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EFFECTS OF ACUTE AND REPEATED ADMINISTRATION OF AMIFLAMINE ON MONOAMINE OXIDASE INHIBITION IN THE RAT

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Abstract—The inhibitory effect on monoamine oxidase (MAO) of the reversible MAO-A inhibitor (+)-4-dimethylamino-2,α-dimethylphenethylamine [amiflamine, FLA 336(+)] was evaluated in the rat after acute and repeated (twice daily for two weeks) oral treatment. MAO activity was measured *ex vivo* in slices from the hypothalamus and the duodenum for both MAO-A and MAO-B. Amiflamine selectively inhibited the A form of MAO after repeated as well as after acute treatment ($ED_{50} \approx 7$ μmoles/kg both acute and repeated). In the brain slices this inhibition corresponded to a decrease in the concentration of 5-hydroxyindoleacetic acid (5-HIAA) and to an increase in the concentration of 5-HT in the hypothalamus, the hippocampus and the striatum. The concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) was decreased in the striatum to the same extent as the decrease in the 5-HIAA concentrations. The effect on the homovanillic acid (HVA) concentration was somewhat weaker as was the increase in the concentration of dopamine. No essential difference was found after acute and chronic treatment on the amine and metabolite levels. The MAO activity returned to normal 24 hours after final dosing. A large difference between the neuronal and the extraneuronal protection against the phenelzine-induced irreversible MAO inhibition in the hypothalamus was found after both acute and repeated treatment. The ED_{50} of the protection within the serotonergic neurons was 1.3 μmoles/kg p.o. (acute) and 0.75 μmoles/kg p.o. (repeated). Amiflamine was 3 times less potent within noradrenergic neurons than within serotonergic neurons. A brain to plasma ratio of about 20:1 was found for amiflamine and its metabolites. The plasma and the brain concentrations of the *N*-demethylated metabolite [FLA 788(+)] exceeded that of amiflamine after a single dose, whereas the *N*,*N*-demethylated metabolite [FLA 668(+)] was found in low concentrations. The effect on MAO-A correlated significantly with the plasma and the brain concentration of FLA 788(+).

Monoamine oxidase [MAO, amine: oxygen oxidoreductase (deaminating) (flavine containing) EC 1.4.3.4] exists in two forms. The type of MAO which is mainly responsible for the deamination of the neurotransmitter amines 5-hydroxytryptamine (5-HT, serotonin) and noradrenaline (NA) is selectively inhibited by clorgyline and is referred to as MAO-A [1, 2]. The other enzyme type (MAO-B) shows greatest affinity for benzylamine and phenethylamine (PEA) and is selectively inhibited by *l*-deprenyl [3]. Tyramine is a substrate for both forms of the enzyme in the rat brain and liver [1] while being mainly deaminated by MAO-A in the rat intestine [4]. The demonstration that MAO exists in multiple forms led to the possibility of developing selective inhibitors which may inhibit the deamination of the transmitter amines 5-HT and NA without markedly affecting the deamination of tyramine [5].

Both clorgyline and *l*-deprenyl inactivate MAO irreversibly by a suicide mechanism [6, 7]. The irreversible action tends to decrease the selectivity of these compounds after long-term treatment [8]. However, when carefully selected low doses of irreversible MAO inhibitors are used the selectivity may partly be maintained [9]. Reversible MAO-A inhibitors, on the other hand, maintain their selectivity and ought therefore to be the drugs of choice in antidepressant therapy (for review see [10]). We have previously described that the (+)-enantiomer of 4-dimethyl-amino-2,α-dimethylphenethylamine [amiflamine, FLA 336(+)], a selective and reversible MAO-A inhibitor, is as potent as clorgyline in the rat brain after oral administration [11]. *In vitro* amiflamine was 2–6 times less potent than its *N*-demethylated metabolite [FLA 788(+)] and about 1000 times less potent than clorgyline.

In order to evaluate the selectivity upon long-term treatment, amiflamine was administered orally to rats twice daily for two weeks. The selectivity on MAO in hypothalamus and duodenum was examined with 5-HT, tyramine and PEA as the substrates, and the effect on the concentration of 5-HT and dopamine (DA) and their metabolites were determined. The relative importance of the metabolite FLA 788(+) for the MAO inhibition after amiflamine treatment is discussed.

MATERIALS AND METHODS

MAO inhibition and amine levels

Dosing schedule. Amiflamine was administered orally to male Sprague–Dawley rats (160–170 g at the beginning of the experiment) either in a single

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dose or twice daily (7 am and 8 pm) for 14 days. Water and food were supplied *ad lib.* but the animals were starved for 12 hr before the last dosing. The compounds, dissolved in deionized water, were given orally (2 ml/kg). Three doses of amiflamine (1.5, 7 and 30 μmoles/kg) were given. The animals were decapitated with a guillotine 2, 6 or 26 hr after the last administration.

Blood sampling. Blood was taken in heparinized tubes at the time of the decapitation and the plasma was stored at −20° until used for assay of amiflamine, FLA 788(+) and FLA 668(+) concentrations.

MAO inhibition—slices. The activity of MAO was measured in slices from the hypothalamus and the duodenum using as substrates ^{14}C-5-HT, ^{3}H-tyramine and ^{14}C-PEA at a final concentration of each compound of 0.1 μM. Slices, 100 mg of the duodenum for all three substrates and the hypothalamus using 5-HT and tyramine and 40 mg of the hypothalamus using PEA, were prepared. The slices were incubated at 37° in 2.0 ml Krebs–Henseleit's buffer pH 7.4, containing 5.5 mM glucose, 1.1 mM asorbic acid and 0.13 mM EDTA. The determination of the deamination of 5-HT and tyramine was performed simultaneously in the same slice preparation, since the deamination of one amine did not interfere with that of the other at the low substrate concentrations used [11].

The incubation time was 5 min with 5-HT and tyramine as substrate and 90 sec when PEA was the substrate. Under these conditions the rate of deamination was linear. The uptake of ^{14}C-5-HT and ^{3}H-tyramine in the hypothalamic slices was determined simultaneously with the deamination as described previously [12]. More than 95% of the total acid metabolites found in duodenum slices were present in the incubation medium and negligible amounts remained in the slice. The reaction was in these experiments stopped by addition of 4 ml 1 M HCl and 1.5 ml of the acidified medium was taken for extraction of the acids (and alcohols) formed during incubation into 6 ml of ethyl acetate as described previously [11].

The remainders of the brains were stored at −70° until used for assay of amiflamine, FLA 788(+) and FLA 668(+) concentrations.

Analysis of 5-HT, 5-HIAA, DA, DOPAC and HVA. The concentration of 5-HT and 5-HIAA in striatum, hippocampus and hypothalamus and of DA, DOPAC and HVA in stratum was performed by a HPLC method with electrochemical detection [13]. The rats used in this experiment were not the same as those used for MAO studies in slices but were identically dosed.

Analysis of amiflamine, FLA 788(+) and FLA 668(+). The plasma and the brain concentrations of amiflamine, FLA 788(+) and FLA 668(+) were determined by a mass fragmentographic method (Högberg *et al.*, to be published).

Protection against phenelzine-induced MAO inhibition

Dosing schedule. Amiflamine was administered in various doses once or twice daily (7 am and 5 pm) for 14 days orally to male Sprague–Dawley rats (160–170 g at the beginning of the experiment). Phenelzine, 4 mg/kg s.c., was given 1, 24 or 48 hr after the final amiflamine administration. The animals were decapitated with a guillotine 24 or 48 hr after the phenelzine dose.

MAO activity in synaptosomes. The MAO activity in synaptosomes from hypothalamus was determined intraneuronally and extraneuronally as described previously [14] using ^{14}C-5-HT (1×10^{-7}M final concentration) and ^{14}C-NA (2.5×10^{-7}M final concentration) as substrates. The results are expressed as % protection of the phenelzine induced MAO inhibition (for further methodological details, see [14]).

Compounds

S-(+)-4-dimethylamino-2,α-dimethylphenethylamine [amiflamine, FLA 336(+)] (+)-hydrogen tartrate, (+)-4-methylamino-2,α-dimethylphenethylamine [FLA 788(+)] (+)-hydrogen tartrate and (+)-4-amino-2,α-dimethylphenethylamine [FLA 668(+)] (+)-hydrogen-ditartrate were synthesized by Dr. L. Florvall (Astra Läkemedel AB, Södertälje, Sweden). Phenelzine sulphate was obtained from Warner–Lambert (Morris Plains, NJ). 5-Hydroxytryptamine (side chain 2-[^{14}C]) creatinine sulphate (specific activity 60 mCi/mmole) and l-noradrenaline [8-^{14}C] hydrogen tartrate (specific activity 57 mCi/mmole) were purchased from The Radiochemical Centre (Amersham, U.K.) and tyramine [^{3}H(G)] hydrochloride (specific activity 9.6 Ci/mmole) and β-phenethylamine [ethyl-7-[^{14}C]) hydrochloride (specific activity 48.25 mCi/mmole) from New England Nuclear (Boston, MA).

RESULTS

Concentrations of amiflamine and its metabolites in plasma and brain. The plasma concentrations of the metabolite FLA 788(+) exceeded those of amiflamine at all doses and times examined (with the exception of 2 hr after repeated treatment with the highest dose) (Table 1). Very low plasma concentrations of the primary amine metabolite FLA 668(+) were found at all doses. The ratio between the plasma concentrations of FLA 788(+) and amiflamine decreased with increasing amiflamine doses. Whereas the concentrations of the parent compound in plasma were higher after repeated treatment, those of FLA 788(+) were almost the same as after a single dose of amiflamine 2 hr after the last administration. After 24 hr only low concentrations of amiflamine and FLA 788(+) were detected even at the highest dose.

The brain concentrations of amiflamine and its metabolites were 13 to 58 times higher than the corresponding plasma concentrations (Table 2). At steady state, the brain concentrations of amiflamine were twice (highest dose) to 12 times (lowest dose) higher than those obtained after a single dose. For FLA 788(+) the corresponding ratios between repeated and acute brain concentrations were much less, being almost 1 at the two highest doses. Thus, the brain concentrations of amiflamine exceeded those of FLA 788(+) at the highest dose after acute and at the two higher doses after chronic treatment. There were highly significant correlations between

Table 1. Plasma concentrations of amiflamine, FLA 788(+) and FLA 668(+) after a single dose and after administration twice daily for 14 days of amiflamine

Dose (μmoles/kg)	Time after last administration (hr)	Plasma concentration, nmoles/ml*					
		Acute			Chronic		
		Amiflamine	FLA 788(+)	FLA 668(+)	Amiflamine	FLA 788(+)	FLA 668(+)
1.5	2	< 0.01	0.05 ± 0.02	0.01 ± 0.002	0.02 ± 0.002	0.10 ± 0.01	< 0.01
7		0.05 ± 0.004†	0.30 ± 0.01†	0.02 ± 0.001†	0.14 ± 0.02‡	0.38 ± 0.01‡	0.02 ± 0.001‡
30		0.89 ± 0.12†	1.20 ± 0.05†	0.03 ± 0.002†	1.46 ± 0.16‡	1.05 ± 0.03‡	0.03 ± 0.001‡
7	6	0.01 ± 0.001§	0.11 ± 0.01§	0.02 ± 0.001§	0.02 ± 0.004	0.16 ± 0.01	< 0.01
30		0.23 ± 0.05§	0.68 ± 0.01§	0.03 ± 0.002§	0.41 ± 0.03	0.79 ± 0.03	0.02 ± 0.001
30	24	< 0.01	0.03 ± 0.01	< 0.01	0.04 ± 0.01	0.13 ± 0.01	0.01 ± 0.001

* Each value is the mean ± S.E.M. from 10 rats except for † 25 rats, ‡ 20 rats, § 15 rats.

Table 2. Brain concentrations of amiflamine, FLA 788(+) and FLA 668(+) after a single dose and after administration twice daily for 14 days of amiflamine

Dose (μmoles/kg)	Time after last administration (hr)	Brain concentration, nmoles/g*					
		Acute			Chronic		
		Amiflamine	FLA 788(+)	FLA 668(+)	Amiflamine	FLA 788(+)	FLA 668(+)
1.5	2	0.05 ± 0.01	0.8 ± 0.04	0.1 ± 0.01	0.6 ± 0.1	2.7 ± 0.3	0.2 ± 0.02
7		2.8 ± 0.2†	5.1 ± 0.5†	0.4 ± 0.02†	8.1 ± 0.9†	6.6 ± 0.8†	0.3 ± 0.01†
30		25.7 ± 3.5†	16.2 ± 2.0†	1.2 ± 0.2†	59.9 ± 2.8†	15.3 ± 1.1†	0.9 ± 0.1†
7	6	0.7 ± 0.03	2.0 ± 0.04	0.02 ± 0.02	nd	nd	nd
30		5.6 ± 0.3	7.5 ± 0.1	0.6 ± 0.1	nd	nd	nd
30	24	< 0.07	0.3 ± 0.04	< 0.07	< 0.07	1.9 ± 0.3	0.1 ± 0.01

* Each value is the mean ± S.E.M. from 5 rats except for † 10 rats.
nd, Not determined.

Table 3. Inhibition of deamination of ^{14}C-5-HT, ^{3}H-tyramine and ^{14}C-PEA in slices from rat hypothalamus using 1×10^{-7}M of the amines (amiflamine was given orally in a single dose or twice daily for 2 weeks)

Dose (μmoles/kg p.o.)	Time after last administration (hr)	Percentage inhibition of deamination*					
		Acute			Chronic		
		^{14}C-5-HT	^{3}H-tyramine	^{14}C-PEA	^{14}C-5-HT	^{3}H-tyramine	^{14}C-PEA
1.5	2	19 ± 4‡	10 ± 5	nd	13 ± 2†	4 ± 8	nd
7		49 ± 3†‡	29 ± 3†‡	nd	39 ± 2†‡§	37 ± 4†‡§	
30		72 ± 2†‡	46 ± 2†‡	5 ± 5	67 ± 1†‡§	40 ± 1†‡§	10 ± 5
7	6	24 ± 2‡	18 ± 2‡	nd	19 ± 3‡	13 ± 3‡	nd
30		60 ± 2‡	39 ± 2‡	nd	55 ± 2‡	30 ± 4‡	nd
30	24	− 3 ± 5	0 ± 4	11 ± 8	2 ± 3	− 3 ± 4	− 18 ± 9‡

* Each group is the mean ± S.E.M. of 5 animals except for † 10 animals.
‡ $P < 0.05$ compared with controls, § $P < 0.05$ compared with acute treatment (Student's *t*-test).
nd, Not determined.

Table 4. Inhibition of deamination of ^{14}C-5-HT, ^{3}H-tyramine and ^{14}C-PEA in slices from rat duodenum using 1×10^{-7}M of the amines (amiflamine was given orally in a single dose or twice daily for 2 weeks)

Dose (μmoles/kg p.o.)	Time after last administration (hr)	Percentage inhibition of deamination*					
		Acute			Chronic		
		^{14}C-5-HT	^{3}H-tyramine	^{14}C-PEA	^{14}C-5-HT	^{3}H-tyramine	^{14}C-PEA
1.5	2	8 ± 10	− 5 ± 11	nd	9 ± 7	6 ± 9	nd
7		51 ± 2†‡	39 ± 3†‡	nd	36 ± 4†§	34 ± 9†‡	nd
30		77 ± 2†‡	54 ± 3†‡	− 11 ± 4	64 ± 3†‡§	41 ± 3†‡§	1 ± 12
7	6	26 ± 2‡	15 ± 8	nd	15 ± 8	8 ± 8	nd
30		69 ± 3‡	52 ± 3‡	nd	64 ± 1‡	46 ± 2‡	nd
30	24	3 ± 8	1 ± 6	− 17 ± 22	− 33 ± 17§	− 56 ± 16‡§	11 ± 9

* Each group is the mean ± S.E.M. of 5 animals except for † 10 animals.
‡ $P < 0.05$ compared with controls, § $P < 0.05$ compared with acute treatment (Student's *t*-test).
nd, Not determined.

the plasma and the brain concentrations of amiflamine and of FLA 788(+) formed ($r = 0.98$ and 0.99 respectively, $P < 0.001$).

MAO inhibition in slices. The decrease in the deaminating activities in hypothalamic and duodenal slices from rats treated with amiflamine using low (0.1 μM) concentrations of 5-HT, tyramine and PEA as substrates was determined. Tables 3 and 4 show that amiflamine selectively inhibited the A form of MAO since the deamination of PEA was not affected in either acute or repeated experiments. In both hypothalamus and duodenum the inhibition of the deamination of 5-HT and tyramine increased with increasing dose. The inhibition was stronger at 2 hr than at 6 hr after administration and returned to normal after 24 hr. Inhibition after the chronic administration tended to be somewhat lower than that after a single dose. Quite interestingly, the deaminating activities in the duodenal slices tended to be higher compared with the controls 24 hr after the last administration. This increase was significant for the tyramine deamination.

Correlations between plasma or brain concentrations and MAO inhibition. The inhibition of the 5-HT deamination in the brain slices showed strong positive correlations with the concentrations of FLA 788(+) in the plasma (Fig. 1A) and in the brain (Fig. 1B) after administration of amiflamine. Although similar correlation coefficients were obtained for acute and repeated treatments, the slopes of the regression lines seem to differ. Thus, repeated amiflamine treatment tended to result in reduced MAO inhibition.

Protection against phenelzine-induced MAO inhibition. Reversible MAO inhibitors protect the enzyme against irreversibly acting MAO inhibitors by competing for the active site of the enzyme [15–17]. Recently we reported that amiflamine preferably protected MAO-A against phenelzine-induced inhibi-

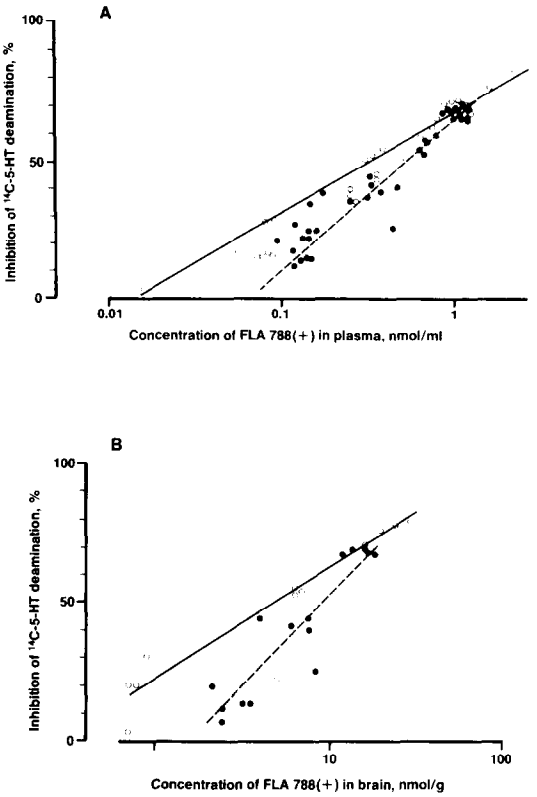

Fig. 1. The relationship between the inhibition of the deamination of ^{14}C-5-HT by hypothalamic slices and the plasma (A) and brain (B) concentrations of FLA 788(+) after acute (○,-) (A) $r = 0.94$, $P < 0.001$, (B) $r = 0.88$, $P < 0.001$; and chronic (●,---) (A) $r = 0.93$, $P < 0.001$, (B) $r = 0.94$, $P < 0.001$ administration of amiflamine to rats. The rats were sacrificed 2 and 6 hr after the last administration of amiflamine, which was given twice daily at the doses 1.5, 7 and 30 μmoles/kg p.o.

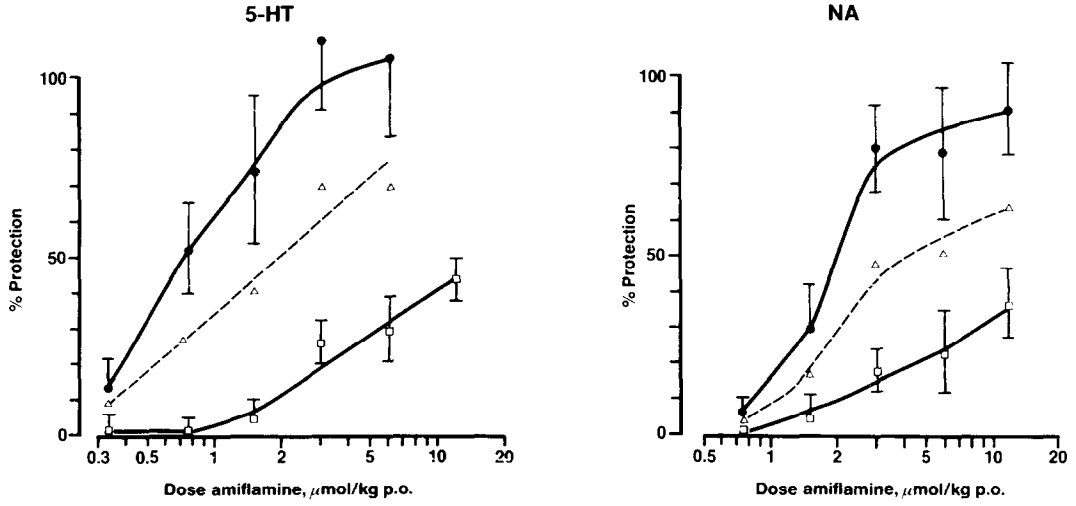

Fig. 2. Protection by amiflamine against the irreversible MAO inhibition produced by phenelzine in rats. Various doses of amiflamine were administered twice daily for 14 days and phenelzine sulphate, 4 mg/kg s.c., was injected 1 hr after the last dose. The rats were sacrificed 48 hr later and the deamination of ^{14}C-5-HT (1×10^{-7}M) and ^{14}C-NA (2.5×10^{-7}M) in crude synaptosomal preparations of hypothalamus was determined in absence and presence of alaproclate (3×10^{-6}M) for the 5-HT deamination and of maprotiline (3×10^{-6}M) for the noradrenaline deamination as described in [14]. Intraneuronal (●), extraneuronal (□) and average (△) protection. Each value is the mean ± S.E.R. from 5 rats.

tion within the serotonergic neurons and to a lesser degree MAO-A within the noradrenergic neurons in the rat hypothalamus [14]. MAO-A outside these neurons was considerably less protected. The results suggested that amiflamine and/or FLA 788(+) was accumulated in the monoaminergic neurons by the membranal uptake mechanism, since pretreatment of the rats with norzimeldine or desipramine decreased the MAO inhibitory effect intraneuronally. We have now examined the effect of 14 days treatment of the rats with amiflamine on the protection of the neuronal and extraneuronal MAO-A against phenelzine in the rat hypothalamus (Fig. 2). In accordance with the acute effect there was a large difference between the protection of the neuronal MAO compared with extraneuronal MAO. The ED_{50} for the protection within the serotonergic neurons was 0.75 μmoles/kg p.o. 1 hr after the last administration compared to a value 1.3 μmoles/kg obtained after a single dose. The corresponding ED_{50} values for the protection of MAO within noradrenergic neurons was 2.0 (repeated) and 4.0 (acute) μmoles/kg p.o. The ED_{50} for the extraneuronal protection after chronic treatment was above 12 μmoles/kg p.o.

Amiflamine either *per se* or as its most active metabolite FLA 788(+) appears to be accumulated into the serotonergic and the noradrenergic neurons (as shown by Fig. 2) via the membranal uptake mechanism. Therefore the duration of the MAO inhibition was examined after a single dose or after the final administration of 14 days' treatment twice daily with three different doses of amiflamine (Table 5). After single doses of 3 and 15 μmoles/kg no inhibitory effect on the 5-HT deamination in hypothalamic synaptosomes was observed 48 hr later. No protection against the phenelzine effect was obtained when phenelzine was injected 24 hr after the amiflamine administration (Table 6). However, 60 μmoles/kg of amiflamine protected the serotonergic MAO when given 24 hr but not when administered 48 hr before phenelzine. After chronic treatment the protection against the phenelzine-induced MAO inhibition was observed 24 hr but not 72 hr after the last 15 μmoles/kg dose. At

Table 5. Duration of the inhibition of the deamination of ^{14}C-5-HT in serotonergic synaptosomes in the rat hypothalamus

	Dose μmoles/kg p.o.	Time (hr) after last amiflamine	Inhibition of deamination of 5-HT (%)	
			Neuronal	Extraneuronal
Single	3	48	− 16 ± 10	− 5 ± 2
	15	48	− 7 ± 8	− 5 ± 6
	60	48	− 24 ± 6	− 7 ± 3
	60	72	− 24 ± 1	− 2 ± 2
Chronic	3	48	10 ± 7	9 ± 6
	15	48	17 ± 4	12 ± 3
	15	96	− 10 ± 4	7 ± 2
	60	48	56 ± 9*	1 ± 5
	60	72	41 ± 10*	− 2 ± 4

* $P < 0.01$ compared with controls (Student's *t*-test).

Amiflamine was given in a single dose or repeatedly twice daily for 14 days. Each value is the mean ± S.E.M. from 5 rats.

Table 6. Duration of the protection against phenelzine induced MAO inhibition

	Dose μmoles/kg p.o.	Time (hr) between last dose of amiflamine and phenelzine	Protection against phenelzine (%)	
			Neuronal	Extraneuronal
Single	3	24	4	0
	15	24	2	3
	60	24	68	8
	60	48	0	4
Chronic	3	24	0	10
	15	24	35	18
	15	48	0	0
	60	24	98	0
	60	48	70	0

Amiflamine was given in a single dose or repeatedly twice daily for 14 days. Phenelzinc, 4 mg/kg s.c. was injected at various times after the final amiflamine administration. Each value is the mean from 5 rats, which were sacrificed 24 hr after phenelzine.

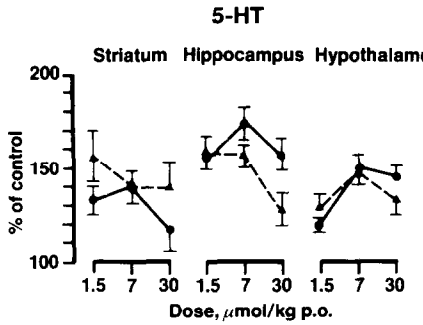

Fig. 3. The concentration of serotonin (5-HT) in striatum, hippocampus and hypothalamus 2 hr after the last administration of amiflamine given in a single dose (●) or twice daily for 14 days (△) at 1.5, 7 and 30 μmoles/kg p.o. Each value is the mean ± S.E.M. (vertical bars) of 5 rats.

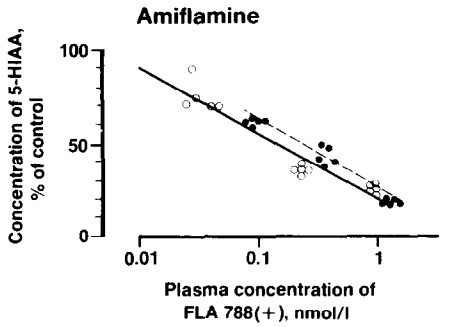

Fig. 5. The relationship between the concentration of 5-hydroxyindoleacetic acid (5-HIAA) in hypothalamus and the concentration of FLA 788(+) in plasma 2 hr after a single (○,-) ($r = 0.96$, $P < 0.001$) and the last dose of amiflamine after 14 days treatment (●,---) ($r \times 0.94$, $P < 0.001$) twice daily in the doses 1.5, 7 and 30 μmoles/kg p.o.

60μmoles/kg, amiflamine almost completely protected MAO 24 hr after the final dose and a marked protection was obtained also after 48 hr. At this high dose the deamination of serotonin in the serotonergic synaptosomes was significantly reduced 48 and 72 hr after the final dose.

Effect on brain serotonin and 5-HIAA concentrations. Amiflamine treatment of the rats with the dose 1.5 μmoles/kg p.o. produced increase in the serotonin concentrations in hippocampus, striatum and hypothalamus (Fig. 3). No real difference was obtained between acute and repeated treatment in this respect. In hypothalamus, 7 μmoles/kg produced a higher response than did the low dose whereas in the other two regions the increases were similar. At the highest dose (30 μmoles/kg p.o.) the degree of increase in serotonin concentrations after repeated treatment (hippocampus and hypothalamus) and acute treatment (striatum) appeared to be lower than after 7 μmoles/kg p.o. The serotonin concentrations in all three regions were similar to that of the controls 24 hr after a single dose and were in some cases lower than the controls in the repeated study.

Amiflamine produced similar dose-dependent decreases in the 5-HIAA concentrations in hippocampus, striatum and hypothalamus when measured 2 hr after the last administration (Fig. 4). No difference between single and repeated treatments or between the regions were observed. The 5-HIAA concentrations were normal 24 hr after the last administration of the two lowest doses but were significantly reduced after the highest dose, particularly after repeated administration (20% reduction compared with controls). The decrease in 5-HIAA was highly correlated to the plasma concentration of FLA 788(+) (Fig. 5).

Effects on the dopamine metabolism in striatum. The concentrations of DA in striatum were less elevated by amiflamine than were those of 5-HT in the same region (Fig. 6). The lowest dose examined did not cause any effect after a single administration whereas repeated amiflamine produced a significant elevation of the DA level. The two higher doses increased the DA concentrations after single administration whereas repeated treatment had no effect.

The concentration of DOPAC in striatum was dose dependently decreased by amiflamine in a very similar manner after acute and repeated administration (Fig. 6). The decrease in DOPAC levels was almost the same as those in 5-HIAA in the same region. The concentration of HVA in striatum was less affected than was that of DOPAC.

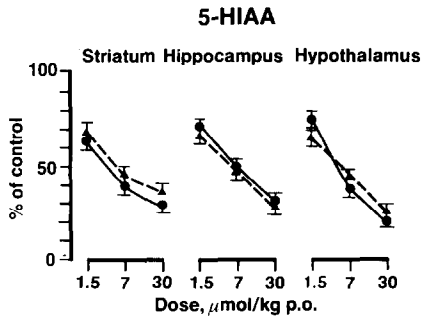

Fig. 4. The concentration of 5-hydroxyindoleacetic acid (5-HIAA) in striatum, hippocampus and hypothalamus 2 hr after the last administration of amiflamine given in a single dose (●) or twice daily for 14 days (△) at 1.5, 7 and 30 μmoles/kg p.o. Each value is the mean ± S.E.M. (vertical bars) of 5 rats (the same as in Fig. 3).

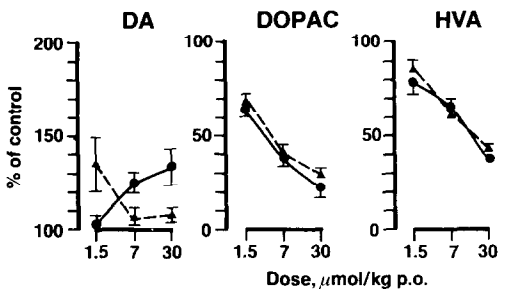

Fig. 6. The concentrations of dopamine (DA), DOPAC and HVA in the striatum of rats treated with a single dose (●) or repeated doses (△) (twice daily for 14 days) of amiflamine. The rats were sacrificed 2 hr after the last administration. Each value is the mean ± S.E.M. (vertical bars) of 5 rats (the same as in Fig. 4).

The concentrations of DA, DOPAC and HVA all approached control values 24 hr after the final administration of amiflamine.

DISCUSSION

In a previous study it was shown that amiflamine and its metabolite FLA 788(+) are potent reversible and selective MAO-A inhibitors in the rat brain [11]. The high potencies of these compounds are not only due to a favourable distribution to the brain but also to an accumulation of the compounds into serotonergic and to a lesser degree into noradrenergic neurons, whereby the inhibition becomes considerably higher inside than outside these neurons [14]. The present study shows that this neuronal selectivity is retained after repeated treatment for two weeks of the rats with amiflamine. The reversibility of the MAO inhibition was shown both in the slice experiments and in the phenelzine protection experiments. Due to the accumulation of amiflamine and/or FLA 788(+) in the serotonergic neurons the recovery from the MAO inhibition in these neurons was delayed after very high (25–50 times higher than the ED_{50} value obtained within serotonergic neurons when amiflamine was administered one hour prior to phenelzine) repeated doses of amiflamine.

The high plasma and brain levels of the metabolite FLA 788(+) compared with those of the parent compound at a low dose of amiflamine indicate that this metabolite is mainly responsible for the MAO inhibition *in vivo*. In accordance with this hypothesis the MAO inhibition observed with the slice technique was highly significantly correlated with the brain and plasma levels of FLA 788(+). This view is also further supported by the higher *in vitro* inhibitory potency of FLA 788(+) than of amiflame [11]. It seems likely therefore that FLA 788(+) is accumulated within the serotonergic and, albeit to a lesser degree, within the noradrenergic nerve terminals and thereby produces the high MAO inhibition therein. The contribution of amiflamine itself to the MAO inhibition *in vivo* is difficult to estimate from the experiments hitherto performed.

A dose-dependent metabolism of amiflamine is indicated by the different ratios of amiflamine to FLA 788(+) at different amiflamine doses. The half-lives of amiflamine and FLA 788(+) are difficult to determine from the results in this study. A more detailed pharmacokinetic study, including an estimate of drug absorption has to be performed in order to obtain all the pharmacokinetic information about amiflamine.

The decrease in the brain levels of the deaminated metabolites of the biogenic amines could be supposed to give a direct measure of the degree of MAO inhibition. However, it was found that a dose of amiflamine which produces about 50% protection against phenelzine-induced MAO inhibition in the serotonergic nerve terminals in hypothalamus decreased the 5-HIAA concentration by only 20–30%. Several factors may contribute to this apparent discrepant finding. (1) Part of the 5-HIAA formation occurs outside the neurons, (2) a part of the 5-HIAA in the brain at the time of the sacrifice (2 hr) was formed before the amiflamine administration, (3) 50% protection against phenelzine does not necessarily mean 50% inhibition of MAO with 5-HT as the substrate since the relative affinities of the two substrates (5-HT and phenelzine), the free neuronal concentrations of the two substrates and the different time courses of the decrease in 5-HIAA and the protection of MAO against phenelzine after the amiflamine administration have to be accounted for, (4) the increase in the 5-HT concentration within the serotonergic neurons after amiflamine may contribute to the protection against phenelzine.

A factor that obviously influences the amine levels and the formation of deaminated amine metabolites is negative feed-back mechanisms which decrease the pre-synaptic activity. This is most likely the explanation of the observation that the dopamine metabolites DOPAC and HVA decreased as rapidly as did 5-HIAA after amiflamine administration in spite of a lower MAO inhibition in the dopaminergic neurons compared to that in the serotonergic neurons [14]. This larger sensitivity of the dopamine system towards down-regulation of the pre-synaptic activity after MAO inhibition is also observed for other reversible MAO-A inhibitors, e.g. cimoxatone [18], Ro 11-1163 [19] and CGP 11305 A [20], neither of which have selectivity for MAO within a given neuronal population (Ask *et al.*, unpublished observations). These inhibitors decrease the dopamine metabolites DOPAC and HVA considerably to a greater extent than 5-HIAA [18–20].

No preference of amiflamine for MAO within dopaminergic neurons has been found previously [14]. Hence the larger decrease in the concentration of DOPA than that of HVA does not necessarily indicate such a neuronal preference but may be due to a higher turnover of the former.

The increase in the concentration of 5-HT probably corresponds better to an MAO inhibition within serotonergic neurons than a decrease in that of 5-HIAA which may largely be of extraneuronal sources. Hence the significant increase in the 5-HT concentrations up to 150% of the control values in the brain regions examined 2 hr after 1.5 μmoles/kg of amiflamine is in accordance with the preferred inhibition of the serotonergic MAO. The findings that higher doses diminished the increase in the 5-HT concentration in these regions may in part be due to release of 5-HT at these doses since amiflamine and/or FLA 788(+) transported by the membranal 5-HT uptake mechanism may induce a counter-transport of 5-HT in the opposite direction. Feed-back regulated inhibition of the synthesis of 5-HT may also contribute to this effect.

In accordance with a lower effect on the dopaminergic MAO the dopamine concentration in striatum was not elevated as much as the 5-HT concentration in the same region. Decreased rate of synthesis of dopamine may also occur after MAO inhibition.

The results obtained show that amiflamine retains its MAO-A and serotonergic selectivity and reversibility upon prolonged treatments of rats.

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