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SHORT COMMUNICATION

Effects of tetrahydro-β-carbolines on monoamine oxidase and serotonin uptake in mouse brain

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Although it has been known for some time that β-carboline compounds are constituents of hallucinogenic plants [1], these compounds have attracted interest recently because of the reports that enzymes are present in brain which can form β-carbolines *in vitro* [2–6]. Therefore, the possibility exists that β-carbolines may be formed *in vivo*. This is particularly interesting since McIsaac *et al.* [7] reported that 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC; this compound could also be named 6-methoxy-tetrahydro-norharman) almost doubled brain serotonin (5-hydroxytryptamine, 5-HT) without affecting norepinephrine or 5-hydroxyindoleacetic acid (5-HIAA). We have verified the 5-HT elevation after 6-MeO-THBC [8, 9]. In view of the fact that 5-HT is involved in many neurobiological processes [10], this interaction between 6-MeO-THBC and 5-HT takes on added significance.

6-MeO-THBC could affect brain 5-HT several other ways, e.g. increased synthesis, decreased degradation, decreased reuptake, or increased storage of 5-HT. These may take place via membrane or enzymatic processes and/or increased availability of substrates. For example, other β-carbolines such as harman and harmine have been shown to be reversible inhibitors of monoamine oxidase (MAO) [11], which appears to be dependent upon structure [12]. Ho *et al.* [13] reported that 6-MeO-THBC was only a very weak inhibitor of liver MAO *in vitro*, and later Ho, *et al.* [14] reported no effect of 6-MeO-THBC on brain MAO *in vivo*. We, however, had preliminary evidence using the kynuramine disappearance assay [15] that 6-MeO-THBC did inhibit brain MAO *in vitro*. Because of these apparently conflicting data, we decided to investigate further the effects of 6-MeO-THBC on MAO activity. As part of our more general study of 6-MeO-THBC on the 5-HT system we also determined its action on 5-HT uptake.

Female CFl mice (Carworth, New City, N.Y.), 60 to 90-days-old, were used. The hydrochloride form of 6-MeO-THBC was synthesized according to the method of Ho

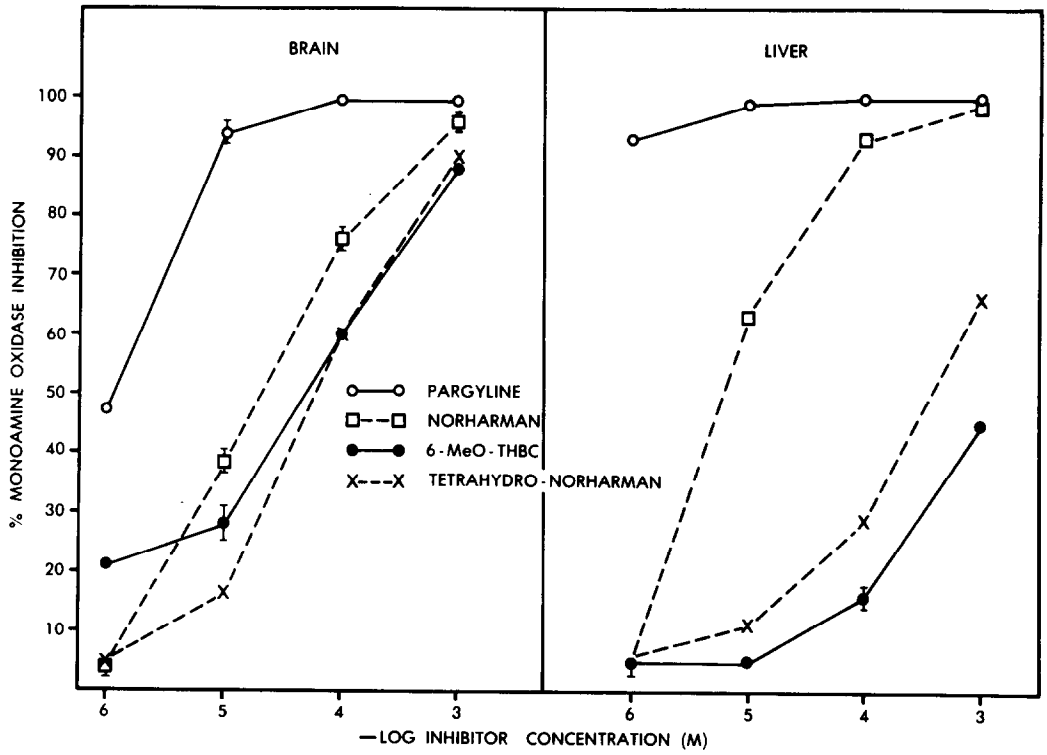

Fig. 1. Effect of various concentrations of drugs on monoamine oxidase activity in brain and liver of CFl mice. Each point represents the mean of four or five experiments, and bars represent ± S. E. M. Standard errors less than 1.0 are not given. Incubation was carried out as described in the text. The mean ± S. E. M. control values for MAO activity were 0.0642 ± 0.0008 nmole ^{14}C-metabolites/min/mg of brain and 0.1095 ± 0.0086 nmole ^{14}C-metabolites/min/mg of liver.

et al. [13], and the purity was checked by melting point determination, elemental analysis, and thin-layer chromatography. Other drugs used were pargyline HCl (gift of Abbott Laboratories), imipramine and chlorimipramine (gift of GEIGY Pharmaceuticals), Lilly 110140 (gift of Lilly Research Laboratories), norharman HCl (Sigma), and tetrahydro-norharman HCl (noreleagnine HCl, Sigma).

MAO was assayed in fresh whole brain and liver by the method of Wurtman and Axelrod [16] as described by Nagatsu [17] using [^{14}C]tryptamine bisuccinate (47.0 mCi/m-mole, New England Nuclear). Tissue was homogenized in 0.15 M KCl and an aliquot containing 1.0 mg tissue was used per tube. The drugs (concentrations given as free base) were preincubated with the incubation mixture (total volume = 0.3 ml) for 10 min at 37°, the isotopic compound was added, incubation was continued for 20 min, and the reaction was stopped with 2 N HCl. Six ml of toluene was added, the tube was shaken and centrifuged, 3 ml of the toluene layer was added to 10 ml of the scintillation mixture, and radioactivity was measured.

The uptake of [^{3}H]5-HT creatinine sulfate (16 Ci/m-mole, Amersham/Searle) into a synaptosomal suspension from whole brain was determined by the method of Kuhar, *et al.* [18]. The drugs were preincubated in the incubation medium (total vol. = 4.0 ml) containing 0.3 ml of synaptosomal suspension for 5 min at 37°, the isotopic compound was added, and the incubation was continued for 4 min. The assay was linear with time and tissue concentration. The reaction was stopped by placing the tubes in an ice water bath. The tubes were centrifuged, incubation medium was aspirated, pellets were solubilized, transferred to scintillation vials, and radioactivity was counted after adding scintillation fluid. The concentration of [^{3}H]5-HT in the reaction mixture was 3.0×10^{-9} M.

The data *in vitro* for MAO (Fig. 1) show that pargyline, a known MAO inhibitor, inhibits MAO in both brain and liver to a greater extent than any of the β-carbolines. In the β-carboline group, the tetrahydro-β-carbolines are more effective inhibitors in brain than in liver, whereas the reverse is true for norharman. In general these structure activity data support the results *in vitro* of McIsaac and Estevez [12]. The present data show that this structure–activity relationship is more pronounced in liver than in brain. Furthermore, Ho *et al.* [13] had reported that, in liver, the methoxy group decreased the inhibitory activity of β-carbolines. The present study suggests this is also somewhat more pronounced in liver than in brain.

Table 1. Per cent inhibition of monoamine oxidase activity*

	Brain	Liver
Pargyline	96.8 ± 1.3	98.2 ± 0.25
Tetrahydro-norharman	6.8 ± 2.7	4.0 ± 2.8
6-MeO-THBC	8.5 ± 1.7	0.0 ± 0.0

* Per cent inhibition of monoamine oxidase activity in CFl mice 2 hr after an intraperitoneal injection of 100 mg/kg of either pargyline HCl, tetrahydro-norharman HCl or 6-MeO-THBC HCl. Incubation was carried out as described in the text. Numbers in the table are mean values ± S. E. M. from four experiments. The mean ± S. E. M. control values for MAO activity are 0.0474 ± 0.0027 nmole ^{14}C-metabolites/min/mg of brain and 0.0690 ± 0.0031 nmole ^{14}C-metabolites/min/mg of liver.

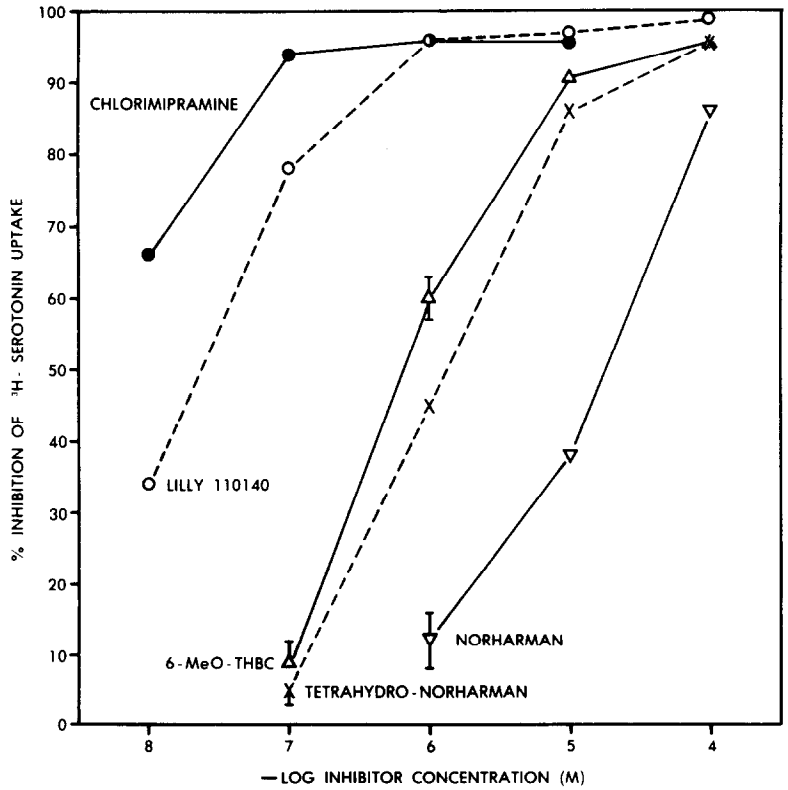

Fig. 2. Effect of various concentrations of drugs on inhibition of [^{3}H]serotonin uptake into a synaptosomal suspension from mouse brain. Each point represents the mean of four to five experiments, and bars represent ± S. E. M. Standard errors less than 1.0 are not given. Incubation was carried out as described in the text. The mean ± S. E. M. control value of [^{3}H]serotonin uptake was $1183 \pm 50 \times 10^{-15}$ mole/4 min/2.1 mg of protein.

In a separate experiment, 6-MeO-THBC, tetrahydronorharman, or pargyline (100 mg/kg of HCl salt, 0.02 ml/g) or 0.9% saline (0.02 ml/g) was injected intraperitoneally into mice. They were killed 2 hr later, and MAO was measured as above but without the preincubation. In both brain and liver, pargyline produced almost complete inhibition of MAO whereas the inhibition produced by 6-MeO-THBC and tetrahydronorharman was less than 9 per cent (Table 1). It remains to be determined whether these compounds inhibit one or all of the multiple forms of MAO which have been reported to exist [19]. Further structure–activity relationships on the effects of these drugs also need elucidating.

The effects of the various drugs under investigation on the uptake of [^{3}H]5-HT are shown in Fig. 2. Tetrahydronorharman and 6-MeO-THBC inhibit [^{3}H]5-HT uptake 45 and 60 per cent respectively, at 10^{-6} M. This is less than the inhibition produced by the known uptake inhibitors Lilly 110140 and chlorimipramine but more than that for norharman. In a separate experiment, 6-MeO-THBC (100 mg/kg of HCl salt, 5 mg/ml, 0.02 ml/g) or 0.9% saline (0.02 mg/g) was injected 2 hr before mice were killed and the brains were removed and assayed for [^{3}H]5-HT uptake as described above but without preincubation. The mean ± S. E. inhibition of uptake in animals injected with 6-MeO-THBC was 83.4 ± 1.6 per cent (the mean ± S.E. control value of [^{3}H]serotonin uptake was 1128 ± 43×10^{-15} mole/4 min/2.1 mg of protein). Although we are not presently able to assay the concentration of 6-MeO-THBC in brain after injection, the result of this experiment *in vivo* suggests that 6-MeO-THBC gets into brain at a concentration high enough to produce inhibition of uptake. Characterization of the type of inhibition of uptake (competitive or noncompetitive) has not yet been determined. Inhibition of 5-HT uptake into synaptosomes by the β-carbolines tetrahydroharman and 6-hydroxytetrahydroharman has been reported by Tuomisto and Tuomisto [20], but they did not use any norharman or tetrahydro-norharman compounds.

In summary, our data show that the tetrahydro-β-carbolines, 6-MeO-THBC and tetrahydro-norharman, produce inhibition of MAO *in vitro* and inhibition of 5-HT uptake *in vitro* and *in vivo*. The uptake inhibition takes place at a lower concentration than MAO inhibition and thus seems to be the stronger effect. Whether the uptake inhibition alone or in combination with MAO inhibition could produce the elevated levels of brain 5-HT reported after injection of 6-MeO-THBC is not clear. An increase in brain 5-HT concentration after the 5-HT uptake blocker chlorimipramine has been reported by Halaris *et al.* [21]. It is also of interest that they found that chlorimipramine inhibited brain MAO *in vitro* (30–40 per cent at 10^{-4} M) but not *in vivo*. Ho *et al.* [22] have suggested that the elevation in 5-HT after 6-MeO-THBC may be the result of increased activity of peripheral 5-hydroxytryptophan decarboxylase. This could enhance the formation of plasma 5-HT which, if increased sufficiently, might cause a concentration gradient from the blood to the brain tissue.

The present data do confirm the interaction of 5-HT with β-carbolines and would take on added significance if β-carbolines were to be formed *in vivo*. For example, they could act as neuroregulatory agents modulating the metabolism of endogenous 5-HT. As was mentioned above, many of the β-carbolines are hallucinogenic [1], and it is of interest that LSD, a powerful hallucinogen, also affects the metabolism of 5-HT [23].

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