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Effects of 6-methoxytetrahydro-β-carboline on 5-hydroxytryptamine binding in rat brain

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6-MeOTHBC competes for 5-HT binding sites in rat brain in-vitro and in-vivo. The β-carboline is significantly more active at the type 1 [^{3}H]-5-HT, than the type 2 [^{3}H]spiperone receptors, in-vitro. Following injection, 6-MeOTHBC significantly decreases [^{3}H]-5-HT binding in the cortex. The ineffectiveness on [^{3}H]spiperone binding in-vivo corresponds with the low affinity in-vitro. The effect of 6-MeOTHBC on central 5-HT binding may be a significant aspect of its 5-HT-ergic activity.

A decade ago the compound 6-methoxytetrahydro-β-carboline (6-MeOTHBC) (I) was found in our laboratory to double the 5-hydroxytryptamine (5-HT) concentration in rodent brain without affecting levels of either the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), or noradrenaline (McIsaac et al 1972). Subsequently, 6-MeOTHBC was shown to inhibit MAO, especially type A (Meller et al 1977), and to inhibit 5-HT uptake into synaptosomes (Buckholtz & Boggan 1977); therefore, either of these actions could lead to the observed increase in central 5-HT concentrations.

I

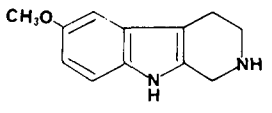

FIG. 1. Chemical structure of 6-methoxytetrahydro-β-carboline.

* Correspondence.

The stimulus properties of 6-MeOTHBC show some similarity to those of lysergic acid diethylamide (LSD) (White et al 1982) which, in turn, are mediated at least in part by activity at 5-HT receptors. Because 6-MeOTHBC selectively affects 5-HT neurotransmission, has an LSD-like behavioural effect, and contains an indole nucleus, it seemed worthwhile to examine its 5-HT receptor binding properties. In the work presented here, we tested the ability of 6-MeOTHBC to compete for 5-HT receptors as defined by [^{3}H]5-HT and [^{3}H]spiperone binding (Peroutka & Snyder 1979).

Methods

Male Sprague-Dawley rats (220–240 g) were used. The hydrochloride form of 6-MeOTHBC was synthesized in our laboratory as previously described (Ho et al 1968). [^{3}H]5-Hydroxytryptamine creatinine sulphate (28·7 Ci mmol^{-1}) and [^{3}H]spiperone (29·9 Ci mmol^{-1}) were obtained from New England Nuclear. Rats were decapitated, the cortex and striatum quickly removed, frozen on dry ice and stored at −80 °C until assayed (within 1–2 weeks). For the in-vivo experiments, groups of 6 rats were injected with 50 mg kg^{-1} i.p. of 6-MeOTHBC or 0·9% NaCl (saline) and killed 2 h later. The cortex was removed and frozen as above.

To measure 5-HT type 1 receptor binding (Peroutka & Snyder 1979), tissues were homogenized in 40 volumes of 0·05 M Tris-HCl buffer, pH 7·7, with a Brinkman Polytron, centrifuged at 48 000*g* for 10 min, and washed once with fresh buffer. Pellets were then

Table 1. Effect of 6-MeOTHBC on 5-HT binding in-vitro in two rat brain areas. Inhibition of binding was measured using drug concentrations ranging from 10^{-9} to 10^{-4} M. The concentration of the ligands were 4 nM[^{3}H]5-HT and 0·4 nM-[^{3}H]spiperone. Specific binding was defined as the excess over blanks taken in the presence of 8 μM and 1 μM D-LSD, respectively. IC50 values were derived by Log-probit analysis; 95% confidence intervals given in parentheses. Tissues were pooled from 8–9 rats for the determination of both 5-HT type 1 and 5-HT type 2 receptor binding in the two brain areas.

	IC50 (M)	
Brain area	5-HT$_1$ ([^{3}H]-5-HT)	5-HT$_2$ ([^{3}H]-spiperone)
Cortex	$3{\cdot}9 \times 10^{-7}$ ($2{\cdot}6 \times 10^{-6}$–$6{\cdot}0 \times 10^{-8}$)	$2{\cdot}1 \times 10^{-5}$ ($1{\cdot}3 \times 10^{-5}$–$3{\cdot}5 \times 10^{-5}$)
Striatum	$9{\cdot}2 \times 10^{-7}$ ($8{\cdot}6 \times 10^{-6}$–$9{\cdot}8 \times 10^{-8}$)	$1{\cdot}0 \times 10^{-4}$ ($1{\cdot}6 \times 10^{-4}$–$6{\cdot}7 \times 10^{-5}$)

resuspended in 100 volumes 0·05 M Tris-HCl containing 4 mM $CaCl_2$, 1 μM pargyline and 0·1% ascorbic acid. Following a 10 min preincubation at 37 °C, 2 ml aliquots were incubated with [^{3}H]5-HT (4 nM) for 10 min at 37 °C. Samples were rapidly filtered through Whatman GF/B glass filters and the tubes and filters rinsed 3 times with ice cold Tris buffer. Non-specific binding was determined in the presence of 8 μM unlabelled D-LSD and was subtracted from all values. To determine the IC50, 6-MeOTHBC (10^{-9} to 10^{-4} M) was added before the ligand. All assays were run in triplicate. For the in-vivo studies, triplicate assays were done on cortex from 6 controls and 6 drug-treated animals. Binding values are expressed as pmol g^{-1} tissue.

Tissues for [^{3}H]spiperone 5-HT type 2 receptor binding (Peroutka & Snyder 1979) were homogenized in 10 volumes of 0·05 M Tris-HCl, pH 7·5. Pellets were resuspended a third time in 40 volumes of Tris-HCl, pH 7·5, incubated for 10 min at 37 °C and centrifuged. The final pellet was taken up in 100 volumes of Tris-HCl, pH 7·7, containing $CaCl_2$, pargyline and ascorbic acid. The homogenates were pre-incubated for 15 min, then cooled on ice another 15 min. Aliquots of 1 ml were incubated with [^{3}H]spiperone (0·4 nM) for 15 min at 37 °C. Blanks for non-specific binding contained 1 μM of D-LSD. Filtration and washing were the same as for ^{3}H-5-HT binding.

In order to compare values obtained by us with those published by others (Peroutka et al 1981) IC50 values for methysergide, a 5-HT antagonist, were also determined under our assay conditions.

Results

The IC50 values for 6-MeOTHBC in two brain areas are shown in Table 1. The β-carboline is much more active in displacing [^{3}H]5-HT in-vitro than [^{3}H]spiperone. Somewhat higher concentrations of 6-MeOTHBC were required to produce 50% inhibition of [^{3}H]5-HT and [^{3}H]spiperone binding in striatum than in cortex.

The IC50 values for methysergide were 100 nM for type 1, and 4 nM for type 2 receptors using our assay methods. This is in agreement with the values reported by Peroutka et al (1981) for methysergide. It and several other 5-HT antagonists all have a higher affinity for the type 2 receptors.

Table 2. Effect of an acute injection of 6-MeOTHBC on 5-HT binding in the cortex. Rats 220–240 g were injected i.p. with 50 mg kg^{-1} of 6-MeOTHBC or saline, and killed 2 h later. Specific binding was determined using the same concentration of ligands and D-LSD blanks as in Table 1. Each value is the mean ± s.e.m. from six rats. All assays were run in triplicate. Student's *t*-test was used for statistical analysis.

	pmol g^{-1}	
	[^{3}H]5-HT	[^{3}H]Spiperone
Controls	5·72 ± 0·54	5·79 ± 0·49
6-MeOTHBC	3·00 ± 0·19* (−47·5%)	5·31 ± 0·24 (−8·3%)

* Significant effect of 6-MeOTHBC ($P < 0{\cdot}01$).

Following injection of 6-MeOTHBC, there is a significant decrease (−47·5%) in [^{3}H]5-HT binding in the cortex (Table 2). The insignificant effect on [^{3}H]spiperone binding is in agreement with the low affinity showed in-vitro. The decrease in binding to 5-HT type 1 receptors following an acute injection is apparently due to the drug itself occupying some of the receptors. At 2 h when the elevation of brain 5-HT is at its peak, unchanged [1-^{14}C]6-MeOTHBC accounts for 81 to 84% of total radioactivity in brain (Ho et al 1972). The inhibitory effect in-vitro is consistent with a direct drug action on 5-HT binding sites.

Discussion

There is evidence that β-carbolines may be endogenous to brain and other tissues (Shoemaker et al 1978; Barker et al 1979; Rommelspacher et al 1979). Previous studies indicate that β-carbolines act selectively to increase 5-HT-ergic function (McIsaac et al 1972; Buckholtz & Boggan 1976, 1977; Meller et al 1977; Rommelspacher & Subramanian 1979). In contrast to an earlier report (McIsaac et al 1972), Buckholtz (1980), recently reported that 6-MeOTHBC did decrease 5-HIAA in mouse brain after treatment with 12·5 to 100 mg kg^{-1}. Increased 5-HT and decreased 5-HIAA concentrations have been associated with agonist activity at 5-HT receptors and with hallucinogenesis (Rosecrans et al 1967; Brawley & Duffield 1972). Additionally, it has been reported that 6-MeOTHBC has the ability to mimic the stimulus properties of D-LSD (White et al 1982). The present study demonstrates that 6-MeOTHBC competes with 5-HT at central binding sites, possibly an important aspect of its 5-HT activity.

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Repeated treatment with antidepressant drugs potentiates the locomotor response to (+)-amphetamine

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The therapeutic effect of antidepressant drugs only becomes apparent after about two weeks of treatment. We have found previously that various antidepressants —amitriptyline, imipramine, iprindole, maprotiline, mianserin, nisoxetine, zimelidine, as well as thioridazine and levomepromazine—given chronically but not acutely, potentiate the clonidine-induced aggressiveness in mice, probably via increased reponsiveness of the α_1-adrenergic system (Maj et al 1980, 1981, 1982b). We have also demonstrated that in mice treated chronically (but not acutely) with antidepressants, reserpine stimulates the locomotor activity immediately after its administration (in the phase of amine release) (Maj et al 1983). These results may indicate that noradrenaline (NA] as well as dopamine (DA), i.e. the increased responsiveness of DA system, may be involved in the effects observed after prolonged administration of the antidepressants. Hence, we have examined how given chronically they affect the action of (+)-amphetamine on locomotor activity in mice. According to Spyraki & Fibiger (1981), desipramine given chronically enhances the locomotor hyperactivity induced by (+)-amphetamine or apomorphine. Recently, repeated treatment with citalopram has been found to potentiate the locomotor response to (+)-amphetamine in rats (Hyttel, personal communication).

The antidepressants we tested were chosen for their various pharmacological profiles: imipramine and amitriptyline block the neuronal uptake of NA and 5-HT; maprolitine and (+)-oxaprotiline are selective NA uptake inhibitors (Maître et al 1971; Mishra et al 1981; Delini-Stula et al 1982); (−)-oxaprotiline does not inhibit NA uptake (Mishra et al 1981); zimelidine inhibits 5-HT uptake and, at higher doses, NA uptake also (Ross et al 1981); citalopram and fluvoxamine are selective 5-HT uptake inhibitors (Christensen et al 1977; Claassen et al 1977; Pawłowski et al 1981; Maj et al 1982a); mianserin and iprindole, both atypical, do not inhibit the neuronal uptake of amines (Gluckman & Baum 1969; van Riezen et al 1981). For comparison, cocaine, a monoamine uptake inhibitor, and phentolamine, a NA antagonist, were also studied.

According to Spyraki & Fibiger (1981), chronic desipramine potentiates the locomotor reponse to (+)-amphetamine and apomorphine given in a narrow (low) dose range. We had observed that, given chronically, imipramine and citalopram potentiate the locomotor effect of low doses of (+)-amphetamine, therefore, in

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