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MONOAMINE OXIDASE INHIBITION IN BRAIN AND LIVER PRODUCED BY β-CARBOLINES: STRUCTURE–ACTIVITY RELATIONSHIPS AND SUBSTRATE SPECIFICITY

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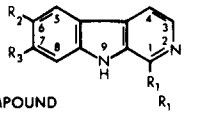
Abstract—The ability of a series of β-carboline compounds to inhibit monoamine oxidase (MAO) was studied in homogenates of mouse brain and liver. The *in vitro* structure–activity study with tryptamine as substrate showed the following to affect the degree of MAO inhibition: methyl group at carbon-1; methoxy or hydroxy group at carbon-6 or 7; ring saturation. In general, there was greater *in vitro* inhibition of brain MAO than of liver MAO. 6-Methoxy-1,2,3,4-tetrahydro-β-carboline produced greater MAO inhibition with serotonin as the substrate than with β-phenylethylamine both *in vitro* and *in vivo*, suggesting that it is a relatively selective inhibitor of MAO type A.

In 1972, McIsaac *et al.* [1] reported that 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC, 6-MeO-tetrahydronorharman) elevated brain serotonin (5-hydroxytryptamine, 5-HT) without affecting brain 5-hydroxindoleacetic acid (5-HIAA) or brain norephinephrine (NE). We confirmed that 6-MeO-THBC elevated brain 5-HT but also found a decrease in 5-HIAA [2, 3] due, in part, to the monoamine oxidase (MAO) inhibiting properties of the compound [4]. Our data conflict with those of Ho *et al.* [5] who had shown that 6-MeO-THBC did not inhibit brain MAO *in vivo* and was a poor *in vitro* inhibitor of MAO in liver [6]. These differences remain to be explained.

We [4] reported that, with respect to 6-MeO-THBC, norharman, and tetrahydronorharman, differences in amount of MAO inhibition depended upon the compound being tested and the tissue (brain or liver) from which the enzyme was taken. Other β-carbolines such as harmine, harmaline, and harman (Fig. 1) have been shown to be reversible inhibitors of liver MAO acting on 5-HT [7]. McIsaac and Estevez [8] reported that methoxylated compounds were equipotent with unsubstituted β-carbolines and that the unsaturated compounds were most active in inhibiting liver MAO with tyramine as substrate, whereas the tetrahydro compounds were least active. Ho *et al.* [6] showed that in liver, with tryptamine as substrate, the methoxy group at C-6 (Fig. 1) decreased inhibitory activity as did a methyl group at C-1. Because of these variations in data, assay, substrate, and tissue from laboratory to laboratory we examined more fully the structure-activity relationship among a greater number of β-carbolines (Fig. 1) and the nature of the difference in the ability of the β-carbolines to inhibit MAO from brain and liver.

We also determined substrate specificity in the MAO inhibitory properties of selected β-carbolines, especially 6-MeO-THBC. Different forms of MAO, referred to as types A and B, preferentially deaminate different substrates [9]. For example, 5-HT is a preferred substrate for type A, β-phenylethylamine (PEA) for type B, and tyramine and tryptamine are substrates common in both types. In addition, MAO inhibitors such as clorgyline and deprenyl are relatively selective for types A and B respectively [9]. The studies mentioned above by Ho's group used tyramine or tryptamine as substrate and we used trypt-

β - CARBOLINE



COMPOUND	R_1	R_2	R_3
HARMAN	CH_3	H	H
6-METHOXY-HARMAN	CH_3	OCH_3	H
NORHARMAN	H	H	H
HARMINE	CH_3	H	OCH_3
HARMOL	CH_3	H	OH

3,4 - DIHYDRO - β - CARBOLINE

COMPOUND	R_1	R_2	R_3
6-METHOXY-HARMALAN	CH_3	OCH_3	H
HARMALINE	CH_3	H	OCH_3
HARMALOL	CH_3	H	OH

1,2,3,4 - TETRAHYDRO - β - CARBOLINE

COMPOUND	R_1	R_2	R_3
6-METHOXY-TETRAHYDRO-β-CARBOLINE (6-METHOXY-TETRAHYDRONORHARMAN)	H	OCH_3	H
TETRAHYDRONORHARMAN	H	H	H
TETRAHYDROHARMAN	CH_3	H	H
6-METHOXY-TETRAHYDROHARMAN	CH_3	OCH_3	H
6-HYDROXY-TETRAHYDROHARMAN	CH_3	OH	H
TETRAHYDROHARMINE	CH_3	H	OCH_3
TETRAHYDROHARMOL	CH_3	H	OH

Fig. 1. Structures of β-carbolines.

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amine. Since harmaline preferentially inhibits MAO type A [9] and since 5-HT is the preferential substrate for type A, we considered that the full inhibitory capability of 6-MeO-THBC might not have been manifest with tryptamine or tyramine as substrate.

MATERIALS AND METHODS

Subjects. Female CF1 mice (Carworth), 45–75 days old, were used.

Drugs and reagents. Drugs which were purchased and their sources are as follows: Sigma Chemical Company—harman HCl, 6-methoxy-harman, 6-methoxy-tetrahydroharman in norharman HCl, tetrahydronorharman HCl (noreleagine HCl), serotonin creatine sulfate, tryptamine HCl; Regis Chemical Company—6-methoxy-harmalan, 6-hydroxy-tetrahydroharman, harmine HCl, harmaline HCl dihydrate, tetrahydroharmine (leptaflorine), harmol HCl, harmalol HCl; ICN Pharmaceuticals—tetrahydroharman, tetrahydroharmol, β-phenylethylamine HCl. 6-MeO-THBC HCl was synthesized according to the method of Ho *et al.* [6]. The following drugs were gifts from their respective sources: pargyline (Abbott Labs); clorgyline (May and Baker Ltd., Dagenham, England); deprenyl (Dr. J. Knoll, Semmelweiss University, Budapest, Hungary). Radioactive substrates were purchased from New England Nuclear. They were tryptamine-bisuccinate [side chain-2-^{14}C] (53 mC/m-mole, 0.52 mg/ml), 5-hydroxytryptamine binoxalate [2-^{14}C] (48.5 mC/m-mole, 0.54 mg/ml), and β-phenylethylamine [1-^{14}C]hydrochloride (9.86 mC/m-mole).

Preparation of mouse brain and liver homogenates. Mice were decapitated and whole brain or liver was homogenized with a Brinkman Polytron homogenizer in cold 0.15 M KCl. The homogenate was diluted with 0.15 M KCl to make 10 mg tissue/ml and 0.1 ml of diluted homogenate containing 1 mg tissue was used for each assay tube as the enzyme preparation.

In vitro and *in vivo* procedures. For the *in vitro* assays, various concentrations of the drugs (molar) were used in the reaction mixture (see below). For the *in vivo* studies, drugs (doses calculated as salts; see Fig. 4 and Table 2) were injected i.p. and animals were killed at various times after drug administration.

Monoamine oxidase assays. For the structure-activity studies with [^{14}C]tryptamine as substrate, MAO was assayed by the method of Wurtman and Axelrod [10] as described by Nagatsu [11]. The incubation mixture contained 100 μl enzyme preparation, 50 μl phosphate buffer (1.0 M, pH 7.4), 50 μl water or various concentrations of drug, 50 μl tryptamine HCl (1.0 mM), and 50 μl tryptamine-2-[^{14}C]bisuccinate (diluted in 0.01 N HCl to contain 0.01 mg/ml). The final substrate concentration was 0.17 mM. Reaction mixtures were preincubated at 37° for 10 min with drug solution or water, 50 μl of cold and 50 μl of radioactively labeled substrate were added, and incubation was continued for 20 min. The reaction was terminated by adding 0.2 ml of 2 N HCl. Reaction blanks were produced by adding 0.2 ml of 2 N HCl to the reaction mixture before adding the isotope. Deaminated metabolites were extracted into 6 ml of toluene by mixing on a Vortex for 30 sec, and the tube was then centrifuged. Three ml of the toluene layer was added to 10 ml of the scintillation cocktail (40 ml Liquifluor (New England Nuclear) per 1 L-toluene) and radioactivity was measured with a Beckman liquid scintillation counter.

For the *in vitro* and *in vivo* studies on substrate specificity, MAO was determined using the method of Robinson *et al.* [12]. The incubation mixture contained 200 μl Na_2HPO_4-KH_2PO_4 buffer (0.5 M, pH 7.4), 100 μl water or various concentrations of drug, 100 μl enzyme, 50 μl nonradioactive substrate and 50 μl radioactively labeled substrate. The substrates used were 5-HT and PEA. The radioactive 5-HT binoxalate was diluted in 0.01 N HCl to contain 0.01 mg/ml and the non-radioactive compound was 5-HT creatinine sulfate (1 mM). The radioactive PEA was dissolved in water to give 0.023 mg/ml and the non-radioactive PEA was 1 mM. The final substrate concentration was 0.1 mM for both 5-HT and PEA. Reaction mixtures were preincubated at 37° for 5 min with drug solution or water in the *in vitro* experiments and with water in the *in vivo* experiments, 50 μl of cold and 50 μl of radioactive substrate were added, and incubation was continued for 20 min. For the blank, an aliquot of the homogenate was boiled for 10 min and 100 μl used as the enzyme source. At the end of the incubation, the reaction mixture was pipetted directly onto the Amberlite CG-50 column which was washed immediately with 2 ml deionized water. The effluent and wash containing the radioactive deaminated products were collected in a scintillation vial. Ten ml of scintillation fluid (consisting of 500 ml Triton X-100, 1 L-toluene, 6 g PPO, and 300 mg POPOP) were added and the radioactivity was measured with a Beckman liquid scintillation counter.

Data analysis. MAO activity was expressed as a percentage of the control tube containing water in place of inhibitor after counts from the blank were subtracted from tubes containing inhibitor and control tubes. Control MAO activity was also calculated as nmoles ^{14}C-metabolites/min/mg brain as described by Nagatsu [11]. EC50s were determined graphically by plotting concentration vs per cent inhibition on semi-log paper and interpolating the concentration at which 50 per cent inhibition occurred.

RESULTS

Structure-activity studies. Plots of per cent MAO inhibition vs inhibitor concentrations in brain and liver are shown in Fig. 2, and EC50s for brain are shown in Table 1. In liver the most potent inhibitor among the β-carbolines tested was norharman followed by harman; thus, the presence of a methyl group at C-1 (Fig. 1) decreased inhibitory potency. For both norharman and harman, as the ring is saturated (compare tetrahydronorharman and tetrahydroharman respectively), potency decreases. Finally, addition of a methoxy group at C-6 further decreases potency. In brain, the relationship is not the same. First, harman is a more potent inhibitor than norharman. Saturation of harman leading to tetrahydroharman reduces potency, and a further reduction is produced by addition of a methoxy group at C-6 as in liver. However, with norharman, although saturation to tetrahydronorharman does reduce potency, addi-

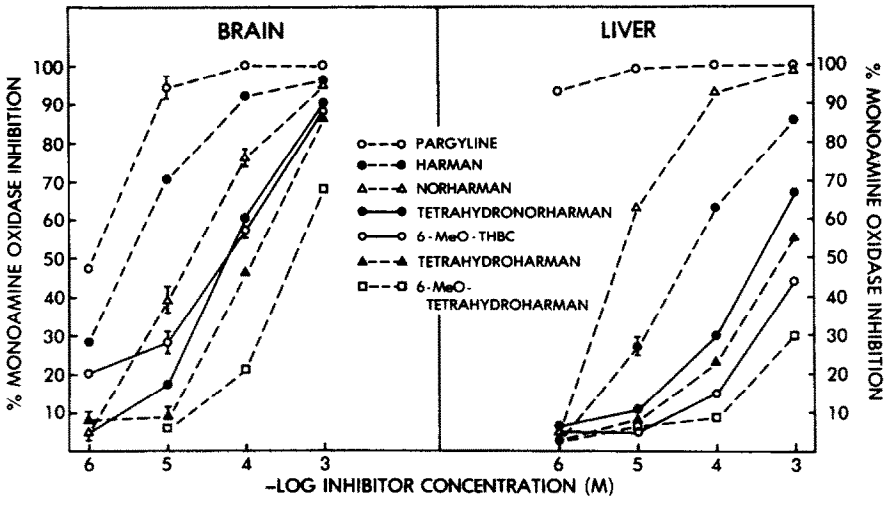

Fig. 2. Effects of various drugs on MAO activity in mouse brain and liver. MAO activity was measured according to Nagatsu [11] with tryptamine as substrate as described in the text. Each point represents the mean of four determinations and bars represent ± S.E. S.E.'s less than 1.0% are not shown. The mean ± S.E. control values for MAO activity were 0.0598 ± 0.0027 nmoles ^{14}C metabolites/min/mg brain, and 0.0959 ± 0.0073 nmoles ^{14}C metabolites/min/mg liver.

tion of a methoxy group at C-6 (6-MeO-THBC) produces no further reduction.

Harmine and harmaline were the most potent inhibitors of brain MAO of all the β-carbolines tested and pargyline, a non β-carboline MAO inhibitor used for comparison, showed a degree of inhibition similar to that of harman. With respect to C-6 vs C-7 substitution, a methoxy group at C-7 led to greater MAO inhibition than a methoxy group at C-6, regardless of the degree of ring saturation (e.g. harmine vs 6-MeO-harman, harmaline vs 6-MeO-harmalan, and tetrahydroharmine vs 6-MeO-tetrahydroharman).

Table 1. *In vitro* inhibition by drugs of the deamination of tryptamine by mouse brain MAO

Drug	EC_{50} (M)
Harman	3.3×10^{-6}
6-MeO-Harman	3.1×10^{-6}
6-MeO-Harmalan	1.8×10^{-5}
Tetrahydroharman	1.2×10^{-4}
6-MeO-Tetrahydroharman	4.2×10^{-4}
6-OH-Tetrahydroharman	9.1×10^{-4}
Norharman	2.0×10^{-5}
Tetrahydronorharman	5.8×10^{-5}
6-MeO-THBC	5.8×10^{-5}
Harmine	8.0×10^{-8}
Harmaline	6.0×10^{-8}
Tetrahydroharmine	1.4×10^{-5}
Harmol	5.8×10^{-6}
Harmalol	1.0×10^{-5}
Tetrahydroharmol	1.0×10^{-5}
Pargyline	1.1×10^{-6}

Data (EC_{50}) are expressed as concentration of drug which inhibited MAO activity by 50 per cent as determined from semi-log plots of inhibitor concentration vs MAO inhibition. Assays were performed according to Nagatsu [11] with tryptamine as substrate as described in the text. Each value represents the mean of four determinations. The mean ± S.E. control value for MAO activity was 0.0598 ± 0.0027 nmoles ^{14}C metabolites/min/mg brain.

Substrate specificity. Plots of per cent brain MAO inhibition vs inhibitor concentrations with either 5-HT or PEA as substrates are shown in Fig. 3. The degree of MAO inhibition depended on the substrate. Clorgyline, harmaline and 6-MeO-THBC were more potent inhibitors of MAO with 5-HT as substrate than with PEA. In addition, 6-MeO-THBC showed greater MAO inhibition with 5-HT than with tryptamine (Fig. 2) as substrate. On the contrary, deprenyl and pargyline were more potent inhibitors with PEA than with 5-HT.

In vivo *studies.* With 5-HT as substrate, the MAO inhibition produced by i.p. injection of 6-MeO-THBC (100 mg/kg) was significant by 15 min, approached a maximum of about 27 per cent by 1 hr, stayed at this level from 1 to 4 hr and declined by 24 hr. Clorgyline, on the other hand, reached a 91 per cent level of inhibition by 15 min and was still at a very high level (80 per cent) at 24 hr after injection (Fig. 4). A determination of substrate specificity with 5-HT and PEA as substrates after i.p. drug administration showed that 6-MeO-THBC and harmaline produced greater inhibition with 5-HT, and deprenyl produced greater inhibition with PEA (Table 2).

DISCUSSION

Structure-activity studies. (1) Methyl group at C-1. We confirmed the data of Ho *et al.* [6] that placement of a methyl group on C-1 of tetrahydronorharman, 6-MeO-THBC and norharman decreased the ability to inhibit MAO from liver. The same is true for brain except that harman was a more potent inhibitor than norharman. The reason for this reversal is not known.

(2) Saturation. In general, the aromatic compounds are more potent inhibitors of brain MAO than are the tetrahydro compounds, with the dihydro compounds being intermediate (Fig. 2, Table 1). This trend has been reported previously for MAO from liver [6, 8]. There was some inconsistency in the order

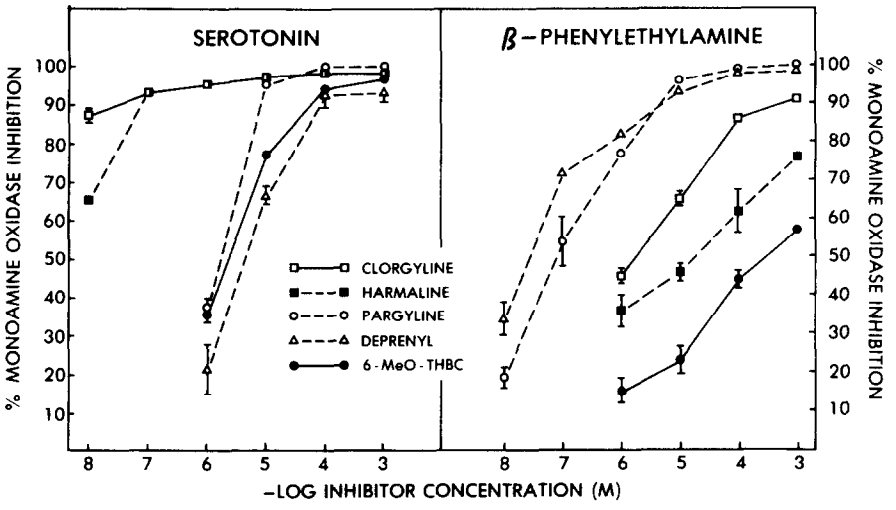

Fig. 3. Effects of various drugs on MAO activity in mouse brain with serotonin or β-phenylethylamine as substrate. MAO activity was measured according to Robinson *et al.* [12] as described in the text. Each point represents the mean of four determinations and bars represent ± S.E. S.E.'s less than 1.0% are not shown. The mean ± S.E. control values for MAO activity were 0.0708 ± 0.0025 nmoles ^{14}C metabolites/min/mg brain for serotonin and 0.0394 ± 0.0018 nmoles ^{14}C metabolites/min/mg brain for β-phenylethylamine.

of MAO inhibitory potency in both brain and liver. For example, in brain harmine and harmaline were not very different in MAO inhibitory potency (Table 1; [7, 13]); harmol was more potent than harmalol which was not different from tetrahydroharmol (Table 1), although Ozaki *et al.* [14] reported a difference between the latter two. Pletscher *et al.* [13] found no difference between harmol and harmalol.

(3) Substitution at C-6. McIsaac and Estevez [8] reported that in liver introduction of a hydroxyl group at C-6 decreased potency. We found this same general relationship in brain (Table 1). Introduction of a methoxy group at C-6 of β-carbolines also decreased the ability of the compounds to inhibit liver MAO (Fig. 2; [6, 8]). In brain, however, the relationship was not so clear (Fig. 2, Table 1). For example, although 6-MeO-tetrahydroharman was less potent than tetrahydroharman, 6-MeO-THBC was equipotent with tetrahydronorharman and 6-MeO-harman was equipotent with harman.

(4) Substitution at C-7. The present data from brain (Table 1) showed that methoxy substitution at C-7 produced a more potent MAO inhibitor (harmine) than the unsubstituted compound (harman) or the compound with C-6 substitution (6-MeO-harman). Previous studies using liver have reported the same [7, 14] or opposite [8] results.

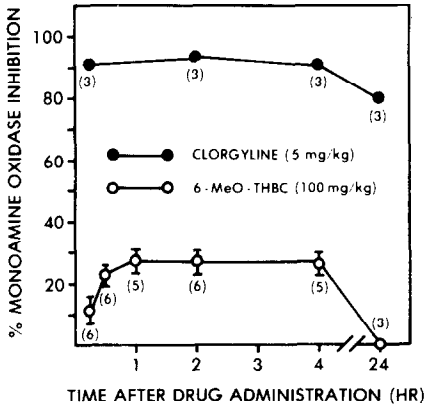

Fig. 4. Inhibition of mouse brain MAO at various times after i.p. injection of 6-MeO-THBC (100 mg/kg) or clorgyline (5 mg/kg). MAO activity was measured according to Robinson *et al.* [12] with serotonin as substrate as described in the text. Values are means ± S.E. from 3–6 separate determinations (number of determinations given in parentheses). S.E.'s less than 2.0% are not shown. The mean ± S.E. control value for MAO activity was 0.0413 ± 0.0014 nmoles ^{14}C metabolites/min/mg brain.

Liver vs brain. With the exception of norharman, the β-carbolines produced greater inhibition of brain MAO than of liver MAO. Other investigators have shown the same pattern for harmine or harmaline, i.e., greater inhibition of MAO in brain than in liver [15, 16]. These tissue differences in MAO-inhibiting properties of drugs may be the result of (1). differences between brain and liver in the forms of MAO; (2). differences between brain and liver in the localization or proportions of MAO's and/or differences in the amount of drug reaching sites critical for MAO inhibition in the *in vivo* studies. Although there is as yet no definitive information for these speculations with respect to β-carbolines, Hartman and Udenfriend [17], using immunological techniques, have found a bovine brain mitochondrial MAO that is not present in liver mitochondria.

Substrate specificity. Tryptamine is a common substrate for MAO types A and B and we considered that previous studies with tryptamine as the substrate [4] may not have have measured the full potential of the MAO inhibiting property of 6-MeO-THBC. Thus, we determined the MAO inhibitor effects of 6-MeO-THBC *in vitro* with 5-HT and PEA as substrates. 6-MeO-THBC was compared to clorgy-

Table 2. Apparent *in vivo* inhibition of the deamination of serotonin and β-phenylethylamine by mouse brain MAO

Drug	Dose (mg/kg)	Per cent MAO inhibition Substrate: Serotonin	Per cent MAO inhibition Substrate: β-Phenylethylamine
6-MeO-THBC	100	22.5 ± 3.0	13.4 ± 3.2
Deprenyl	5	17.2 ± 2.9	65.8 ± 1.9
Harmaline	5	54.7 ± 1.2	38.0 ± 3.2

Mice were killed 2 hr after the i.p. injection of the drugs (doses given as salts). MAO activity was measured according to Robinson *et al.* [12] as described in the text. Numbers in the table are mean values ± S.E. from 3–6 separate determinations. The mean ± S.E. control values for MAO activity were 0.0413 ± 0.0014 nmoles ^{14}C metabolites/min/mg brain for serotonin and 0.0376 ± 0.0017 nmoles ^{14}C metabolites/min/mg brain for β-phenylethylamine.

line and harmaline (relatively selective inhibitors of MAO type A) and deprenyl and pargyline (relatively selective inhibitors of MAO type B) [9]. 6-MeO-THBC produced the same pattern of inhibition as clorgyline and harmaline and may be a relatively selective inhibitor of MAO type A. We found the same patterns of substrate specificity for clorgyline, deprenyl, harmaline, and pargyline as have been reported previously in brain [16, 18–21]. The magnitude of the MAO inhibition by clorgyline (Fig. 3) was quite large even at 10^{-8} M concentration (i.e. about 88 per cent). Some studies have reported inhibition to decline between 10^{-7} and 10^{-8} M so that inhibition is low at 10^{-8} M (e.g. 20 per cent [19]; 10 per cent [20]), but other studies have reported higher values for MAO inhibition at 10^{-8} M (e.g. 70 per cent [18]; 60 per cent [22]). Our results with deprenyl (i.e., about 70 per cent inhibition at 10^{-5} M with PEA) are comparable to those of Meyerson *et al.* [20] with benzylamine as substrate.

In vivo *studies.* The fact that we measured greater MAO inhibition *in vitro* with 5-HT as substrate than with tryptamine led us to reevaluate our previous *in vivo* results [4] with only tryptamine as substrate. We did find (Fig. 4) greater inhibition at 2 hr with 5-HT as substrate (26 per cent) than we found previously [4] with tryptamine (8.5 per cent). In contrast to 6-MeO-THBC, the inhibition produced by clorgyline rose faster and remained higher for a longer time. This type of rapid, sustained MAO inhibition with clorgyline has been reported previously [21, 23]. We also examined the *in vivo* substrate specificity by comparing 5-HT and PEA substrates using the inhibitors 6-MeO-THBC, deprenyl, and harmaline (Table 2). The *in vivo* specificity paralleled that *in vitro*, with 6-MeO-THBC and harmaline showing greater inhibition with 5-HT and deprenyl showing more inhibition with PEA. Fuentes and Neff [24] have reported similar results after examining the *in vivo* inhibition of rat brain MAO produced by deprenyl, harmaline, and other compounds with 5-HT and PEA as substrates.

General remarks. We demonstrated structure-activity relationships in brain for the inhibition of MAO by β-carbolines which, with some exceptions, paralleled those previously reported for liver. Our special interest in 6-MeO-THBC led us to investigate substrate-specific MAO inhibition produced by this compound, and we did find greater inhibition with 5-HT as substrate. Previous work by ourselves [4] and others [5, 6] with tryptamine, a substrate common to MAO types A and B, may have underestimated the MAO inhibitory potency of 6-MeO-THBC. This MAO inhibition in combination with inhibition of 5-HT uptake in brain [4, 25], thereby interfering with access to MAO, could account for at least part of the increase in brain 5-HT concentration produced by 6-MeO-THBC [1] and might also be involved in the behavioral effects of 6-MeO-THBC [2, 3]. It should be noted, however, that inhibition of 5-HT uptake occurs at lower concentrations (EC50 for 5-HT uptake was 6.3×10^{-7} M [4]) than does inhibition of MAO (even with 5-HT as substrate) and is thus more likely to have physiological significance.

A great deal of recent interest in the tetrahydro-β-carbolines has been generated by reports that enzymatic preparations from brain can convert indoleamines to tetrahydro-β-carbolines in the presence of 5-methyltetrahydrofolic acid [26–31]. The possibility exists that these compounds could be formed *in vivo*, and evidence for *in vivo* formation of 6-MeO-THBC in rat hypothalamus has recently been reported [32]. Perhaps 6-MeO-THBC as well as other β-carbolines could be neuroregulatory agents through their effects on uptake and MAO.

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