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Substrate- and stereoselective inhibition of human brain monoamine oxidase by 4-dimethylamino-α,2-dimethylphenethylamine (FLA 336)

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Monoamine oxidase (MAO, monoamine O_2: oxidoreductase, EC 1.4.3.4) is thought to exist as two catalytically active forms, termed MAO-A and MAO-B, where the -A form of the enzyme is inhibited by low concentrations of the acetylenic inhibitor clorgyline, and the -B form inhibited by low concentrations of deprenyl (Johnston 1968; Knoll & Magyar 1972). According to this definition, in the human brain, 5-hydroxytryptamine (5-HT) is metabolized by MAO-A alone, whereas benzylamine is metabolized by MAO-B alone (Tipton et al 1973; White & Glassman 1977). In both human and rat brain, the MAO-A is thought to be localized predominantly, but not exclusively, in the neuronal tissue, whereas the -B form of the enzyme is predominantly non-neuronal in nature (Student & Edwards 1977; Oreland et al 1980; Fowler et al 1980b).

Monoamine oxidase inhibitors have long been used for the treatment of certain types of depression which are not responsive to treatment by the tricyclic inhibitors of noradrenaline uptake (for discussion, see e.g. Pare 1979). One of the drawbacks of treatment with classical MAO inhibitors is the so-called 'cheese effect'—a pressor response to the tyramine found in various foodstuffs (Blackwell & Marley 1966). The MAO-B selective inhibitor deprenyl has been reported not to have this effect (Knoll 1976; Elsworth et al 1978), but this compound does not appear to be useful in the treatment of depression (Mendis et al 1981). The phenethylamine analogue FLA 336 (4-dimethylamino-α, 2-dimethylphenethylamine), an MAO-A selective inhibitor in the rat, also appears to be without an appreciable 'cheese' reaction (Ask, Högberg, Schmidt, Kiessling, Ross, text in preparation). In the present study, the effect of the optical isomers of this compound upon the activity of human brain MAO-A and -B has been studied. In addition, the principal metabolite of FLA 336(+) has been investigated.

Brains were taken at autopsy from 7 individuals (age range 23–79 years), without histories of neurologic, psychiatric, or liver disease. The brain regions studied (hypothalamus, medulla oblongata, thalamus, nucleus accumbens, cortex gyrus cinguli and frontal cortex) were dissected from the brains as described by Adolfsson et al (1979), put into air-tight packages, and frozen at −70 °C. The frozen brain regions were pulverized in a mortar filled with liquid nitrogen. The tissues were not allowed to thaw during this procedure. The frozen powders were placed in tubes, weighed, and kept frozen. Before use, the powders were resuspended in 'sucrose buffer' (0·25 M sucrose, 10 mM potassium phosphate, pH 7·8) to a protein concentration of 1·5 mg ml^{-1}. This homogenizing procedure, which allows the rapid storage of autopsy material, has been shown not to affect the substrate-specificity or kinetic properties of either MAO-A or -B (Fowler et al 1980b).

Monoamine oxidase activity was assayed radiochemically by the method of Callingham & Laverty (1973), as modified by Fowler et al (1979), with [^{14}C]-5-HT and [^{14}C]benzylamine as substrates for MAO-A and -B, respectively. All assays were carried out under an atmosphere of air, and, unless otherwise shown, at a substrate concentration of 50 μM. Specific activities of MAO, corrected for the efficiencies of extraction of the deaminated metabolites into the organic layer of the assay medium (see Fowler et al 1980a), are expressed as nmol (of substrate metabolized) (mg protein)$^{-1}$ min^{-1}. Protein concentrations were determined by the method of Markwell et al (1978) because this method is not affected by high concentrations of sucrose. Human serum albumin was used as the protein standard.

The radioactive substrates for MAO, 5-hydroxytryptamine-[side chain-2-^{14}C]binoxalate and benzylamine [methylene-^{14}C]hydrochloride were obtained from New England Nuclear (Boston, Mass., U.S.A.) and ICN Pharmaceuticals Inc. (Irvine, CA, U.S.A.) respectively. (+)- and (−)-FLA 336 and (+)-4-monomethylamino-α,2-dimethylphenethylamine (FLA 788 (+)), the principal metabolite of FLA 336 (+), were

Table 1. Kinetic parameters of MAO-A and MAO-B towards 5-HT and benzylamine, respectively, in 6 regions of human brain. K_m and V_{max} values were calculated from linear regression analysis of duplicate determinations of activity at six substrate concentrations (15, 20, 30, 50, 75 and 100 μM), plotted as S/v against S. In all cases, the correlation coefficients of the regression lines were higher than r = 0·95. K_m and V_{max} values are given as means ± s.e.m. for determinations in brain regions from three individuals.

	MAO-A (5-HT)		MAO-B (Benzylamine)	
	K_m (μM)	V_{max} (nmol mg prot.$^{-1}$ min^{-1})	K_m (μM)	V_{max} (nmol mg prot.$^{-1}$ min^{-1})
Hypothalamus	28 ± 4	2·87 ± 0·47	33 ± 2	4·73 ± 0·32
Medulla oblongata	31 ± 3	1·24 ± 0·27	29 ± 4	2·91 ± 0·70
Thalamus	29 ± 5	1·79 ± 0·41	32 ± 1	2·98 ± 0·76
Nucleus accumbens	23 ± 2	1·49 ± 0·47	28 ± 7	3·69 ± 0·99
Cortex gyrus cinguli	27 ± 3	1·89 ± 0·56	28 ± 5	2·86 ± 1·16
Frontal cortex	24 ± 6	1·59 ± 0·55	29 ± 2	2·19 ± 0·92

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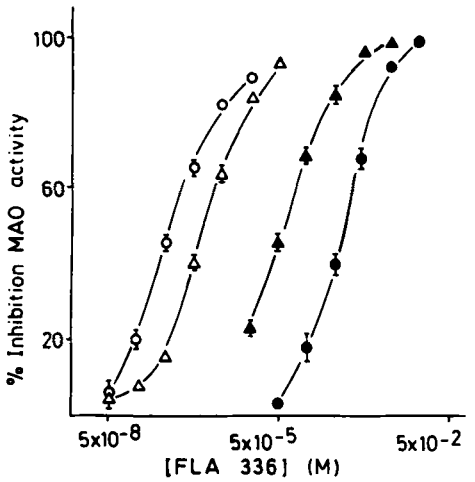

FIG. 1. The effect of FLA 336(+) and FLA 336(—) upon the in vitro activity of human hypothalamic MAO. Each point represents the mean ± s.e.r. of duplicate determinations of the % inhibition of MAO activity produced in 3 homogenates by the inhibitors. The samples were set to an assay protein concentration of 0·38 mg ml^{-1}. Substrates and inhibitors used: 5-HT as substrate (50 μM), FLA 336(+) (○) and FLA 336(—) (△) as inhibitor; benzylamine (50 μM) as substrate, FLA 336(+) (●) and FLA 336(—) (▲) as inhibitor.

gifts from Astra Läkemedel AB, Södertälje, Sweden. All other reagents used were standard laboratory reagents of analytical grade wherever possible.

The kinetic parameters of human brain MAO-A and -B in six brain regions are shown in Table 1. The K_m values for both MAO-A towards 5-HT and MAO-B towards benzylamine are very similar across the brain, a result consistent with a previous conclusion that the variation in the activity of MAO-B from brain region to brain region and from individual to individual was due, with the possible exception of the medulla oblongata, to a variation in the concentration of otherwise identical active centres of this enzyme form (Fowler et al 1980b).

The activity of monoamine oxidase of human brain appeared to be inhibited in a substrate- and stereo-selective manner by the phenethylamine analogue FLA 336. The (+)-isomer inhibited the activity of MAO-A with an IC50 concentration of about 0·8 μM, whereas the MAO-B activity was inhibited by much higher concentrations of this enantiomer (IC50 values about 600 μM) (Table 2). The (−)-isomer appeared to be 3–4 times less potent than the (+)-form as an inhibitor of MAO-A activity, but 9–10 times more potent as an inhibitor of MAO-B activity (Table 2). The data for the hypothalamus as enzyme source are shown in Fig. 1. Thus, the MAO-A selective inhibition found for FLA 336 (+) in the rat (Ask et al, text in preparation) can also be demonstrated in the human brain. Furthermore, the potency of the FLA 336 (+) isomer as an inhibitor of MAO-A is approximately the same across the brain (Table 2).

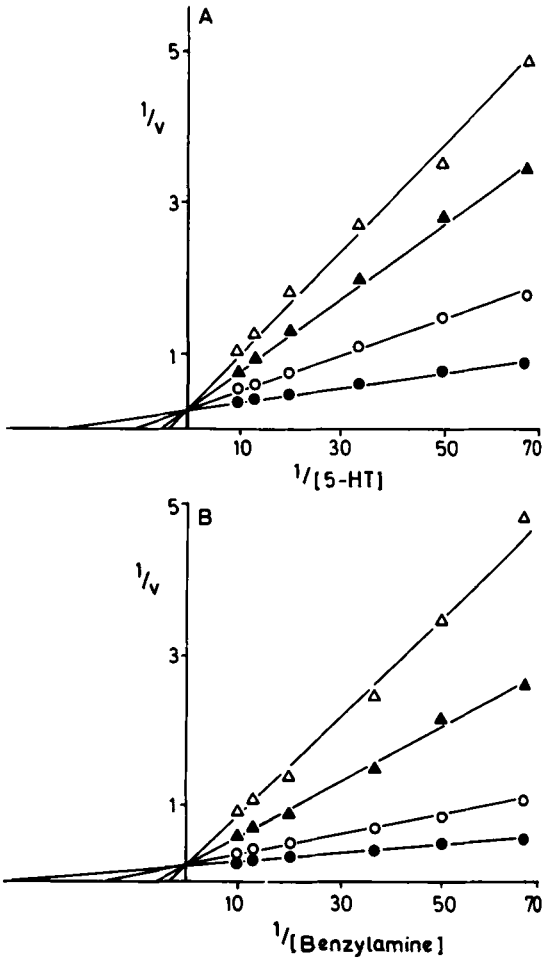

FIG. 2. Double reciprocal plots of the MAO activity in the absence and presence of FLA 336(+). Ordinates: 1/initial velocity (in nmol mg $protein^{-1}$ min^{-1}); abscissae: 1/substrate concentration in mM. All points shown are the means of duplicate determinations of MAO activity in three human hypothalamus homogenates. Substrates used were: Panel A: 5-HT, in the absence (●) and presence of 0·5 μM (○), 1·5 μM (▲) and 2·5 μM (△) FLA 336(+); panel B: benzylamine, in the absence (●) and presence of 0·5 mM (○), 1·5 mM (▲) and 2·5 mM (△) FLA 336(+).

The inhibition of MAO-A and -B by FLA 336(+) was not changed when the brain regions were incubated with the inhibitor for 30 min at 37 °C before addition of substrate to assay for activity. In addition, dilution experiments (performed as described by Fowler et al 1977), indicated that the inhibition of both forms of the enzyme by FLA 336(+) was fully reversible. The inhibition of MAO-A and -B activity of the hypothalamus by FLA 336(+) appeared to be competitive in nature (Fig. 2), with K_i values for this inhibitor of 0·4 and 200 μM, for MAO-A and MAO-B, respectively

Table 2. IC50 values for the inhibition of human brain MAO-A and -B by FLA 336(+), FLA 336(—), and FLA 788(+). The IC50 values were determined from duplicate determinations of the inhibition of MAO-A (towards 50 μM 5-HT) and MAO-B (towards 50 μM benzylamine) by 4–6 concentrations of the compounds. Concentrations of the inhibitors were chosen so that the inhibition of the MAO activities were in the range 10–90% (for example, see Fig. 1), and the IC50 values determined from semilogarithmic plots of the data. Values given in the text represent means ± s.e.m. for determinations in brain regions from three individuals.

	IC50 value (μM)	
FLA 336 (+)	MAO-A	MAO-B
Hypothalamus	0·66 ± 0·03	695 ± 104
Medulla oblongata	1·02 ± 0·13	560 ± 83
Thalamus	0·80 ± 0·05	568 ± 42
Nucleus accumbens	0·94 ± 0·11	665 ± 21
Cortex gyrus cinguli	0·92 ± 0·07	659 ± 143
Frontal cortex	0·81 ± 0·04	589 ± 28
FLA 336 (—)		
Hypothalamus	2·57 ± 0·03	67 ± 4
Medulla oblongata	2·75 ± 0·15	64 ± 8
FLA 788 (+)		
Hypothalamus	0·19 ± 0·01	499 ± 46

Table 3. The kinetics of inhibition of human brain MAO-A and -B by FLA 336(+). K_I values for hypothalamus were calculated from the data shown in Fig. 2 by replotting of the individual data by the method of Dixon (1953). For all six brain regions, the K_I values were also calculated from the individual IC50 values (shown in Table 2) and the individual K_m values of the MAO-A and -B towards the respective amine substrates used in the study (shown in Table 1) by the method of Cheng & Prusoff (1973). Values shown in the Table represent the means ± s.e.m. The substrates used were: MAO-A, 5-HT; MAO-B, benzylamine.

	K_I value (μM) of FLA 336(+) towards:	
	MAO-A	MAO-B
Method of Dixon:		
Hypothalamus	0·39 ± 0·03	206 ± 16
Method of Cheng & Prusoff:		
Hypothalamus	0·24 ± 0·03	279 ± 54
Medulla oblongata	0·38 ± 0·03	205 ± 39
Thalamus	0·28 ± 0·03	220 ± 19
Nucleus accumbens	0·30 ± 0·04	235 ± 42
Cortex gyrus cinguli	0·32 ± 0·01	243 ± 75
Frontal cortex	0·25 ± 0·03	216 ± 18

(Table 3). Assuming a competitive interaction, K_I values can be calculated for the other brain regions from the data given in Tables 1 and 2 by the method of Cheng & Prusoff (1973). These values are in good agreement with the K_I values calculated for the hypothalamus (Table 3).

The data are therefore consistent with the hypothesis that FLA 336(+) is an extremely potent, reversible, competitive inhibitor of MAO-A in the human brain. Such MAO-A selective inhibition was to be expected, since other α-methyl substituted monoamines, such as amphetamine and mexiletine, have also been shown to be competitive selective inhibitors of MAO-A in rat tissues (Mantle et al 1976; Callingham 1977).

The nature of the active centres of both MAO-A and -B appear to be stereospecific. In rat tissues, it has been shown that L-(−)-adrenaline is oxidized at a faster rate than D-(+)-adrenaline (Blaschko et al 1937). Stereospecificity is also seen for tyramine, when enantiomers are created by the replacement of one of the α-methylene hydrogens with deuterium (Belleau et al 1960). A variety of inhibitors, including amphetamine (Fuller & Walters 1965) and deprenyl (Knoll & Magyar 1972) have been shown to act stereoselectively. The most marked cases of stereoselectivity have been found for both substrates and inhibitors: in the rat liver, (−)-phenylethanolamine is a substrate for MAO-A, whereas the (+)-enantiomer is metabolized by MAO-B (Williams 1977); and in the rat brain the (+)-enantiomer of 2,3-dichloro-α-methylbenzylamine is an MAO-A selective inhibitor whereas the (−)-isomer is an MAO-B selctive inhibitor (Fuller & Hemrick 1978). The data shown in Table 2 would also suggest that human brain MAO-A and -B have the same stereospecific requirements as the MAO enzyme forms found in animal tissues.

In initial experiments FLA 788(+), the principal metabolite of FLA 336(+), was studied. This compound also appeared to inhibit MAO-A activity selectively, with an IC50 value rather lower than that of the parent compound (Table 2).

In conclusion, the data presented in this paper indicate that FLA 336(+) is a useful inhibitor of MAO-A in the human brain, especially since this compound does not give the 'cheese effect' often found with MAO inhibitors (Ask et al, text in preparation). Since it has been postulated that the 5-hydroxytryptaminergic system of the brain is deficient in depressives (see Murphy et al 1978; Oreland 1979), an inhibitor selective for the form of MAO responsible for the metabolism of this neurotransmitter may prove to have considerable antidepressant properties.

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Elevation of 3,4-dihydroxyphenylacetic acid concentration by L-5-hydroxytryptophan in control and fluoxetine-pretreated rats

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5-Hydroxytryptophan (5-HTP), the immediate precursor to 5-hydroxytryptamine (5-HT), has been reported to increase acutely the concentration of dopamine metabolites in brain (Awazi & Guldberg 1978; Everett 1979). This effect may occur via conversion of 5-HTP to 5-HT within the dopamine neuron and displacement of vesicular dopamine by the 5-HT (Ng et al 1972; Butcher et al 1972), but a possible alternative explanation is that 5-HTP elevates the concentration of 5-HT at a 5-HT synapse influencing dopamine turnover. Consistent with the latter possibility are reports that 5-HT uptake inhibitors increase dopamine metabolite concentrations in haloperidol-treated rats (Waldmeier & Delini-Stula 1979) and that (+)-fenfluramine, a 5-HT releaser, elevates dopamine metabolites in rat striatum in control rats but not in rats pretreated with *p*-chlorophenylalanine or with a 5-HT uptake inhbitor to prevent the 5-HT activating effect of (+)-fenfluramine (Crunelli et al 1980).

Different effects of pretreatment with a 5-HT uptake inhibitor would be predicted depending on which of the above mechanisms were involved. 5-HT uptake inhibition would not be expected to alter the decarboxylation of 5-HTP within dopamine neurons and should not influence the 5-HTP effect on dopamine neurons if the first of the above mechanisms were correct. On the other hand, uptake inhibition should enhance the action of 5-HT on receptors at 5-HT synapses by preventing its inactivation by neuronal uptake. Several actions of 5-HTP thought to be mediated by 5-HT synapses are enhanced by uptake inhibition. For instance, pretreatment of rats with fluoxetine, a 5-HT uptake inhibitor, potentiates the suppression of food intake in rats (Goudie et al 1976), the elevation of serum corticosterone (Fuller et al 1975a) and prolactin (Krulich 1975) in rats, the suppression of REM sleep in cats and rats (Slater et al 1978), and the lowering of blood pressure in hypertensive rats (Fuller et al 1979) by 5-HTP.

The experiment described in this communication was done to see if fluoxetine pretreatment would alter the ability of 5-HTP to increase the concentration of the dopamine metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), in rat brain. Male Wistar rats (130–150 g) from Harlan Industries, Cumberland, Indiana, were

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