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Monoamine oxidase inhibition and brain amine metabolism after oral treatment with toloxatone in the rat

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Rats were administered toloxatone 100 mg kg^{-1} p.o., and killed 0·5 to 8 hours later. Toloxatone reversibly inhibited type A MAO, but did not affect the activity of type B MAO in whole brain. Cerebral concentrations of noradrenaline, dopamine and 5-hydroxytryptamine were increased after toloxatone, while their metabolite concentrations were reduced. Synaptosomal uptake processes of these amines were not altered by toloxatone.

Toloxatone, 5-(hydroxymethyl)-3-(3-methylphenyl)-2-oxazolidinone, is a potential antidepressant agent which is currently undergoing clinical trials. Previous biochemical studies in vitro and ex vivo after intravenous injection in the rat have shown that toloxatone reversibly inhibits the activity of monoamine oxidase (MAO, E.C. 1.4.3.4.) using 5-hydroxytryptamine (5-HT) as substrate, but is much less active when β-phenethylamine (PEA) is used as substrate (Kan et al 1978). Since 5-HT is a preferential substrate for type A MAO, and PEA for type B MAO (Houslay & Tipton 1974), toloxatone appears to be a reversible inhibitor of type A MAO. The present study attempts to confirm this profile after oral administration, and to determine the relationship between MAO inhibition and the metabolism of the amines 5-HT, noradrenaline (NA) and dopamine (DA) in the rat brain.

Some inhibitors of MAO have been found to block the neuronal uptake of NA, DA (Horn & Snyder 1972; Baker et al 1978), or 5-HT (Buckholtz & Boggan 1976; Fuller et al 1976). Therefore, we have also determined whether toloxatone affects the uptake of the brain amines.

Groups of 6 male Charles River rats (125–150 g) were used. All rats were fasted for 18 h before the experiment. The rats were administered orally either the solvent (0·5% methylcellulose, MC, Fluka A.G., in tap water, 10 ml kg^{-1}) or toloxatone (100 mg kg^{-1}) in suspension in MC. The animals were decapitated at 0·5, 1, 2, 4 and 8 h after treatment and the brains were rapidly dissected, cut into two halves (sagittal section) and frozen in liquid nitrogen. The samples were kept at −20 °C until analysis.

Oxidative deamination of 5-HT and PEA was assayed as described by Wurtman & Axelrod (1963), with minor modifications. Aliquots (0·25 ml) of half brain homogenates (1 g per 10 ml of phosphate buffer 0·1 M pH 7·40) were incubated at 37 °C in air with either ^{14}C-5-HT (66·5 nCi) or ^{14}C-PEA (74·3 nCi) in a total volume of 1 ml, the final concentration of each substrate being 10 μM. In both cases, final tissue dilution was 0·025 g ml^{-1}. Incubation times were 5 and 2 min for 5-HT and PEA respectively. After acidification deaminated metabolites were extracted in toluene–ethyl acetate (1:1, v/v) and counted by liquid scintillation in toluene-PPO.

Oxidative deamination of NA was measured by the following technique (Robinson et al 1968; Goridis & Neff 1971): 60 μl aliquots of homogenates were incubated for 30 min with ^{14}C-NA (53·7 nCi) in a total volume of 0·25 ml, the final concentration of the substrate being 13·1 μM. In this case, the final tissue dilution was 0·024 g ml^{-1}. After protein precipitation, the deaminated metabolites of NA were separated by passage through a column of Amberlite (Na^+ form) and counted by liquid scintillation in Aquasol (NEN).

Enzymatic reactions were linear with both time and tissue concentration for all three substrates.

Brain concentrations of NA and DA were determined according to the method of Shellenberger & Gordon (1971). The NA metabolite 3-hydroxy-4-methoxy-phenylethyleneglycol sulphate (MOPEG-SO_4) was extracted and analysed by the technique of Meek & Neff (1972), and the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) by a slight modification of the method of Crow et al (1977). 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA) were assayed as described by Curzon & Green (1970). The uptake of H^3-NA into rat hypothalamic synaptosomes, and H^3-DA and H^3-5-HT into rat striatal synaptosomes were analysed by a

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combination of the methods of Koe (1976) and Wong & Bymaster (1976). The conditions used produced an uptake of all three amines which was linear with respect to time and synaptosome concentration.

Fig. 1 shows the time-course of inhibition of 5-HT, NA and PEA deamination by rat brain MAO after acute oral treatment with toloxatone (100 mg kg^{-1}). For both type A substrates, 5-HT and NA (Houslay et al 1976) maximal inhibition (73 and 79% respectively) occurred 30 min after administration and decreased rapidly until 8 h, where no inhibitory drug effect could be detected. In contrast, PEA oxidation was very slightly inhibited (maximum 6%, Fig. 1).

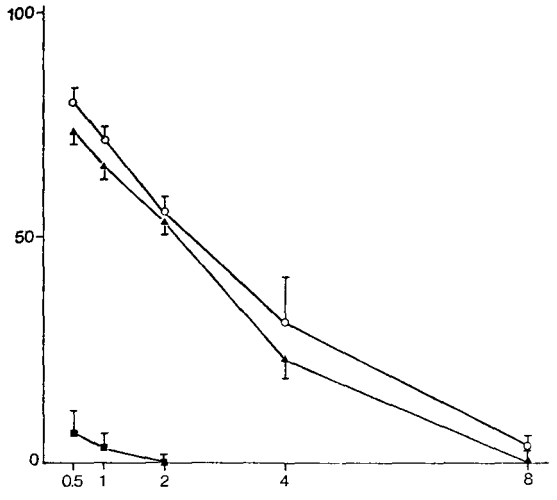

FIG. 1. In vivo effect of toloxatone on type A and B MAO activities in rat brain. Rats were treated with 0·5 % MC (control groups) or toloxatone (100 mg kg^{-1}) orally, 0·5, 1, 2, 4 and 8 h before death. Percentage MAO inhibition (ordinate) using ^{14}C-5-HT (▲), ^{14}C-NA (○) or ^{14}C-PEA (■) as substrate is shown at various times in hours (abscissa) after treatment with toloxatone. Each point represents the mean ± s.e.m. of six animals.

The effect of toloxatone on brain amines and metabolites is shown in Table 1.

The concentration of 5-HT in the rat brain was significantly elevated for 2 h after treatment with 100 mg kg^{-1} toloxatone, and brain 5-HIAA concentrations were reduced for 4 h. The maximum increase in 5-HT was 37%, and the maximum fall in 5-HIAA was 29%. Brain concentrations of NA and DA rose more slowly than 5-HT, the maximum increase being observed 2 h after injection for NA (+30%) and 4 h after injection for DA (+ 26%). Similarly, brain concentrations of MOPEG-SO_4 and DOPAC were reduced by toloxatone, the maximum being 36% for MOPEG-SO_4 and 63% for DOPAC. The toloxatone-induced changes in amines and their metabolites lasted 2 to 4 h, and were absent 8 h after treatment. Toloxatone only inhibited the uptake of NA and DA at very high concentrations (IC50 300 μM), whereas tranylcypromine produced a marked inhibition of NA and DA uptake at much lower concentrations (IC50 0·4 and 4 μM respectively).

Studies with tranylcypromine suggest that the amount of MAO in the rat brain is in excess of that needed for normal functioning, so that a large degree (even 85%) of inhibition may not result in the biochemical or pharmacological changes which are seen after total inhibition of MAO (Green & Youdim 1975). The changes that we have observed in the metabolism of brain transmitters indicate that oral treatment of rats with 100 mg kg^{-1} toloxatone produces a functionally important inhibition of MAO in vivo. A knowledge of the amplitude and time-course of the changes in the three amines and their metabolites is particularly important for a

Table 1. Changes in brain amines and metabolites in rats treated orally with 100 mg kg^{-1} toloxatone. Values are presented as percentage ± s.e.m. of the concentrations in rats treated only with the solvent. Statistical differences were evaluated by the Dunnett test, * $P<0·05$, ** $P<0·01$. Concentrations in solvent-treated rats (100% values) were: NA, 307 ± 17 ng g^{-1}; DA, 838 ± 30 ng g^{-1}; 5-HT, 533 ± 18 ng g^{-1}; MOPEG-SO_4, 163 ± 7 ng g^{-1}; DOPAC, 105 ± 5 ng g^{-1}; 5-HIAA, 390 ± 10 ng g^{-1}.

	Brain Concn (% of controls ± s.e.m.)					
h†	NA	DA	5-HT	MOPEG-SO_4	DOPAC	5-HIAA
0·5	106 ± 9	111 ± 4	137 ± 6**	99 ± 6	52 ± 5**	82 ± 3**
1	119 ± 9	113 ± 5*	134 ± 5**	67 ± 7**	39 ± 3**	71 ± 3**
2	130 ± 8**	113 ± 6*	131 ± 5**	64 ± 5**	37 ± 4**	71 ± 3**
4	127 ± 5**	126 ± 5**	114 ± 8	75 ± 7*	38 ± 4**	78 ± 8*
8	109 ± 4	108 ± 4	107 ± 7	88 ± 11	74 ± 8	92 ± 6

† Time after treatment

reversible inhibitor since the values of MAO activity obtained in the study of the inhibition of the enzyme, and the time-course of this inhibition, are dependent on the conditions of tissue dilution used in the assay, and thus do not represent absolute determinations of the inhibition. The results obtained on 5-HT, NA and PEA metabolism are consistent with toloxatone being a reversible inhibitor of type A MAO, and the changes in brain concentrations of DA and DOPAC suggest that DA is deaminated by a type A MAO in rat brain. Some authors (Braestrup et al 1975; Waldmeier et al 1976) have concluded that DA is preferentially deaminated in rat brain by type A MAO, whereas other studies (Yang & Neff 1974) have also reported an elevation of rat brain DA after administration of deprenyl, a specific inhibitor of type B MAO. Although the concentrations of the three amines were increased after toloxatone, and the concentrations of their metabolites decreased, the percentage increase of each amine differed from the percentage decrease of its metabolite. One important reason for this is the difference between the values in untreated rats of each amine and metabolite (see legend of Table 1). Furthermore, the factors determining the accumulation of the amines, such as turnover rate, and extent of feed-back inhibition of synthesis (Carlsson et al 1976) are different from those determining the disappearance of the metabolites, which include diffusion processes, active transport mechanisms, and, in the case of DOPAC, subsequent enzymatic conversion to homovanillic acid and the conjugate DOPAC-SO_4.

Studies on brain synaptosomes indicate that toloxatone cannot be considered an inhibitor of the uptake processes of NA, DA or 5-HT, in contrast to other MAO inhibitors such as tranylcypromine, pheniprazine, phenelzine and l-deprenyl, which inhibit the uptake of catecholamines into synaptosomes or slices (Horn & Snyder 1972; Baker et al 1978; Knoll & Magyar 1972; Braestrup et al 1975), and quipazine and tetrahydro-β-carbolines, which inhibit the uptake of 5-HT (Buckholtz & Boggan 1976; Fuller et al 1976).

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