



## MONOAMINE OXIDASE INHIBITORY EFFECTS OF SOME 4-AMINOPHENETHYLAMINE DERIVATIVES

MIGUEL REYES-PARADA,\* MA. CECILIA SCORZA,† RODOLFO SILVEIRA,†  
 FEDERICO DAJAS,‡ GUSTAVO COSTA,‡ KEITH F. TIPTON§ and BRUCE K. CASSELS\*||

\*Department of Chemistry, Faculty of Sciences, Universidad de Chile, Casilla 653, Santiago, Chile;  
 †Cell Biology Division, IIBCE, Av. Italia 3318, Montevideo, Uruguay; ‡Neurochemistry Division,  
 IIBCE, Av. Italia 3318, Montevideo, Uruguay; and §Biochemistry Department, Trinity College,  
 Dublin 2, Ireland

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**Abstract**—The *in vitro* and *ex vivo* monoamine oxidase (MAO) inhibitory effects of ( $\pm$ )-4-dimethylamino- $\alpha$ -methyl-phenethylamine (4-DMAA) and ( $\pm$ )-4-methylamino- $\alpha$ -methyl-phenethylamine (4-MAA) were reassessed, in comparison with the previously unstudied achiral parent compound, 4-dimethylaminophenethylamine (4-DMAPEA) and with a salt of 4-DMAA enriched in the levo isomer, (“-”)-4-DMAA, using amiflamine [*S*-(+)-4-dimethylamino- $\alpha$ ,2-dimethylphenethylamine] as positive control. The *in vitro* studies confirmed that 4-amino- $\alpha$ -methylphenethylamine derivatives are highly selective and reversible MAO-A inhibitors. Furthermore, (“-”)-4-DMAA was less active than the racemic mixture. The side chain-unsubstituted compound, 4-DMAPEA, proved to be a nonselective and reversible MAO inhibitor. The *ex vivo* results, in which catecholamines, serotonin (5-HT) and their metabolites were measured in two brain regions after *i.p.* administration, confirmed the results obtained *in vitro*. These results are consistent with the suggestion that the 4-amino group contributes to MAO inhibitory effects of  $\alpha$ -methyl-phenethylamines, and show that the presence and orientation of an  $\alpha$ -methyl side chain substituent may be important when determining the potency and selectivity of these compounds. All compounds tested could be quantified by HPLC with electrochemical detection.

**Key words:** antidepressants; phenylethylamine derivatives; phenylisopropylamine derivatives; stereo-selectivity

MAO|| (EC 1.4.3.4, amine: O<sub>2</sub> oxidoreductase), which catalyses the oxidative deamination of a variety of monoamines, can be subdivided, on the basis of substrate and inhibitor selectivity, into two isoforms: MAO-A and MAO-B. The A form of the enzyme selectively oxidizes serotonin (5-HT) and is selectively and irreversibly inhibited by clorgyline. The B form selectively oxidizes PEA and benzylamine and is inhibited by 1-deprenyl [see 1,2]. Non-selective and irreversible MAO inhibitors (MAOIs), have been used successfully in the treatment of depression. However, their adverse interactions with dietary amines, such as tyramine, have limited their use [3]. Selective and reversible MAO-A inhibitors are interesting compounds with potential action as antidepressant drugs which appear to interact only weakly with dietary tyramine [4]. Although at present many substances are known to inhibit the A form of the enzyme selectively, extensive and rational

molecular series, which could lead to quantitative structure-activity relationships (QuaSARs), are lacking. Florvall *et al.*, however, synthesized and evaluated more than 45 4-aminophenethylamine derivatives as MAOIs [5-7]. We have now reassessed the *in vitro* and *ex vivo* MAOI effects of some members of this series of compounds comparing them with the previously unstudied achiral parent compound, 4-DMAPEA and with a salt of 4-DMAA enriched in the levo isomer, (“-”)-4-DMAA. The well-studied selective MAO-A inhibitor, amiflamine [*S*-(+)-4-dimethylamino- $\alpha$ , 2-dimethyl-phenethylamine] [8-11], was included for comparative purposes. The structures of these compounds are shown in Fig. 1.

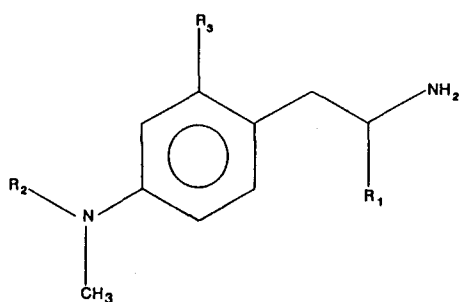
### MATERIALS AND METHODS

**Compounds.** All chemicals used were of the highest grade commercially available. THF and ACN were Merck HPLC grade. Monoamines, metabolites and PEA were from the Sigma Chemical Co. (Poole, U.K.). Amiflamine was kindly donated by Astra A.B. (Sweden).

4-DMAA, 1 and 4-DMAPEA, 2 were synthesized following a published sequence [5, 12, 13]. Briefly, 4-dimethylaminobenzaldehyde was condensed with nitroethane or nitromethane, respectively, and the corresponding  $\beta$ -nitrostyrenes obtained were reduced over several days with LiAlH<sub>4</sub>, using dry THF as solvent. 4-MAA, 3 was prepared by the procedure

|| Corresponding author. Tel. + 56 2 271 2865—branch 302; FAX + 56 2 271 3888.

¶ Abbreviations: ACN, acetonitrile; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 4-DMAA, 4-dimethylamino- $\alpha$ -methyl-phenethylamine; 4-DMAPEA, 4-dimethylamino-phenethylamine; ED, electrochemical detection; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; 4-MAA, 4-methylamino- $\alpha$ -methyl-phenethylamine; MHPG, 3-methoxy-4-hydroxy-phenylglycol; MAO; monoamine oxidase; MAOI, monoamine oxidase inhibitor; NA, noradrenaline. PEA, 2-phenethylamine; and THF, tetrahydrofuran.



COMPOUND	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
4-DMAPEA	H	CH <sub>3</sub>	H
4-DMAA	CH <sub>3</sub>	CH <sub>3</sub>	H
4-MAA	CH <sub>3</sub>	H	H
Amiflamine	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>

Fig. 1. Chemical structures of 4-DMAA, 4MAA, 4-DMAPEA and amiflamine.

of Florvall *et al.* [14]. In the cases of 1 and 2, the salts formed with the usual inorganic acids (HCl, HBr, H<sub>2</sub>SO<sub>4</sub>) were hygroscopic and, therefore, the more convenient tartrates were used. The (*R,R*)-tartrate, which precipitated in high yield from an isopropanol solution of ( $\pm$ )-4DMAA, was not appreciably enriched in either enantiomer, judging from the <sup>1</sup>H NMR spectrum of the recovered base after adding (+)- $\alpha$ -methoxy- $\alpha$ -trifluoromethyl-phenylacetic acid [15], and is referred to hereafter as ( $\pm$ )-4-DMAA tartrate. After five recrystallizations of this salt in methanol, the product was enriched in the (–)-enantiomer (enantiomeric ratio approximately 5:3, calculated similarly, [15]), and is referred to as (–)-4-DMAA tartrate.

*In vitro studies.* In these studies the potency, selectivity and reversibility of MAO inhibition was evaluated. For this purpose, a crude mitochondrial suspension, obtained from the brain of male IIBCE rats weighing 180–230 g, was used. The rats were decapitated, the whole brain was dissected out and the cerebellum was discarded. The tissue was homogenized in 10 vol. of 0.32 M sucrose (glass-teflon homogenizer). The homogenate was centrifuged at 800 g for 10 min at 4°. The supernatant was carefully decanted and centrifuged at 10,000 g for 20 min at 4°. The pellet obtained was rehomogenized in the original volume of 0.32 M sucrose and the centrifugation was repeated. The pellet obtained was resuspended in 10 mL of 0.1 M sodium phosphate buffer, pH 7.4, divided into 1 mL aliquots in small test tubes and kept at 4° until use, the same day. The protein content was determined according to Lowry *et al.* [16] with BSA as standard. The mitochondrial MAO activities were determined using preferential substrates for MAO-A (5-HT) and MAO-B (PEA). HPLC with electrochemical

detection (HPLC-ED) was used for determining MAO-A, and the activity of MAO-B was measured luminometrically [17].

To determine the inhibition of MAO-A, the incubation mixture consisted of 50  $\mu$ L of a 50  $\mu$ M 5-HT solution (giving a final concentration of  $2.5 \times 10^{-6}$  M, in a final volume of 1 mL); 400  $\mu$ L of 0.1 M sodium phosphate buffer, pH 7.4, the inhibitor to be tested at appropriate concentrations in 50  $\mu$ L of distilled water; and 500  $\mu$ L of the mitochondrial suspension. The reaction was started by the addition of the mitochondrial suspension. This mixture was incubated for 10 min at 37° in a shaking water bath in open test tubes. The reaction was stopped by adding 800  $\mu$ L of 1 M HClO<sub>4</sub>. The mixture was then centrifuged at 15,000 g for 5 min at 4°, and 50  $\mu$ L of supernatant was injected into the HPLC system. Control experiments were performed without inhibitor. Blanks were run without mitochondrial suspension. Amiflamine was used as a positive control. In all cases the volume adjustments were made with 0.1 M phosphate buffer (pH 7.4). Each experiment was performed in triplicate. The heights of the chromatographic peaks of 5-HT and its main MAO metabolite, 5-HIAA were used to calculate MAO-A activity. Product formation was found to be linear with time for at least 10 min and with enzyme concentration under these conditions (data not shown).

A C18 reverse phase column (ODS 250 mm  $\times$  4.6 mm, BIOPHASE, U.S.A.), an amperometric detector (BAS LC-3A), and a two-channel graphic recorder (BAS), were used to analyse the reaction mixtures. The mobile phase flow rate was 1 mL/min, and its composition was 31.5 g citric acid; 956 mL bi-distilled water; sufficient 12 N NaOH to bring the pH value to 3; 200 mg sodium octyl sulfate (SOS); ACN and THF in different proportions for the different drugs. The respective volumes of ACN and THF per litre were 40 and 30 mL for 4-DMAA, 45 and 30 mL for 4-DMAPEA, 45 and 11.2 mL for 4-MAA and 40 and 50 mL for AM. Detector sensitivity was 20 nA, and the oxidation potential was fixed at 850 mV.

Experiments to determine the inhibition of MAO-B were performed by luminometry, using PEA as a preferential substrate for the enzyme. This method is adapted from a previously reported procedure [17]. The activity of MAO-B was determined by measuring the height of the luminometric peak and interpolating it on a calibration curve drawn for different H<sub>2</sub>O<sub>2</sub> concentrations. The incubation mixture consisted of 20  $\mu$ g horseradish peroxidase in 50  $\mu$ L distilled water; 50  $\mu$ L of a  $10^{-3}$  M PEA solution (giving a final concentration of 37  $\mu$ M in a final volume of 1.3 mL); the inhibitor to be tested, at an appropriate concentration in 50  $\mu$ L of distilled water; 800  $\mu$ L of 68 mM Tris-HCl buffer, pH 8, and 132  $\mu$ M luminol in buffer solution. The assays were begun by the addition of 300  $\mu$ L of the mitochondrial suspension to the mixture in the luminometer cell, and the steady state luminescence was determined after 5 min. The luminometer, an LKB Wallac 1250 with a single channel LKB Bromma 2210 recorder, was thermostated at 37°. Control experiments were carried out without inhibitors. Phenelzine, a non-

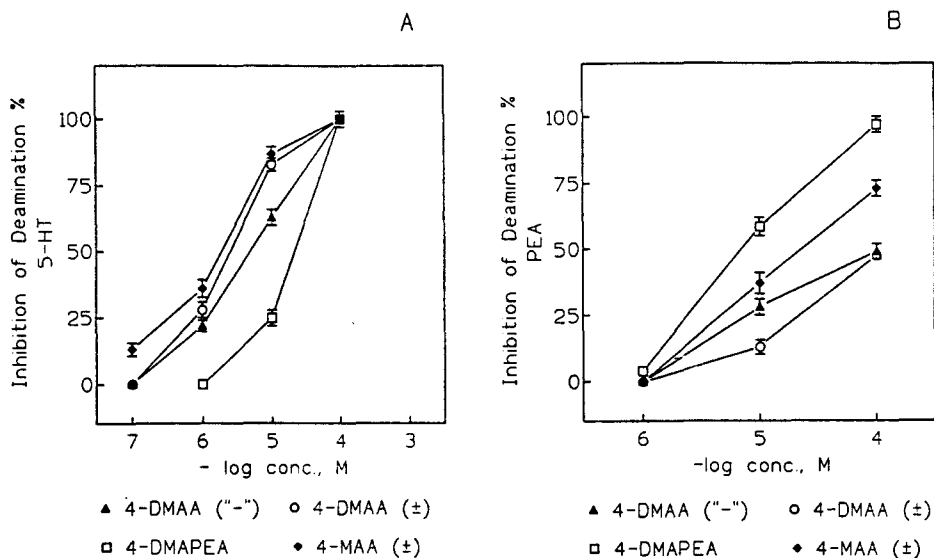


Fig. 2. Inhibition of monoamine oxidase A and B represented by the percentage of inhibition of deamination of 5-HT (A) and PEA (B) measured by HPLC-ED and luminometry, respectively. The experiments were performed without enzyme-inhibitor preincubation. Mitochondria were prepared from whole rat brain. Each point is the mean  $\pm$  SEM of three determinations.

selective MAOI, was also used as a positive control of inhibition. Blank runs were done without PEA. In all cases volume adjustments were made with 68 mM Tris-HCl buffer, pH 8. Results are reported as the mean of three successive experiments.

Time-courses of the MAO-A or MAO-B inhibition by the drugs were assessed by preincubating the reaction mixture with either compound at an appropriate concentration, for 10, 30 and 60 min. After preincubation, MAO-A and MAO-B activities were measured under the same conditions described above.

The reversibility of the inhibitory process for both forms of MAO was assessed by repeated washing of the mitochondrial suspension [18]. MAO-A or MAO-B incubation mixtures were preincubated for 10 min with drugs at appropriate concentrations. The preparations were then washed three times (centrifugation and resuspension) with 0.1 M sodium phosphate buffer, pH 7.4. Finally, MAO activities were measured again, using HPLC-ED for MAO-A and luminometry for MAO-B. Control samples, in which the inhibitor solution was replaced by an equal volume of water, were treated in the same way.

**Ex vivo studies.** To evaluate the effects of ( $\pm$ )-4-DMAA and (" - ") -4-DMAA, four groups of male IIBCE rats weighing 200–220 g were used. Three of these groups were injected i.p. with equimolar doses (39.5  $\mu$ mol of free base) of ( $\pm$ )-4-DMAA tartrate, (" - ") -4-DMAA tartrate, or amiflamine tartrate. The control group was injected with saline. The volume injected was 1 mL/kg in each case. Rats were decapitated 6 and 24 hr after injection, and the dorsal raphe nuclei and the hippocampus were immediately dissected out. The tissues were kept at  $-70^\circ$  until use, not more than 3 days later.

Table 1. The effects of preincubation on the inhibition of MAO-A by 4-aminophenethylamine derivatives

Compound [ $10^{-4}$ M]	Percent of inhibition of MAO-A Preincubation time (min)		
	10	30	60
4-DMAA ( $\pm$ )	100 $\pm$ 2	96 $\pm$ 2	98 $\pm$ 3
4-DMAA (" - ")	97 $\pm$ 3	99 $\pm$ 1	98 $\pm$ 1
4-DMAPEA	98 $\pm$ 2	8 $\pm$ 4*	0*
4-MAA ( $\pm$ )	100 $\pm$ 2	100 $\pm$ 1	100 $\pm$ 1

Crude mitochondrial suspensions, obtained from rat brain, were preincubated with inhibitors in 100  $\mu$ L sodium phosphate buffer, pH 7.4, at  $37^\circ$  for the times indicated. Percent inhibition of the deamination of 5-HT (2.5  $\mu$ M) was determined by HPLC-ED. The values are means  $\pm$  SEM of triplicate determinations. The specific MAO-A activity of the control, determined with 5-HT, was 1.05 nmol product formed/min/mg protein.

\*  $P < 0.05$  Student's *t*-test, compared with 10 min preincubation.

The effects of the drugs on levels of monoamines and their metabolites were measured by HPLC-ED. The tissue was suspended in 0.1 M HClO<sub>4</sub> (100  $\mu$ L/mg of tissue), sonicated, and finally centrifuged at 10,000 *g* for 5 min at  $4^\circ$ . Fifty microlitres of the supernatant was injected into the chromatograph. 5-HT, DA, NA, MHPG, HVA, DOPAC, and 5-HIAA were measured. A standard solution containing a known amount of the substances to be measured was run at the beginning and at the end of each experiment, and by this procedure the correction factors for the amine and acid metabolites

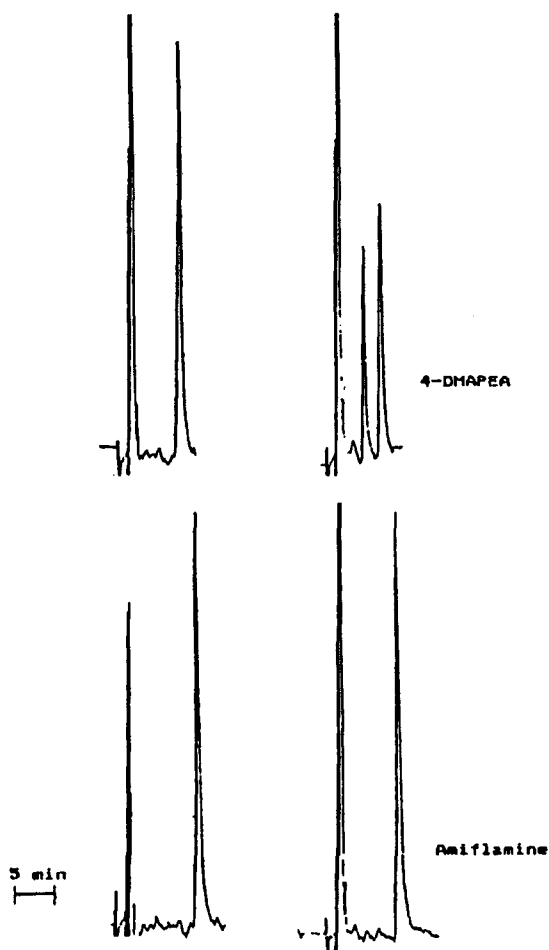


Fig. 3. HPLC elution profiles for 4-DMAPEA (upper) and amiflamine (lower) before (right) and after (left) incubation with the mitochondrial preparation. Fifty micrograms of inhibitor was incubated with the mitochondrial suspension for 5 min. Separation was by HPLC with electrochemical detection as described in the text.

were determined. All experiments were performed with three separate rats. The detector potential was fixed at 850 mV. The sensitivity of the amperometric detector was 5 nA and the mobile phase flow rate was 1 mL/min. The mobile phase used consisted of 31.5 g citric acid, 956 mL distilled water, 12 N NaOH to pH 3, 200 mg SOS, 45 mL ACN, and 20 mL THF.

In all experiments, statistical analysis was performed using the PRESTA software package (paquete de Procesamiento ESTADístico, versión 1.1 for PCs, 1989, V. Abaira and J. Zaplana. FISSS, Madrid, Spain).

## RESULTS AND DISCUSSION

### In vitro studies

Figure 2A shows the MAO-A inhibitory activities of each of the drugs tested. All derivatives showed strong, concentration-dependent inhibition of MAO-A. The achiral derivative 4-DMAPEA (approx.  $I_{50}$  =

20  $\mu$ M) also exhibited this effect clearly, although to a lesser extent than its chiral congeners. The different potencies observed for ( $\pm$ )- and ("–")-4-DMAA ( $I_{50}$  values of approx. 2 and 5  $\mu$ M, respectively) reflect the importance of the spatial distribution of the side chain substituents in their interaction with the enzyme, and indicate the (+)-isomer to be the more potent one. This observation, and the fact that the 4-methylamino-derivative was more active (approx.  $I_{50}$  = 1.2  $\mu$ M), show the same tendency as the results published by Ask *et al.* [8, 19] for amiflamine and its more active N-demethylated metabolite, the (+)-isomer being the more potent in both cases.

Figure 2B shows the MAO-B inhibitory effect of the drugs tested. Here again, there are differences between the 4-DMAA isomers, but it is clear that both of them, which gave  $I_{50}$  values >100  $\mu$ M, as well as their demethylated analogue 4-MAA (approx.  $I_{50}$  = 20  $\mu$ M), are relatively weak inhibitors of MAO-B activity. In contrast, the achiral derivative 4-DMAPEA showed relatively strong MAO-B inhibition (approx.  $I_{50}$  = 10  $\mu$ M). These results support previous observations that such arylisopropylamine derivatives are selective MAO-A inhibitors [5], and at the same time show that the parent phenethylamine derivative, studied here for the first time, is a non-selective MAO inhibitor. The different behavioural profile of the phenylisopropylamines and the phenethylamine derivative is thus very probably associated with the presence and orientation of a methyl group on the side chain [see 20].

Table 1 shows the effects of enzyme-inhibitor preincubation (10, 30 and 60 min) on MAO-A inhibition produced by these compounds. In all cases, except for 4-DMAPEA, the MAO-A inhibition was not time dependent, since the degree of inhibition did not change even after the longer incubation time. In the case of 4-DMAPEA, the inhibition observed after 10 min of preincubation with the enzyme was abolished after 30 min of preincubation. These results suggest that 4-DMAPEA is probably metabolized in the presence of the mitochondrial suspension used in these studies. It is known that  $\alpha$ -methyl substitution of primary amines prevents them from acting as substrates for MAO [21] and thus it is possible that 4-DMAPEA may be a substrate for the enzyme whereas its  $\alpha$ -methyl derivatives are not. As shown in Fig. 3, a decrease in the size of the peak of 4-DMAPEA and the appearance of a new peak in the HPLC chromatogram after incubation with the mitochondrial suspension confirmed the conclusion that metabolism was occurring. No such changes were seen with the other compounds. Further work would be necessary to indicate the enzyme(s) involved.

Table 2 shows the results obtained in the reversibility studies. The inhibition of MAO-A activity resulting from 10 min of preincubation with the drugs was essentially completely reversed after three washes of the preparations. Essentially the same results were obtained in the studies with MAO-B, i.e. the inhibition was not time-dependent, except in the case of 4-DMAPEA whose inhibitory effect disappeared after 30 min of preincubation (Table 3).

Table 2. Reversibility of the inhibition of MAO-A produced by 4-aminophenethylamine derivatives as determined by repeated washing

Compound	Percent of control values			
	Before washing		After washing	
	5-HIAA	5-HT	5-HIAA	5-HT
4-DMAA ( $\pm$ )	33 $\pm$ 3*	186 $\pm$ 12*	71 $\pm$ 3*	104 $\pm$ 4
4-DMAA ("–")	22 $\pm$ 3*	173 $\pm$ 5*	100 $\pm$ 1	95 $\pm$ 1
4-DMAPEA	7 $\pm$ 2*	180 $\pm$ 12*	86 $\pm$ 1	100 $\pm$ 3
4-MAA ( $\pm$ )	16 $\pm$ 1*	210 $\pm$ 6*	87 $\pm$ 2	94 $\pm$ 2

Crude mitochondrial suspensions were preincubated for 10 min with inhibitor (10  $\mu$ M except for 4-DMAPEA where the concentration was 100  $\mu$ M) and then the preparation was washed three times by centrifugation and resuspension. MAO-A activity of the preparation and of the control experiments was measured using 5-HT (2.5  $\mu$ M) by HPLC-ED. Each value is the mean  $\pm$  SEM of triplicates.

\*  $P < 0.05$  Student's *t*-test, compared with control values.

Table 3. The effects of preincubation on the inhibition of MAO-B by 4-aminophenethylamine derivatives

Compound [10 <sup>-4</sup> M]	Percent of inhibition of MAO-B	
	Preincubation time (min)	
	10	30
4-DMAA ( $\pm$ )	37 $\pm$ 3	35 $\pm$ 1
4-DMAA ("–")	41 $\pm$ 2	40 $\pm$ 3
4-DMAPEA	97 $\pm$ 3	0*
4-MAA ( $\pm$ )	73 $\pm$ 2	69 $\pm$ 1

Crude mitochondrial suspensions, obtained from rat brain, were preincubated with inhibitors in 100  $\mu$ L sodium phosphate buffer, pH 7.4, at 37° for the times indicated. Percent inhibition of the deamination of PEA (37  $\mu$ M) was determined by luminometry. The values are means  $\pm$  SEM of triplicate determinations.

\* $P < 0.05$  Student's *t*-test, compared with 10 min preincubation.

Furthermore, MAO-B activity was completely recovered after repeated washing (data not shown). These results indicate that MAO inhibition by all drugs tested is reversible.

#### Ex vivo studies

Figure 4 shows the monoamine (NA and 5-HT) and metabolite (5-HIAA) levels in the dorsal raphe nuclei 6 hr after injection. NA and 5-HT increased with respect to control levels, more markedly in the case of 5-HT. Again, both 4-DMAA preparations (racemic and levorotatory) showed different potencies, with the racemate being the most potent. Surprisingly, not only were the 5-HIAA levels not lower than the control level, as might have been expected, but in the case of ( $\pm$ )-4-DMAA, an increase was observed. This effect might result from the great increase in the 5-HT level which could then be deaminated by MAO-B [22]. On the other hand, if the inhibition of MAO-A were competitive,

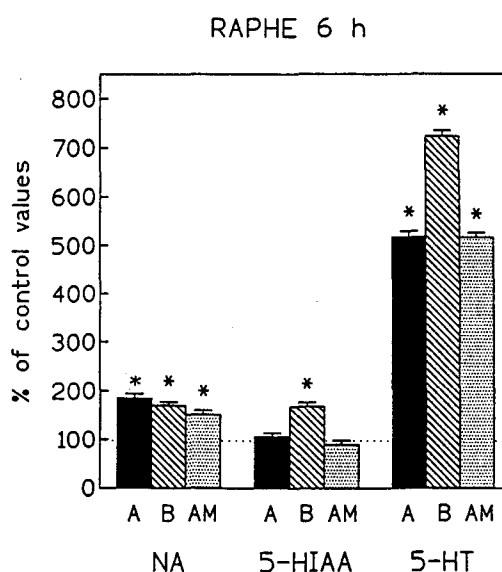


Fig. 4. Effects of ("–")-4-DMAA (A), ( $\pm$ )-4-DMAA (B) and amiflamine (AM) on monoamines (NA and 5-HT) and metabolite (5-HIAA) levels in dorsal raphe nuclei, 6 hr after i.p. administration. Each bar represent the mean  $\pm$  SEM of three rats. (\*Significant difference,  $P < 0.05$ , Student's *t*-test.)

an increase in substrate level might suffice to make partial deamination possible, even under inhibitory conditions [see 23].

Figure 5a shows the effects of drugs on hippocampus 6 hr after injection. An increase in NA levels was also observed in this tissue. 5-HT levels also rose, most markedly with amiflamine, although this effect was less pronounced than in the dorsal raphe nuclei. MAO-A inhibition is clearly reflected by the decrease in 5-HIAA levels. As observed in the raphe, the drug which induced the greatest increase in the substrate level (amiflamine in this

## HIPPOCAMPUS

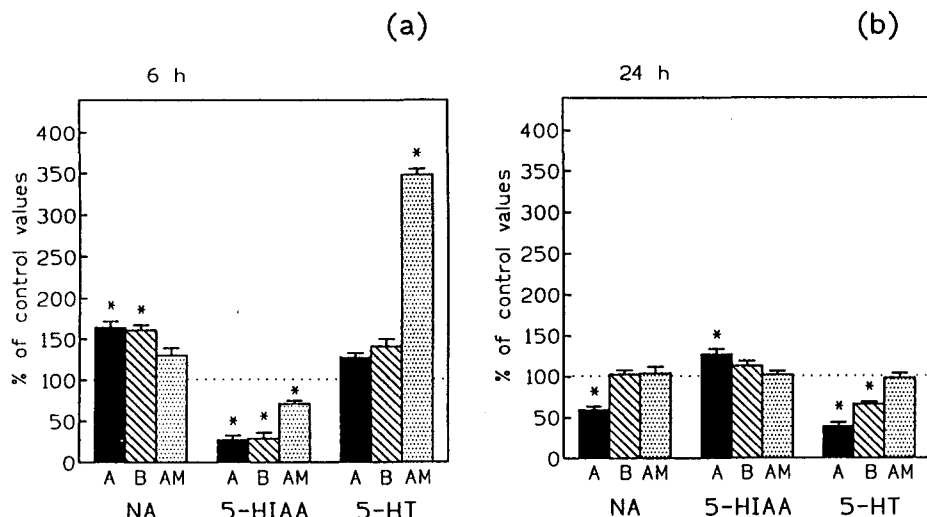


Fig. 5. Effects of ("–")-4-DMAA (A), ( $\pm$ )-4-DMAA (B) and amiflamine (AM) on NA, 5-HT and 5-HIAA levels in hippocampus, 6 (a) and 24 hr (b) after i.p. administration. Each bar represents the mean  $\pm$  SEM of three rats. (\*Significant difference,  $P < 0.05$ , Student's *t*-test.)

case), was the one which produced the smallest decrease in the metabolite level. It is important to note the remarkable difference between the effects of amiflamine on NA and 5-HT levels. Ask *et al.* [19, 24, 25, see also 9] reported that amiflamine was highly selective for serotonergic neurons, and the present results are consistent with such behaviour. These results also suggest that 4-DMAA does not show such neuron selectivity. This property, associated with the presence of a methyl group at position 2 of the aromatic ring in the amiflamine molecule, suggests that further study of related compounds could provide interesting insights regarding the structural requirements of the presynaptic reuptake site in serotonergic neurons.

Neither the raphe nuclei nor the hippocampus showed significant changes in MHPG, DA, HVA or DOPAC levels 6 hr after injection (data not shown).

Figure 5b shows NA, 5-HIAA and 5-HT levels in hippocampus 24 hr after injection. The concentration of all compounds had returned to levels close to the control values by this time. These *ex vivo* results confirm the reversibility observed *in vitro*. They are also consistent with the suggestion that the 4-amino group in such phenethylamine derivatives could be important for their MAO inhibitory potencies [5, 6]. The observations that the 4-methylamino derivative (4-MAA) had greater potency and that the racemic compound was a better inhibitor than the material enriched in the (–) isomer, are in accord with the tendencies reported by Ask *et al.* for the behaviour of amiflamine and its N-demethylated metabolite [8, 19] in mitochondrial suspensions prepared from hippocampus.

In the course of the MAO-A inhibition experi-

ments, it was found that 4-aminophenethylamines can be detected electrochemically at an anodic potential of 850 mV. Although the arylamino group, whether mono- or dimethylated, might not be expected to be oxidized so easily, it is known [26] that good electron donor groups such as methoxyls lower the oxidation potential of this kind of molecule and, in particular, a 4-amino group is more effective than a 4-methoxy group in this regard. This phenomenon is presumably due to an increase in the energy of the highest occupied molecular orbital (HOMO), from which an electron is removed when the molecule is oxidized at the anode. The fact that the amino group is a very good electron donor may, therefore, explain the electrochemical behaviour of amiflamine and its analogues. Such a property in clinically useful drugs could be of great value for analytical purposes.

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