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Serotonergic Mediation of Tetrahydro-β-Carboline

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SCHECHTER, M. D. *Serotonergic mediation of tetrahydro-β-carboline*. PHARMACOL BIOCHEM BEHAV 24(5) 1209–1213, 1986.—Rats were trained to discriminate between the stimulus properties of tetrahydro-β-carboline (THBC) and its vehicle in a two-lever, food-motivated operant task. By steadily increasing the training dose, the discrimination was attained at 20.0 mg/kg THBC. Dose-response experiments subsequently indicated that decreasing doses of THBC produced decreased discrimination and generated an ED50=3.63 mg/kg. Administration of the serotonergically-active drug, fenfluramine, produced THBC-appropriate responding in a dose-responsive manner. In addition, LSD and yohimbine produced partial generalizations in the THBC-trained rats. These data suggest that the discriminative stimulus properties of THBC are mediated by serotonergic neurons in the central nervous system.

Tetrahydro-β-carboline LSD Fenfluramine Yohimbine Serotonin Drug discrimination

THE tricyclic compound 1,2,3,4 tetrahydro-β-carboline (THBC) has been reported to be endogenously present in the brain of rats [2, 10, 22, 28] and in the platelets, urine [9,10], brain and liver of man [1]. This compound, also known as tetrahydronorharmane and tryptoline, is a condensation product of tryptamine and formaldehyde and, as such, has been observed to interact relatively specifically with central serotonergic (5-HT) systems in numerous ways. The possible mechanisms involved in this interaction include: (a) inhibition of 5-HT uptake mechanisms [3, 11, 24]; (b) inhibition of Type A monoamine-oxidase, for which 5-HT is a preferred substrate [5]; (c) direct stimulation of 5-HT postsynaptic receptors [16,19]; and (d) release of 5-HT [25,30]. Any, or all, of these mechanisms can lead to a THBC-induced increase in brain concentrations of serotonin but no increase in either norepinephrine or dopamine as initially reported in 1972 to occur with 6-methoxy-THBC [15]. Furthermore, various pharmacological effects of THBC indicate that its actions are mediated by serotonergic systems [4,23].

Within a discriminative stimulus (DS) paradigm, a subject comes under stimulus control of a drug whereby correct operant responses in a choice situation are contingent upon which drug was previously administered. Research that employs the DS procedure indicates a direct relationship between the central effects of the drug and its ability to serve as a DS [18]. Although one report [17] indicated that rats trained to discriminate LSD from saline would respond, in 69% of the trials, upon the LSD-correct lever after administration of 7.1 mg/kg THBC, THBC has never been evidenced to be capable of controlling discriminative behavior.

Thus, the purposes of the present investigation were to attempt to train rats to discriminate the stimulus properties of THBC in the DS paradigm and to employ various serotonergically-active drugs to provide an accurate estimate of its *in vivo* mechanism of action upon that neurochemical system.

METHOD

Subjects

Twelve experimentally-naive male Sprague-Dawley rats, weighing 171–224 g at the beginning of experimentation, were used. They were housed in individual living cages and their weights were adjusted, by daily rationing of commercial rat chow, to approximately 80 to 85% of their free-feeding weights as determined by daily weighing of two control free-feeding rats purchased at the same time as experimental animals from the supplier (Zivic-Miller, Allison Park, PA). Water was continuously available in the home cages which were kept at a constant temperature (20–22°C) and maintained on a 12-hour light/12-hour dark daily cycle.

Apparatus

The experimental space consisted of eight identical standard rodent operant chambers (Lafayette Instruments Corp., Lafayette, IN) each equipped with two operant levers located 7 cm apart and 7 cm above the grid floor. A food pellet receptacle was mounted 2 cm above the grid floor at an equal distance between the two levers. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and 9 W house-light. Solid-state programming equipment (Med Associates, E. Fairfield, VT) was used to control and record the sessions and was located in an adjacent room.

Discriminative Training

Drug discrimination training was based upon procedures

described in detail elsewhere [27]. There were two training phases. In the first phase, the food-deprived rats learned to press the lever indicating vehicle (control) administration and received a food reward (45 mg Noyes pellet) for each correct response, fixed ratio 1 (FR1) schedule. This schedule was made progressively more difficult, in daily 15 min sessions, over 10 days until an FR10 schedule was achieved. Throughout lever press training, all rats received daily intraperitoneal (IP) injections of THBC vehicle (V) 30 min prior to being placed into the two-lever operant box. Immediately following the attainment of the FR10 schedule after vehicle administration, the opposite lever was activated and rats received a food reward for each correct response, fixed ratio 1 (FR1) schedule, after the IP administration of an equal volume of vehicle (1 ml/kg body weight) containing 5 mg/kg THBC. (Without previous investigations using THBC as the drug to control discriminative responding, the initial training dose was chosen as a result of one previous DS study [17] employing this drug. The plan was to double this dose after every two weeks of training until discrimination between it and its vehicle could be reliably attained.) Daily sessions of 15 min duration were continued over 8 days with drug administration until an FR10 schedule was reached. In order to minimize effects due to any possible position preference, the rats were divided into two subgroups (n=6). For one subgroup, responding on the left lever was reinforced by delivery of food pellets in every session following drug injection, whereas the other group was reinforced with food after responding on the right lever following drug injection. Responses on the opposite lever were reinforced with food pellets after vehicle injection.

Phase II discrinination training then began. Subjects were trained 5 days per week with reinforcement in a pseudorandom sequence. Thus, in each two week period, there were five days with drug lever (D) and five days with vehicle lever (V) correct. The pattern was D,V,V,D,D; V,D,D,V,V. The training criterion was reached when the animal selected the appropriate lever, according to the drug state imposed, on eight of ten consecutive sessions.

Dose-Response Relationships

As the rats attained the discriminative training criterion at the 20 mg/kg dose of THBC (see the Results section), testing and training sessions of 15 min duration, with alternating administrations of 20 mg/kg THBC or its vehicle, were continued on Mondays, Wednesdays and Fridays. It was intended that if a rat was observed to make more than two incorrect first lever selections in any of 10 consecutive maintenance sessions, the data on that rat's performance would be deleted from the results. This, however, did not occur. On Tuesdays and Thursdays, the rats were injected IP with lower (1.25–10.0 mg/kg) doses of THBC and, 30 min later, were placed into the experimental chamber. They were allowed to lever press, without receiving reinforcement, until ten presses were made on either lever. To preclude training at a drug dose different than employed to train the animals, the rats were immediately removed from the experimental chamber once the total responses on one lever reached 10 presses. Each of these lower doses of THBC was tested in each animal on two occasions with each test preceded both by a drug (20 mg/kg THBC) and a vehicle maintenance session. The lever first pressed ten times was designated as the "selected" lever and the percentage of rats choosing the drug-correct lever constitutes the quantal measurement (below).

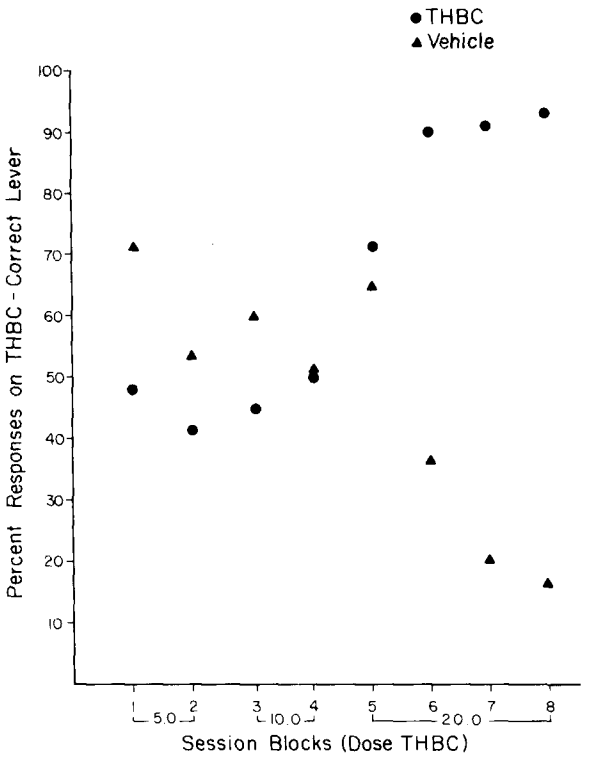

FIG. 1. Learning curve for rats (n=12) trained to discriminate THBC from its vehicle. Ordinate: Percent of selected lever responses on the THBC-correct lever; Abscissa: Session blocks with each block consisting of 5 THBC training trials and 5 vehicle sessions, according to the sequence: D,V,V,D,D; V,D,D,V,V. Dose of THBC (in mg/kg) in brackets was doubled every two weeks (see the Method section).

Substitution Tests

Subsequently, testing days (Tuesdays and Thursdays) were used to investigate the ability of the THBC-trained rats to discriminate numerous drugs evidenced to act upon serotonergic neurons, viz., LSD, fenfluramine, and yohimbine, at doses reported in the literature to produce behavioral effects. Each dose of these drugs was administered IP and the rats were tested, in extinction, 20 min after injection. Upon making 10 responses on either lever, the animal was removed from the operant chamber.

Drugs

The hydrochloride salt ofTHBC was prepared by dissolving THBC (Norleagnine, Sigma) in absolute ethanol modified with concentrated HCl to a final pH of 3.8 and recrystallization was induced at 4°C. Fenfluramine was generously supplied by Dr. David N. Johnson, A. H. Robins Research Laboratories, Richmond, VA, yohimbine HCl was purchased from Sigma Chemical Company, St. Louis, MO, and LSD tartrate was obtained from the NIDA. All drugs were dissolved in saline and administered IP at an equal volume of 1 ml/kg.

Measurements and Statistics

The lever pressed 10 times first was designated as the

TABLE 1

DOSE-RESPONSE AND SUBSTITUTION RESULTS IN RATS (n=12) TRAINED TO DISCRIMINATE THBC

Treatment	Dose (No. trials) mg/kg	Quantal	Quantitative (SD)
Vehicle	— (22)	15.5	27.2 (4.7)
THBC	20.0 (22)	92.6	83.4 (7.0)
	10.0 (2)	79.2	70.5 (5.4)
	5.0 (2)	54.2	55.6 (7.1)
	2.5 (2)	50.0	50.9 (12.0)
	1.25 (2)	15.0	22.7 (11.1)
Fenfluramine	2.0 (2)	95.8	77.2 (1.7)*
	1.0 (2)	58.3	53.8 (5.4)
	0.5 (2)	25.0	29.8 (23.4)
LSD	0.24 (2)	82.4	62.1 (1.9)
	0.10 (2)	25.0	37.4 (3.4)
Yohimbine	5.0 (2)	72.2	64.6 (1.4)
	2.5 (2)	50.0	49.6 (10.2)

*Non-significant difference from quantitative measurement after THBC training dose (t-test of means).

"selected" lever. The percentage of rats selecting the lever appropriate for the training drug was the quantal measurement of discrimination. In addition, a quantitative measurement of discriminative performance, measured by the animals' distribution of responses on the two levels prior to delivery of the first 10 responses, was used. This measure was calculated as 100 × number of correct responses divided by the number of correct and incorrect responses when one lever was pressed 10 times. The advantages in using both measurements have been discussed by Stolerman and D'Mello [29]. The quantal data for the dose-response experiments were analyzed by the method of Litchfield and Wilcoxon [13] which employs probit vs. log-dose effects and generates ED50s and tests for parallelism.

RESULTS

Acquisition of Discrimination

Initial training with 5 mg/kg THBC on the pseudo-random schedule (see the Method section) during the first two weeks of training produced 48.3% THBC-appropriate responses, whereas vehicle administration resulted in 71.7% of responses on this lever (Fig. 1).This result was most likely caused by the fact that the rats had most previously been trained to lever press on the drug lever to the FR10 schedule over the preceding 8 days. However, this result, i.e., greater percentage responding upon the drug lever after vehicle than after drug, persisted for 8 weeks of training and until the 20 mg/kg dose of THBC was administered. At that time, i.e., on the 9th and 10th week of training (session block 5, Fig. 1), the administration of THBC began to produce greater percentage responding on the THBC-correct lever than did its vehicle. This appropriate discriminative performance continued and increased for the next 6 weeks (session blocks 6, 7, and 8, Fig. 1) at which time all the rats attained the 8 of 10 consecutive correct lever choice criterion (see the Method section).

Dose-Response Relationship

Once the discrimination criterion was attained in all rats, interspersed maintenance sessions with 20 mg/kg THBC produced 92.6% of lever selections upon the THBC-correct lever, whereas administration of its vehicle produced 15.5% of selections upon this lever (or 84.5% selections upon the opposite vehicle-appropriate lever). Decreasing doses of THBC, each administered on two test days, produced decreased discriminative responding both in terms of quantal and quantitative measurements (Table 1) and generated a standard dose-response curve for each measurement. Analysis of these curves by the method of Litchfield and Wilcoxon [13] yielded an ED50=3.63 mg/kg (95% confidence range: 2.16–6.09 mg/kg) for the quantal data and a similar ED50 of 3.56 (1.80–7.13) mg/kg for the quantitative data.

Substitution Tests

The results of administering various drugs to THBC-trained rats, to test for their similarity to the discriminative effects of THBC, appear in Table 1. Fenfluramine at a dose of 2.0 mg/kg IP produced 95.8% of selected lever responses upon the THBC-appropriate lever and a quantitative measure non-significantly different from that calculated after the administration of the THBC training dose. Decreasing doses of fenfluramine produced decreasing quantal and quantitative discriminative performance. Analysis of the dose-reponse curve for fenfluramine [13] yielded an ED50=0.78 (95% confidence range: 0.56–1.08) mg/kg and when this dose-response curve was compared to that of the THBC dose-response curve [13] the two curves were shown not to be parallel, i.e., calculated t=6.34>critical t=3.18.

Substitution tests with 0.24 mg/kg LSD, in two trials, produced 82.4% first choice (selected lever) responses upon the THBC-correct lever and the highest dose of yohimbine (5.0 mg/kg) produced 72.2% responses upon this lever.

DISCUSSION

The present study indicates that tetrahydro-β-carboline (THBC) can serve as the drug to control discriminative responding in a two-lever food-motivated operant task. By starting with a 5 mg/kg dose of THBC and doubling the training dose every two weeks, it was found that 20 mg/kg THBC is the appropriate dose to control discriminative responding. Once rats were trained to the discriminative criterion, decreasing doses of THBC produced decreased discriminative performance and generated a standard dose-response relationship with an ED50=3.63 mg/kg.

Substitution tests with various doses of fenfluramine indicated that the discriminative stimuli of these two drugs, i.e., fenfluramine and THBC, are similar as suggested previously [17]. However, the non-parallel nature of the two dose-response curves suggests that THBC and fenfluramine may not be acting by similar mechanisms or upon similar receptors [12].

Substitution tests with the highest dose of (0.24 mg/kg) LSD produced 82.4% selected lever choices on the THBC-lever. Nielsen *et al.* [17] trained rats to discriminate 0.1 or 0.24 mg/kg LSD from saline in a similar paradigm and reported that 7.1 mg/kg THBC produced 69% responding upon the LSD-appropriate lever and this effect was blocked by pretreatment with the 5-HT synthesis inhibitor p-chlorophenylalanine. More recently, rats trained to discriminate 0.1 ml/kg LSD selected the LSD-appropriate lever

on 77.6% of trials after the IP administration of 10 mg/kg THBC (J. C. Winter, personal communication). These behavioral and additional biochemical studies led to the suggestion that THBC has either a direct 5-HT agonist action or acts via 5-HT release, like fenfluramine. The present study suggests a symmetrical, although partial, transfer of discrimination between LSD and THBC. Likewise, yohimbine which has been shown to produce generalization in rats trained to discriminate LSD [6] generally produces THBC-appropriate responding.

The neurochemical basis of the discriminative effects of fenfluramine [8, 14, 31] and LSD [7,32] have repeatedly been shown to reside in the serotonergic neurons. While yohimbine is commonly described as an α_2-adrenergic antagonist, there is evidence that it may, in addition, act as an agonist at serotonergic receptors [6, 20, 26]. Although there is no simple model for functional regulation of 5-HT or its subtypes of receptors [21], the present study evidences the serotonergic mediation of the discriminative properties of THBC. Further research will be required to determine which subtype of receptor plays a major role in the behavioral effects of this substance.

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REFERENCES

1. Barchas, J. D., G. R. Elliot, J. D. Armaral, E. Erdilyi, S. O'Connor, M. Bowden, K. H. K. Brodie, P. A. Bergen, J. Renson and R. J. Wyatt. Triptolines, formation from tryptamines and 5-MTHF by human platelets. *Arch Gen Psychiatry* **31:** 862–867, 1974.
2. Barker, S. A., R. E. Harrison, G. B. Brown and S. T. Christian. Gas chromatographic/mass spectrometric evidence for the identification of 1,2,3,4-tetrahydro-β-carboline as an in vivo constituent of rat brain. *Biochem Biophys Res Commun* **87:** 146–154, 1979.
3. Buckholtz, N. S. Brain serotonin and 5-hydroxyindole-acetic acid concentrations and serotonin synthesis following tetra-β-carboline administration in mice. *Naunyn Schmiedebergs Arch Pharmacol* **314:** 215–221, 1980.
4. Buckholtz, N. S. Neurobiology of tetrahydro-β-carbolines. *Life Sci* **27:** 893–903, 1980.
5. Buckholtz, N. S. and W. O. Boggan. Monoamine oxidase inhibition in brain and liver produced by β-carbolines. Structure-activity relationships and substrate specificity. *Biochem Pharmacol* **26:** 1991–1996, 1977.
6. Colpaert, F. C. Cross generalization with LSD and yohimbine in the rat. *Eur J Pharmacol* **102:** 541–544, 1984.
7. Colpaert, F. C., C. J. E. Niemegeers and P. A. J. Janssen. A drug discrimination analysis of lysergic acid diethylamide (LSD): *In vivo* agonist and antagonist effects of purported 5-hydroxytryptamine antagonist and of pirenperone, an LSD-antagonist. *J Pharmacol Exp Ther* **221:** 206–214, 1982.
8. Gourdie, A. J. Discriminative stimulus properties of fenfluramine in an operant task. An analysis of its cue functions. *Psychopharmacology (Berlin)* **53:** 97–102, 1977.
9. Honecker, H. and H. Coper. Tetrahydronorharmane (THN) and 6-hydroxynorharmane: Physiological components in platelets and urine of man. *Naunyn Schmiedebergs Arch Pharmacol* **302:** R63, 1978.
10. Honecker, H. and H. Rommelspacher. Tetrahydronorharmane (tetrahydro-β-carboline), a physiologically ocurring compound of indole metabolism. *Naunyn Schmiedebergs Arch Pharmacol* **305:** 135–141, 1978.
11. Komulainen, H., J. Tuomisto, M. M. Airaksinen, I. Kari, P. Peura and L. Pollari. Tetrahydro-β-carbolines and corresponding tryptamines: *In vitro* inhibition of serotonin, dopamine and noradrenaline uptake in rat brain synaptosomes. *Arch Pharmacol Toxicol* **46:** 299–307, 1980.
12. Levine, R. R. *Pharmacology: Drug Actions and Reactions*, 2nd edition. Boston: Little, Brown, and Co., 1978, p. 175.
13. Litchfield, J. T. and F. Wilcoxon. A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther* **96:** 99–113, 1949.
14. McElroy, J. F. and R. S. Feldman. Discriminative stimulus properties of fenfluramine: Evidence for serotonergic involvement. *Psychopharmacology (Berlin)* **83:** 172–178, 1984.
15. McIsaac, W. M., D. Taylor, K. E. Walker and B. T. Ho. 6-Methoxy-1,2,3,4-tetrahydro-β-carboline—a serotonin elevator. *J Neurochem* **19:** 1203–1206, 1972.
16. Müller, W. E., K. J. Fehske, H. D. Borbe, U. Wollert, C. Nanz and H. Rommelspacher. On the neuropharmacology of harmane and other β-carbolines. *Pharmacol Biochem Behav* **14:** 693–699, 1981.
17. Nielsen, E. B., F. J. White, A. M. Holohean, P. M. Callahan and J. B. Appel. Behavioral and biochemical evidence for serotonergic actions of tetrahydro-β-carbolines. *Life Sci* **31:** 2433–2439, 1982.
18. Overton, D. A. State-dependent learning produced by addicting drugs. In: *Opiate Addiction, Origin and Treatment*, edited by S. Fisher and A. M. Freedman. Washington, DC: D. H. Winston, 1973, pp. 61–75.
19. Pannier, L. and H. Rommelspacher. Actions of tetrahydronorharmane (tetrahydro-β-carbolines) on 5-hydroxytryptamine and dopamine-mediatd mechanism. *Neuropharmacology* **20:** 1–8, 1980.
20. Papeschi, R., T. L. Sourkes and M. B. H. Youdim. The effects of yohimbine on brain serotonin metabolism, motor behavior and body temperature of the rat. *Eur J Pharmacol* **15:** 318–326, 1971.
21. Petroutka, S. J. and S. H. Snyder. Multiple serotonin receptors: Differential binding of ^{3}H-serotonin, ^{3}H-lysergic acid diethylamide and ^{3}H-spiroperidol. *Mol Pharmacol* **16:** 687–699, 1979.
22. Rommelspacher, H. and B. Greiner. Formation of β-carbolines: a new metabolic pathway of indoles in the brain of rats. *Naunyn Schmiedebergs Arch Pharmacol* **302:** R60, 1978.
23. Rommelspacher, H., H. Kauffman, C. H. Cohnitz and H. Coper. Pharmacological properties of tetrahydronorharmane (tryptoline). *Naunyn Schmiedebergs Arch Pharmacol* **298:** 83–91, 1977.
24. Rommelspacher, H., S. Strauss and C. H. Cohnitz. Inhibition of 5-hydroxytryptamine uptake by tetrahydronorharmane in vivo. *Naunyn Schmiedebergs Arch Pharmacol* **303:** 229–233, 1978.
25. Rommelspacher, H. and N. Subramannian. Tetrahydronorharmane modulates the depolarization-induced efflux of 5-hydroxytryptamine and dopamine and is released by high potassium concentration from rat brain slices. *Eur J Pharmacol* **56:** 81–86, 1979.
26. Sanghvi, I. and S. Gershon. Similarities between behavioral and pharmacological actions of yohimbine and 5-hydroxytryptophan in the conscious dog. *Eur J Pharmacol* **11:** 125–129, 1970.
27. Schechter, M. D. Lack of effect of choline and narcotic antagonists upon apomorphine discrimination. *Eur J Pharmacol* **68:** 187–191, 1980.
28. Shoemaker, D. W., J. T. Cummins and T. G. Bidder. β-Carbolines in rat arcuate nucleus. *Neuroscience* **3:** 233–239, 1978.

29. Stolerman, I. P. and G. D. D'Mello. Role of training conditions in discrimination of central nervous system stimulants by rats. *Psychopharmacology (Berlin)* **73:** 295–303, 1981.
30. Thomas, T. N., N. S. Buckholtz and J. W. Zemp. 6-Methoxy-1,2,3,4-tetrahydro-β-carbolines effects on retinal serotonin. *Life Sci* **25:** 1435–1442, 1979.
31. White, F. J. and J. B. Appel. A neuropharmacological analysis of the discriminative stimulus properties of fenfluramine. *Psychopharmacology (Berlin)* **73:** 110–115, 1981.
32. Winter, J. C. Stimulus properties of phenethylamine hallucinogens and lysergic acid diethylamide: The role of 5-hydroxytryptophan. *J Pharmacol Exp Ther* **204:** 416–423, 1978.