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Monoamine Oxidase Inhibitory Properties of Some Methoxylated and Alkylthio Amphetamine Derivatives

STRUCTURE–ACTIVITY RELATIONSHIPS

Ma. Cecilia Scorza, Cecilia Carrau,* Rodolfo Silveira,* Gerald Zapata-Torres,† Bruce K. Cassels† and Miguel Reyes-Parada*‡*

*División Biología Celular, Instituto de Investigaciones Biológicas Clemente Estable, CP 11600, Montevideo, Uruguay; and †Departamento de Química, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

ABSTRACT. The monoamine oxidase (MAO) inhibitory properties of a series of amphetamine derivatives with different substituents at or around the *para* position of the aromatic ring were evaluated. In *in vitro* studies in which a crude rat brain mitochondrial suspension was used as the source of MAO, several compounds showed a strong (IC_{50} in the submicromolar range), selective, reversible, time-independent, and concentration-related inhibition of MAO-A. After i.p. injection, the compounds induced an increase of serotonin and a decrease of 5-hydroxyindoleacetic acid in the raphe nuclei and hippocampus, confirming the *in vitro* results. The analysis of structure–activity relationships indicates that: molecules with amphetamine-like structure and different substitutions on the aromatic ring are potentially MAO-A inhibitors; substituents at different positions of the aromatic ring modify the potency but have little influence on the selectivity; substituents at the *para* position such as amino, alkoxyl, halogens, or alkylthio produce a significant increase in the activity; the *para*-substituent must be an electron donor; bulky groups next to the *para* substituent lead to a decrease in the activity; substituents located at positions more distant on the aromatic ring have less influence and, even when the substituent is a halogen (Cl, Br), an increase in the activity of the compound is obtained. Finally, the MAO-A inhibitory properties of some of the compounds evaluated are discussed in relation to: (a) potential antidepressant activity, and (b) their reported hallucinogenic, neurotoxic, or anxiolytic effects. BIOCHEM PHARMACOL 54;12:1361–1369, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. monoamine oxidase; MAO inhibitors; amphetamine derivatives; structure–activity relationships; phenylisopropylamine derivatives; antidepressants

MAO§ (EC 1.4.3.4, monoamine:O_2 oxidoreductase), which catalyzes the oxidative deamination of a variety of monoamines such as catecholamines and 5-HT, exists in two different forms (MAO-A and -B) that are distinguishable by their substrate selectivity, inhibitor sensitivity, and amino acid sequence [1–3]. Non-selective and irreversible MAOIs have been used as effective antidepressant drugs. However, severe side-effects, such as hypertensive crisis ("cheese effect") after the ingestion of tyramine-rich food, have limited their use [4–6]. A better understanding of the differing activities of the two enzymatic isoforms has led to the development of a new generation of antidepressants based on the selective and reversible inhibition of MAO-A. This class of compounds exhibits antidepressant actions while it appears to interact only weakly with dietary tyramine [7–9].

Several phenylisopropylamines, including amphetamine itself, have been evaluated as MAOIs [10]. Generally speaking, substituents on the aromatic ring of the phenylisopropylamine molecule (in particular at the *para* position), such as amino groups [11–13], halogens [14], hydroxyl [15] and methoxyl groups [16, 17], lead to an increase in the potency and selectivity towards MAO-A, with respect to the parent compound. However, except for the studies of Ross and Florvall's group [11, 12, 17–20], no extensive molecular series of MAOIs have been studied

‡ Corresponding author: Dr. Miguel Reyes-Parada, Instituto de Investigaciones Biológicas Clemente Estable, División Biología Celular, Avenida Italia 3318, CP 11600, Montevideo, Uruguay. Tel. (598-2) 471616 (Br 115); FAX (598-2) 475548/472603; e-mail: reyes@iibce.edu.uy

§ *Abbreviations:* MAO, monoamine oxidase; MAOI, MAO inhibitor; 5-HT, serotonin; DMAPEA, 4-dimethylaminophenethylamine; 5-HIAA, 5-hydroxyindoleacetic acid; SOS, sodium octyl sulfate; DMAPAA, 4-dimethylaminophenylacetic acid; PCA, *p*-chloroamphetamine; FLA-527, 2,5-dimethoxy-4-dimethylaminoamphetamine; MTA, 4-methylthioamphetamine; ETA, 4-ethylthioamphetamine; ITA, 4-isopropylthioamphetamine; 2,4-DMA, 2,4-dimethoxyamphetamine; DOTFM, 2,5-dimethoxy-4-trifluoromethylamphetamine; 4-EtOA, 4-ethoxyamphetamine; 4-MetOA, 4-methoxyamphetamine; 3,4-DMA, 3,4-dimethoxyamphetamine; 2,5-DMA, 2,5-dimethoxyamphetamine; ALEPH-2, 2,5-dimethoxy-4-ethylthioamphetamine; ALEPH-1, 2,5-dimethoxyl-4-methylthioamphetamine; TMA-2, 2,4,5-trimethoxyamphetamine; TMA, 3,4,5-trimethoxyamphetamine; DOB, 2,5-dimethoxy-4-bromoamphetamine; DOI, 2,5-dimethoxy-4-iodoamphetamine; DOM, 2,5-dimethoxy-4-methylamphetamine; DON, 2,5-dimethoxy-4-nitroamphetamine; DOA, 2,5-dimethoxy-4-aminoamphetamine; 2-Br-4,5-DMA, 2-bromo-4,5-dimethoxyamphetamine; 5-Br-2,4-DMA, 5-bromo-2,4-dimethoxyamphetamine; 2-NO_2-4,5-DMA, 2-nitro-4,5-dimethoxyamphetamine; 2-Br-4,5-MDA, 2-bromo-4,5-methylenedioxyamphetamine; 2-NO_2-4,5-MDA, 2-nitro-4,5-methylenedioxyamphetamine; 2-Cl-4,5-MDA, 2-chloro-4,5-methylenedioxyamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4,-methylenedioxymethamphetamine; Rn, raphe nuclei; and Hc, hippocampus.

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that could contribute to a better understanding of the structural requirements necessary to design new potent and selective amphetamine-like MAOIs.

Methoxylated amphetamines have been the object of many structure–activity relationship studies [see Refs. 21–23 for reviews] because of their great therapeutic and abuse potential, and the high affinity and selectivity for 5-HT receptors and the 5-HT uptake carrier exhibited by some of them [for recent reviews see Refs. 23 and 24]. In these studies, it has also been shown that the *para* position appears to play an essential role in the potency and selectivity of these drugs.

On the basis of these precedents, in this work the *in vitro* selectivity, time dependency, and reversibility of the inhibition of MAO-A and MAO-B induced by a series of phenylisopropylamines, mainly methoxylated derivatives, were studied. Furthermore, the *ex vivo* effects of some compounds were assessed by measuring the endogenous levels of monoaminergic neurotransmitters and their corresponding MAO metabolites in different rat brain regions. Finally, some structure–activity relationships for the series of drugs studied were established.

MATERIALS AND METHODS

Chemicals

All the chemicals used were of the highest grade commercially available. Tetrahydrofuran (THF) and acetonitrile (ACN) were Merck HPLC grade. Amphetamine sulfate, PCA, monoamines, metabolites, aldehyde dehydrogenase from yeast (AldDH) and β-nicotinamide adenine dinucleotide (β-NAD) were from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). DMAPAA was from Aldrich (Milwaukee, WI, U.S.A.). Amiflamine and FLA-527 were donated by ASTRA AB (Södertälje, Sweden) and *l*-deprenyl was donated by Prof. J. Knoll (Semmelweis University of Medicine, Hungary). MTA, ETA, ITA, 2,4-DMA, and DOTFM were synthesized and donated by Dr. David E. Nichols (Purdue University, U.S.A.). All other compounds used in this study were synthesized following published procedures [Ref. 25 unless stated otherwise]. 3,4-DMA, MDA, 2,5-DMA, DOB, 2-Br-4,5-DMA, 5-Br-2,4-DMA, 2-Br-4,5-MDA, 2-Cl-4,5-MDA, DON, 2-NO_2-4,5-DMA, and 2-NO_2-4,5-MDA were available in our laboratory from previous work [26–29].

Other compounds used in this study included 4-EtOA [30], 4-MetOA, ALEPH-2, ALEPH-1, TMA-2, TMA, DOI, DOM, DOA [31], and MDMA. All amphetamine derivatives were stored and used as salts: hydrochlorides, hydrobromides, or nitrate (in the case of DON). Their identity was checked by ^{1}H NMR and their purity by elemental analysis.

Enzymatic Assays

The effects of the phenylisopropylamine derivatives on MAO-A or MAO-B activities were studied using a crude rat brain mitochondrial suspension. The tissue was prepared from whole rat brain (after discarding the cerebellum) of male IIBCE rats weighing 200–240 g as described previously [13]. The mitochondrial MAO activities were determined by HPLC with electrochemical detection (HPLC-ED), using selective substrates for the A form (5-HT) and B form (DMAPEA) [13, 32].

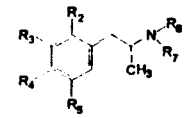
To determine the inhibition of MAO-A, the incubation mixture consisted of 25 μL of a 50 μM 5-HT solution (final concentration 2.5 μM in a final volume of 0.5 mL); 225 μL of 0.1 M sodium phosphate buffer, pH 7.4; the inhibitor to be tested at the appropriate concentration (10 nM–100 μM) in 50 μL of distilled water; and 200 μL of the mitochondrial suspension. For MAO-B, the incubation mixture consisted of 25 μL of a 100 μM DMAPEA solution (final concentration 5 μM in a final volume of 0.5 mL); 105 μL of 0.1 M sodium phosphate buffer, pH 7.4; AldDH (0.8 units) and β-NAD (0.6 μmol) dissolved in 200 μL of phosphate buffer; the inhibitor to be tested at the appropriate concentration (10 nM–100 μM) in 50 μL of distilled water; and 120 μL of the mitochondrial suspension. The reaction was started by the addition of the mitochondrial suspension. The mixtures were incubated for 10 min (MAO-A) and 5 min (MAO-B) at 37° in a shaking water bath in open test tubes. The reaction was stopped by adding 200 μL of 1 M $HClO_4$. The mixture was then centrifuged at 15,000 × *g* for 5 min at 4°, and 50 μL of supernatant was injected into the HPLC system. Control experiments were performed without inhibitor, and blanks were run without mitochondrial suspension. In all cases, volume adjustments were made with 0.1 M phosphate buffer. Each concentration of drug was assayed in triplicate. The heights of the chromatographic peaks of 5-HT, DMAPEA, and their respective main MAO metabolites, 5-HIAA and DMAPAA, were used to calculate MAO activity. Product formation was found to be linear with incubation time and enzyme concentration under the experimental conditions described (data not shown). The IC_{50} values were determined from plots of inhibition percentage, calculated in relation to a sample of the enzyme treated under the same conditions without inhibitors, versus −log [I].

Chromatographic Conditions

A C18 reverse phase column (ODS 250 × 4.6 mm, BIOPHASE, U.S.A.), an amperometric detector (BAS LC-3A), and a two-channel graphic recorder (BAS) were used to analyze the reaction mixtures. The mobile phase flow rate was 1 mL/min, and its composition was 31.5 g citric acid; 908 mL bidistilled water; sufficient 12 N NaOH to bring the pH value to 3; 200 mg SOS; 42 mL ACN; and 50 mL THF. Detector sensitivity was 10 nA, and the oxidation potential was fixed at 0.85 V using a glassy carbon working electrode versus an Ag/AgCl reference electrode.

Time–courses of the MAO-A or MAO-B inhibition by the drugs were assessed by preincubating the reaction mixture with different compounds at an appropriate con-

TABLE 1. Structural descriptions of the amphetamine derivatives tested



Compound	R_2	R_3	R_4	R_5	R_7	R_8
1) Amiflamine	CH_3	H	$N\text{-}(CH_3)_2$	H	H	H
2) *l*-Deprenyl	H	H	H	H	$CH_2\text{–}C\equiv CH$	CH_3
3) Amphetamine	H	H	H	H	H	H
4) ETA	H	H	$S\text{-}CH_2\text{-}CH_3$	H	H	H
5) MTA	H	H	$S\text{-}CH_3$	H	H	H
6) ITA	H	H	$S\text{-}CH\text{-}(CH_3)_2$	H	H	H
7) 4-EtOA	H	H	$O\text{-}CH_2\text{-}CH_3$	H	H	H
8) 4-MetOA	H	H	$O\text{-}CH_3$	H	H	H
9) PCA	H	H	Cl	H	H	H
10) 3,4-DMA	H	$O\text{-}CH_3$	$O\text{-}CH_3$	H	H	H
11) 2,5-DMA	$O\text{-}CH_3$	H	H	$O\text{-}CH_3$	H	H
12) 2,4-DMA	$O\text{-}CH_3$	H	$O\text{-}CH_3$	H	H	H
13) ALEPH-2	$O\text{-}CH_3$	H	$S\text{-}CH_2\text{-}CH_3$	$O\text{-}CH_3$	H	H
14) ALEPH-1	$O\text{-}CH_3$	H	$S\text{-}CH_3$	$O\text{-}CH_3$	H	H
15) DOTFM	$O\text{-}CH_3$	H	CF_3	$O\text{-}CH_3$	H	H
16) FLA 527	$O\text{-}CH_3$	H	$N\text{-}(CH_3)_2$	$O\text{-}CH_3$	H	H
17) TMA-2	$O\text{-}CH_3$	H	$O\text{-}CH_3$	$O\text{-}CH_3$	H	H
18) TMA	H	$O\text{-}CH_3$	$O\text{-}CH_3$	$O\text{-}CH_3$	H	H
19) DOB	$O\text{-}CH_3$	H	Br	$O\text{-}CH_3$	H	H
20) DOM	$O\text{-}CH_3$	H	CH_3	$O\text{-}CH_3$	H	H
21) DON	$O\text{-}CH_3$	H	NO_2	$O\text{-}CH_3$	H	H
22) DOI	$O\text{-}CH_3$	H	I	$O\text{-}CH_3$	H	H
23) DOA	$O\text{-}CH_3$	H	NH_2	$O\text{-}CH_3$	H	H
24) 2Br-4,5-DMA	Br	H	$O\text{-}CH_3$	$O\text{-}CH_3$	H	H
25) 5Br-2,4-DMA	$O\text{-}CH_3$	H	$O\text{-}CH_3$	Br	H	H
26) $2NO_2$-4,5-DMA	NO_2	H	$O\text{-}CH_3$	$O\text{-}CH_3$	H	H
27) 2Br-4,5-MDA	Br	H	$CH_2\text{-}O\text{-}CH_2$		H	H
28) $2NO_2$-4,5-MDA	NO_2	H	$CH_2\text{-}O\text{-}CH_2$		H	H
29) 2Cl-4,5-MDA	Cl	H	$CH_2\text{-}O\text{-}CH_2$		H	H
30) MDMA	H	$CH_2\text{-}O\text{-}CH_2$		H	H	CH_3
31) MDA	H	$CH_2\text{-}O\text{-}CH_2$		H	H	H

centration, for 30 and 60 min. After preincubation, MAO-A and MAO-B activities were measured under the same conditions described above [13].

The reversibility of the inhibitory process for both forms of MAO was assessed by repeated washing of the mitochondrial suspension. MAO-A or MAO-B mixtures were preincubated for 10 min with drugs at appropriate concentrations. Then the preparations were washed three or four times (centrifugation and resuspension) with 0.1 M sodium phosphate buffer, pH 7.4. Finally, MAO activities were measured again, using HPLC-ED. Control samples, in which the inhibitor solution was replaced by an equal volume of water, were treated in the same way [13].

In all cases, the protein content was determined according to Lowry *et al.* [33] with BSA as standard.

Ex Vivo *Studies*

To evaluate the *in vivo* effects of the drugs, monoaminergic neurotransmitter levels were quantified in different rat CNS regions after the peripheral administration of the compounds. Groups of male HBCE rats (N = 6–8) weighing 200–220 g were injected i.p. with equimolar doses (39.5 μmol of free base) of the drugs. The control group (N = 10) was injected with saline. The volume injected was 1 mL/kg in each case. Rats were decapitated 1 and 6 hr after injection, and the dorsal Rn and Hc were dissected out immediately. The tissues were kept at −70° until used (not more than 5 days later). The levels of monoamines and their metabolites were measured by HPLC-ED as previously described [13].

Statistical Analysis

The statistical significance was determined using Student's *t*-test. In all cases, the significance level was found to be $P < 0.05$.

RESULTS

Table 1 summarizes the structures of the amphetamine derivatives studied.

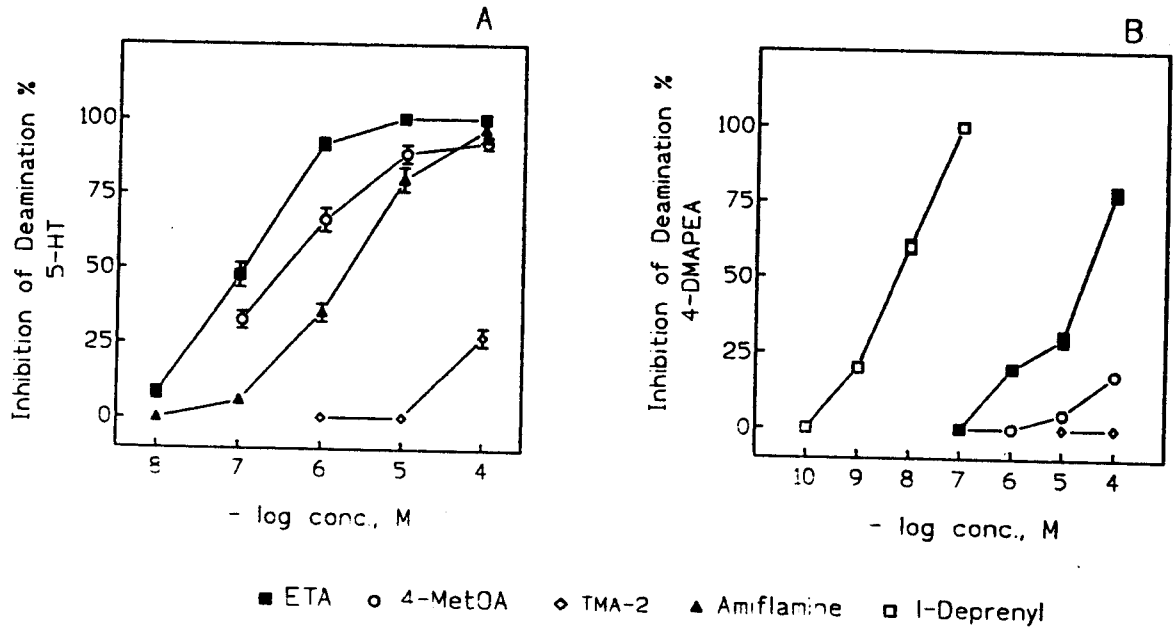


FIG. 1. Effects of some amphetamine derivatives as MAOIs. Inhibition of MAO-A and -B is represented by the percentage of inhibition of deamination of 5-HT (A) and DMAPEA (B), as measured by HPLC-ED. The experiments were performed without enzyme-inhibitor preincubation. Mitochondrial suspension was prepared from whole rat brain. Each point is the mean ± SD of three determinations.

In Vitro *Studies*

In these studies, the effects of the compounds on the MAO activities were evaluated. Figure 1 shows an example of the curves obtained for the inhibition of MAO-A (panel A) and MAO-B (panel B) by some of the compounds. Amiflamine and *l*-deprenyl were included as a positive control for the inhibition of MAO-A and MAO-B, respectively. Several compounds showed a strong, selective, and concentration-dependent inhibition of MAO-A.

Table 2 summarizes the IC_{50} values for the inhibition of both forms of MAO by the amphetamine derivatives tested. It is noteworthy that most of the compounds (including amphetamine itself) were clearly selective as MAO-A inhibitors. Additionally, several derivatives (4–8, 12) were more potent than amiflamine, the reference MAO-A inhibitor amphetamine derivative, with their IC_{50} values in the submicromolar range. Moreover, some drugs were completely devoid of MAO inhibitory properties.

Table 3 shows the effects of enzyme-inhibitor preincubation (0, 30, and 60 min) on MAO-A and -B inhibition produced by some compounds. All drugs, except clorgyline and *l*-deprenyl, inhibited enzymatic activity independently of time, since the degree of inhibition was not changed even after the longest incubation time. In the case of clorgyline and *l*-deprenyl, as expected for suicide inhibitors, the inhibition observed after 30- and 60-min preincubation was significantly greater than that observed with no preincubation.

The results obtained in the reversibility studies are shown in Table 4. The inhibition of MAO-A after a 10-min preincubation with the drugs was, in most of the cases, reversed significantly after three washes of the preparation. In the case of ETA, four washes were needed to

TABLE 2. IC_{50} Values for MAO inhibition

Compound	IC_{50} (μM) MAO-A	MAO-B
1) Amiflamine	2.0	NE
2) *l*-Deprenyl	50	0.007
3) Amphetamine	11	NE
4) ETA	0.1	29
5) MTA	0.2	NE
6) ITA	0.4	8.1
7) 4-EtOA	0.2	> 100
8) 4-MetOA	0.3	NE
9) PCA	4.0	NE
10) 3,4-DMA	20	NE
11) 2,5-DMA	> 100	NE
12) 2,4-DMA	0.6	NE
13) ALEPH-2	3.2	NE
14) ALEPH-1	5.1	NE
15) DOTFM	NE	NE
16) FLA 527	NE	NE
17) TMA-2	NE	NE
18) TMA	NE	NE
19) DOB	100	NE
20) DOM	24	NE
21) DON	NE	NE
22) DOI	43	NE
23) DOA	NE	NE
24) 2Br-DMA	9.3	NE
25) 5Br-DMA	13	NE
26) $2NO_2$-DMA	NE	NE
27) 2Br-MDA	13	64
28) $2NO_2$-MDA	NE	78
29) 2Cl-MDA	6.3	38
30) MDMA	30	NE
31) MDA	9.3	NE

The IC_{50} values were calculated from the inhibition versus −log concentration curves, with 4–5 concentrations of the compounds. Each concentration was tested in triplicate. NE = no effect at 100 μM.

TABLE 3. Effects of preincubation on the inhibition of MAO-A and MAO-B by amphetamine derivatives

	Percent of inhibition of MAO-A and MAO-B					
	Preincubation time (min)					
	MAO-A			MAO-B		
Compound	0	30	60	0	30	60
ETA [5×10^{-7} M]	88.0 ± 2.7	92.0 ± 3	93.2 ± 2.6	79.8 ± 1.1	79.2 ± 1.2	77.0 ± 1.4
MTA [10^{-6} M]	78.0 ± 1.6	75.2 ± 3.6	79.0 ± 4.3	ND	ND	ND
4-EtOA [10^{-6} M]	81.7 ± 2.4	82.5 ± 1.3	85.1 ± 1.3	76.9 ± 0.5	76.8 ± 1	78.6 ± 0.6
ALEPH-2 [10^{-5} M]	74.3 ± 0.5	77.4 ± 2.5	80.2 ± 1.6	ND	ND	ND
2-Br-4,5-DMA [10^{-5} M]	51.4 ± 2.6	50.5 ± 4.4	51.6 ± 1	ND	ND	ND
TMA-2 [10^{-4} M]	27.0 ± 1.6	26.4 ± 5.2	27.2 ± 3.6	0	0	0
2-NO_2-4,5-MDA [10^{-4} M]	ND	ND	ND	47.0 ± 1	40.5 ± 5.2	47.5 ± 2.3
4-MetOA [10^{-4} M]	ND	ND	ND	13.2 ± 3.4	10.1 ± 3	12.5 ± 5.4
Clorgyline [10^{-8} M]	17.9 ± 5.4	75.0 ± 4.6*	74.4 ± 1*	ND	ND	ND
l-Deprenyl [10^{-7} M]	ND	ND	ND	0	65.0 ± 3*	76.6 ± 2.2*

Crude mitochondrial suspensions were preincubated at 37° for the times indicated, with each compound at a concentration that, without preincubation, did not produce total inhibition of the enzyme. Percent inhibition of deamination of 5-HT (2.5 μM) for MAO-A and DMAPEA (5 μM) for MAO-B was determined by HPLC-ED. Clorgyline and *l*-deprenyl were used as a positive control. Values are means ± SD of triplicate determinations. ND = not determined.

* $P < 0.05$ compared with 0-min preincubation (Student's *t*-test).

observe a slight but significant reversal of the enzyme inhibition. The inhibitory effect of the irreversible MAO-A inhibitor clorgyline was present after three or four washes. For most of the compounds, MAO-A activity showed different degrees of recovery after washes.

Ex Vivo *Studies*

Table 5 shows the effects of some amphetamine derivatives on the 5-HT and 5-HIAA levels in two brain regions, 1 and 6 hr after their peripheral administration. In the Rn, all compounds tested induced a significant increase in 5-HT levels 1 hr after injection, whereas a significant decrease in 5-HIAA was observed in all cases. Since 5-HT is mainly metabolized by MAO-A, the changes observed in the content of the monoamine and its main MAO metabolite are probably due to inhibition of the enzyme activity. This is also supported by the fact that amiflamine produced basically the same effects.

Recovery of 5-HT and 5-HIAA levels to near control values was observed in most cases 6 hr after drug administration. These *ex vivo* findings are in agreement with the reversibility observed *in vitro*. Nevertheless, 6 hr after administration of amiflamine and MTA, increased 5-HT levels were still found, an effect that was even more pronounced than the effect observed after 1 hr.

In a region rich in serotonergic nerve terminals such as the Hc, most of the compounds elicited the same changes observed in the Rn. Thus, in Hc 1 hr after injection, MAO-A inhibition by drugs was evidenced by an increase in 5-HT and/or a decrease in 5-HIAA levels.

As was seen in the Rn, a reversal of the effects of some compounds was observed after 6 hr. Nevertheless, once again amiflamine and MTA produced an increase in 5-HT and a concomitant 5-HIAA reduction which persisted 6 hr after their administration. Like amiflamine, ETA maintained an increased 5-HT level until 6 hr after administration, although 5-HIAA returned to levels close to the control value at this time.

TABLE 4. Reversibility of the inhibition of MAO-A produced by some amphetamine derivatives as determined by repeated washing

	Percent of inhibition of MAO-A	
Compound [10^{-4} M]	Before washing	After washing
MTA	100	69.1 ± 0.6*
4-EtOA	100	42.7 ± 8.1†
4-MetOA	100	5.8 ± 3.1‡
ALEPH-2	97.3 ± 1.3	28.6 ± 5.3*
2-Br-4,5-DMA	89.1 ± 3.1	9.1 ± 1.8‡
ETA	100	100
Clorgyline	100	100
ETA§	100	85.1 ± 1.6†
Clorgyline§	100	100

Crude mitochondrial suspension was preincubated for 10 min with inhibitor, 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 by HPLC-ED using 5-HT as selective substrate. Each value is the mean ± SD of triplicates.

*†‡ Significantly different from "before washing" values (as determined by Student's *t*-test): * $P < 0.01$, † $P < 0.05$, and ‡ $P < 0.001$.

§ Experiments with ETA and clorgyline were washed four times by centrifugation and resuspension.

DISCUSSION

In this paper, we report the MAO inhibitory properties of a series of amphetamine derivatives with substituents such as halogens, alkylthio and methoxyl groups at or around the *para* position. Our results show that some of these modifications on the amphetamine structure can lead to an increase in the potency of the derivatives as MAO-A inhibitors, as compared with the parent compound. Although all derivatives have a close structural similarity to the monoaminergic neurotransmitters metabolized by both

TABLE 5. Effects of some derivatives on 5-HT and 5-HIAA endogenous levels in the raphe nuclei and hippocampus

Compound	5-HIAA		5-HT	
	1 hr	6 hr	1 hr	6 hr
Raphe nuclei				
Amiflamine	20.1 ± 17.5*	34.0 ± 27.0*	240.4 ± 133.0*	322 ± 178.0*†
4-EtOA	48.2 ± 14.5*	103.3 ± 19.1†	187.0 ± 27.0*	74.3 ± 14.0*†
4-MetOA	34.2 ± 15.0*	99.2 ± 24.0†	165.0 ± 31.0*	92.1 ± 36.7†
MTA	38.0 ± 8.1*	80.1 ± 11.3*†	127.3 ± 6.8*	180.5 ± 31.3*†
ETA	50.7 ± 7.5*	130.0 ± 22.6*†	176.5 ± 35.5*	131.0 ± 23.4*†
Saline	432.6 ± 30		810 ± 61.5	
Hippocampus				
Amiflamine	40.0 ± 14.6*	37.0 ± 36.5*	171.1 ± 12.6*	189.5 ± 23.1*†
4-EtOA	69.5 ± 13.7*	98.5 ± 10.3†	157.0 ± 27.8*	90.1 ± 6.6†
4-MetOA	68.0 ± 16.5*	101.2 ± 17.0†	128.0 ± 18.7*	116.8 ± 8.0†
ETA	58.6 ± 10.7*	105.8 ± 10.4†	127.9 ± 11.4*	131.3 ± 22*
MTA	63.3 ± 4.7*	45.6 ± 8.4*†	93.9 ± 7.7	123 ± 23.3*†
Saline	260.3 ± 30		365 ± 70.2	

Animals were injected i.p. with an equimolar dose of the compounds (39.5 μmol/kg). Each value represents the mean ± SD (N = 6–10 in each group), expressed as a percent of the control group treated with saline. The values of the control group are expressed in ng/g of tissue.

* Significant difference ($P < 0.05$, Student's *t*-test) of treated group from the saline group.

† Significant difference ($P < 0.05$, Student's *t*-test) of the 6-hr group from the 1-hr group.

forms of MAO, they presented a clear selectivity for MAO-A. These results suggest that MAO-B may have more stringent structural requirements, presumably at the active site, to accept structures differing from those of its physiological substrates. In our case, this precluded an analysis of the structural requirements necessary for a compound to exhibit MAO-B inhibitory properties.

Regarding the potency of the derivatives as MAO-A inhibitors, the most potent compounds (ETA, 4-EtOA, MTA, 4-MetOA, and ITA) were obtained when only the *para* position of the aromatic ring was substituted. The addition of bulky substituents adjacent to this position decreased potency. Thus, the presence of a second methoxyl group at the *meta* position produced a compound 60-fold less potent than the monosubstituted derivative (see 4-MetOA vs 3,4-DMA). Moreover, two substituents occupying both *meta* positions completely abolished the activity of the compound (4-MetOA vs TMA). Substituents localized at more distant positions from the *para* carbon atom had less influence on the activity of MAO-A inhibitors (4-MetOA vs 2,4-DMA), whereas compounds with groups at positions other than *para* presented less activity than the parent molecule (amphetamine vs 2,5-DMA). Interestingly, the presence of two methoxyl groups at positions 2 and 5 induced a greater decrease in the activity than might be expected from the analysis of the influence of each substituent separately. For instance, 3,4-DMA was approximately 60-fold less potent than 4-MetOA, whereas 2,4-DMA had a potency similar to that of the monosubstituted derivative. However, when three methoxyl groups were placed at position 2, 4, and 5 in the same molecule, the inhibitory activity disappeared completely (TMA-2). Similar trends were observed in the case of PCA vs DOI or DOB (PCA has approximately the same potency as an MAO-A inhibitor as the *para* brominated and iodinated derivatives [14, 34, 35]), and to a lesser extent in the cases of MTA vs ALEPH-1 and ETA vs ALEPH-2.

The introduction of a halogen atom at position 2 increased the potency as an MAO-A inhibitor (2Br-4,5-DMA vs 3,4-DMA; 2Cl-4,5-MDA vs MDA). This last finding is in agreement with the results obtained by Florvall *et al.* for the amiflamine series [11, 12, 18].

On the other hand, some electron donor character of the substituents at the *para* position seems to be necessary to inhibit the enzyme, because strongly electron accepting groups like trifluoromethyl or nitro completely abolished the activity of the compounds (ALEPH-1 vs DON, DOM vs DOTFM). Nevertheless, the electronic properties of the *para* substituents seemed to have an optimum that was reached in the sulfur-containing compounds (MTA vs 4-MetOA, ETA vs 4-EtOA, ALEPH-2 vs TMA-2).

Regarding the size of the *para* substituent, our results show that ethoxy- or ethylthio-substituted compounds were more effective than methoxyl- or methylthio-substituted derivatives (4-EtOA vs 4-MetOA, ETA vs MTA, ALEPH-2 vs ALEPH-1). This indicates that the enzyme may better accommodate compounds with larger substituents at the *para* position. However, when the alkyl chain contained more than two carbon atoms, a decrease in potency was seen (ETA vs ITA).

No changes were observed in MAO-A and -B inhibition after different preincubation times (0, 30, and 60 min), indicating that the blockade of both forms of MAO was not time dependent and suggesting that the compounds were reversible MAOIs. Reversibility studies confirmed this assumption, showing a significant recovery of MAO-A activity after repeated washing of the preparation. These results indicate that the *in vitro* MAO inhibition by these amphetamine derivatives is reversible and essentially different from that produced by suicide inhibitors like clorgyline or *l*-deprenyl. In addition, these data also show that the inhi-

bition was not due to substrate competition, since the inhibitors were not metabolized by the enzyme, but rather resulted from a real blockade of enzyme activity.

The *ex vivo* studies essentially confirmed the *in vitro* observations. Some of the most potent MAO-A inhibitors as evaluated *in vitro* induced significant and reversible increases and decreases of 5-HT and 5-HIAA levels, respectively. These effects were observed in both serotonergic cell bodies and nerve terminal enriched regions, probably reflecting the MAO-A inhibition. Regarding the duration of the effects, most of the compounds were short-acting, eliciting only minor or no changes in brain indol levels 6 hr after their administration. The long-lasting effects of amiflamine (Table 5) have been related to its neuron-selective action [20]. This compound and/or its *N*-demethylated metabolite can be transported into the serotonergic neurons via the 5-HT uptake carrier and is, therefore, more active within these neurons compared with other neurons and cells. Some other analogues, e.g. MTA which also produced changes in 5-HT and 5-HIAA 6 hr after its injection, may have this neuron-selectivity, as well.

The MAOI properties of the compounds evaluated here are basically in agreement with previously reported data. Thus, the I_{50} values described here for amphetamine [10], amiflamine, FLA 527 [11], PCA [14], and 4-MetOA [16] are similar to those published by different authors using diverse methodologies. Additionally, the structure–activity relationships obtained showed similar tendencies compared with previous reports in terms of selectivity [12, 19], the importance of the substituent at the *para* position of the aromatic ring of the amphetamine molecule [11–14, 16, 19], the nature of the substituent [11, 14, 16, 17, 20, 36, 37], and the type of interaction with the enzyme [10, 12, 16–18, 20, 36].

In summary, our results show that: molecules with an amphetamine-like structure and different substitutions on the aromatic ring are potentially MAO-A inhibitors; substituents at different positions of the aromatic ring modify the potency but have little influence on the selectivity; substituents at the 4 (*para*) position such as amino, alkoxyl, halogens, or alkylthio produce a significant increase in the activity; the *para*-substituent must be an electron donor; bulky groups next to the *para* substituent lead to a decrease in the activity; substituents located at more distant positions on the aromatic ring have less influence and, even when the substituent is a halogen (Cl, Br), an increase in the activity of the compound is obtained; for the evaluated series, the ideal lipophilic character and size of the *para*-substituent were found for the alkylthio derivatives; and the MAO-A inhibition induced by the derivatives studied was reversible in all cases.

Several additional considerations should be made about the compounds that were studied. Some of the drugs evaluated, such as 4-MetOA, DOB, DOI, DOM, ALEPH-1, or ALEPH-2, have been described as having hallucinogenic properties in humans [25]. Classically, these effects have been associated with the activation of 5-$HT_{2A/2C}$ receptor subtypes [38–40]. Our results show that although some of these compounds have strong MAOI properties, this activity should not play a dominant role in the production of psychedelic effects, since it does not correlate with the hallucinogenic potency reported in humans (as is the case for their 5-$HT_{2A/2C}$ affinity) [25, 38, 41]. Nevertheless, interactions with the enzyme should be considered when subjective differences in the reported effects of this class of compounds in humans are analyzed.

On the other hand, compounds like MDA, MDMA, and PCA are recognized serotonergic neurotoxins [23, 42–44], whereas MTA, which was reported to have effects similar to those of MDMA in a drug discrimination paradigm and some commonalities in the mechanism of action, i.e. serotonin release properties [23, 24, 45], did not elicit 5-HT neurotoxicity. It may be possible that the differences between MTA and MDMA are related to their different activities as MAO-A inhibitors. In this case, the enzyme inhibition produced by MTA and the concomitant increase of the neurotransmitter in the surroundings of the nerve terminal might prevent the initial rapid decrease of 5-HT, which seems to be a necessary condition to produce the long-term neurotoxicity induced by compounds such as MDA or MDMA [46]. This would not be true in the case of PCA, which has considerable MAO-A inhibitory activity, but there is some evidence indicating that PCA and MDMA serotonergic neurotoxicity is initiated by different mechanisms [46–48].

As reported, MTA is a potent and selective non-neurotoxic serotonin releasing and uptake blocking agent [23, 45]. Our results indicate that this compound is also a potent and selective MAO-A inhibitor. Therefore, MTA combines in the same molecule the two characteristic features of the most recently developed antidepressants, making this derivative an interesting candidate for further evaluation in this sense.*

Finally, it has been been reported recently that another compound, ALEPH-2, exhibits an anxiolytic-like profile in rodents, as evaluated in different behavioral models, and possesses an unusual pharmacological profile [49, 50]. Its MAO-A inhibitory properties demonstrated in this work should be regarded in the framework of the analysis of its putative anxiolytic action, considering the acknowledged superposition that exists between the activities of anxiolytics and antidepressants [51].

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* Marona-Lewicka D and Nichols DE, The effect of selective serotonin releasing agents in the chronic mild stress model of depression in rats. In: 26th Annual Meeting, Society for Neuroscience Abstracts, Vol. 22, Part 1, Washington, DC, 16–21 November 1996, p. 181.

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