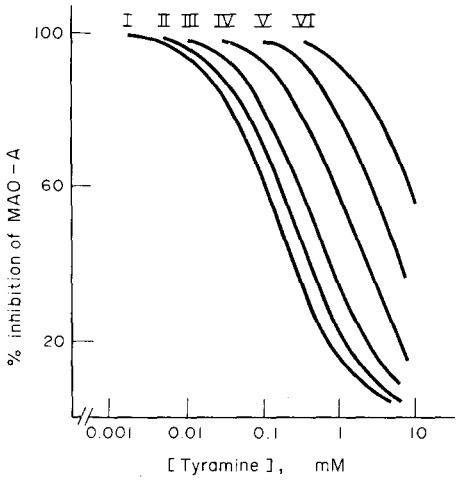

Fig. 5. Theoretical plots of the % inhibition of rat intestinal MAO-A activity by a competitive inhibitor. Curves were calculated for different tyramine concentrations from the formulae:

$$v = \frac{V_{max}}{1 + \frac{K_m}{[\text{tyramine}]}\left(1 + \frac{[\text{inhibitor}]}{K_i}\right)}$$

and % inhibition $= 100 - \left(\frac{v\,(\text{in presence of inhibitor})}{v\,(\text{in absence of inhibitor})} \times 100\right)$, assuming a K_m of MAO-A towards tyramine of 120 μM. Inhibitor concentrations were taken as the following: $1/3K_i$ (I), K_i (II), $3K_i$ (III), $10K_i$ (IV), $30K_i$ (V) and $100K_i$ (VI).

These results are of interest with respect to the "cheese reaction", a pressor effect of tyramine found in foodstuffs after treatment with classical MAO inhibitors such as tranylcypromine and phenelzine (see Refs 4–6 for reviews). One mechanism for the "cheese effect" is if ingested tyramine, instead of being metabolised by the intestinal MAO, passes into the circulation, it would then be able to liberate noradrenaline from nerve endings and produce the pressor effect (see Refs 4–6 for reviews). It should be stressed, however, that other mechanisms have been postulated including the notion that the "cheese effect" is a pharmacological effect entirely distinct from MAO inhibition [31].

If the "cheese reaction" is indeed the result of intestinal MAO inhibition, then certain predictions can be made for different classes of MAO inhibitors. Human intestinal MAO is mainly of the A-type [32], and the MAO-B-selective irreversible inhibitor *l*-deprenyl is without significant "cheese reaction" effect [32, 33] whereas the MAO-A-selective irreversible inhibitor clorgyline has a considerable "cheese reaction" [33]. On the other hand, the "cheese effect" found after administration of the competitive inhibitor FLA 336(+) (4-dimethylamino-α,2-dimethylphenethylamine) is much smaller than that found for clorgyline [34], and a similar result has been found in some preliminary studies in man with toloxatone where no tyramine potentiation was found after treatment for 7 days at a daily dose of 1000 mg [35]. One explanation for this lower level of "cheese reaction" is that the binding of a competitive (but not non-competitive) reversible inhibitor can be displaced by high concentrations of substrate. Such a displacement can be calculated, and is shown graphically in Fig. 5 for a substrate with a K_m of 120 μM (the value of rat intestinal MAO-A towards tyramine). At a concentration of tyramine of 100 μM (which could be found in the proximal part of the intestine after eating a mature cheese, such as Camembert, which has a very high concentration of tyramine [5]), there is considerable displacement of inhibitor binding by the tyramine, provided the inhibitor concentration does not exceed $3 \times K_i$ (Fig. 5). In consequence, the concentration of tyramine reaching the circulation would be lower than that found if no inhibitor binding displacement had occurred. Although this hypothesis is purely speculative, it does provide an explanation as to why some MAO-A-selective inhibitors have lower "cheese effects" than others.

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Note added in proof.—Recently Veryovkina *et al.* [*Vop med. Khim.* **28**, 88 (1982)] have also reported that high concentrations of tyramine are deaminated by both forms of MAO in the rat intestine.