contract tile activity of pyrimidine nucleotides. These results suggest that the cerebral arteries of the dog can be stimulated to contract by UTP and UDP, presumably, through interaction with a tissue component or receptor system which appears to be complementary to the uracil part and to the phosphate chain (2 or 3 phosphates in length) of the pyrimidine nucleotide molecule.

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REFERENCES

BURNSTOCK, G. (1972). *Pharmac. Rev.*, **24**, 509–581.
HASHIMOTO, K., KUMAKURA, S. & TANEMURA, I. (1964). *Arzneimittel-Forsch.*, **14**, 1252–1254.
NIELSEN, K. C. & OWMAN, C. H. (1971). *Brain Res.*, **27**, 33–42.
URQUILLA, P. R., VAN DYKE, K. & TRUSH, M. (1977). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **36**, 1035.

Monoamine oxidase inhibitory properties of 5-hydroxymethyl-3-*m*-tolyloxazolidin-2-one (toloxatone)

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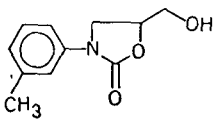
This paper reports on the monoamine oxidase (MAO, EC 1.4.3.4.) inhibiting properties *in vitro* and *in vivo*, of a new oxazolidin-2-one derivative with antidepressant activity in animals (Gouret, Mocquet & others, 1977) and potential clinical efficacy in man (Martin, 1973). A comparison has been made with clorgyline, a specific inhibitor of MAO A (Johnston, 1968) and (±)-deprenyl, a specific inhibitor of MAO B (Knoll & Magyar, 1972) (for a review see Houslay, Tipton & Youdim, 1976).



In vivo *studies*. Male Charles River rats, 125–150 g, were injected intravenously with drugs dissolved in saline and decapitated at preselected times after administration (Fig. 3). Their brains were rapidly removed and homogenized (Ultraturrax) in 10 volumes (w/v) of ice cold 0·2 M phosphate buffer pH 7·40. MAO activity was measured using ^{14}C-5-HT ([2-^{14}C]-5-hydroxytryptamine creatinine sulphate, 0·43 μCi, specific activity 54–58 mCi mmol^{-1}, the Radiochemical Centre, Amersham) or ^{14}C-β-PEA ([1-^{14}C]-β-phenethylamine hydrochloride, 0·39 μCi, specific activity 51 mCi mmol^{-1}, New England Nuclear) as substrates according to a procedure adapted from Wurtman & Axelrod (1963). Briefly: samples (0·5 ml) of brain homogenates were incubated at 37° in air with 0·45 ml of phosphate buffer and 0·050 ml of ^{14}C-5-HT or ^{14}C-β-PEA. Incubation times were respectively 10 and 5 min when MAO activity was linear with respect to both time and protein concentration. Then, 0·2 ml of 4 N HCl and 7 ml of toluene was added to each tube which was mechanically shaken (10 min) and centrifuged (3000 *g*, 5 min). Four ml of the upper organic phase was counted in 10 ml of a scintillation mixture of 2,5-diphenyl oxazole (PPO) in toluene (0·4%, w/v). The blank was boiled homogenate. Protein was measured according to Lowry, Rosebrough & others (1951), with bovine serum albumin as a standard.

For the measurement of concentrations of toloxatone and its metabolites in brain, groups of 3 rats were injected with the drug labelled with ^{14}C in the carbonyl group (50 mg. kg^{-1}, 20 μCi, specific activity 5 mCi mmol^{-1}, ICN Pharmaceuticals) and killed 5, 30, 60, 120 and 240 min later. Brains were rapidly removed, frozen at −20° and lyophilized. Samples (50 mg) were combusted in an oxidizer, the $^{14}CO_2$ absorbed in a scintillation mixture of methanol–toluene–phenethylamine–bidistillated water (22:40:33:50 v/v) containing PPO (0·4%), and the radioactivity measured by liquid scintillation counting.

In vitro *studies*. The specificity of the MAO inhibitory (MAOI) effect of toloxatone was compared *in vitro* with clorgyline HCl (M & B) and (±)-deprenyl HCl. Aliquots (0·5 ml) of whole rat brain homogenates in 10 volumes (w/v) of 0·2 M phosphate buffer pH 7·40 were preincubated at 22° for 15 min with various concentrations of MAO inhibitors in a total volume of 0·95 ml. MAO activity was assayed as already described using ^{14}C-5-HT or ^{14}C-β-PEA and the per cent inhibition plotted against inhibitor concentration using the log-probit representation. In these conditions, the concentration of a particular inhibitor producing 50% inhibition of MAO activity (IC 50) with either 5-HT or β-PEA as substrate, was determined graphically.

Kinetic parameters (Michaelis constant, Km; maximal velocity Vmax; inhibitory constant, Ki) of MAO inhibition by toloxatone were determined from double reciprocal plots using rat brain stems rapidly dissected

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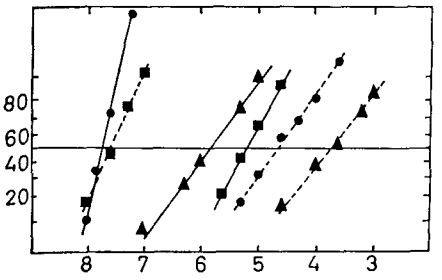

FIG. 1. Comparative effects of toloxatone (▲), clorgyline (●) and (±)-deprenyl (■) on both type A and B monoamine oxidase activities on rat brain. Log probit plots of *in vitro* inhibition (%) of MAO activity with 5-HT (——) or β-PEA (– – – –) as substrate. Aliquots of rat brain homogenates (7·48 mg protein) were incubated with 8·62 μm ^{14}C-5-HT or 9·40 μM ^{14}C-β-PEA as described in the text. Without inhibitor, the amount of deaminated products was 1·93 ± 0·04 nmol and 3·96 ± 0·02 nmol for 5-HT and β-PEA respectively. Ordinate: enzyme inhibition (%). Abscissa: Log [inhibitor] (M).

at 4° according to Glowinski & Iversen (1966) and then homogenized in 20 volumes of 0·2 M phosphate buffer pH 7·40. For Km and Vmax, samples (0·1 ml) were incubated 20 min at 37° with ^{14}C-5-HT, 8–38 μM, in the absence or presence of drug (10 μM). For Ki, samples were incubated in the same conditions with drug 0·5–3 × 10^{-5} M, in the presence of 25 or 49 μM ^{14}C-5-HT. The final volume was always 1·0 ml. The reaction was linear with both time and protein concentration. At the end of the incubation 0·2 ml of 4 N HCl was added and deaminated metabolites extracted. Enzyme velocity was expressed in pmol ^{14}C-5-HT catabolized in 20 min. For each representation the curve of best fit was obtained by linear regression analysis.

Reversibility of the MAOI effect of toloxatone was studied in brainstems from 5 rats, pooled and homogenized in 20 volumes of 0·2 M phosphate buffer pH 7·40 containing EDTA 1 mM. Homogenate (5 ml) was incubated 15 min at 22° without (control) or with toloxatone (5·0 × 10^{-6} M) or clorgyline (5·0 × 10^{-9} M). The final volume was 10 ml; MAO activity was then monitored as before using 0·2 ml of each homogenate. After dialysis (18 h at 4°) against 100 volumes of phosphate buffer 0·02 M pH 7·40, EDTA 1 mM, MAO activity was similarly estimated.

Table 1. *Substrate preferences of toloxatone and selected MAO inhibitors.* Concentrations of inhibitor producing 50% inhibition of the oxidation of the substrate by rat brain homogenates (IC 50) were obtained from log probit plots. The ratios represent the concentration of a particular inhibitor producing 50% inhibition of MAO with β-PEA as substrate divided by concentration of the same inhibitor producing 50% inhibition of MAO with 5-HT as substrate.

Inhibitor	IC 50 (M)		Ratio
	5-HT	β-PEA	
Toloxatone	1·4 × 10^{-6}	2·0 × 10^{-4}	143
Clorgyline	1·8 × 10^{-8}	2·2 × 10^{-5}	1222
(±)-Deprenyl	6·5 × 10^{-6}	2·6 × 10^{-8}	0·004

Fig. 1 shows the effect of toloxatone on the deamination of ^{14}C-5-HT and ^{14}C-β-PEA by brain homogenates; with ^{14}C-5-HT as substrate, the molar concentration producing 50% inhibition (IC 50) was 1·4 × 10^{-6} M. In similar conditions, clorgyline was 100 times more potent as an inhibitor of MAO A (IC 50 = 1·8 × 10^{-8} M). With ^{14}C-β-PEA as substrate, deamination was inhibited by much higher molar concentrations of drug (IC 50 = 2·0 × 10^{-4} M). With the same substrate (±)-deprenyl showed much stronger MAO inhibiting properties (IC50 = 2·6 × 10^{-8} M). The ratio IC50 β-PEA/IC50 5-HT calculated for each compound (Table 1) shows toloxatone to preferentially inhibit type A enzyme although less selectively than clorgyline.

The kinetics of MAO inhibition *in vitro* with rat brain stem (an area containing mostly type A MAO activity, Suzuki & Yagi, 1976) were measured with ^{14}C-5-HT as substrate. MAO activity was non-competitively inhibited (maximal velocity was decreased by 80%) by toloxatone (10 μM), with apparent Ki value of 4·8 μM (Dixon's determination; Fig. 2 B).

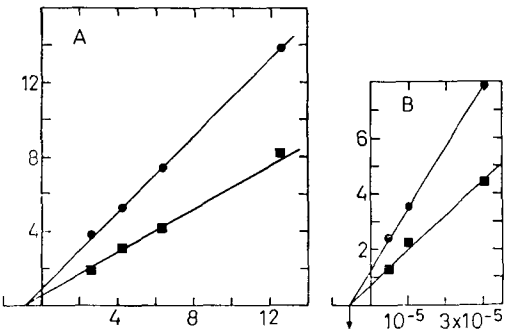

FIG. 2. *In vitro* effect of toloxatone on type A MAO activity of the rat brain stem. A. Double reciprocal plot of MAO activity *vs* ^{14}C-5-HT concentrations. MAO activity was estimated with various concentrations of ^{14}C-5-HT (8; 16; 24; 38 μM) in the absence (■) or in the presence (●) of toloxatone (10 μM). The velocity V is expressed in pmol of ^{14}C-5-HT catabolized in 20 min in the presence of 0·1 ml of rat brain stem homogenate containing 0·250 mg of protein. Each point is the mean of four determinations. Ordinate: 1/V × 10^{-2} $pmol^{-1}$. Abscissa: 1/[^{14}C-5-HT] × 10^{-2} μM^{-1}. B. Dixon plot of the inhibition of MAO activity by toloxatone. MAO activity was estimated with two different concentrations of ^{14}C-5-HT : 25 (●) and 49 μM (■) in the presence of various concentrations of toloxatone. Each point is the mean of four determinations. Ordinate: 1/V × 10^{-2} $pmol^{-1}$. Abscissa: [toloxatone] (M). Arrow: Ki = 0·48 × 10^{-5}M.

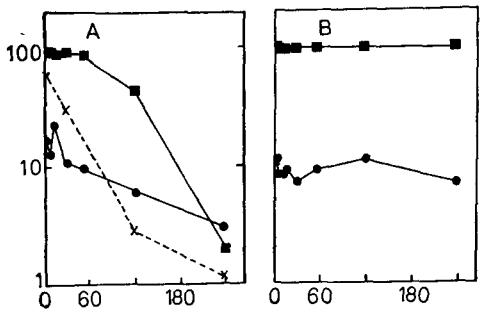

FIG. 3. *In vivo* effects of toloxatone and clorgyline on type A and B MAO activities in rat brain. A. Time course inhibition (%) of MAO activity (left hand ordinate) using either ^{14}C-5-HT (■) or ^{14}C-β-PEA (●) as substrate. Groups of 3 rats were injected with saline (control groups) or toloxatone (50 mg kg^{-1}) by the intravenous route (i.v.) 3, 5, 10, 15, 30, 60 and 240 min before death. Then, form A and B MAO activities were estimated. Each point is the mean of three rats. Total radioactivity (toloxatone + metabolites), expressed as μg equivalent to the unchanged drug g^{-1} of fresh tissue (also left hand ordinate and same intervals) was measured after administration of 20 μCi of toloxatone (50 mg kg^{-1}, i.v.) (– – –X– – –). In control groups, 0·99 ± 0·03 and 2·82 ± 0·09 nmol of deaminated metabolites were produced with respectively 7·80 μM 5-HT and 8·4 μM β-PEA. Abscissa: Time (min). B. Time course of MAO activity inhibition with either ^{14}C-5-HT (■) or ^{14}C-β-PEA (●) as substrate after single administration of clorgyline (2 mg kg^{-1}, hydrochloride, i.v.). Experimental conditions as in A. In control group 1·44 ± 0·015 and 2·98 ± 0·07 nmol of deaminated metabolites were produced with respectively 5-HT (8·0 μM) or β-PEA (7·60 μM) as substrate. Ordinate: % inhibition of MAO. Abscissa: Time (min).

The results of the *in vivo* experiments suggested that toloxatone possesses relatively specific type A MAOI activity. The time course of MAO inhibition with either ^{14}C-5-HT or ^{14}C-β-PEA as substrate is shown in Fig. 3A. A correlation was found between the rate of recovery of MAO activity (^{14}C-5-HT as substrate) and the disappearance of the compound and its metabolites as determined by brain total radioactivity measurements in rats treated with ^{14}C-toloxatone. But as the drug inhibition is reversible and dilution occurs in the homogenization procedure, the extent of inhibition of MAO can not be precisely assessed. In contrast clorgyline (2 mg kg^{-1}, i.v., single dose) completely inhibited type A MAO up to 4 h after administration (Fig. 3B). In these conditions, β-PEA deamination was slightly inhibited (7 to 10%) and remained constant until the fourth hour. Maître, Delini-Stula & Waldmeier (1976) have demonstrated that clorgyline (10 mg kg^{-1}, s.c.) inhibited rat brain MAO A activity irreversibly with a half time inhibition of 12 days.

Our results demonstrate that toloxatone acts as a reversible MAO inhibitor both *in vivo* and *in vitro*; after dialysis, the MAO inhibitory activity of toloxatone in the rat brain stem was markedly decreased (−65%). In contrast, the inhibitory effect of clorgyline remained unchanged.

Metabolic studies in two different mammalian species as well as in man have demonstrated that toloxatone is extensively metabolized (Malnoë & Strolin Benedetti, 1975). In the rat brain, at least two hydroxyphenyl derivatives as well as the unchanged drug have been identified. In some *in vitro* experiments on rat brain homogenates, unlabelled MAO substrates and labelled toloxatone have been used to demonstrate by t.l.c. that only unchanged drug and no metabolites were present. It is clear therefore that, the *in vitro* MAO inhibiting properties of toloxatone are due to unchanged drug. However, *in vivo* a possible interaction of brain metabolites with the enzyme cannot be ruled out.

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REFERENCES

GLOWINSKI, J. & IVERSEN, L. L. (1966). *J. Neurochem.*, **13**, 655–669.

GOURET, C., MOCQUET, G., COSTON, A. & RAYNAUD, G. (1977). *J. Pharmac. (Paris)*, **8**, 333–350.

HOUSLAY, M. D., TIPTON, K. F. & YOUDIM, M. B. H. (1976). *Life Sci.*, **19**, 467–478.

JOHNSTON, J. P. (1968). *Biochem. Pharmac.*, **17**, 1285, 1297.

KNOLL, J. & MAGYAR, K. (1972). *Adv. Biochem. Psychopharmac.*, **5**, 393–408.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). *J. biol. Chem.*, **19**, 265–275.

MAÎTRE, L., DELINI-STULA, A. & WALDMEIER, P. C. (1976). *In: Monoamine oxidase and its inhibition.* A CIBA foundation symposium, vol. 39, pp. 247–267. Amsterdam: Elsevier.

MALNOË, A. & STROLIN BENEDETTI, M. (1975). Abstracts of the Sixth International Congress of Pharmacology, Helsinki, Finland, July 20–25, p. 132.

MARTIN, M. (1973). *L'information psychiatrique*, **49**, 1023–1027.

SUZUKI, O. & YAGI, K. (1976). *Experientia*, **32**, 13–14.

WURTMAN, R. J. & AXELROD, J. (1963). *Biochem. Pharmac.*, **12**, 1439–1441.