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A monoamine oxidase-B inhibitor, MD 780236, metabolized essentially by the A form of the enzyme in the rat

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In-vivo studies on the metabolism of [^{14}C]MD 780236 a short-acting selective type B MAO inhibitor in the rat showed the acid to be the major metabolite in plasma and urine, whereas it was minor in brain, where the alcohol was the major metabolite. Pretreatment with SKF 525-A did not modify the metabolite profile in brain, but benserazide decreased the alcohol. Pretreatment with (−)-selegiline had no effect, but clorgyline or clorgyline with (−)-selegiline significantly decreased the alcohol and increased the primary amine metabolite in brain. In-vivo results suggest that MAO-A is the enzyme responsible for the metabolism of MD 780236. This was confirmed by in-vitro studies. Rat brain homogenates extensively metabolized the drug, with the aldehyde being the major metabolite formed (28% of the total radioactivity in the incubation mixture after 60 min incubation). The acid (12%) was more important than the alcohol (4%) in-vitro. The addition of all metabolites originating from possible MAO activity gave 46% when the incubation was carried out at pH 7·4 and 82% at pH 8·8. The presence of NADPH or NAD$^+$ did not alter the relative amounts of metabolites formed. Total metabolites originating from MAO activity in the presence of (−)-selegiline accounted for 40% of total radioactivity, whereas in the presence of clorgyline they accounted for 8% and in the presence of both clorgyline and (−)-selegiline they were reduced to 3%, compared with 45% in controls. As a further proof of the importance of MAO-A in the metabolism of MD 780236, rats were pretreated with clorgyline 1 h before the drug and MAO-B inhibition measured at different times ex-vivo in brain and liver. The short-lasting phase of inhibition of MAO-B disappeared after pretreatment with clorgyline, and inhibition at 24 h was as high as that at 1 h. These results demonstrate the importance of the A form of MAO for the metabolism of MD 780236.

MD 780236, 3-[4-((3-chlorophenyl)methoxy)-phenyl]-5-[(methylamino)methyl]-2-oxazolidinone methane sulphonate is a new molecule having MAO-B inhibiting properties in-vitro, ex-vivo and in-vivo (Strolin Benedetti et al 1982b,c; Dostert et al 1983). Detailed in-vitro studies (Tipton et al 1982) have shown that MD 780236 is a selective irreversible inhibitor as well as a substrate of the B form of MAO. From preliminary metabolic studies in the rat (Strolin Benedetti & Dow 1982), the acid derivative produced by deamination was found to be a major metabolite. The aim of the present work was therefore to study whether MAO is the enzyme system responsible in-vitro and in-vivo for the formation of the acid and other possible metabolites, such as the aldehyde and alcohol derivatives, and if so to what extent the A or B form participate in the metabolism of the molecule. If MD 780236 is initially demethylated by enzyme systems such as the microsomal monooxygenases, to produce the primary amine and if this is a substrate of MAO, then the above metabolites would also be formed.

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MATERIALS AND METHODS

Chemicals

[^{14}C]MD 780236 methane sulphonate (spec. act. 23 mCi mmol^{-1}) was synthesized with the ^{14}C label in the carbonyl group of the oxazolidinone ring by C.E.A., Saclay, France and was shown by t.l.c. to have a radiochemical purity of >96%. Phenylethylamine hydrochloride, β-[ethyl-1-^{14}C]([^{14}C]PEA, spec. act. 48 mCi mmol^{-1}) was obtained from New England Nuclear, USA. Clorgyline, non-radioactive MD 780236 and potential metabolites (Fig. 1), were synthesized by the Dept. of Organic Chemistry, Delalande Research Centre. The hydrated form of the aldehyde was prepared and used as reference compound because of the higher stability of the adduct. (−)-Selegiline (deprenyl) was a gift from Chinoin, Budapest, Hungary. Benserazide was a gift from F. Hoffmann La Roche and Co., Ltd, Basle, Switzerland. SKF 525-A was a gift from Smith Kline & French Labs, Ltd, Welwyn Garden City, U.K. Tranylcypromine, NAD$^+$, NADPH and semicarbazide were obtained from Sigma, U.S.A. All other reagents were of analytical grade.

Animal treatment
Male Sprague Dawley rats (Charles River, CD, France), 150 g, fasted overnight, were used.

Metabolism in-vivo
Effect of time
Rats were treated with [^{14}C]MD 780236 (5 mg kg^{-1}, orally, expressed as the free base; 50 μCi/animal). Rats were housed in metabolism cages to collect urine (in ice-cooled containers) and faeces separately. Groups of four animals were decapitated 2, 4 and 8 h after drug administration, and amounts of radioactivity, unchanged drug and major metabolites in brain, plasma and urine (pooled 0–2, 0–4 and 0–8 h samples) were determined. At 4 h drug absorption was complete, metabolism already extensive, and brain levels of radioactivity still high enough to allow accurate quantitation of metabolites; this death time was therefore used in all subsequent in-vivo studies.

Effect of pretreatments
Groups of 4 rats were pretreated with the following compounds: SKF 525-A (75 mg kg^{-1} orally), (−)-selegiline (5 mg kg^{-1} orally), (−)-selegiline (5 mg kg^{-1} orally) + clorgyline (10 mg kg^{-1} orally), clorgyline (10 mg kg^{-1} orally), tranylcypromine (2 mg kg^{-1} orally) and benserazide (150 mg kg^{-1} i.p.). Doses are expressed in terms of the hydrochloride salts. One hour later, the animals were given [^{14}C]MD 780236 (5 mg kg^{-1}) and were killed 4 h after this second treatment. Urine (0–24 h) was collected in a further group of rats pretreated with SKF 525-A and in a group of controls.

Separation and quantitation of metabolites
Brains were rapidly excised, rinsed in 0·9% NaCl (saline), blotted, weighed and homogenized in methanol (5 ml) containing non-radioactive reference compounds (1 mg ml^{-1}) with an Ultra-Turrax (Janke & Kundel, K.G.) homogenizer. Radioactivity was measured on a 200 μl aliquot of homogenate which was dissolved in 1 ml Soluene (Packard Instrument Co. Inc.) and counted in 10 ml Dimilume (Packard Instrument Co. Inc.) using an Intertechnique SL3000 (Intertechnique, France) liquid scintillation counter. Quenching was corrected by the external standard technique. Homogenates were shaken for 15 min, centrifuged and an aliquot (500 μl) of methanol chromatographed on silica gel 60 F_{254} t.l.c. plates (E. Merck, Darmstadt, F.R. Germany) using the following solvent system n-butanol–glacial acetic acid–water (60:20:20, by vol.). Blood was collected in heparinized tubes and radioactivity was determined on 100 μl of plasma in 10 ml. Unisolve (Koch-Light Laboratories Ltd, Colnbrook, U.K.). Plasma (500 μl) was lyophilized and taken up in methanol (1·5 ml) containing non-radioactive reference compounds. An aliquot (500 μl) of the methanol extract was chromatographed in the same system as for brain. Urinary radioactivity was measured on an aliquot (100 μl) and urine was diluted in methanol (1/50 v/v) containing the reference compounds, and chromatographed as described above.

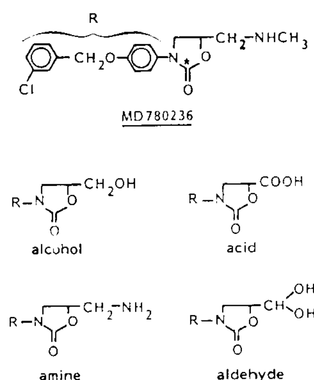

FIG. 1. Structures of MD 780236 and potential metabolites. * Indicates the position of the label in [^{14}C]MD 780236.

Radioactivity on t.l.c. plates was localized by autoradiography using Kodak NS-2T, X-ray film (Kodak-Pathé, France) and radioactive zones compared with the zones corresponding to the non-radioactive reference compounds, which were visualized at 254 nm. Radioactive zones corresponding to unchanged drug and metabolites were removed from plates and chromatographed separately in other solvent systems, and good agreement was found in R_F values compared with those of reference compounds; all subsequent work therefore relied on the localization of radioactive zones by the visualization at 254 nm of their non-radioactive reference compounds. Zones corresponding to unchanged MD 780236 and its demethylated primary amine, aldehyde, acid and alcohol metabolites were removed from the plates, shaken with methanol (2 ml) and the radioactivity determined after the addition of Unisolve (10 ml). The remaining areas of the t.l.c. plates were also removed and counted to give the totality of radioactivity deposited. The amounts of unchanged drug and metabolites in

brain, plasma and urine were expressed as a percentage of the administered radioactivity.

Metabolism in-vitro

Effect of time

Brains of untreated rats were homogenized in 0·1 M phosphate buffer (pH 7·4) to give a 20% w/v suspension. [^{14}C]MD 780236 (0·13 μCi) was added to 1 ml aliquots of homogenates to give a final concentration of 5×10^{-6} M. The effect of time on the formation of metabolites was studied in the following manner. Homogenates (1 ml) +[^{14}C]MD 780236 (5×10^{-6} M) were incubated in duplicate by agitation at 37 °C in presence of air for 0, 5, 10, 15, 30, 45 and 60 min.

Effect of pH, NADPH and NAD+

Brains of untreated rats were homogenized on 0·1 M phosphate buffer (pH 7·4) or 0·1 M pyrophosphate (pH 8·8) to give a 20% w/v suspension. Incubations of homogenate (1 ml) and [^{14}C]MD 780236 (5×10^{-6} M) were carried out in duplicate at pH 7·4 in the presence of 0·1 mM NADPH or at pH 8·8 in the presence of 0·25 mM NAD$^+$ at 37 °C for 1 h (Tipton et al 1981).

Effect of inhibitors

Incubations of homogenates (1 ml) and [^{14}C]MD 780236 (5×10^{-6} M) were carried out at 37 °C for 1 h in the presence of tranylcypromine (4×10^{-4} M) or semicarbazide (9×10^{-4} M) along with a boiled homogenate blank and a time zero blank (reaction stopped with methanol immediately after the addition of drug to homogenate).

Effect of (−)-selegiline, clorgyline or a mixture of both compounds

Incubations of homogenate (1 ml) and [^{14}C]MD 780236 (5×10^{-6} M) were carried out in duplicate at 37 °C for 1 h in the presence of (−)-selegiline (4×10^{-7} M) or clorgyline (4×10^{-7} M) or a mixture of both compounds at the same concentrations, along with a time zero blank.

After in-vitro incubations the reaction was stopped by the addition of methanol (2 ml) containing non-radioactive reference compounds. The subsequent steps for the separation and quantification of reaction products were as described for brain in the in-vivo studies.

MAO-B inhibition ex-vivo—rat liver and brain homogenates

This was determined as described previously (Strolin Benedetti et al 1982b,c) with [^{14}C]PEA as substrate in rats pretreated with clorgyline (10 mg kg^{-1} orally) 1 h before MD 780236 (5 mg kg^{-1} orally) and killed at different times after MD 780236.

RESULTS

Changes with time in the amounts of total radioactivity, unchanged drug and metabolites in plasma, urine and brain 2, 4 and 8 h after MD 780236 are shown in Figs 2a–c respectively. In plasma (Fig. 2a) the acid was the major metabolite present at all times studied, and represented about 2% of the dose/5 ml at 2 h. The alcohol was present in much smaller amounts, whereas unchanged drug, aldehyde and primary amine were detectable but present in very small amounts. In urine (Fig. 2b) the metabolite profile was similar to that in plasma, with the acid again the major metabolite. Urinary excretion of the acid metabolite was 5% of the dose 4 h after drug administration. The metabolite profile in the brain was very different (Fig. 2c), with the acid the least important metabolite present. The alcohol was the major metabolite in the brain, representing 0·2% of the dose at 4 h. The relative importance of other

Table 1. Effect of pretreatment with SKF 525-A (75 mg kg^{-1}, orally), benserazide (150 mg kg^{-1}, i.p.), (−)-selegiline (5 mg kg^{-1}, orally) or (−)-selegiline (5 mg kg^{-1}, orally) + clorgyline (10 mg kg^{-1}, orally) on the amount of total radioactivity, MD 780236 and major metabolites, in rat brain after [^{14}C]MD 780236 (5 mg kg^{-1}, orally). Animals were pretreated 1 h before and killed 4 h after MD 780236.

	% of the dose/brain (mean ± s.d., n = 4)				
	Control	SKF 525A	Benserazide	(−)-Selegiline	(−)-Selegiline + clorgyline
MD 780236	0·12 ± 0·03	0·13 ± 0·04	0·13 ± 0·04	0·16 ± 0·01	0·15 ± 0·02
Acid	0·03 ± 0·01	0·03 ± 0·01	0·03 ± 0·01	0·03 ± 0·01	0·03 ± 0·01
Alcohol	0·30 ± 0·05	0·31 ± 0·08	0·20 ± 0·04*	0·27 ± 0·04	0·19 ± 0·03*
Amine	0·08 ± 0·02	0·06 ± 0·02	0·07 ± 0·01	0·11 ± 0·02	0·17 ± 0·01**
Radioactivity	0·70 ± 0·10	0·71 ± 0·17	0·57 ± 0·13	0·73 ± 0·04	0·70 ± 0·06

* $P < 0·05$, ** $P < 0·001$, Student's *t*-test.

compounds was in the order unchanged drug, primary amine, aldehyde and lastly acid derivative. No differences with time were noted in the relative amounts of metabolites in plasma, urine and brain.

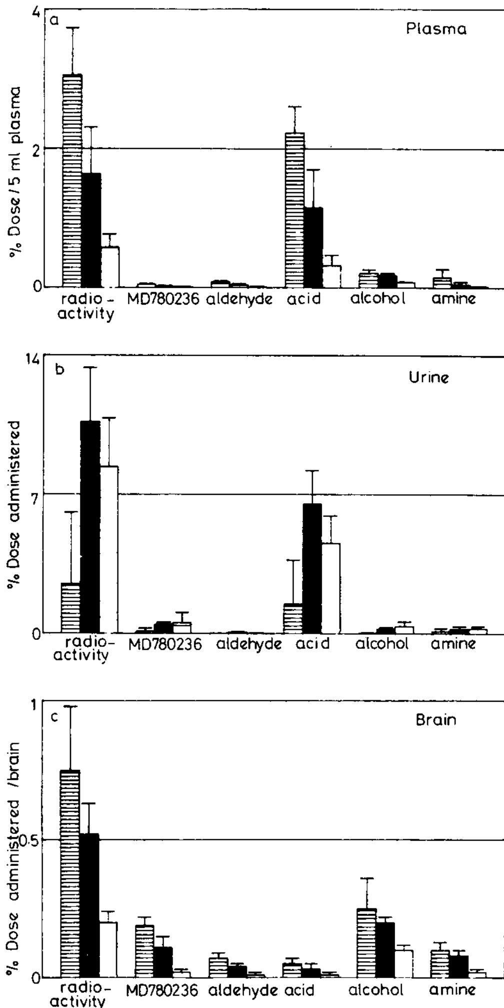

FIG. 2. (a) Total radioactivity, MD 780236, aldehyde, acid, alcohol and amine metabolites in rat plasma, 2 (hatched), 4 (solid) and 8 (open columns) hours after [^{14}C]MD 780236 (5 mg kg^{-1} orally). Results are expressed as percentage of the dose/5 ml plasma (mean with s.d., n = 4).
(b) Total radioactivity, MD 780236, aldehyde, acid, alcohol and amine metabolites in rat urine after [^{14}C]MD 780236 (5 mg kg^{-1} orally). Results are expressed as percentage of the dose administered (mean with s.d., n = 4). Urine samples correspond to 0–2 (hatched), 0–4 (solid) and 0–8 (open columns) hour collections.
(c) Total radioactivity, MD 780236, aldehyde, acid, alcohol and amine metabolites in rat brain, 2 (hatched), 4 (solid) and 8 (open columns) hours after [^{14}C]MD 780236 (5 mg kg^{-1} orally). Results are expressed as percentage of the dose administered/brain (mean with s.d., n = 4).

Pretreatment with SKF 525-A did not modify the proportions of unchanged drug and metabolites in the brain (Table 1). In 24 h urine (results not shown) the amounts of unchanged drug and alcohol metabolite were decreased but the amounts of the major urinary metabolite, the acid derivative, and of the primary amine were unchanged. Benserazide significantly decreased the amounts of the alcohol metabolite in rat brain. Pretreatment with (−)-selegiline at a dose which almost totally inhibits the B form of MAO (Strolin Benedetti et al 1982c) had no effect on the amounts of unchanged drug or metabolites in rat brain (Table 1), whereas pretreatment with both (−)-selegiline and clorgyline resulted in a significant decrease in the amount of the alcohol metabolite and a significant increase in the primary amine metabolite (Table 1). Tranylcypromine pretreatment produced a significant decrease in total radioactivity in the brain (Table 2), therefore no statistical analysis was carried out on the amounts of individual metabolites.

Table 2. Effect of pretreatment with clorgyline (10 mg kg^{-1} orally) or tranylcypromine (2 mg kg^{-1} orally) on the amount of total radioactivity, MD 780236 and major metabolites, in rat brain after [^{14}C]MD 780236 (5 mg kg^{-1} orally). Animals were pretreated 1 h before and were killed 4 h after MD 780236.

	% of the dose/brain (mean ± s.d., n = 4)		
	Control	Clorgyline	Tranylcypromine
MD 780236	0·08 ± 0·02	0·11 ± 0·03	0·07 ± 0·02
Acid	0·03 ± 0·01	0·02 ± 0·01	0·01 ± 0·01
Alcohol	0·19 ± 0·01	0·14 ± 0·02**	0·05 ± 0·02
Amine	0·07 ± 0·01	0·12 ± 0·03*	0·07 ± 0·01
Aldehyde	0·05 ± 0·02	0·05 ± 0·01	0·02 ± 0·01
Radioactivity	0·49 ± 0·06	0·51 ± 0·06	0·25 ± 0·05***

* $P < 0·02$, ** $P < 0·01$, *** $P < 0·005$, Student's t-test.

Pretreatment with clorgyline at a dose which almost totally inhibits the A form of MAO (Strolin Benedetti et al 1982c) resulted in a significant decrease in the amount of the alcohol metabolite and a significant increase in the primary amine metabolite; total radioactivity, unchanged drug, acid and aldehyde metabolites were all unchanged (Table 2).

The above results suggested that MAO-A was the enzyme responsible for the metabolism of MD 780236. To complete these in-vivo observations, a series of in-vitro experiments was carried out.

In-vitro metabolism of MD 780236 by rat brain homogenates showed a time-dependent decrease in unchanged drug (50% decrease in 60 min), with corresponding production of metabolites (Fig. 3). The most important metabolite formed in-vitro was the aldehyde (28% of the total radioactivity in the

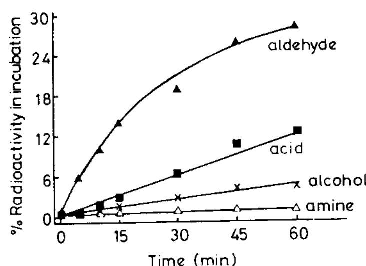

FIG. 3. Time-dependence of in-vitro metabolism of [14C]MD 780236 (5×10^{-6} M) by rat brain homogenates (20%, w/v) in 0·1 M phosphate buffer (pH 7·4) incubated at 37 °C.

incubation mixture after 60 min incubation). The acid (12%), alcohol (4%) and primary amine metabolites (1%) were also formed in-vitro, although in less important quantities.

The addition of all metabolites originating from possible MAO activity gave 44% of the total radioactivity in the incubation mixture after 60 min incubation.

Table 3. Effect of buffer and incubation in the presence of NAD+ and NADPH on the in-vitro metabolism of [14C]MD 780236 (5×10^{-6} M) by rat brain homogenates. Amounts of MD 780236 and major metabolites are expressed as a percentage of radioactivity in the incubation mixture. Results are the means of two separate incubations. Homogenates (20%, w/v) were prepared in phosphate (pH 7·4) or pyrophosphate (pH 8·8) buffer and incubated for 1 h at 37 °C.

Buffer	0·1 M phosphate pH 7·4		0·1 M pyrophosphate pH 8·8	
Incubations conditions		NADPH (1×10^{-4} M)		NAD+ ($2·5 \times 10^{-4}$ M)
	% of radioactivity in the incubation mixture			
MD 780236	42·8	43·8	6·3	12·8
Acid	4·6	4·8	21·8	20·8
Alcohol	3·6	3·8	4·2	4·4
Amine	1·0	0·8	1·6	2·1
Aldehyde	37·8	35·5	56·0	48·8

Formation of the acid and alcohol metabolites was linear over 60 min although their production appeared to lag behind that of aldehyde formation. Linearity for the formation of the aldehyde was limited to the first 15 min of incubation. Levels of the primary amine were too low to accurately assess linearity.

Increasing the pH of the incubation mixture from 7·4 to 8·8 greatly increased metabolism (Table 3), with greater formation of the aldehyde and acid. The addition of all metabolites originating from possible MAO activity gave 46% of the total radioactivity in the incubation mixture at pH 7·4 and 82% at pH 8·8. The presence of NADPH or NAD+ did not alter the relative amounts of metabolites formed.

The decrease in the amount of unchanged drug with time appears to be enzymatic as boiling the homogenate before incubation stopped the reaction (Table 4). The reaction appears to be due to MAO activity as the presence of tranylcypromine produced complete inhibition. Other amine oxidases are unlikely to be involved as semicarbazide did not inhibit the reaction. Incubation in the presence of (–)-selegiline inhibited the metabolism of MD 780236 to a very small extent, if any (Table 5), whereas the presence of clorgyline alone or in combination with (–)-selegiline led to almost complete inhibition of metabolism. Total metabolites originating from MAO activity in the presence of (–)-selegiline accounted for 40% of total radioactivity, compared with 45% in the controls, whereas with clorgyline they accounted for 8% of total radioactivity. Metabolites originating from MAO activity were reduced to 3% in the presence of both clorgyline and (–)-selegiline. Similar results were obtained when inhibitors were preincubated for 20 min before incubation with MD 780236.

As a further proof for the importance of MAO-A in the metabolism of MD 780236, and to confirm the

Table 4. Effect of tranylcypromine (4×10^{-4} M) or semicarbazide (9×10^{-4} M) on the in-vitro metabolism of [14C]MD 780236 (5×10^{-6} M) by rat brain homogenates (20%, w/v) in 0·1 M phosphate buffer (pH 7·4) incubated at 37 °C. Amounts of MD 780236 and major metabolites are expressed as a percentage of the radioactivity in the incubation mixture.

Incubation conditions		Boiled homogenate		Tranylcypromine (4×10^{-4} M)	Semicarbazide (9×10^{-4} M)
Time of incubation (h)	0	1	1	1	1
	% of radioactivity in the incubation mixture				
MD 780236	91·9	91·6	54·5	91·6	53·7
Acid	0·4	0·5	9·5	0·5	7·3
Alcohol	0·3	0·2	3·9	0·3	5·1
Amine	0·3	0·5	0·7	0·8	0·7

Table 5. Effect of (−)-selegiline, clorgyline or a mixture of both compounds on the in-vitro metabolism of [^{14}C]MD 780236 (5×10^{-6} M) by rat brain homogenates (20%, w/v) in 0·1 M phosphate buffer (pH 7·4) incubated at 37 °C. Results are the means of 2 separate incubations.

Incubation conditions		(−)-Selegiline (4×10^{-7} M)		Clorgyline (4×10^{-7} M)	(−)-Selegiline + clorgyline
Time of incubation (h)	0	1	1	1	1
	% of radioactivity in the incubation mixture				
MD 780236	93·1	45·8	50·8	82·5	89·6
Acid	0·2	14·4	13·3	1·7	0·7
Alcohol	0·2	5·1	4·2	2·4	0·5
Amine	0·2	1·0	2·0	1·1	1·1
Aldehyde	0·6	25·2	22·8	3·5	1·4

results from the above metabolism studies, rats were pretreated with clorgyline 1 h before the drug and MAO-B inhibition measured at different time intervals ex-vivo in brain and liver. The short-lasting phase of ex-vivo inhibition of MAO-B disappeared, after pretreatment with clorgyline, and inhibition at 24 h and 48 h was almost as high as that at 1 h after MD 780236 (Fig. 4).

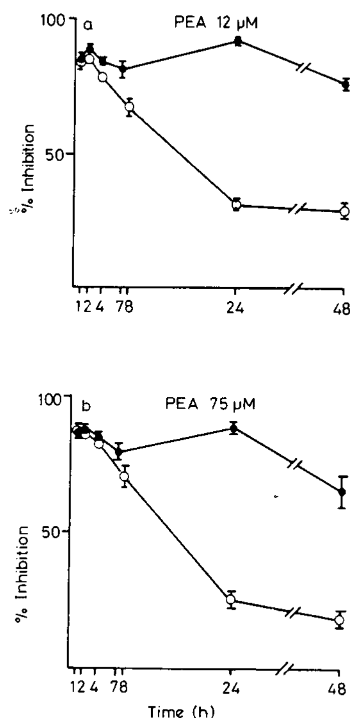

FIG. 4. Ex-vivo inhibition of brain (a) and liver (b) MAO-B in rats treated with MD 780236 (5 mg kg^{-1} orally) (○) or pretreated with clorgyline (10 mg kg^{-1} orally) (●) 1 h before MD 780236. The percentage inhibition of MAO-B by MD 780236 was calculated using non-treated rats as controls. The percentage inhibition of MAO-B by MD 780236 after pretreatment with clorgyline was calculated using clorgyline-pretreated rats as controls.

DISCUSSION

MD 780236 is substantially metabolized in-vivo to form its aldehyde, alcohol and acid derivatives. It has been previously demonstrated that the acid derivative is practically devoid of MAO inhibitory properties, whereas the alcohol is a potent reversible inhibitor of MAO-B (Dostert et al 1983). Although the acid is the major metabolite in plasma and urine, it is present in negligible amounts in brain, where the alcohol is more important. These striking differences in metabolite profiles could be due to several factors. The acid might be formed in important amounts in the brain in-vivo but be preferentially cleared by an active transport system similar to that for the acidic metabolites of endogenous amines. This is supported by in-vitro studies, with rat brain homogenates, where the acid is more important than the alcohol, at least in the experimental conditions used.

Alternatively, in-vivo concentrations of MD 780236 and its aldehyde may vary in different tissues and affect the relative activities of aldehyde dehydrogenase and reductase for the aldehyde as has been shown for aldehydes formed from biogenic amines (Turner et al 1974). Why the hydrated aldehyde in the brain is important in-vitro and not in-vivo, is unlikely to be due to lack of cofactors for aldehyde dehydrogenase or reductase as the addition of NAD$^+$ or NADPH in-vitro did not alter the amounts of the aldehyde. The experimental conditions in-vitro may favour the hydration of the aldehyde, whereas in-vivo, enzymatic attack would predominate.

The metabolism of MD 780236 is only slightly modified by pretreatment of rats with SKF 525-A, an inhibitor of microsomal monooxygenases (Netter 1980). Important modifications of the concentrations of the primary amine, demethylated derivative of MD 780236, by SKF 525-A might have been expected. The lack of effect of SKF 525-A suggests that *N*-demethylation is a minor metabolic pathway or

that both unchanged drug and primary amine are substrates of MAO with similar affinities.

Pretreatment experiments with benserazide, a potent inhibitor of L-dopa decarboxylase, were carried out because this compound also inhibits an amine oxidase widely distributed in the rat, particularly in cardiovascular tissues. This enzyme, which is resistant to inhibition in-vitro by concentrations of clorgyline sufficient to inhibit both MAO-A and -B activities completely, has been called clorgyline-resistant amine oxidase (CRAO) and seems to be a copper and pyridoxal phosphate-dependent enzyme (Clarke et al 1982).

From the results of the present work, a possible involvement of this enzyme system in the metabolism of MD 780236 cannot be totally excluded, as there is a slight decrease of the alcohol derivative in rats pretreated with benserazide. However, results on the metabolism of MD 780236 obtained in-vivo with brain homogenates in the presence of semicarbazide, another inhibitor of CRAO, suggest that the involvement of this enzyme is minimal. Semicarbazide was used to check for a possible effect of CRAO derived from traces of vascular tissues on the brain metabolism of MD 780236 (Lewinsohn et al 1978, 1980).

Pretreatment of rats with clorgyline, a specific MAO-A inhibitor, significantly decreased the percentage of the alcohol and increased that of the primary amine, whereas pretreatment of rats with (−)-selegiline, a specific MAO-B inhibitor, did not produce any significant modification. These results are in favour of MAO-A being the enzyme responsible for the formation of the metabolites originating from deamination, although a small participation of MAO-B in the metabolism of the molecule cannot be excluded.

Results from in-vitro metabolic studies strongly confirm the in-vivo data. The fact that MD 780236 is extensively metabolized by rat brain in-vitro, a tissue containing very low levels of monooxygenases (Sasame et al 1977; Guengerich & Mason 1979; Marietta et al 1979) and CRAO (Lewinsohn et al 1978), but high activities of MAO, particularly of MAO-A (Strolin Benedetti et al 1982a), further favours the involvement of MAO in the metabolism of the molecule. The important increase of metabolites originating from deamination of MD 780236, with changes in pH, is also in agreement with MAO being the enzyme system involved, as it has been suggested that the un-ionized form of the substrate is the form metabolized by the enzyme (Fowler & Oreland 1982).

Moreover, in-vitro metabolism of MD 780236 in the brain in the presence of selective inhibitors of the A or B form of the enzyme has demonstrated that the A form is mainly responsible for the metabolism of the molecule, although the B form seems to have a slight involvement.

Another molecule which has been described as an irreversible inhibitor, as well as a substrate, of MAO is phenelzine (Clineschmidt & Horita 1969a,b). Unfortunately, non-selective inhibitors of MAO, at least at the doses administered, were used in that study, so that it is not possible to distinguish whether one form of the enzyme preferentially metabolizes phenelzine.

MD 780236 is a short-acting inhibitor of MAO-B in ex-vivo experiments (Strolin Benedetti et al 1982b), but when rats are pretreated with clorgyline the inhibition of brain and liver MAO B by MD 780236 is long-lasting. These results conclusively demonstrate the importance of the A form of MAO for the metabolism of the molecule, not only in brain but also in peripheral tissues. In a parallel study (Tipton et al 1982) the K_m of MD 780236 for MAO-A was calculated and a value of 1·1 μM was found using a rat liver mitochondrial preparation that had been incubated with (−)-selegiline to inhibit the B form of the enzyme (pH 7·2).

In conclusion, although MD 780236 is a selective inhibitor of the B form of MAO, it appears to be metabolized essentially by the A form.

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