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BEHAVIORAL AND BIOCHEMICAL EVIDENCE FOR SEROTONERGIC ACTIONS OF TETRAHYDRO-β-CARBOLINES

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Summary

In two groups of rats trained to discriminate LSD (0.1 mg/kg or 0.24 mg/kg) from saline, tetrahydro-β-carboline (THBC; 1-12 mg/kg as free base) and its derivative 6-methoxy-THBC (1-12 mg/kg as free base) substituted partially for LSD. The substitution of THBC for 0.1 mg/kg of LSD was analyzed further with antagonism tests in 16 animals and was attenuated by the serotonin (5-HT) antagonist BC-105 (pizotifen; 3 mg/kg) but not by the dopamine (DA) antagonist haloperidol (0.1 mg/kg). It was abolished by pretreatment with the 5-HT synthesis inhibitor p-chlorophenylalanine (100 mg/kg/day for 3 days). In addition, THBC was found to inhibit ^{3}H-LSD binding to homogenates of rat frontal cortex with an IC_{50} value of 4 μM which is similar to that previously reported for other 5-HT agonists. These data indicate that THBCs exert potent 5-HT agonist actions. Since THBCs have recently been found in mammalian brain and other tissues, the present results are of interest in relation to a possible role of these substances in endogenous psychosis.

β-Carbolines are known to affect several neurotransmitter systems in the brain; that is, depending on the particular structure of the β-carboline molecule these compounds interact preferentially with acetylcholine, benzodiazepine (BZD), dopamine (DA), opiate or serotonin (5-HT) receptors (1-3). A subclass of the β-carbolines, the 1,2,3,4-tetrahydro-β-carbolines (THBCs; also referred to as tetrahydroisoquinolines, tetrahydronorharmans or tryptolines) have been identified as constituents of the active substances found in various plants used by South American Indians for their psychotomimetic and hallucinogenic properties (1). Recently, THBCs have been discovered in mammalian brain and other tissues (4-7); this has lead to speculations about a possible role for these substances in human psychopathology (1, 8-10). There is growing evidence that THBC as well as 6-methoxy-THBC act principally through 5-HT mechanisms (3, 11-16).

In recent years, drug discrimination procedures have been used with considerable success to evaluate the in vivo mechanisms of action of many psychoactive compounds (17-19). Subtle behavioral effects can be detected with this method thereby providing a means of elucidating the unique pharmacology of a drug or of differences among drugs, in cases where other methods have been inconclusive (20). The drug discrimination procedure typically involves training

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subjects to respond on a designated manipulandum following administration of the drug in question (the training drug) and on another manipulandum following the drug vehicle (or another drug). Eventually, the choice behavior of the subject is controlled by the effect of the drug (as it is perceived by the subject) in the absence of any other cue. Moreover, since drug cues have been shown to have a high degree of pharmacological specificity, especially when combined with extensive pharmacological manipulations (e.g., antagonism testing) (17-19), they may provide an accurate estimate of relevant, in vivo mechanisms.

In view of the possible psychotomimetic effects of THBCs and the likelihood that these compounds are serotonergic, the present experiments were designed to determine the ability of THBC and 6-MeO-THBC to mimic the discriminative stimulus properties of the potent hallucinogen d-lysergic acid diethylamide (LSD) which are mediated specifically by 5-HT neuronal systems (21-24). In addition, the ability of THBC to displace ^{3}H-LSD binding to homogenates of rat frontal cortex was investigated.

Methods

Thirty-two male Sprague-Dawley rats weighing 350-400 grams were used. The animals were housed individually in a room of constant temperature (21-23°C) and relative humidity (40-60%). Room lights were on from 0700 to 1900 hours; food and water were available ad libitum, except as noted (below).

LSD-discrimination procedure. Details of these methods have been described elsewhere (23-26). Eight commercially-available operant chambers for rodents (BRS/LVE Model 143-24), equipped with two levers and a dipper, located equidistant from the two levers, were used. The animals were deprived to 80-85% of their free-feeding body weight, by restricting water intake to that received in the chamber, and trained to respond under a fixed ratio (FR) schedule of 20 responses for water reinforcers (0.1 ml). Following an intraperitoneal (i.p.) injection of LSD, responding was reinforced only on a designated (drug) lever, whereas responding on the opposite lever had no programmed consequences; following saline injections (i.p.), responding was reinforced only on the lever opposite to the drug lever. One group of animals (N=16) was trained to discriminate 0.1 mg/kg of LSD, whereas another group (N=16) was trained initially to discriminate 0.16 mg/kg of LSD; this dose was raised gradually during the course of training to a final dose of 0.24 mg/kg. Stable, high (>95% correct) accuracy of discrimination was evident after ca 35 sessions in both groups. The percent correct responses on the lever appropriate to the injection of LSD or saline was calculated as the number of correct responses (i.e., 20) x 100 divided by the sum of the number of correct and incorrect responses during the first FR.

Every 2-4 days, test sessions, in which the ability of other doses of LSD or of other compounds to substitute for LSD, or to antagonize these effects, were conducted. LSD dose-response and THBC substitution tests were conducted in both the 0.1 mg/kg and in the 0.24 mg/kg LSD-trained groups, whereas further antagonism testing was conducted in the 0.1 mg/kg LSD-trained group only. Test sessions were terminated after a total of 20 responses had been made on either lever, or when a maximum of 25 minutes had elapsed. Free access to water was allowed for 5-10 minutes at least 1 hour following a test session. Only data from animals which made at least 10 responses and from sessions during which at least half of the animals responded were used. Upon completion of all other drug testing, one additional week of LSD-saline discrimination training was given, ending with a saline session; 100 mg/kg/day of p-chlorophenylalanine (PCPA) was given for the next three days thereafter, followed by four days with no further treatment or testing. A saline test session was then conducted, followed by a THBC test session.

Drug solutions were made as follows and injected i.p. in a volume of 1 ml/kg, 15 minutes before test sessions, except as indicated otherwise. BC-105 maleate (pizotifen; Sandoz) in deionized (DI) water; chlordiazepoxide HCl (Hoffman-LaRoche) dissolved in oil solvent (Hoffman-LaRoche), injected 30 minutes before test; clonazepam (Hoffman-LaRoche) in suspension of DI water with 1% Tween 80 (Sigma), in DI water; diazepam (Hoffman-LaRoche); ampules of 5 mg/ml diluted with propyleneglycol injected 30 minutes before test; haloperidol (McNeil) diluted with saline from ampules of 5 mg/ml, injected 1 hour before test; harmaline HCl (Sigma) in DI water; harmane HCl (Sigma) in DI water; LSD bitartrate (NIDA) in 0.9% saline; THBC 2HCl or 2HBr (noreleagnine; Sigma) and 6-MeO-THBC (free base; Drs. Steven Barker and H. Rommelspacher) in 15% propyleneglycol in DI water adjusted to pH 3.5-4.0 with HCl. All doses refer to the salts except for the THBCs and PCPA which refer to the free bases.

^{3}H-LSD receptor binding assay. Three experimentally-naive rats were killed by decapitation, the brains rapidly removed and placed on ice. The frontal cortex was dissected and homogenized in 20 vol of TRIS HCl buffer (0.05 M, pH 7.4) using a Brinkman Polytron (setting 8 for 10 seconds). The homogenate was centrifuged at 15,000 g for 10 minutes, the resulting pellet was resuspended as the process was repeated three more times. Between the second and third washes, the tissue homogenate was incubated for 10 minutes at 37°C. The final pellet was resuspended in 20 vol of modified TRIC HCl including 0.1% ascorbic acid, 4mM $CaCl_2$ and 10 mM pargyline. Aliquots (100 µl) of the membrane suspension were incubated for 10 minutes at 37°C with 3 nM/100 µl of ^{3}H-LSD (58.7 Ci/mM; N.E.N., Boston, MA) and different concentration of THBC. Specific binding was defined as the difference between binding in the presence and absence of 1.0 µM unlabelled LSD (27), and represented 60-80% of the total radioactivity bound. Incubation was terminated by vaccuum filtration through Whatman GF/B filters followed by three 5 ml washes with cold buffer. Radioactivity was extracted overnight in 10 ml of scintillation liquid and measured by liquid scintillation spectrometry (60% efficiency). Experiments were conducted in triplicate with six concentrations of THBC. Values represent the means of three determinations which varied by less than 15%.

Results

Figure 1 shows the dose-response curves of LSD in the two LSD-trained groups of animals; the slopes and positions of the curves were dependent on the dose of the training drug (0.1 or 0.24 mg/kg). THBC and 6-MeO-THBC substituted partially for LSD with almost equal potencies; the extent of these substitutions depended on the training dose of LSD.

The effects of various pharmacological manipulations in the group trained to discriminate 0.1 mg/kg of LSD (from saline) are summarized in Table 1. The substitution of THBC for LSD was attentuated by the 5-HT receptor antagonist BC-105, but was unaffected by the DA-antagonist haloperidol. The blocking effect of the potent benzodiazepine clonazepam on the THBC substitution was intermediate, although this was only based on a single dose of the drug. Benzodiazepines (chlordiazepoxide, diazepam and clonazepam) neither substituted nor blocked the effects of LSD (data not shown). The two aromatic β-carbolines, harmane and harmaline, were also tested, a maximum of 54% substitution to 0.1 mg/kg of LSD was found with the highest dose of harmaline whereas harmane produced only 22% LSD responding; drug-induced tremors precluded testing of higher doses. Pretreatment with PCPA, which inhibits 5-HT synthesis at the rate limiting step tryptophan hydroxylase (28), strongly attenuated the THBC substitution for LSD, but had no effect on the animal's ability to discriminate saline from LSD.

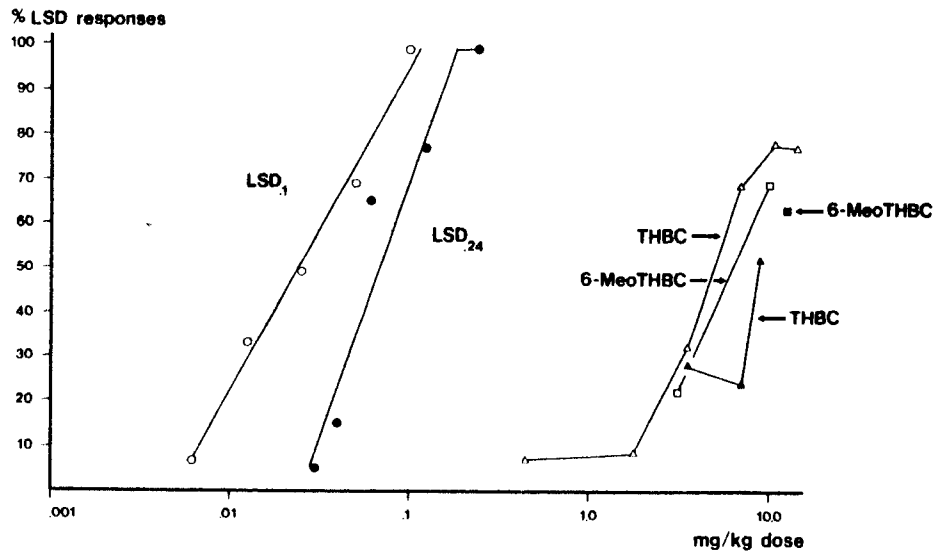

Figure 1

The effect of LSD and THBCs in rats trained to discriminate the stimulus properties of LSD 0.1 or .24 mg/kg from saline. Open symbols equal .1 mg/kg LSD training dose; filled symbols equal .24 mg/kg LSD training dose.

TABLE 1

Drug treatment	% LSD-lever responses	n/N
THBC 7.1 mg/kg	69 ± 9 ** a, b	16/16
THBC 7.1 mg/kg + BC-105 3 mg/kg	18 ± 7 * a, ** c	16/16
THBC 7.1 mg/kg + Haloperidol 0.1 mg/kg	63 ± 10 ns c	15/15
THBC 7.1 mg/kg + Clonazepam 0.25 mg/kg	41 ± 9 ** a, * c	15/15
PCPA + Saline	14 ± 8 ns a	14/14
PCPA + THBC 7.1 mg/kg	12 ± 7 ns a	13/14
Harmaline 8 mg/kg	54 ± 10 ** a, b	10/15
Harmane 3 mg/kg	22 ± 9 * a	15/15

Effects of pharmacological manipulations in animals trained to discriminate 0.1 mg/kg of LSD from saline. Shows average % LSD-lever responses ± S.E.M. and (n/N) the number of animals responding (n) out of the number of animals tested (N). *: $p < 0.05$; **: $p < 0.01$; a: compared to saline; b: compared to 0.1 mg/kg LSD; c: compared to 7.1 mg/kg of THBC; ns: not statistically significant, (paired t-tests).

Finally, THBC was found to inhibit ^{3}H-LSD binding (3 nM) to homogenates of rat frontal cortex. An IC_{50} value of 4 μM was determined by log-probit analysis. Under the present assay conditions, the dissociation constant (K_d) for ^{3}H-LSD binding is 3 nM (29); thus, the K_i value for THBC inhibition of ^{3}H-LSD binding is 2 μM ($K_i = IC_{50}/(1+^3H\text{-LSD}/K_d)$).

Discussion

While the mechanism of action underlying the hallucinogenic potency of LSD in humans is unknown, considerable evidence suggests that 5-HT neuronal systems play a major role in other pharmacological and behavioral effects of the drug--at least in animals (30, 31). This has been confirmed by drug discrimination procedures in that putative 5-HT receptor antagonists attenuate the stimulus properties of LSD and related hallucinogens whereas other neurotransmitter antagonists (or agonists) do not (23, 24).

Although the drug discrimination model does not imply that drugs such as lisuride which mimics LSD (20) necessarily have hallucinogenic properties in humans, it does suggest that such agents and LSD may have similar mechanisms, in this case involving post-synaptic 5-HT receptors (21-24, 31-33). Thus, the finding that THBC and its derivative 6-MeO-THBC substitute for LSD may be interpreted as meaning that these compounds have 5-HT agonist actions. Although these substitutions were not complete, the fact that their extent depended upon the training dose of LSD indicates that the animals were not responding "randomly", since it has previously been found that the dose of training drug is an important determinant for the magnitude (and perhaps the substrate) of pharmacological interactions in the drug discrimination procedure (24, 25, 34, 35). Indeed, direct or indirect 5-HT agonists often do not produce complete substitution for LSD (26, 36), a fact which may indicate that the LSD cue is a compound stimulus, perhaps mediated by multiple 5-HT receptors, or that THBCs exert less than the full extent of LSD's actions (36). The antagonism of THBC by BC-105 may indicate that the common action of THBC and LSD involves stimulation of 5-HT receptors because BC-105 also antagonizes LSD (33, 36).

THBCs can increase 5-HT neurotransmission through a variety of neuronal mechanisms: they can inhibit synaptosomal 5-HT uptake mechanisms (12, 14), inhibit type A monoamine oxidase (MAO-A) for which 5-HT is a preferred substrate (11), directly stimulate 5-HT receptors (2, 37) and, upon entry into 5-HT cells, stimulate the release of 5-HT (15, 16). This multiplicity of actions suggests many possible mechanisms through which THBCs could elicit discriminable effects which are similar to those of LSD. Fortunately, some of these possibilities can be ruled out. Fluoxetine (38), citalopram (39), and chlorimipramine (40) are more potent and specific than THBCs at inhibiting 5-HT uptake (14, 41), yet none of these agents substitute reliably for either LSD (23) or the discriminably similar 5-HT agonist quipazine (26). Therefore, it is doubtful that the ability of THBCs to inhibit 5-HT uptake is sufficient to cause substitution for LSD. Since harmaline and harmane are considerably more potent than THBCs as inhibitors of MAO-A (11) but are less effective than THBCs at mimicking LSD, it is also unlikely that this mechanism is responsible for the observed effect.

That THBCs may have a direct 5-HT agonist action is suggested by the finding that THBC is capable of inhibiting specific ^{3}H-LSD binding to rat frontal cortex with a potency which resembles that of 5-HT and the 5-HT agonist 6-methoxy-N,N-dimethyltryptamine (27,42). THBC also displaces specific ^{3}H-5-HT binding to rat frontal cortex at similar concentrations (IC_{50} = 4 μM) (9). However, the present results favor the hypothesis that the LSD-like discriminative effect of THBC is mediated by its ability to release 5-HT from presynaptic vesicles since

pretreatment with doses of the 5-HT synthesis inhibitor PCPA which are known to deplete 5-HT levels (28) abolished the substitution of THBC for LSD; similar treatment has been found to enhance the discriminability of LSD (32). Thus, with respect to the drug discrimination situation, the effects of THBC may closely resemble those of another 5-HT releasing agent, fenfluramine (26, 36, 43).

The extent to which the aromatic β-carbolines, harmane and harmaline resemble THBC with respect to mimicking LSD is unclear from the present experiments due to the limited dose range that was possible to test. Evidence from receptor binding studies supports the view that these compounds probably do not induce LSD-like discriminable effects, since the affinity of aromatic β-carbolines to 5-HT receptors is much lower than that of THBCs (2). However, these compounds interact with other receptor systems (2, 9), some of which may be related to reported hallucinogenic effects in humans (i.e., muscarinic acetylcholine and opiate receptors).

Many β-carbolines, although not THBCs, also have high affinity for BZD receptors (2, 9, 44). However, the discriminative stimulus properties of hallucinogens do not apparently involve BZD receptors, since neither chlordiazepoxide, clonazepam nor diazepam had significant effects on the LSD cue; the weak antagonistic effect of clonazepam on the THBC substitution for LSD, may reflect possible 5-HT antagonistic effects generally attributed to BZDs (45). However, hallucinogens and BZDs do have certain similar effects, most notably that they both release punished responding (45, 46).

In summary, the present experiments present the first evidence for LSD-like effects of THBCs. Given the presence of these compounds in human brain, these data may have important implications for a possible role of THBCs in psychopathology.

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