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Inhibition of monoamine oxidase in 5-hydroxytryptaminergic neurones by substituted *p*-aminophenylalkylamines

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1 A series of substituted *p*-aminophenethylamines and some related compounds were examined with regards to the inhibition of monoamine oxidase (MAO) *in vivo* inside and outside 5-hydroxytryptaminergic neurones in the rat hypothalamus. This was recorded as the protection against the irreversible inhibition of MAO produced by phenelzine by determining the remaining deaminating activity in the absence and presence of citalopram using a low (0.1 μM) concentration of [^{14}C]-5-hydroxytryptamine (5-HT) as substrate.

2 Some of the phenethylamines were much more potent inside than outside the 5-hydroxytryptaminergic neurones. This neuronal selectivity was antagonized by pretreatment of the rats with norzimeldine, a 5-HT uptake inhibitor, which indicates that these compounds are accumulated in the 5-HT nerve terminals by the 5-HT pump.

3 Selectivity was obtained for compounds with dimethyl, monomethyl or unsubstituted *p*-amino groups. An isopropyl group appears to substitute for the dimethylamino group but with considerably lower potency. Compounds with 2-substitution showed selectivity for aminergic neurones and this effect decreased with increased size of the substituent. The 2,6-dichloro derivative FLA 365 had, however, no neuronal selective action but was a potent MAO inhibitor. Substitutions in the 3- and 5-positions decreased both potency and selectivity.

4 Prolongation of the side chain with one methylene group abolished the preference for the MAO in 5-hydroxytryptaminergic neurones although the MAO inhibitory potency remained. The selectivity disappeared by increasing the α-substituent to an ethyl group but remained for the α,α-dimethyl substituted derivatives.

5 It is concluded that compounds which are (1) transported by the 5-HT pump and (2) potent reversible MAO-A inhibitors produce pronounced inhibition of MAO in 5-hydroxytryptaminergic neurones.

Introduction

The development of specific inhibitors of the transport mechanisms responsible for the re-uptake of released transmitter amines in the monoaminergic nerve terminals, has made it possible to measure the deamination of radiolabelled biogenic amines inside specific aminergic synaptosomes when a low concentration of the substrate is added to a synaptosomal preparation (Ross & Ask, 1980; Ask *et al.*, 1982c; 1983; 1984). Thus, the difference between the deamination of the added substrate in the absence and presence of the uptake inhibitor is a measure of the deamination of the substrate after being transported into these synaptosomes. This technique has also made it possible to examine whether irreversible monoamine oxidase (MAO) inhibitors have any preference for the enzyme inside the specific aminergic nerve terminals compared with that outside these terminals (Fagervall & Ross, unpublished observations). Since, reversible MAO inhibitors protect the enzyme against irreversible inhibitors, it is also possible to determine the *in vivo* affinity of the former for MAO inside and outside the aminergic neurones if the enzyme assay is performed with a synaptosomal preparation, in the absence and presence of selective inhibitors of amine uptake, at a time when the reversible effect has terminated (Ross & Ask, 1980; Ask *et al.*, 1982a; 1983; 1984).

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In previous studies we have observed that some reversible MAO-A inhibitors protect MAO within the aminergic neurones in the rat brain against phenelzine at lower doses than required to protect MAO outside these neurones (Ask *et al.*, 1983; 1984). Of particular interest were the findings that amiflamine and N-desmethylamiflamine ((+)-FLA 788) showed significant preference for MAO inside 5-hydroxytryptaminergic and noradrenergic nerve terminals in the rat hypothalamus whereas N,N-didesmethylamiflamine ((+)-FLA 668) was a very selective inhibitor of MAO in noradrenergic nerve terminals (Ask *et al.*, 1983; 1984). Since pretreatment of the rats with norzimeldine or desipramine which are selective inhibitors of 5-hydroxytryptamine (5-HT) and noradrenaline uptake respectively, antagonized the preference for MAO of these monoaminergic nerves, the neuronal selectivity appears to be due to accumulation of the inhibitors within the aminergic nerve terminals via the membranous uptake mechanisms. These findings indicate that it may be possible to develop MAO inhibitors with a specific action within a certain amine system. Hence, a compound has to fulfil two requirements in order to be a neuronally selective MAO inhibitor in a specific amine system: it has to be (1) a potent MAO-A inhibitor and (2) transported by the amine pump. In the present study we have re-examined a series of previously published compounds structurally related to amiflamine (Florvall *et al.*, 1978; Ask *et al.*, 1982b) with regard to their inhibitory action on MAO in 5-hydroxytrypaminergic nerve terminals in the rat hypothalamus *in vivo*. In a separate study the corresponding effects on MAO in catecholaminergic nerve terminals are described (Ask *et al.*, 1985).

Methods

Male Sprague-Dawley rats weighing 160–200 g were used. They were housed in groups of four or five and were freely supplied with food and water. Since the test compounds were administered orally the rats were deprived of food the night before the administration. Phenelzine sulphate, 4 mg kg^{-1} s.c., was injected one hour after the administration of the test compound; the rats were then again allowed food. They were killed by decapitation 48 h after the phenelzine injection, the hypothalami were dissected out and homogenized in 20 volumes of ice-cold 0.25 M sucrose in small all-glass homogenizers. The homogenates were centrifuged at 800 *g* for 10 min and the supernatants used in the assay. Each compound was examined in four or more doses using five (in some experiments four) rats at each dose.

The inhibition of rat brain mitochondrial MAO *in vitro* was determined as described by Ask *et al.* (1982b) with [^{14}C]-5-HT (50 μM) and [^{14}C]-phenethylamine (PEA) (2.5 μM) as substrates.

The deamination of [^{14}C]-5-HT by the synaptosomal preparation was determined as described previously (Ask *et al.*, 1983) with some modifications. Fifty microlitres of the synaptosomal preparation, corresponding to 2.5 mg wet weight of tissue, was added to glass-stoppered centrifuge tubes containing 925 μl of Krebs-Henseleits buffer, pH 7.4, equilibrated with 93.5% O_2 and 6.5% CO_2, and containing glucose 5.6 mM, ascorbic acid 1.1 mM, Na_2 EDTA 0.13 mM, maprotiline 1 μM and amfonelic acid 0.3 μM. Each preparation was examined in duplicate in the absence and presence of citalopram 0.12 μM. After 10 min incubation in a water bath at 37°C, 25 μl of [^{14}C]-5-HT (0.1 μM final concentration) was added and the incubation was continued for 10 min. The reaction was stopped by addition of 1 ml of 1 M HCl. The [^{14}C]-5-hydroxyindoleacetic acid (5-HIAA) formed was extracted into 6 ml of ethyl acetate by vigorous shaking for 2 × 1 min in a multi-tube vortexer (model 2601, Scientific Manufacturing Industries). After centrifugation 4 ml of the organic layer were taken into 10 ml Econofluor (NEN) and 1 ml ethanol and the radioactivity measured in a Packard Tri Carb scintillation counter. The rate of deamination is expressed as nmol [^{14}C]-5-HIAA formed per g wet weight of tissue per 10 min incubation. Under the conditions used the deamination was linear for at least 10 min.

The deamination of [^{14}C]-5-HT in the presence of citalopram was taken as that occurring outside 5-HT synaptosomes. The difference between the values obtained in the absence and presence of citalopram was taken as a measure of the MAO activity occurring within 5-HT synaptosomes. Citalopram itself had no MAO inhibitory effect at the concentration used (cf. Hyttel, 1982).

The *in vivo* inhibition of MAO by the test compound was calculated from the phenelzine protection experiments as described by Green & ElHait (1980) according to the formula:

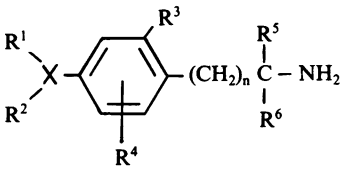
$$\left(1 - \frac{\ln \frac{100}{t}}{\ln \frac{100}{p}}\right) \times 100$$

in which t is the mean deaminating activity in the synaptosomal preparations from rats treated with the test compound + phenelzine, expressed as % of the control activity, and p is the corresponding activity in synaptosomes from the phenelzine-treated rats.

The difference between the inhibition inside and outside the 5-hydroxytryptaminergic neurones for each dose was determined using the Mann-Whitney U-test. Neuronal selectivity was regarded to occur if at

Table 1 Chemical structures of the compounds examined in the present study

Compound



		n	*X*	R^1	R^2	R^3	R^4	R^5	R^6
FLA 299		1	CH	CH_3	CH_3	H	H	CH_3	H
FLA 289		1	N	CH_3	CH_3	H	H	CH_3	H
FLA 558		1	N	CH_3	CH_3	F	H	CH_3	H
FLA 314		1	N	CH_3	CH_3	Cl	H	CH_3	H
FLA 405		1	N	CH_3	CH_3	Br	H	CH_3	H
(±)-FLA 336									
(+)-FLA 336	a	1	N	CH_3	CH_3	CH_3	H	CH_3	H
(−)-FLA 336									
FLA 365		1	N	CH_3	CH_3	Cl	6-Cl	CH_3	H
FLA 417		2	N	CH_3	CH_3	Cl	H	CH_3	H
FLA 450		1	N	CH_3	CH_3	Cl	H	C_2H_5	H
FLA 463		1	N	CH_3	CH_3	Cl	H	CH_3	CH_3
FLA 717		1	N	CH_3	CH_3	CH_3	H	CH_3	CH_3
FLA 384		1	N	CH_3	CH_3	H	3-CH_3	CH_3	H
(+)-NBF 003		1	N	CH_3	CH_3	CH_3	5-Br	CH_3	H
FLA 727		1	N	CH_3	H	H	H	CH_3	H
(+)-FLA 788		1	N	CH_3	H	CH_3	H	CH_3	H
(+)-NBF 008		1	N	CH_3	H	CH_3	5-Br	CH_3	H
RAN 113		1	N	CH_3	H	CH_3	3-CH_3	CH_3	H
FLA 334		1	N	H	H	H	H	CH_3	H
(±)-FLA 668									
(+)-FLA 668		1	N	H	H	CH_3	H	CH_3	H
(−)-FLA 668									
(+)-NBF 021		1	N	H	H	CH_3	5-Br	CH_3	H

a = Amiflamine.

least three of the doses showed a significantly larger inhibition inside than outside the neurones.

Compounds

The chemical structures of the compounds studied are given in Table 1. The absolute configurations of amiflamine (+)-FLA 336, (+)-FLA 788, (+-FLA 668, NBF 003, NBF 008 and NBF 021 are the S-forms (Hjertén *et al.*, 1983). All FLA and NBF compounds were synthesized by L. Florvall and M.-L. Persson and RAN 113 by R. Sandberg, at Astra Läkemedel, Södertälje, Sweden, where norzimeldine dihydrochloride was also synthesized. Maprotiline hydrochloride was a gift from Ciba-Geigy AG, Basel, Switzerland and citalopram hydrobromide from Lundbeck and Co A/S, Copenhagen, Denmark. Phenelzine sulphate was donated by Warner Lambert, Morris Plains, N.J. U.S.A. Amfonelic acid was bought from Research Biochemical Incorporated, Wayland, Mass., U.S.A. and 5-hydroxytryptamine [sidechain-2-^{14}C]-creatinine sulphate (specific activity 61 mCi mmol^{-1}) from Amersham International plc, England.

Results

Monoamine oxidase inhibition in vitro

The compounds were tested for their MAO inhibitory potencies *in vitro* using a rat brain mitochondrial preparation and [^{14}C]-5-HT (50 μM) or [^{14}C]-phenethylamine (2.5 μM) as substrates. The results for some of the compounds have been published previously but are included in Table 2 in order to facilitate a comparison with the *in vivo* results. All compounds were selective MAO-A inhibitors but with varying degrees of selectivity. Least selective and potent compound was 4-isopropylamphetamine (FLA 299).

The (−)-enantiomer of amiflamine ((−)-FLA

Table 2 Inhibition of rat brain monoamine oxidase (MAO)-A (50 μM 5-hydroxytryptamine (5-HT)) and MAO-B (2.5 μM phenethylamine (PEA)) *in vitro* and of MAO inside and outside 5-hydroxytryptaminergic nerve terminals in the rat hypothalamus *in vivo*

Compound	*MAO inhibition* In vitro IC_{50} (μM) 5-HT	PEA	In vivo ED_{50} (μmol kg^{-1} p.o.) Intra neuronal	Extra neuronal
FLA 299	12.5	240	33	380*
FLA 289	3.7	400	2.5	30
FLA 558	1.2[a]	120	0.45	3.0
FLA 314	0.21[a]	80	2.2	40*
FLA 405	0.22[a]	100	3.4	12
FLA 336	2.7[a]	440	2.3	15
Amiflamine	0.8[a]	>1000	1.1	5.2
(−)-FLA 336	3.0[a]	125	8.5	100*
FLA 365	0.013[a]	180	3.5	4.8
FLA 417	0.045[a]	21	8.5	7.8
FLA 450	0.38[a]	75	14	20
FLA 463	1.2[a]	700	8.5	24
FLA 717	12	2100	8.0	100
FLA 384	8.0	650	7.5	300*
(+)-NBF 003	1.1	480	15	36
FLA 727	0.55	1500	0.7	11
(+)-FLA 788	0.13[b]	>1000	0.8	5.0
(+)-NBF 008	0.09	320	7.6	12
RAN 113	1.1	5400	8.0	40
FLA 334	7.2	1600	50	>144 (5%)
FLA 668	1.6	2500	15	>78 (13%)
(+)-FLA 668	1.5	>1000	>51 (29%)	>51 (13%)
(−)-FLA 668	7.5	1300	27	>>125 (0%)
(+)-NBF 021	0.8	190	28	45

The inhibition was determined using the phenelzine-synaptosomal technique as described in Methods. [a]Values taken from Ask *et al.* (1982b). [b]Values taken from Ask *et al.* (1982c). *Extrapolated value.

336) was much less selective than the (+)-enantiomer (amiflamine), mainly due to higher potency in inhibiting the B-form. This observation is in accordance with previous findings (Fowler & Oreland, 1981; Ask *et al.*, 1982c). Similar steric differences were observed for the enantiomers of FLA 668. The most selective inhibitor was 4-dimethylamino-2,6-dichloro-α-methylphenethylamine (FLA 365) mainly due to its high A-inhibitory potency. Next to this compound in potency was 4-dimethylamino-2-chloro-α-methylphenylpropylamine (FLA 417). Among the other compounds N-desmethylamiflamine ((+)-FLA 788) was 5 times more potent than amiflamine, which was two times more potent than N,N-didesmethylamiflamine ((+)-FLA 668). The 2-bromo (FLA 405) and 2-chloro (FLA 314) derivatives were more potent than the 2-fluoro (FLA 558), 2-methyl (FLA 336) and the unsubstituted (FLA 289) compounds. A bromo substitution in the ortho position to the *p*-amino group (NBF 003, NBF 008, NBF 021) did not change the potency or the selectivity. The α-ethyl substituted derivative (FLA 450) was almost as potent as the α-methyl compound (FLA 314), whereas the α,α-dimethyl derivatives (FLA 463 and FLA 717) were 4 to 6 times less active than their corresponding α-methyl compounds (FLA 314 and FLA 336).

Inhibition of the synaptosomal deamination of [^{14}C]-5-hydroxytryptamine by citalopram

Citalopram is a very selective 5-HT uptake inhibitor (Hyttel, 1982) and is therefore suitable for determining how much of the deamination of [^{14}C]-5-HT (0.1 μM) occurs inside and outside the 5-HT synaptosomes. It was found that about 50% of the deamination occurred in the 5-HT synaptosomes and the inhibition of 5-HT uptake was obtained in the same dose range as the inhibition of the deamination. At the concentration chosen (0.12 μM) citalopram had no effect on the deamination of [^{14}C]-noradrenaline and [^{14}C]-dopamine by hypothalamic and striatal synaptosomes, respectively. Maprotiline (1 μM) or amfonelic acid (0.3 μM) did not affect the deamination of [^{14}C]-5-HT.

Protection against phenelzine in vivo

The *in vivo* affinity of the compounds for MAO-A was evaluated using the phenelzine technique; i.e. the reversible MAO inhibitor protects the enzyme from irreversible inhibition by phenelzine. The assay of the deaminating activity of crude synaptosomal preparations of the hypothalamus was performed with a low concentration of [^{14}C]-5-HT (0.1 μM) in the absence and presence of citalopram. With this technique it is possible to measure the protection against phenelzine both inside and outside the 5-hydroxytryptaminergic neurones (Ask *et al.*, 1983; 1984).

Most of the compounds examined showed sig-

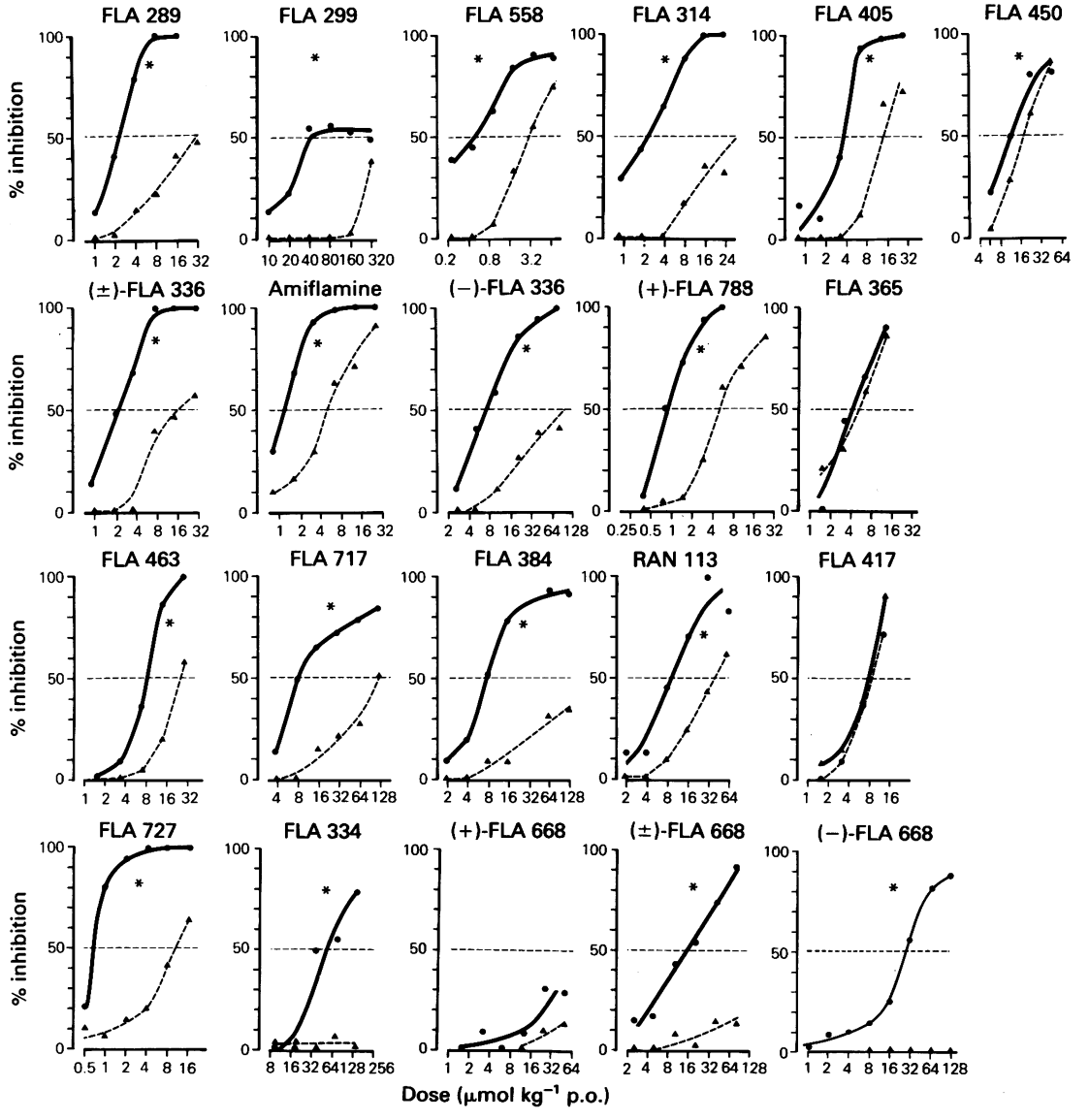


Figure 1 Inhibition of monoamine oxidase (MAO) within (continuous lines) and outside (broken lines) 5-hydroxytryptaminergic nerve terminals in the rat hypothalamus *in vivo*. The inhibition was determined from phenelzine protection experiments as described in Methods. Each value is the mean from groups of 4 or 5 rats. The asterisks denote a significant ($P < 0.05$, Mann-Whitney U-test) difference between neuronal and extraneuronal inhibition for at least three dose levels.

nificantly larger inhibition of MAO inside than outside the 5-HT nerve terminals (Figure 1, Table 2). The following compounds had particularly high selectivity: FLA 334, (−)-FLA 668, FLA 384, FLA 314, FLA 727, FLA 289, FLA 717, FLA 558, amiflamine and N-desmethylamiflamine ((+)-FLA 788) in the order mentioned. The latter three compounds had the highest potency in protecting MAO in the 5-hydroxytryptaminergic neurones in the hypothalamus. High neuronal selectivity was observed both for compounds with high (e.g. FLA 289, FLA 558 and FLA 314) and low (e.g. FLA 717, FLA 384 and FLA 668) *in vivo* potency. Although bromo substitution in the *ortho* position to the *para* amino group (NBF 003, NBF 008, NBF 021) did not change the *in vitro* potency, it reduced the *in vivo* potency and the neuronal selectivity considerable (Table 1). The *in vitro* inhibitory potency on MAO-A with 5-HT as substrate was significantly correlated to the intra-5-hydroxytryptaminergic neurone inhibitory activity *in vivo* in the Spearman rank test ($r_s = 0.41$, $P < 0.05$, $n = 24$).

Interestingly the two most potent MAO inhibitors FLA 365 and FLA 417 had no neuronal selectivity at all. This lack of selectivity did not seem to be dependent on the high *in vitro* potency, since FLA 450 which was ten times less potent *in vitro* was also almost without any neuronal selectivity. The stereospecificity observed for the enantiomers of FLA 336 *in vitro* was also shown *in vivo* (Figure 1). Thus the (+)-enantiomer (amiflamine) was about two times more potent than FLA 336 whereas (−)-FLA 336 had about one tenth of amiflamine's potency. The difference between the enantiomers was twice as large as that found *in vitro*.

FLA 668 showed an even larger stereospecificity than FLA 336 in the *in vivo* test (Table 2). Although considerably less potent than FLA 336, (−)-FLA 668 protected MAO almost completely within the 5-HT nerve terminals at doses which had no effect outside these neurones. The (+)-enantiomer (N,N-didesmethylamiflamine) had, on the other hand, a very slight protective effect for MAO against phenelzine both inside and outside 5-hydroxytryptaminergic neurones.

It was shown previously that the selectivity of amiflamine and N-desmethylamiflamine in protecting MAO within 5-HT nerve terminals was antagonized by pretreatment of the animals with norzimeldine, a selective 5-HT uptake inhibitor (Ask *et al.*, 1982a; 1983; 1984). As shown in Table 3 the neuronal selectivities of FLA 289, FLA 314, FLA 384, FLA 558, FLA 668 and FLA 727 at doses producing 66 to 94% inhibition of MAO in 5-HT nerve terminals in the saline pretreated rats were antagonized by norzimeldine.

Discussion

The results obtained in this study support and extend the conclusions from previous investigations, that it is possible to develop MAO inhibitors with strong effects inside 5-hydroxytryptaminergic neurones in the rat brain at doses which have no or slight effects in non-aminergic neurones or cells (Ask *et al.*, 1982a; 1983; 1984). This preference for the MAO in 5-hydroxytryptaminergic neurones is obviously due to uptake and accumulation of the inhibitors in these neurones,

Table 3 Antagonism by norzimeldine of the prefered inhibition of monoamine oxidase (MAO) in 5-hydroxytryptaminergic nerve terminals in the rat hypothalamus

Compound	*Dose* (μmol kg^{-1} p.o.)	*MAO inhibition* (%) *Saline* (S)		*Norzimeldine* (NZ)	
		N	*EN*	*N*	*EN*
FLA 289	4	75 ± 3	11 ± 4**	32 ± 3††	26 ± 3
FLA 314	3.5	75 ± 2	24 ± 5**	43 ± 15†	37 ± 14
FLA 384	15	73 ± 4	26 ± 5**	36 ± 4††	26 ± 2*
FLA 558	2	66 ± 6	10 ± 5**	53 ± 3†	29 ± 2**
FLA 727	4	94 ± 7	19 ± 5**	41 ± 5††	24 ± 2**
FLA 668	34	66 ± 3	8 ± 2**	36 ± 8††	18 ± 4*

Norzimeldine dihydrochloride, 20 mg kg^{-1} i.p., or saline was given 30 min before the oral administration of the test compound. Phenelzine sulphate, 4 mg kg^{-1} s.c., was injected 1 h after the test compound. The rats were killed 48 h later and the MAO inhibition within (N) and outside (EN) 5-hydroxytryptaminergic neurones was determined from the protection against phenelzine as described in Methods. Each value is the mean ± s.e.mean from five rats.
* $P < 0.05$, ** $P < 0.01$; EN vs N (Mann-Whitney U-test).
† $P < 0.05$, †† $P < 0.01$; N_{NZ} vs N_S (Mann-Whitney U-test).

since the selective 5-HT uptake inhibitor norzimeldine antagonized this preference. Hence the neuronal selectivity indicates that the compound is transported by the membranous 5-HT pump. In the series of compounds examined in the present study the structure-activity relationship obtained for the neuronal selectivity may therefore reflect that for transport by the 5-HT pump. It can be argued that the high MAO inhibitory potencies of FLA 365 and FLA 417 may hide any neuronal selectivity, since the affinity for MAO is probably much higher than that for the 5-HT pump. Diffusion of the compounds may then give sufficient amounts to protect MAO against phenelzine in all cells at doses lower than those in which the compounds are accumulated into the 5-HT nerve terminals. However, the fact that (+)-FLA 788, which has only one tenth of the *in vitro* potency of FLA 365, protected MAO in 5-hydroxytryptaminergic neurones at lower doses than did FLA 365 argues against such an explanation. Hence, the most plausible explanation of the lack of neuronal selectivity for FLA 365 and FLA 417 is that these compounds are not transported by the 5-HT pump. This means that the two chlorine atoms in *ortho* positions (FLA 365) and the prolongation of the side chain with one methylene group (FLA 417) are not favourable for transport by this pump.

The dimethylamino phenethylamine derivatives studied are rapidly N-demethylated in rats *in vivo*, as indicated by the results obtained with amiflamine (Ask *et al.*, 1982c). The desmethylated compounds available for test ((+)-FLA 788, FLA 727 and NBF 008) were considerably more potent MAO inhibitors in the *in vitro* assay than the corresponding tertiary amines but were only slightly more potent in 5-hydroxytryptaminergic neurones *in vivo*. Therefore, it is likely that the desmethylated compounds are mainly responsible for the *in vivo* effects. Since the rate of desmethylation may be affected by the substituents in the molecules, the structure-activity relationships obtained for the neuronal selectivity carry some uncertainty. However, the structure-activity relationship for the neuronal selectivity listed below probably also reflects transport into these terminals. (1) The non-substituted *p*-aminoamphetamine derivatives FLA 289, FLA 727 and FLA 334 had a pronounced selective action. (2) Compounds substituted at 2-position with F, Cl, Br or CH_3 were selective with decreasing effect upon increasing size of the substituent. (3) The 2,6-disubstituted chlorine derivative FLA 365 had no selective action. (4) Compounds substituted with a methyl group at 3-position (FLA 384) or 5-position (RAN 113) had selective actions. The 5-bromo derivatives NBF 003, NBF 008 and NBF 021 had considerably lower *in vivo* potency and very slight neuronal selectivity but similar *in vitro* potency compared to the parent compounds, amiflamine, (+)-FLA 788 and (+)-FLA 668, which indicates reduced transport capability. (5) Changing the α-substituent to an ethyl group (FLA 450) almost abolished the selectivity. The α,α-dimethyl derivatives (FLA 463 and FLA 717) had, on the other hand, selective action. (6) Prolongation of the side chain with one methylene group (FLA 417) abolished the selective inhibition of the MAO within aminergic neurones. (7) The difference observed between the enantiomers of FLA 668 indicates a steric dependency probably in the transport capacity. Thus, the R-(−)-enantiomer, which had lower MAO inhibitory potency *in vitro*, seems to be more efficiently transported than the S-(+)-enantiomer. The difference observed between amiflamine and (−)-FLA 336 may, on the other hand, be explained by their different *in vitro* potencies in inhibiting MAO-A. (8) Since the *p*-isopropyl group in FLA 299 is metabolically more stable than the structurally similar *p*-dimethylamino group the partial but selective protection of the MAO within 5-hydroxytryptaminergic neurones, with FLA 299 indicates that the N,N-dimethylamino derivatives themselves are transported by the 5-HT pump.

The very large selectivity for neuronal MAO observed with FLA 334 and (−)-FLA 668 indicates that it might be possible to develop MAO inhibitors almost specific for a certain aminergic neuronal system. However, the compounds mentioned are not suitable as such, since they have an even higher affinity for noradrenergic and dopaminergic neurones (Ask *et al.*, 1985). Since the amine pumps have different structural requirements for uptake it appears possible, with appropriate substitutions, to develop reversible MAO inhibitors which are transported by only one amine pump and thereby exert a specific action in this neuronal system. None of the present compounds, which were not synthesized with particular attention to neuronal selectivity, achieved this aim (Ask *et al.*, 1985). However, (+)-FLA 668 is quite selective for noradrenergic neurones, being more than 10 times less potent in 5-hydroxytryptaminergic neurones and 5 times less active in dopaminergic neurones.

In order to develop neurone-specific MAO inhibitors information about the affinities for the amine pump and for MAO-A of the compounds is essential. From the results of the present study it appears that the highest neuronal selectivity is obtained for compounds with moderate or even low affinity for the enzyme. This might mean that the affinity for the amine pump should be higher than for MAO-A. Studies are now in progress in which the affinities of the compounds for the 5-HT uptake mechanism and those for MAO-A are measured in the same synaptosomal preparation *in vitro*.

The ultimate goal for the development of neurone-specific MAO inhibitors is the clinical uses of such compounds. It is hoped that this specificity might decrease side-effects. However, it is not usually possi-

ble beforehand to know with certainty which amine system should be affected to obtain a specific therapeutic effect. Hence, specific MAO inhibitors of each aminergic neuronal system are of great theoretical interest. Such compounds might also be valuable as tools for examining the pharmacological importance of intraneuronal MAO (cf Fowler *et al.*, 1984).

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