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SPECIES DIFFERENCES IN THE INTERACTIONS OF THE ANTICONVULSANT MILACEMIDE AND SOME ANALOGUES WITH MONOAMINE OXIDASE-B

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Abstract—Oxidation of the anticonvulsant drug milacemide [2-*n*-(pentylamino)acetamide] by monoamine oxidase-B (MAO-B) has been reported to be important in terminating its activity. Comparison of the oxidation of this compound by MAO-B preparations from ox and rat liver showed the former enzyme to have a significantly higher K_m value towards this substrate. In keeping with this, the K_i values for milacemide acting as a competitive inhibitor of these enzymes showed it to have a lower affinity for ox liver MAO-B. Comparative studies on the time-dependent inhibition of the two enzymes also showed a lower sensitivity of that from the ox liver. Studies with a series of analogues involving replacement of pentylamino group of milacemide showed marked differences between the sensitivities of the two enzymes. The largest differences were shown by the compound 2(4-(3-chlorobenzoxy)phenethylamino)acetamide which gave IC_{50} values of 0.051 ± 0.008 and 4.1 ± 0.8 μM with the rat and ox enzymes, respectively, when activities were assayed without prior enzyme-inhibitor preincubation. When the enzyme and inhibitor were incubated for 60 min at 37° before assay these values fell to 0.027 ± 0.002 and 3.5 ± 0.4 μM, respectively. These marked differences prompted a study of the inhibition of MAO-A and MAO-B from human liver and brain, mouse brain and rat brain as well as MAO-B from ox liver by milacemide and α-methylmilacemide. There were no significant differences in the sensitivities of any of the mitochondrial MAO-A preparations studied towards these compounds. However, MAO-B from human brain and liver mitochondria resembled that from ox liver in being less sensitive to inhibition than the rat and mouse enzymes. Purification of the ox liver MAO-B did not significantly affect its interactions with milacemide and α-methylmilacemide. The marked species differences reported here raise questions concerning the validity of rodent model systems, that have frequently been used for assessing the *in vivo* and *in vitro* actions of milacemide and its analogues, for the situation in the human.

Key words: convulsions; 5-hydroxytryptamine; monoamine oxidase; 2-(*n*-pentylamino)acetamide; 2-phenylethylamine; species differences; tyramine

The anticonvulsant drug milacemide (2-*n*-(pentylamino)acetamide) [1–4] has been shown to be a substrate for the enzyme MAO-B¶, which catalyses its oxidative cleavage to form pentanal, glycinamide and H_2O_2 [5, 6]. It has been suggested that this production of glycinamide, which would subsequently be deamidated to form glycine in the brain, accounts for its anticonvulsant behaviour [5]. However, the report that the MAO-B inhibitor l-deprenyl only partially prevented the anticonvulsant effects of milacemide [2] suggests that the situation might be more complicated than that. The demonstration that α-methylmilacemide, which is not a substrate for MAO-B and does not act as a significant glycine precursor, is also an effective anticonvulsant [7], might suggest that the action of MAO-B may be important for terminating the activity of milacemide rather than for initiating it. Additional evidence that the formation of glycine in the brain through the action of MAO is not the major factor in the anticonvulsant actions of milacemide has been obtained from studies of the behaviour of a series of analogues of this compound in which the pentylamino moiety had been replaced by groups based on the structures of known monoamine oxidase substrates and inhibitors where the anticonvulsant potency did not correlate with ability to act as a substrate for MAO [8]. Furthermore, it appears that the glycinamide portion of the molecule may not be essential for anticonvulsant activity since the alaninamide derivative (*S*)-2-(4-(3-fluorobenzyloxy)-benzylamino)propionamide and its enantiomeric counterpart were also shown to be effective anticonvulsants [9].

These data suggest that milacemide itself possesses anticonvulsant activity and focus attention on the possibility that the anticonvulsant actions of milacemide may be terminated by the activity of monoamine oxidase in the tissues, although the possibility that glycine liberation may make some contribution to the anticonvulsant actions cannot be completely excluded for this specific compound. Degradation by monoamine oxidase may itself be limited since milacemide behaves as a mechanism-based inhibitor, as well as a substrate, of the enzyme

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¶ Abbreviations: 5-HT, 5-hydroxytryptamine; MAO, monoamine oxidase.

[6, 10]. The known differences in MAO-B levels in the brains of different species [11], thus might lead to variations in responses to this drug. This could be of importance since the anticonvulsant activities of this compound have usually been assessed in rodents. Indeed its effectiveness in humans suffering from various forms of epilepsy is not conclusively established [12]. The situation within the human might be further complicated by the age-dependent increases in the activity of MAO-B in the brain [13].

Although the anticonvulsant potencies of drugs such as milacemide are frequently assessed using rodent model systems, such as the ability to antagonize bicuculline-induced convulsions and death in mice [7], investigations on its metabolism have often used a different species, such as the rat or ox. Clearly, such comparisons are based on the assumption that the enzyme from different species will behave identically. The close similarities between the primary amino acid sequences, deduced from cDNA sequences, of MAO-B from different species [14] have given some grounds to suppose that such an assumption might be valid. Furthermore, many published values for the kinetic constants and specificities of MAO-B from different sources do not reveal many gross differences other than those that might be explained by experimental variations. However, it has been reported that there are significant differences between the behaviour of human tissue MAO-B and that enzyme from rat brain in its deamination of some substrates and inhibition by tricyclic antidepressants [15, 16]. This marked species difference between rodent and primate species has important implications for comparative studies of drugs affecting or acting through amine metabolic pathways.

In the present paper we report the results of experiments showing profound species difference in the actions of MAO-B towards milacemide and its analogues which suggest that it may not be possible to extrapolate data obtained in one species to the situation in another.

MATERIALS AND METHODS

5-Hydroxytryptamine-(side chain-2-^{14}C) creatine sulphate, tyramine-(2-^{14}C) HCl and 2-phenylethylamine-(ethyl-1-^{14}C) hydrochloride were obtained from Amersham International or New England Nuclear. L-Deprenyl was kindly given by Prof. J Gaál, Chinoin Pharmaceutical Co. Ltd (Budapest, Hungary). The analogues of milacemide (compound **I**) and the following acetamide derivatives: 2-(benzylamino)acetamide (compound **II**), 2-(phenethylamino)acetamide (compound **III**), 2-(2-indol-3-yl)-ethylamino)acetamide (compound **IV**), 2-(2-(5-methoxyindol-3-yl)ethylamino)acetamide (compound **V**), 2-(2-(4-chlorobenzamido)ethylamino)-acetamide (compound **VI**), 2-(2-benzamidoethylamino)acetamide (compound **VII**) and 2-(4-(3-chlorobenzyloxy)phenethylamino)acetamide (compound **VIII**) were prepared as described previously [8]. α-Methylmilacemide (2-[(1-methyl)-pentyl]aminoacetamide) was synthesized as described before [7].

Rat ox and human liver mitochondria were prepared by the procedure of Kearney *et al.* [17]. Human brain was obtained within 12 hr of death and the frontal lobe cerebral cortex was dissected out and used in the preparation of mitochondria. Mitochondrial fractions from human cerebral cortex and rat brain were prepared by essentially the same procedure except that the tissue was homogenized in 10, rather than three volumes (v/w) of 5 mM Tris-HCl buffer, pH 7.4, containing 0.32 M sucrose and centrifugations of 1000 *g* for 6 min and 15,000 *g* for 10 min were used to sediment the crude nuclear and mitochondrial fractions, respectively. In all cases the crude mitochondrial fractions were washed once, by sedimentation and resuspension, and the pellets obtained were suspended in 0.1 M potassium phosphate buffer, pH 7.2, and stored in small aliquots at −20° until required. Mitochondria pretreated with l-deprenyl or clorgyline [18] were used as a source of MAO-A and -B activities, respectively.

Purified ox liver MAO-B was prepared by the method of Salach [19]. Aldehyde dehydrogenase (EC 1.2.1.3) was prepared from ox liver as previously described [20] and one unit of activity is defined as the amount that catalyses the formation of 1 μmol product/min at 37° in the presence of 500 μM NAD^+ and 3 mM acetaldehyde. The molar extinction coefficient of NADH at 340 nm was taken to be 6220/M/cm [21]. The activity of MAO towards milacemide was determined at 37° by a coupled spectrophotometric assay, in which the formation of NADH was followed continuously at 340 nm as the aldehyde product was further oxidized by aldehyde dehydrogenase [6]. The assay mixture contained 80 mM potassium phosphate buffer, pH 7.2, 500 μM NAD^+, 0.015 units of aldehyde dehydrogenase, the enzyme preparation and the milacemide analogue at the concentrations indicated. The activity of aldehyde dehydrogenase was shown not to be rate limiting under any of the assay conditions used. MAO-A activity towards 5-HT (100 μM), MAO-B activity towards 2-phenylethylamine (10 μM) or, in preparations containing no MAO-A activity, 150 μM tyramine was determined at 37° by the radiochemical procedure previously described [18]. Kinetic parameters and IC_{50} values (the concentrations of inhibitor giving rise to 50% inhibition under the stated conditions) were determined by non-linear regression. Linear transformations, such as the double-reciprocal plot are used for illustrative purposes only. Protein concentrations were determined by the method of Markwell *et al.* [22].

The reversibility of inhibition of purified MAO-B by milacemide was also studied by dilution [8]. The binding of [^{3}H]-labelled pargyline was used to determine the concentrations of MAO-B by the procedures previously described for soluble and membrane-bound preparations of the enzyme [23, 24].

RESULTS

As shown in Fig. 1 milacemide acts as a time-dependent inhibitor of the activity of purified MAO-B from ox liver but the sensitivity towards inhibition

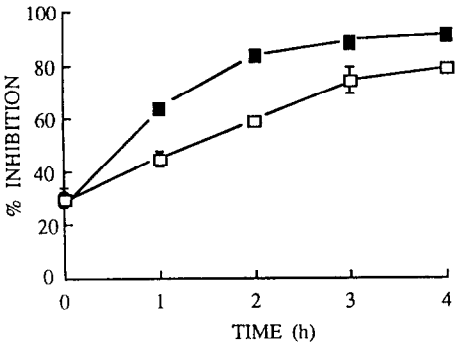


Fig. 1. Time courses of inhibition of rat and ox liver MAO-B by milacemide. Milacemide (□, 250 μM; or ■, 170 μM) was incubated at 37° for the indicated time with purified ox liver MAO-B (5 μg protein/mL; □) or rat liver mitochondria (■, 0.2 mg protein/mL) before the activity towards 10 μM 2-phenylethylamine was determined. Percentage inhibition was calculated with respect to samples preincubated for the same periods in the absence of inhibitor. Data from Ref. 8, for the behaviour of rat liver mitochondria, are included for comparative purposes. Each point is the mean ± range from triplicate determinations in two separate experiments.

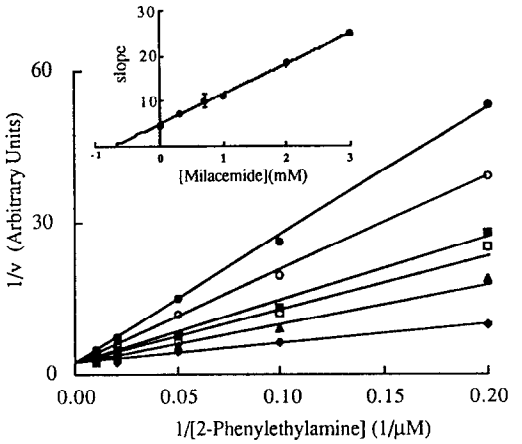


Fig. 3. Kinetics of the inhibition of purified MAO-B from ox liver by milacemide. Initial rates were measured in the presence of the indicated concentrations of 2-phenylethylamine and in the presence of (◆, 0; ▲, 0.3 mM; □, 0.7 mM; ■, 1 mM; ○, 2 mM; and ●, 3 mM) milacemide. Each point is the mean of duplicate determinations. In each case, the errors (range), were no more than 10% of the mean values. The error bars have been omitted for clarity. **Inset**: The dependence of the slopes obtained from the double-reciprocal plots on the milacemide concentration. The intercept of the extrapolated line on the milacemide concentration axis, gives a value of $-K_i$. Each point is the slope ± SE as determined by non-linear regression.

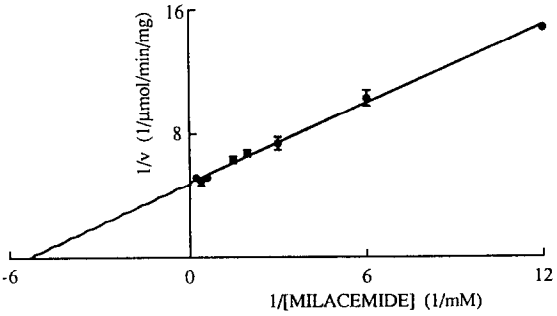


Fig. 2. The effects of milacemide concentration on the activity of ox liver MAO-B. The initial rates of oxidation of milacemide by purified MAO-B were measured at 37° over the indicated range of concentrations. The results are presented as a double reciprocal plot for illustrative purposes. Each point is the mean ± range of duplicate determinations from a single experiment.

appears to be less than that of MAO-B from rat liver mitochondria. After the enzyme had been incubated with milacemide for 60 min at 37° the inhibition was shown to be irreversible by the dilution procedure [8]. When assessed as a substrate the K_m value for the ox liver enzyme was 231 ± 38 μM (mean value ± SEM of seven separate determinations; see representative example in Fig. 2) which was significantly higher than the value of 49 ± 4.7 μM (mean value ± SEM of four separate determinations) found for the activity in rat liver mitochondria [6]. The latter value was in close agreement with that obtained by Janssens de Varebeke *et al.* [5] for MAO-B (39 μM) from the same preparation. In the absence of enzyme-inhibitor preincubation milacemide was found to be a linear competitive inhibitor of the purified ox liver enzyme (Fig. 3) with a K_i value of 742 ± 8 μM (mean ± range of two triplicate determinations) which was also higher than the value of 331 ± 185 μM determined for the rat liver enzyme [6].

Since these studies compared the behaviour of the purified enzyme from ox liver with the crude enzyme in a rat liver mitochondrial preparation it was possible that the differences in the sensitivities to inhibition might have arisen from differences in the state of purity of the enzyme. In order to assess this possibility, rat and ox liver mitochondria were prepared in the same way and compared in terms of the inhibition of their MAO-B activities by milacemide. As shown in Table 1, the differences in inhibitor sensitivities was also evident with these preparations and the concentrations of inhibitor necessary to give 50% inhibition (IC_{50} values) for the enzyme in ox liver mitochondria were in close agreement with those obtained for the purified enzyme.

These differences between the inhibitor sensitivities of the ox and rat enzymes prompted an investigation of the inhibition of the enzyme activities in tissues from other species. Table 1 compares the IC_{50} values for the inhibition of MAO-A and MAO-B from human liver and brain, rat liver and mouse brain mitochondria by milacemide without any prior enzyme-inhibitor preincubation and after 60 min

Table 1. Comparison of the IC_{50} values for the inhibition of MAO from different species by milacemide

Preparation	Preincubation time (min)	IC_{50} (μM) MAO-A (N)	IC_{50} (μM) MAO-B (N)
Rat liver	0	163 ± 67 (2)	455 ± 22 (4)
Mitochondria	60	152 ± 56 (2)	75 ± 6 (4)
Mouse brain	0	149 ± 1.5 (1)	530 ± 3.2 (1)
Mitochondria	60	152 ± 0.9 (1)	52 ± 0.2 (1)
Ox liver	0	—	832 ± 2 (2)
Mitochondria	60	—	270 ± 10 (2)
Ox liver	0	—	808 ± 98 (3)*
Pure MAO-B	60	—	241 ± 52 (3)**
Human brain	0	102 ± 20 (2)	980 ± 12 (4)*
Mitochondria	60	87 ± 13 (2)	172 ± 15 (4)**
Human liver	0	99 ± 7 (2)	1250 ± 250 (2)
Mitochondria	60	120 ± 5 (2)	213 ± 23 (2)

All determinations were made in triplicate. Each value represents the mean ± range or SEM of two or more separate determinations (N), as indicated, except, in the mouse experiments where the values shown are the mean ± SE from a single IC_{50} determination where inhibition estimations were made in triplicate. 5-HT (100 μM) and 2-phenylethylamine (10 μM) were used as substrates for the radiochemical determination of MAO-A and -B activities, respectively. Data for rat liver MAO-A from Ref. 8 are included for comparative purposes.

* These values are not significantly different to each other ($P = 0.094$) but are significantly different ($P = 0.009$) to the corresponding 0 time IC_{50} value obtained with rat liver mitochondria.

** These values are not significantly different to each other ($P = 0.202$) but are significantly different ($P = 0$) to the corresponding 1 hr IC_{50} value obtained with rat liver mitochondria.

preincubation at 37° before assay. As can be seen there were no significant differences between the sensitivities of MAO-A in any of the preparations to milacemide. Furthermore, as had previously been shown for the rat liver enzyme [6], the inhibition showed no significant time-dependence. In the case of MAO-B, the rodent enzymes behaved similarly whereas those from ox liver and human liver and brain were significantly less sensitive to inhibition, either with or without prior enzyme-milacemide preincubation.

The compound α-methylmilacemide has previously been shown to be a reversible and competitive inhibitor of rat liver mitochondrial MAO-A and -B [7, 8]. Table 2 shows a comparison of the inhibitor sensitivities of the enzymes from several different sources to inhibition by this compound. No time-dependence was observed for the inhibition of either MAO-A or MAO-B in any of the sources tested.

In our previous work we have examined the interactions of a series of glycinamide derivatives with the monoamine oxidase from rat liver mitochondria as well as their anticonvulsant behaviour in the mouse bicuculline model [7]. In the present study we have compared the sensitivities of mitochondrial MAO-A and -B from rat liver and MAO-B from ox liver to inhibition by these compounds. Representative curves for the inhibition of MAO-B in rat and ox liver mitochondria by 2-(4-(3-chlorobenzyloxy)-phenethylamino)acetamide (compound **VIII**) are shown in Fig. 4. The comparative IC_{50} values obtained in the absence of enzyme-inhibitor preincubation that are shown in Table 3 indicate all the compounds examined with the exception of compounds **IV** and **VIII** were time-dependent inhibitors of ox and rat liver MAO-B. However, the enzyme from ox liver was considerably less sensitive to inhibition by compounds **I**, **II**, **III**, **VI**, **VII** and **VIII**. There was no apparent difference between their sensitivities to inhibition by compound **IV** and the difference between their sensitivities to compound **V**, which is a poor MAO-B inhibitor, was rather small. After preincubation of the enzyme and milacemide analogue for 60 min at 37° the lower sensitivity of ox liver MAO-B to inhibition persisted for compounds **I**, **II**, **VI**, **VII** and **VIII** but there was little or no difference in the extents of inhibition of the two enzymes by compound **III**.

DISCUSSION

Our earlier work on the behaviour of milacemide [6] and its analogues [7, 8] had mainly used rat liver mitochondria as a good source of both MAO-A and -B activities for *in vitro* studies and the mouse as a model for assessing anticonvulsant activity *in vivo*. However, comparisons of the behaviour of MAO-B from rat liver mitochondria with the same enzyme purified from ox liver revealed significant differences. Milacemide was shown to be a poorer substrate of the ox preparation as determined by differences in K_m values. Furthermore, milacemide was a more potent inhibitor of the rat enzyme during the reversible phase (without any prior enzyme-

Table 2. Comparison of the IC_{50} values for the inhibition of MAO from different species by α-methylmilacemide

Preparation	Preincubation time (min)	IC_{50} (μM) MAO-A (N)	IC_{50} (μM) MAO-B (N)
Rat liver	0	241 ± 46 (3)	63 ± 3 (3)
Mitochondria	60	328 ± 92 (3)	69 ± 7 (3)
Mouse brain	0	218 ± 1.5 (1)	54 ± 0.3 (1)
Mitochondria	60	230 ± 2.8 (1)	57 ± 0.2 (1)
Ox liver	0	—	65 ± 2 (2)
Pure MAO-B	60	—	61 ± 10 (2)
Human brain	0	109 ± 12 (2)	93 ± 9 (2)
Mitochondria	60	109 ± 9 (2)	90 ± 8 (2)
Human liver	0	110 ± 11 (2)	113 ± 3 (2)
Mitochondria	60	110 ± 27 (2)	135 ± 25 (2)

5-HT (100 μM) and 2-phenylethylamine (10 μM) were used as substrates for the radiochemical determination of MAO-A and -B activities, respectively. Each value represents the mean ± range or SEM of two or three separate determinations (N), as indicated, in each of which independent determinations were made in triplicate, except, in the mouse experiments where the values shown are the mean ± SE from a single IC_{50} determination where inhibition estimations were made in triplicate. Data for rat liver MAO are included from Ref. 7 for comparative purposes.

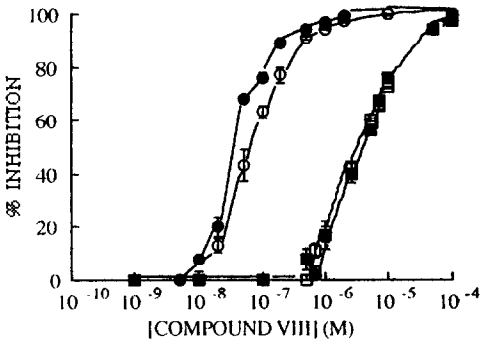


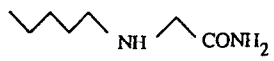
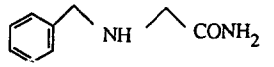
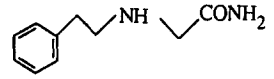
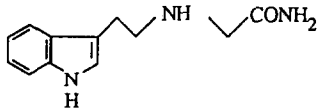
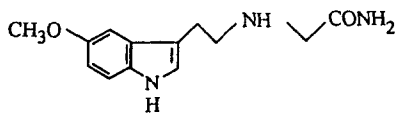
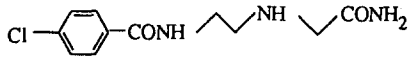
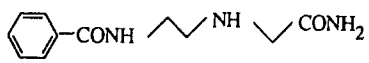
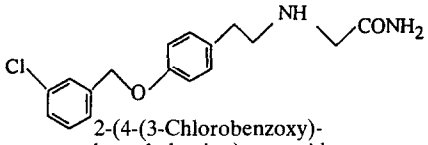
Fig. 4. Comparison of the effects of 2(3-(2-chlorobenzoxy)phenethylaminoacetamide (compound VIII) concentration on the oxidation of 2-phenylethylamine by ox and rat liver mitochondrial MAO-B. Rat liver mitochondria (0.2 mg protein/mL) were incubated with the indicated concentrations of compound VIII for ○, 0 time; or ●, 1 hr at 37° before the activity was determined. Ox liver mitochondria (0.12 mg protein/mL) were incubated in the same way for □, 0 time; or ■, 1 h. Activity was determined with 10 μM 2-phenylethylamine as substrate. Percentage inhibition was calculated with respect to samples preincubated for the same periods in the absence of inhibitor. Each point is the mean ± SEM of triplicate determinations in a single experiment.

inhibitor preincubation), as shown by differences in the K_i values, and it was a better time-dependent inhibitor of the former enzyme. There was a 5.6-fold difference between IC_{50} values for 0 and 1 hr preincubation with the rat liver preparation as opposed to a 2.9-fold difference between corresponding values for the purified MAO-B preparation from ox liver (Table 1). The result was strengthened by an extended incubation of milacemide and purified MAO-B. Inhibition rose to only about 78% after 4 hr, whereas, similar experiments with rat liver mitochondrial MAO-B showed 91% inhibition after 4 hr (Fig. 1).

Since these results involved comparison of the behaviour of a crude preparation of the enzyme in rat liver mitochondria with that purified from ox liver, the possibility that the differences observed might have resulted from the purification of the enzyme from its membrane environment could not be excluded. Milacemide is a relatively lipophilic compound and the solubilisation and delipidation procedures used in the preparation of MAO-B might have affected its access or local concentration at the active site. However, experiments performed with ox liver mitochondria yielded very similar IC_{50} values (Table 1) to those values obtained with purified MAO-B. Furthermore, this difference does not appear to result from non-specific binding of milacemide to other material in the enzyme preparation, since the higher IC_{50} value was obtained with the purified MAO-B preparation and not the more crude preparation.

Although the differences in the specific activities of MAO-B in the crude and purified preparations precluded the use of comparable protein concentrations in activity assays, the amounts of MAO-B added for the rat liver mitochondrial experiments (0.27 pmol MAO-B) were comparable to those used in the experiments with the ox liver preparations (0.7 and 0.6 pmol for the experiments using mitochondria and the purified enzyme, respectively). Hence, it seems apparent that the differences in the behaviour of MAO from rat and ox reflect a genuine functional difference between MAO-B from these two sources, since the comparison of the milacemide response was performed under the same conditions and with similar enzyme preparations from the two

Table 3. The inhibition of rat and ox liver MAO by some milacemide analogues

Analogue	Structure	Preincubation (min)	IC_{50} (μM) Rat MAO-A	Rat MAO-B	Ox MAO-B
I	 2-(n-Pentylamino)acetamide (Milacemide)	0	163 ± 67	257 ± 6	985 ± 15
		60	152 ± 56	53.7 ± 4	283 ± 26
II	 2-(Benzylamino)acetamide	0	42.6 ± 11	490 ± 190	2150 ± 50
		60	47.9 ± 6.3	207 ± 17	880 ± 100
III	 2-(Phenethylamino)acetamide	0	205 ± 75	272 ± 10	613 ± 63
		60	237 ± 64	134 ± 3.4	190 ± 20
IV	 2-(2-(Indol-3-yl)ethylamino)acetamide	0	13.9 ± 2.3	472 ± 97	447 ± 67
		60	13.6 ± 0.9	508 ± 122	441 ± 61
V	 2-(2-(5-Methoxy-3-indol-3-yl)-ethylamino)acetamide	0	144 ± 24	3300 ± 30	2600 ± 10
		60	112 ± 35	1625 ± 40	1410 ± 20
VI	 2-(2-(4-Chlorobenzamido)-ethylamino)acetamide	0	64.3 ± 22	67.9 ± 9.7	1200 ± 100
		60	56.6 ± 22	12.3 ± 2.3	249 ± 20
VII	 2-(2-Benzamidoethylamino)acetamide	0	94.6 ± 3.4	164 ± 64	3900 ± 100
		60	86.5 ± 4.6	20.6 ± 8.1	373 ± 97
VIII	 2-(4-(3-Chlorobenzoxy)-phenethylamino)acetamide	0	2.8 ± 0.1	0.051 ± 0.008	4.1 ± 0.8
		60	2.9 ± 0.3	0.027 ± 0.002	3.5 ± 0.4

Each value represents the mean ± range of two separate experiments, except in the case of compound **V**, where the values shown are the mean ± SE from single IC_{50} determinations where independent inhibition estimations were made either in duplicate (rat experiments) or in triplicate (ox experiments). Tyramine and 5-HT were used as the substrates for MAO-B and MAO-A, respectively and activities were determined radiochemically. Data for rat liver MAO-A and ox liver MAO-B are included from Ref. 8 for comparative purposes.

species. Even more striking differences between the sensitivities of rat and ox liver MAO-B were revealed when their inhibition by the milacemide analogues shown in Table 3 are compared, with the latter preparation being over 10 times less sensitive in the cases of compounds **VI**, **VII**, and about 100 times less sensitive with compound **VIII**.

These species differences prompted studies on the interactions of milacemide and α-methyl milacemide with MAO from mouse tissue, because of the previous use of this animal model in pharmacological studies with milacemide, and human tissue because of the possible importance of milacemide as an anticonvulsant in man. It is notable that there was no apparent difference between the sensitivities of human brain and human liver mitochondrial MAO-B to inhibition by milacemide (Table 1) or α-methyl milacemide (Table 2) suggesting that the differences found here are due to species rather than tissue differences. The IC_{50} values (0 time and 1 hr) for the inhibition of rat liver mitochondrial MAO-B by milacemide closely resemble those obtained with the

mouse preparation and are significantly different ($P < 0.013$), in all cases) to those obtained with the purified ox MAO-B preparation and human brain mitochondrial MAO-B. Similarly, these data indicate that rat and human MAO-B are dissimilar. In contrast, at 0 time and 1 hr preincubation of enzyme and milacemide, the IC_{50} values for the purified ox MAO-B preparation showed no significant difference to human brain mitochondrial MAO-B ($P > 0.094$) in both cases).

The lack of any time-dependent inhibition of either MAO-A or MAO-B by α-methyl milacemide in any of the preparations studied is consistent with our earlier observations that this compound is a reversible and competitive inhibitor of both forms of MAO [7]. Substitution of a methyl group at the α-position of the substrates 2-phenylethylamine, tryptamine and benzylamine produces reversible competitive inhibitors [25] and the α-substituted monoamine mexiletine has also been shown to be a reversible competitive inhibitor of MAO [26]. All those substituted monoamines were found to be MAO-A-selective inhibitors. However, we had previously reported α-methyl milacemide to show slight selectivity towards MAO-B in rat liver [7] and the same was found to be true for the enzyme from mouse brain in the present work. In contrast, this compound showed no significant selectivity as an inhibitor of the two monoamine oxidases when the human liver and brain preparations were examined.

Comparisons of the amino acid sequences for ox [27], human [28] and rat [29] MAO-B reveal considerable similarities. The sequences of ox liver MAO-B peptides show 82% identity to that deduced from the full-length cDNA clone of the rat enzyme and 88% identity to that of the human enzyme. However, such a high degree of similarity does not necessarily imply functional identity. Immunological studies have shown that a MAO-B specific monoclonal antibody raised towards human platelet MAO-B bound to that enzyme in human brain, kidney, lung and liver as well as to purified ox MAO-B and the enzyme from monkey liver and dog liver MAO-B but did not bind to the enzyme from mouse or rat [30]. Human brain MAO-B has been shown to differ from the rat brain enzyme in specificity towards dopamine and some other substrates [16] and in its sensitivity to inhibition by some tricyclic antidepressant drugs [15]. Similarly, differences between the partition ratios of rat and ox liver MAO-B oxidizing the tertiary amine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine have been reported [31].

This marked species difference between rodent and primate species has important implications for comparative studies of drugs affecting or acting through amine metabolic pathways and suggests that it may not be possible to extrapolate data from rat to human tissues. The results reported here would be consistent with the immunological data suggesting a greater similarity between ox and human MAO-B than the enzyme from rodents. Hence, at least in terms of the actions of milacemide and its analogues, it appears that the ox is a better model than the rat or the mouse, for the MAO-B enzyme from human tissue. Unfortunately it is hardly appropriate for *in vivo* anticonvulsant studies. In the case of MAO-A the present studies do not indicate any significant differences between the interactions of the enzyme from the species studied with milacemide and α-methyl milacemide but it is of course possible that studies with other compounds may also reveal functional differences between these enzymes.

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