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Inverse Agonist Properties of the THBC Discriminative Stimulus: Asymmetrical Generalization With FG 7142

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LEIDENHEIMER, N. J. AND M. D. SCHECHTER. *Inverse agonist properties of the THBC discriminative stimulus: Asymmetrical generalization with FG 7142.* PHARMACOL BIOCHEM BEHAV **38**(3) 519–525, 1991.—Male rats were trained to discriminate the stimulus properties of the β-carbolines 1,2,3,4-tetrahydro-β-carboline (THBC) (15.0 mg/kg) or FG 7142 (5.0 mg/kg) from vehicle in a two-lever, food-motivated operant task. Consistent with the serotonergic properties of THBC, administration of the $5HT_{1B}$ agonists TFMPP and mCPP to THBC-trained rats resulted in THBC-appropriate responding. Norharmane, a β-carboline metabolite of THBC, also mimicked the THBC discriminative stimulus. In contrast, the benzodiazepine receptor partial inverse agonist FG 7142, the anxiogenic/convulsant pentylenetetrazole (PTZ), two physiological stressors and the α_2 adrenergic antagonists yohimbine and idazoxan failed to produce THBC-appropriate responding. In the FG 7142-trained rats, THBC and norharmane dose-dependently mimicked the FG 7142 discriminative stimulus. This generalization was not based upon the serotonergic properties of THBC and norharmane since administration of the serotonin agonist mCPP to FG 7142-trained rats failed to produce FG 7142-appropriate responding. The ability of THBC to substitute for the FG 7142 discriminative stimulus was antagonized by the benzodiazepine receptor mixed agonist/antagonist CGS 9896 and the benzodiazepine receptor antagonist RO 15-1788, indicating that THBC produces an inverse agonist stimulus in FG 7142-trained rats. These results suggest that THBC produces a discriminative stimulus which consists of both serotonergic and inverse agonist components.

Drug discrimination THBC FG 7142 Inverse agonist Anxiety Rats β-Carbolines Stimulus properties of drugs

THBC (1,2,3,4-tetrahydro-β-carboline, tetrahydronorharmane or noreleagnine) is a β-carboline endogenously formed in mammals (1,11), the physiological significance of which is unknown. At pharmacological concentrations, THBC enhances serotonergic (5HT) transmission by inhibiting monoamine oxidase (6,18), increasing 5HT release (13,29), blocking 5HT reuptake (4, 5, 7, 15, 28) and by directly stimulating 5HT receptors (22). Additionally, THBC decreases reuptake of dopamine in synaptosomal preparations (7).

The discriminative stimulus properties of THBC are consistent with its neurochemical effects on the 5HT neuronal system. Rats trained to discriminate THBC from vehicle respond in a THBC-appropriate manner following administration of the serotonin releaser fenfluramine (30) or the $5HT_{1B}$ receptor agonists mCPP and TFMPP (31). The lack of generalization of the THBC discriminative cue to either the $5HT_{1A}$ agonists 8-OHDPAT or buspirone indicate that the THBC discriminative stimulus may be associated with a 5HT receptor subtype (31).

In addition to influencing serotonergic transmission, THBC may also modulate GABAergic transmission by acting as an inverse agonist at the benzodiazepine receptor. The behavioral profile of THBC is consistent with that of a benzodiazepine receptor inverse agonist in that THBC produces an "anxiety-like state" in the rat (12), enhances behavioral suppression in conflict models which is antagonized by both diazepam (26) and RO 15-1788 (33) and produces convulsions (27). Additionally, THBC binds with a micromolar affinity to the benzodiazepine receptor (22) and THBC-induced release of [^{3}H]NE and [^{3}H]5HT in the hippocampus is prevented by the benzodiazepine receptor agonist chlordiazepoxide (13). The purpose of the present experiments was to determine if THBC acts as an inverse agonist in the discriminative stimulus paradigm and to assess the similarities between discriminative behavior controlled by THBC and the benzodiazepine receptor partial inverse agonist FG 7142.

METHOD

Subjects

For the experiments in THBC-trained rats, twelve experimentally naive male Sprague-Dawley rats purchased from the Zivic-

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Miller Laboratories (Allison Park, PA), weighing between 125–250 g at the beginning of the experiment, were housed and maintained on a 12-h light (0600–1800)/12-h dark cycle in a room kept at temperatures between 20–22°C. They received water ad lib and a daily rationing of commercial rat chow in their individual home cages. This feeding regimen maintained their weights at approximately 85% of expected free-feeding weight values and provided motivation to learn the appetitive task.

For experiments involving FG 7142-trained animals, two separate groups of rats (n = 8, n = 9), previously trained to discriminate FG 7142 (5.0 mg/kg, IP, 30 min prior to discrimination sessions) (16,17), were used as subjects. The training procedure for these two groups of rats was identical to that described for the THBC training procedure described (below).

Apparatus

Twelve standard rodent operant chambers (Lafayette Instruments Corp., Lafayette, IN) were used as the experimental space. Each chamber contained two levers situated 7 cm apart and 7 cm above a grid floor. A food receptacle was located 2 cm above the grid floor, midway between the two levers. Each operant chamber was enclosed in an unlit, sound-attenuated cubicle with an exhaust fan for ventilation. Solid-state programming equipment (Med Associates, E. Fairfield, VT), located in an adjacent room, was used to control and record discrimination sessions.

A modified operant chamber, with both levers removed and the food delivery hole blocked by a metal plate, served as a novelty chamber and shockbox. The grid floor of this chamber, which was identical to those in the operant chamber used in the discrimination experiments, consisted of 18 metal bars spaced 1 cm apart. A scrambled current was delivered through the grid floor by an A615C Master Shocker (Lafayette Instruments, Lafayette, IN). A Grass SD9 Stimulator (Grass Instruments, Quincy, MA) was employed to trigger the Master Shocker. Unlike the operant chamber used for the discrimination procedure, the shockbox was not enclosed in an unlit sound-attenuated cubicle but was placed in a well-lit laboratory adjacent to the laboratory used for discrimination training/testing.

Training Procedure

Experimentally naive rats (n = 12) were trained to discriminate the stimulus effects of THBC (15.0 mg/kg, IP) from its vehicle. A detailed description of the training procedure has been previously published (16). Briefly, one lever in the operant chamber was designated as the vehicle-appropriate lever and the other was designated as the THBC-appropriate lever. Thirty min prior to each training session, food-deprived rats received an IP injection (1 ml/kg volume) of either THBC (15.0 mg/kg) or vehicle (V). After THBC administration, responses on the THBC-appropriate lever resulted in food reinforcement, whereas responses on the vehicle-appropriate lever did not produce reward. Following vehicle administration, responses on the vehicle-appropriate lever produced reinforcement, whereas responses on the THBC-appropriate lever did not produce reward. The reinforcement schedule was set at a fixed ratio of ten (FR 10), i.e., ten injection-appropriate lever responses yielded one reinforcement (45 mg Noyes pellet). This reinforcement schedule was gradually attained by beginning with an FR 1 schedule (one injection-appropriate lever response yielded one reinforcement) and was gradually increased over a period of one week to the FR 10 schedule. Daily training sessions were terminated when the animal had made 400 injection-appropriate lever responses and received (on the FR 10 schedule) 40 reinforcements. Sessions were conducted once per day according to the following two-week training schedule: THBC, V, V, THBC, THBC; V, THBC, THBC, V, V. The lever which was pressed 10 times first in the session was considered to be the selected lever for that session. For each animal, the training schedule was repeated until the selected lever was injection-appropriate in 8 out of 10 consecutive sessions, twice. Data collection began when all animals had fulfilled this 80% criterion (see the Measurements and Statistics section, below).

THBC Dose-Response Experiments

Once discriminative criterion was attained, the discrimination training regimen was limited to every other day in order to maintain the discrimination criterion. Between THBC and vehicle maintenance days, rats were tested with varying doses of THBC such that each dose was tested twice, once following a THBC maintenance (TM) day and once following a vehicle maintenance (VM) day according to the following schedule: TM, THBC dose 1, VM, THBC dose 1; TM, THBC dose 2, VM, THBC dose 2, etc. This counterbalancing was used to control for any possible residual influence from the previous maintenance day. Thirty min following THBC administration the rats were placed into the operant chamber and immediately removed, without receiving reinforcement, following the tenth response on either lever. Animals were not reinforced on test days to preclude any possible training to a dose of THBC different than that used in training/maintenance. Responses on both the drug and vehicle levers were, however, recorded and used to calculate the quantitative measurement (see the Measurements and Statistics section, below).

Generalization and Antagonism Experiments

Generalization experiments were conducted in which novel test drugs were administered to FG 7142-trained and THBC-trained rats to determine if the discriminative stimulus produced by the novel test drug was similar or dissimilar to that produced by the trained drug. Experiments were also performed in THBC- and FG 7142-trained rats to investigate possible antagonism of either the training drug cue or the cue produced by generalizing test compounds. Each dose of test drug (generalization testing) or test/training drug + antagonist (antagonism testing) was counterbalanced between training drug and vehicle maintenance days using the schedule described (above) for dose-response testing. On test days, animals were removed from the discrimination chamber without reinforcement upon emitting ten responses on either lever. Responses on both the drug and vehicle levers were recorded. All drugs were administered 30 min prior to the discrimination session except for RO 15-1788 and norfenfluramine which were administered 15 and 20 min prior to the session, respectively.

Novelty and Footshock Experiments

For the novelty experiments, rats were injected with vehicle and placed into the modified operant chamber for 20 min, during which time no shocks were delivered. They were removed after 20 min and placed into the operant chambers used for discrimination sessions. After making ten responses on either lever, the rats were removed without receiving reinforcement, and returned to their home cages. Unlike the drug testing experiments which were counterbalanced between maintenance sessions as described above, novelty experiments (by definition) were conducted only once per rat. This was necessary to preclude habituation to novelty (16). Footshock experiments were conducted in a similar manner except that a mild footshock (0.2 mA, 160 ms duration,

330 ms interval) was delivered during the 20-min period in the modified operant chamber (shockbox).

Measurements and Statistics

The number of training sessions required to achieve discriminative stimulus control (criterion performance) is expressed as a sessions-to-criterion (STC) measurement (24). To reach criterion, each rat had to select the injection-appropriate lever in 8 out of 10 consecutive sessions, twice. The first STC (STC 1) indicates the number of sessions required to reach the first of the ten sessions in which 8 out of 10 consecutive sessions were injection-appropriate. The first session in a second series of 8 out of 10 correct consecutive sessions constitutes the STC 2.

The data collected in the drug discrimination sessions is expressed as both quantal and quantitative measurements, each measurement provides an indication of lever preference prior to any reinforcement. The quantal measurement is the percentage of rats selecting the drug lever as their selected lever, i.e., the first lever pressed 10 times. The quantitative measurement is the number of responses on the drug lever divided by the total number of responses on both the drug and vehicle levers at the time that the tenth response is made on either lever. This fraction is expressed as a percentage. Unlike the (all-or-none) quantal measurement, the quantitative measurement accounts for responses on both the selected and unselected levers and, thus, provides a measure of the magnitude, as well as direction, of lever preference. Additionally, parametric statistics may be performed on the quantitative data. The advantages of using both types of measurements are more fully discussed by Stolerman and D'Mello (34).

A test compound was considered to generalize to the training drug if quantal responding following its administration was equal to or greater than 80% on the drug lever. This criterion was based on the performance level set for establishing initial acquisition of the training drug cue, i.e., 80% or greater responding on the drug-appropriate lever. Compounds were considered to partially generalize to the training compound if quantal responding was between 70–80% on the drug-appropriate lever. For generalization testing, the standard deviations for the quantitative measurements reflect the deviation between the two counterbalanced test sessions.

For antagonism testing performed in FG 7142-trained rats, each dose of antagonist was tested twice in each rat, once following a vehicle maintenance session and once following a drug maintenance session. For each rat the quantitative scores from the two antagonism test sessions were averaged. Likewise, individual quantitative scores were averaged from the THBC generalization testing sessions. These individual quantitative scores from the THBC antagonism test sessions and THBC generalization sessions were then tested for differences between group means. This data was subjected to a one-tailed, paired *t*-test, $p<0.05$.

The number of rats used for the discrimination sessions varies in the tables from 5–12. This variability in the number of subjects tested for each condition reflects differences in the initial size of the training groups and the health, viability and reliability of the subjects. If at any time during experimentation an animal's maintenance data fell below the 80% criterion level (less than 80% injection-appropriate responses), the animal's data was dropped from the experiment until responding returned to the 80% criterion level.

Drugs

Drugs used in these experiments were obtained from the following sources: yohimbine, pentylenetetrazole, clonidine, norharmane (Sigma Chemical Company, St. Louis, MO); mCPP HCl [1-(3-chlorophenyl)-piperazine HCl] (Research Biochemicals Incorporated, Natick, MA); RO 15-1788 (Hoffmann-La Roche, Nutley, NJ); CGS 9896 (CIBA-GEIGY, Summit, NJ); THBC (1,2,3,4 tetrahydro-β-carboline) (Aldrich Chemical Company, Milwaukee, WI); norfenfluramine (A.H. Robins Research Laboratories, Richmond, VA), idazoxan (Reckitt & Coleman Pharmaceutical Division, UK). FG 7142, CGS 9896 and RO 15-1788 were suspended in a 2% Tween 80 solution (Sigma Chemical Co., St. Louis, MO) by sonification. Other drugs were dissolved in distilled water. The HCl salt of THBC was prepared by dissolution of THBC in absolute ethanol and acid followed by recrystalization at 4°C.

RESULTS

THBC Discrimination Training and Dose-Response Experiments

The twelve rats trained to discriminate THBC (15.0 mg/kg) from vehicle required 30 daily training sessions to reach discriminative criterion. The STC 1 (±SD) and STC 2 (±SD) were 4.6(4.6) and 14.6(4.6), respectively. The THBC discrimination was dose-responsive with doses of 2.5, 5.0, 10.0 and 15.0 mg/kg producing quantal responding of 33.3, 62.5, 83.3 and 91.7, respectively (Table 1).

Experiments in THBC-Trained Rats

The results of generalization experiments in THBC-trained rats are also presented in Table 1. The highest dose (1.5 mg/kg) of the $5HT_{1B}$ agonist/5HT releaser (25) mCPP was observed to generalize to the THBC discriminative stimulus. Doses of 0.5, 0.75, 1.0 and 1.5 mg/kg of mCPP produced quantal responding of 20.8,27.3, 75.0 and 81.8% respectively, and quantitative responding (±SD) of 26.3 (13.4), 34.3 (9.1), 71.2(8.3) and 68.8 (22.4), respectively. Norfenfluramine, a serotonin releaser (3), produced partial generalization to the THBC cue. In the dose range of 0.5–2.0 mg/kg, norfenfluramine produced maximal THBC-like responding at the 1.5 mg/kg dose with quantal and quantitative (±SD) responding of 75.0% and 71.9%(6.0), respectively. Of the β-carbolines tested in THBC-trained rats, norharmane, the first metabolite of THBC (9), produced partial generalization to the THBC cue in that administration of 5.0, 10.0 and 15.0 mg/kg of norharmane resulted in quantal responding of 12.5, 70.8 and 79.2%. Behavioral disruption precluded testing of this compound at higher doses than those tested. In contrast to the results obtained with mCPP and norharmane, no dose of either FG 7142 or yohimbine was able to produce generalization. Following FG 7142 (5.0–20.0 mg/kg) administration, maximal THBC-appropriate responding was observed at 15.0 mg/kg, which yielded quantal and quantitative responding of 53.8 and 54.2 (1.1)%, respectively. For yohimbine, the maximal THBC-appropriate responding occurred at the 6.0 mg/kg dose which resulted in quantal and quantitative (±SD) responding of 58.3 and 56.7 (10.7), respectively.

Additionally, neither the α_2 adrenergic antagonist idazoxan (10.0 and 15.0 mg/kg) nor the convulsant PTZ (20.0 mg/kg) generalized to the THBC discriminative stimulus. These compounds produced maximal quantal responding of 59.1% (idazoxan) and 63.6% (PTZ). Higher doses of PTZ were not tested due to the convulsive potential of the compound. The physiological stressors novelty and footshock failed to substitute for the THBC cue (Table 2). Quantal and quantitative (±SD) responding following exposure to novelty were 20.0% and 7.1(6.7)%, respectively. Footshock produced quantal and quantitative (±SD) responding

TABLE 1

GENERALIZATION EXPERIMENTS IN RATS TRAINED TO DISCRIMINATE THBC (15.0 mg/kg) FROM ITS VEHICLE

Treatment	Dose (mg/kg)	% Responding on the THBC Lever Quantal	Quantitative (±SD)	N
Vehicle	—	4.2	15.4(6.1)	12
THBC	2.5	33.3	38.6(3.5)	12
	5.0	62.5	60.3(10.9)	12
	10.0	83.3	80.6(0.0)	12
	15.0	91.7	85.6(0.7)	12
mCPP	0.5	20.8	26.3(13.6)	12
	0.75	27.3	34.3(9.1)	11
	1.0	75.0	71.2(8.3)	12
	1.5	81.8	68.8(22.4)	11
Norfenfluramine	0.5	22.8	25.2(8.0)	11
	1.0	63.6	58.2(21.3)	11
	1.5	75.0	71.9(6.0)	10
	2.0	75.0	61.7(10.5)	10
Norharmane	5.0	12.5	19.2(12.4)	12
	10.0	70.8	62.1(7.7)	12
	15.0	79.2	63.9(4.8)	12
FG 7142	5.0	20.8	31.7(10.9)	12
	10.0	25.0	28.9(20.2)	12
	15.0	53.8	54.2(1.1)	12
	20.0	50.0	53.8(6.7)	12
Yohimbine	1.5	0.8	16.3(16.2)	12
	3.0	54.2	48.4(16.3)	12
	4.5	37.5	40.9(4.0)	12
	6.0	58.3	56.7(10.7)	12
	9.0	50.0	50.6(9.3)	12
Idazoxan	10.0	54.6	50.4(14.4)	
	15.0	59.1	59.2(12.9)	11
PTZ	20.0	63.6	60.9(1.2)	11

of 0.0% and 11.1(10.7)%, respectively.

Experiments in FG 7142-Trained Rats

The training data (sessions-to-criterion measurements) and dose-response data for the two groups of FG 7142-trained rats have been previously reported (14,15). The results of generalization testing in rats trained to discriminate FG 7142 (5.0 mg/kg) are presented in Table 3. Of the compounds tested, THBC and norharmane produced FG 7142-appropriate responding, whereas mCPP did not substitute for the FG 7142 cue. Doses of 5.0, 10.0 and 15.0 mg/kg THBC produced quantal responding of 43.8, 75.0 and 92.9% and quantitative responding (±SD) of 46.6(11.7), 63.9(3.0) and 71.1(3.7), respectively. Administration of 5.0, 7.5 and 10.0 mg/kg norharmane resulted in 38.9, 55.6 and 100.0% quantal responding and quantitative responding (±SD) of 45.5(4.7), 54.2(7.6) and 89.0(11.1). Maximal FG 7142-appropriate responding was observed at 1.5 mg/kg of mCPP which resulted in 50.0% quantal and 47.8 (1.7)% quantitative responding (±SD).

The ability of THBC to substitute for the FG 7142 discriminative stimulus was antagonized by both CGS 9896 and RO 15-1788 (Table 4). In CGS 9896 antagonism experiments, administration of THBC (15.0 mg/kg) alone in FG 7142-trained rats produced quantal responding of 90.9 and quantitative responding (±SD) of 67.0(11.2). Coadministration of THBC (15.0 mg/kg) with CGS 9896 (30.0 mg/kg) resulted in quantal responding of 33.3% and quantitative responding of 48.1(7.3); significantly lower than when THBC was tested alone, $p<0.05$. In the exper-

TABLE 2

DISCRIMINATIVE RESPONDING IN THBC-TRAINED RATS FOLLOWING EXPOSURE TO STRESSORS

Treatment	% Responding on the THBC Lever Quantal	Quantitative (±SD)	N
Novelty	20.0	7.1(6.7)	10
Footshock (0.2 mA)	0.0	11.1(10.7)	10

TABLE 3

GENERALIZATION EXPERIMENTS WITH β-CARBOLINES AND SEROTONIN AGONISTS IN RATS TRAINED TO DISCRIMINATE FG 7124

Treatment	Dose (mg/kg)	% Responding on the FG 7142 Lever Quantal	Quantitative (±SD)	N
FG 7142	5.0	88.9	78.7(4.5)	9
THBC	5.0	43.8	46.6(11.7)	8
	10.0	75.0	63.9(3.0)	8
	15.0	92.9	71.1(3.7)	7
mCPP	1.0	25.0	34.6(11.5)	8
	1.5	50.0	47.8(1.7)	8
	2.0	37.5	50.7(2.6)	8
Norharmane	5.0	38.9	45.5(4.7)	9
	7.5	55.6	54.2(7.6)	9
	10.0	100.0	89.0(11.1)	9

TABLE 4

ANTAGONISM OF THE THBC DISCRIMINATIVE STIMULUS IN FG 7142-TRAINED RATS

Treatment	Dose (mg/kg)	% Responding on the FG 7142 Lever Quantal	Quantitative (±SEM)	N
THBC	15.0	90.9	67.0(11.2)	6
THBC+ CGS 9896	15.0 30.0	33.0	48.1(7.3)*	6
THBC	15.0	70.0	62.0(5.5)	5
THBC+ RO 15-1788	15.0 5.0	30.0	32.4(10.3)*	5

*Significantly different from THBC (15.0 mg/kg) administered alone. $p<0.05$.

iment in which RO 15-1788 antagonized the THBC substitution for FG 7142, administration of THBC (15.0 mg/kg) alone produce quantal responding of 70.0% and quantitative responding (±SD) of 62.0(5.5). Coadministration of THBC (15.0 mg/kg) with RO 15-1788 (5.0 mg/kg) resulted in quantal responding of 30.0% and quantitative responding of 32.4(10.3) which was significantly lower than THBC administered alone, $p<0.05$.

DISCUSSION

In the present experiments, rats trained to discriminate THBC generalized to the $5HT_{1B}$ agonists mCPP and TFMPP and partially generalized to the 5HT releaser norfenfluramine and norharmane, THBCs active first metabolite. In contrast, the benzodiazepine receptor partial inverse agonist FG 7142, anxiogenic/convulsant PTZ, α_2 adrenergic antagonists yohimbine and idazoxan and stressful environmental manipulations failed to mimic the THBC stimulus, indicating the specificity of the THBC cue. In other experiments, rats trained to discriminate the interoceptive cue of FG 7142 were shown to generalize, in a dose-responsive manner, to both THBC and norharmane, whereas the serotonergic agonists mCPP failed to produce FG 7142-appropriate responding. The ability of THBC to substitute for the FG 7142 discriminative stimulus was antagonized by the benzodiazepine receptor mixed agonist/antagonist CGS 9896 and antagonist RO 15-1788.

The serotonergic properties of the THBC discriminative stimulus have previously been reported (23, 30, 31). Rats trained to discriminate THBC (20.0 mg/kg) generalized to the 5HT releaser fenfluramine (30) and $5HT_{1B}$ agonists mCPP and TFMPP (31). However, administration of $5HT_{1A}$ agonists failed to produce THBC-appropriate responding. Furthermore, the $5HT_2$ antagonist pirenpirone failed to antagonize the THBC discriminative stimulus (31). The selective generalization of THBC to $5HT_{1B}$ compounds but not to $5HT_{1A}$ drugs and the inability of the $5HT_2$ antagonist pirenpirone to block the THBC cue has led to the speculation that THBC may produce its stimulus effects via $5HT_{1B}$ receptors.

In the present experiment, the THBC discriminative stimulus generalized to the $5HT_{1B}$ agonist mCPP, thus replicating previous findings (31). However, the inability of the 5HT releaser, norfenfluramine, to substitute completely for the THBC discriminative stimulus is inconsistent with the results of Schechter (30), who demonstrated that rats trained to discriminate THBC (20.0 mg/kg) generalize to the 5HT releaser fenfluramine. Norfenfluramine is the first metabolite of fenfluramine and these compounds have been shown to produce similar discriminative cues (2). It is somewhat surprising, in light of this, that norfenfluramine did not completely substitute for the THBC discriminative stimulus. However, three factors may contribute to this apparent discrepancy. First, different training doses were used in the two studies (20.0 mg/kg vs. 15.0 mg/kg). It is possible that the releasing properties of THBC may be apparent at higher doses than those required for stimulation of the $5HT_{1B}$ receptor and, therefore, the higher training dose of THBC would produce a more general and robust serotonergic cue, i.e., both the specific $5HT_{1B}$ agonist and releaser norfenfluramine would produce THBC-appropriate responding. The lower dose could produce a more specific serotonergic cue and therefore be limited in its generalization to norfenfluramine. Secondly, norfenfluramine and fenfluramine have been shown to have differential effects on serotonergic transmission (3) and these differences may account for the observed results. Lastly, the time-course of action of norfenfluramine appears to be more rapid than that of its parent compound (32).

Norharmane produced a partial generalization in THBC-trained rats. This finding is not unexpected since norharmane is the first metabolite of THBC (10). It is possible that THBC may be rapidly metabolized to norharmane and that norharmane, therefore, may contribute to the THBC interoceptive cue. This is an unlikely possibility since norharmane is not detected in the urine of rats until at least four hours following administration of THBC (9). Therefore, the ability of norharmane to engender predominantly THBC-appropriate responding may be related to its effects on serotonergic transmission since, like THBC, norharmane inhibits monoamine oxidase activity and blocks 5HT reuptake (5).

In rats trained to discriminate the benzodiazepine receptor partial inverse agonist FG 7142 both THBC and norharmane dose-responsively generalized to the FG 7142 interoceptive cue. FG 7142 produces an inverse agonist discriminative stimulus (17) which is believed to be anxiomimetic in nature based on the generalization of the FG 7142 cue to both physiological stressors (16) and pentylenetetrazole (17). The ability of THBC and norharmane to produce FG 7142-appropriate responding does not appear to be related to their serotonergic properties since the 5HT agonist mCCP failed to mimic the FG 7142 discriminative cue and, as previously reported, the FG 7142 cue does not generalize to the 5HT agonist norfenfluramine (17). Therefore, the generalization of THBC and norharmane in FG 7142-trained rats may be related to the inverse agonist properties of these compounds.

To investigate the possibility that THBC may act as a benzodiazepine receptor inverse agonist, experiments were performed with the benzodiazepine receptor mixed agonist/antagonist CGS 9896 and antagonist RO 15-1788 in an effort to block the ability of THBC to mimic the FG 7142 cue. Both drugs have been demonstrated to antagonize the FG 7142 cue (17). CGS 9896 and RO 15-1788 antagonized the generalization of FG 7142 to THBC, demonstrating that THBC mimics the FG 7142 cue through an inverse agonist activity at the benzodiazepine receptor. The antagonism of the THBC generalization by RO 15-1788 is especially strong evidence to support this contention since RO 15-1788 has limited intrinsic action itself and is, therefore, likely to be antagonizing the THBC effect at the benzodiazepine receptor level. Indeed, THBC displays a micromolar affinity for the benzodiazepine receptor (22) and there is evidence that THBC may produce benzodiazepine receptor-mediated anxiogenesis (33).

It is possible that the in vivo conversion of THBC to norharmane may underlie the apparent inverse agonist activity of THBC since norharmane has been demonstrated to have inverse agonist properties (19,20) and binds to the benzodiazepine receptor with a two-fold greater affinity than THBC (22). However, as discussed above, the pharmacokinetics of THBC metabolism are not consistent with this possibility (9). It is unlikely, therefore, that the metabolism of THBC to norharmane accounts for the ability of THBC to produce an inverse agonist cue.

In light of the inverse agonist nature of THBC (present study) and its physiological presence in humans (11), it is tempting to speculate that THBC may be an endogenous inverse agonist which mediates anxiety responses. In particular, the inverse agonist activity of THBC might underlie the previously reported generalization of the FG 7142 cue to stressful environmental manipulations (16). To test this hypothesis, these same stressful environmental manipulations were performed in the THBC-trained rats. The inability of the stressors to engender THBC-appropriate responding indicates that it is unlikely that endogenously formed THBC mediates anxiety/stress responses. It is possible, however, that the training dose of THBC used in this study achieved much higher THBC brain concentrations than would be attained physiologically and, therefore, produced a cue that would not be comparable to an interoceptive state produced by THBC in physiological concentrations.

The present experiments demonstrate that an asymmetrical generalization exists between FG 7142 and THBC, i.e., in FG

7142-trained rats, THBC mimics the FG 7142 cue; in THBC-trained rats FG 7142 does not mimic the THBC cue. Asymmetrical generalizations in the discriminative stimulus paradigm are frequently observed between compounds of differing specificities and have been discussed by Wood et al. (35). In this regard, the FG 7142 discriminative stimulus appears to contain one component, i.e., an inverse agonist component, whereas the THBC discriminative stimulus appears to be comprised of at least two components, a dominant serotonergic component and an inverse agonist component. Thus, when THBC is administered to rats trained to discriminate FG 7142, the rats recognize the inverse agonist nature of THBC and respond on the FG 7142 lever. Conversely, when FG 7142 is administered to rats trained to discriminate THBC, the predominantly serotonergic cue is not mimicked by FG 7142.

Parenthetically, the failure of the FG 7142 cue to generalize to mCPP demonstrates the pharmacological specificity of the FG 7142 stimulus. It is, however, interesting to note that mCPP is anxiogenic in humans (8,21) and has been recently shown to produce anxiety-like effects in animal models via $5HT_{1C}$ receptors (14). Thus, on a behavioral level, FG 7142 and mCPP may be expected to produce similar discriminative stimuli. However, it is unknown whether the discriminative stimulus produced by mCPP is based on its anxiogenic properties.

In conclusion, THBC produces a multicomponent discriminative stimulus based on both serotonergic and inverse agonist properties. The 5HT component is predominate in THBC-controlled discriminations, whereas the inverse agonist component is apparent in inverse agonist-controlled discriminations. Furthermore, although THBC is endogenously present in mammals (1,11), and, at pharmacological concentrations possesses inverse agonist properties, it is *unlikely* that THBC functions as an endogenous inverse agonist to mediate anxiety/stress responses.

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