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The Role of Dopamine and Serotonin Receptors in the Mediation of the Ethanol Interoceptive Cue

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SIGNS, S. A. AND M. D. SCHECHTER. *The role of dopamine and serotonin receptors in the mediation of ethanol interoceptive cue.* PHARMACOL BIOCHEM BEHAV **30**(1) 55–64, 1988.—The drug discrimination paradigm was used to evaluate the contribution of dopamine or serotonin receptors in the mediation of the stimulus properties of ethanol. Briefly, rats were trained to discriminate between ethanol (600 mg/kg, IP) and water vehicle. Dose-response relationships were observed for ethanol and rats were tested with various dopamine and serotonin receptor agonists and antagonists. The specific dopamine receptor agonists SKF 38393 (DA_1) and LY 171555 (DA_2) failed to produce appreciable ethanol-like stimulus effects. Furthermore, the dopamine receptor antagonists SCH 23390 (DA_1) and haloperidol (DA_2) did not affect ethanol-appropriate responding when administered in combination with the training dose of ethanol. A number of specific serotonergic receptor ligands were also tested. Quipazine, 5-MeODMT, buspirone, 8-OH-DPAT elicited intermediate ethanol-like stimulus properties in rats. The serotonin receptor blockers pizotifen, pirenperone and (−)propranolol were ineffective in blocking the interoceptive cue produced by 600 mg/kg ethanol. However, TFMPP produced strong ethanol-like discriminative properties and completely substituted for the training dose of ethanol. These results indicate that the stimulus properties of TFMPP are similar to those of a low dose of ethanol.

Ethanol Dopamine Serotonin Drug discrimination Receptors

THE behavioral paradigm of drug discrimination has been extensively used for classifying sedative-hypnotic drugs as being similar or dissimilar to ethanol. This classification is based upon the ability of each of these drugs to produce a discriminative stimulus, or interoceptive cue, similar to that produced by ethanol. In general, sedative-hypnotic drugs display stimulus properties similar to ethanol, whereas drugs which lack CNS depressant effects fail to produce ethanol-like discriminative stimuli in laboratory animals [31]. These studies have been useful for classifying drugs into particular categories with respect to ethanol, but did little to elucidate the neuropharmacological mechanisms that may mediate the ethanol interoceptive cue.

A number of studies have linked the central nervous system (CNS) effects of ethanol with brain dopaminergic activity. Less than 30 min after the administration of a low dose of ethanol (0.5–1.0 g/kg) to laboratory animals, CNS dopamine systems become activated and this results in an increase in dopamine (DA) synthesis and release [29]. In addition, there is evidence that suggests that ethanol affects dopamine receptor function as well. For example, acute ethanol treatment has been shown to stimulate dopamine receptor coupled adenylate cyclase (DA_1) activity in mouse striatum [27], and the nonadenylate cyclase linked dopamine receptor (DA_2) has been reported to be sensitized by ethanol treatment as reflected by an increased sensitivity of rat nucleus accumbens dopamine receptors to the direct application of dopamine [13]. These studies support the hypothesis that ethanol may alter the function of both CNS dopamine receptor subtypes.

Likewise, it has been found that brain serotonin (5-HT) activity is affected by ethanol treatment. Low dose ethanol treatment has been shown to increase [1], decrease [41] and to have no effect upon [8] central nervous system serotonin metabolism. Although there is a paucity of research in the area, administration of drugs which affect central serotonergic activity has been shown to alter the behavioral and neurochemical events elicited by ethanol in animals and man. Ethanol narcosis in mice was potentiated by exogenous administration of serotonin and this effect was attenuated by the serotonergic antagonist, methysergide [7]. In human studies, the indirect serotonergic agonist zimelidine reduced alcohol intake and increased abstinence in alcoholics [40]. Since ethanol appears to be involved with serotonin neurotransmission, serotonin receptors may influence CNS responses to low doses of ethanol.

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The drug discrimination paradigm provides a unique model in which receptor modulation of the interoceptive properties of a drug can be probed using dopamine [59] and serotonin [19] receptor ligands. To date, this approach to understanding the mechanism of ethanol's effects at the receptor level has not been undertaken. Thus, the present study was designed to investigate the effect of drugs, some of which have been hypothesized to be specific at dopamine and serotonin receptors, in the mediation of the stimulus properties of ethanol in rats.

METHOD

Animals

Male Sprague-Dawley rats (Zivic-Miller Laboratories, Allison Park, PA) were used in all operant experiments. They were individually caged in a room on a 12-hr (0600–1800) light/12-hr dark schedule and maintained at a constant temperature (20–22°C) and humidity (40–45%). Tap water was available in the home cage ad lib and their weights were adjusted by daily rationing of approximately 16 g/day rat chow. This facilitated motivation of operant performance for food reward.

Equipment

The experimental space consisted of 10 identical standard rodent operant chambers (Lafayette Instrument 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 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 a 9 W house light. Solid-state programming equipment (Med Associates, E. Fairfield, VT) used to control and record the sessions was located in an adjacent room to preclude any possible external cue effects.

Drug Discrimination Training

The drug discrimination procedure consisted of training rats to press one of two available levers in an operant chamber while the rats were under the influence of the drug state (ethanol) and to press the opposite lever in the nondrug state (vehicle). Thus, each of the two stimuli (drug state and nondrug state) was associated with responding on a particular lever. Training consisted of various phases. Lever pressing for food reward was trained by placing a food-deprived rat into an operant chamber and delivering a food pellet by remote control whenever the exploratory nature of the rat brought it into close proximity of the assigned training lever. The rats soon learned to press levers for reinforcement (45 mg Noyes food pellets) on a graduated (1 to 10) fixed ratio (FR) schedule. Rats were initially administered an intraperitoneal (IP) injection of vehicle (5 ml/kg distilled, deionized water), 10 min later were placed into the operant chamber, and upon pressing the designated lever, received food reinforcement on a FR 1 schedule, i.e., each press resulted in the delivery of one food pellet. The food reinforcement schedule was gradually increased until the rats were pressing the vehicle-appropriate lever on an FR 10 schedule (10 lever presses per pellet). Consecutive daily sessions in which a single vehicle injection was administered were conducted until FR10 responding was stable (approximately 10 daily sessions).

In the next training phase, the rats were administered an equal volume of the training drug (ethanol, 600 mg/kg, IP). Ten min after injection, they were required to press the lever opposite to that which they learned to press after vehicle injection, on an FR schedule, to receive reinforcement. The training continued in daily 15 min sessions proceeding from FR 1 through FR 10 until the ethanol-appropriate lever was pressed on an FR 10 schedule (4–7 sessions). In order to minimize effects due to any possible position preference, the rats in each group were divided into two equally-sized subgroups. For one subgroup, responding on the left lever following ethanol injection was reinforced by the delivery of food pellets, whereas the other subgroup was reinforced with food after responding on the right lever. Responses on the opposite lever in each case was reinforced with food pellets after vehicle injection.

After the rats were pressing both levers on an FR 10 schedule, the last phase of training, i.e., discriminative training, began utilizing a pseudo-random sequence of ethanol (E) or distilled water vehicle (V) administration in the following order: E-V-V-E-E; V-E-E-V-V. Thus, in each two-week period, the rats received 5 ethanol and 5 vehicle administrations. The number of responses on each lever before obtaining the first food pellet was recorded. The first lever pressed 10 times was designated as the "selected" lever. The rats were then allowed to continue lever pressing until 400 responses on the correct lever were made and, thus, 40 food reinforcements (on the FR 10 schedule) were obtained. The rats were required to remain on this training schedule until each animal was able to attain criterion performance. The training criterion was met when the rats "selected" the appropriate lever (according to the drug or nondrug state imposed) correctly in 8 of 10 consecutive daily sessions. The first session of the ten consecutive sessions in which 8 correct selected lever responses were made was designated as the first session-to-criterion (STC_1). To ensure that the discrimination between ethanol and its vehicle was stable, a second 8 correct of 10 consecutive sessions (STC_2) was required before the rats were used in dose-response, substitution or antagonism studies (below).

Dose-Effect Testing

Once training criterion was achieved, the rats were tested with doses of ethanol that were above or below the dose (600 mg/kg) to which they were trained; this allowed a dose-response relationship to be observed. During this series of experiments, the maintenance of the ethanol-vehicle discrimination was assured by administering and testing either 600 mg/kg ethanol or vehicle on every second day. The other doses of ethanol were tested on alternate days according to the following schedule: E-DR_1-V-DR_1-E-DR_2-V-DR_2, etc., where E=600 mg/kg ethanol, V=vehicle, and DR_1=one dose (i.e., 150, 300, 450 or 900 mg/kg) of ethanol and DR_2 is another ethanol dose. Following the time-course after ethanol injection (10 min), the rats were placed into the experimental chamber and were allowed to lever press, without reinforcement, until 10 responses were made on either of the two levers. When 10 responses were made on any one lever, the animal was immediately removed from the experimental chamber to preclude reinforcement at an ethanol dose other than that to which the animals were trained. The lever first pressed 10 times was designated as the "selected" lever. Each ethanol test dose was administered in a random order on 2 occasions with each test session preceded by one vehicle and one ethanol maintenance session. In this way, the

animals' experiences on days preceding test days were counterbalanced with respect to any possible aftereffects that may have been produced by the training conditions.

Agonist and Antagonist Testing

Subsequent to the dose-response experiments, a schedule of substitution and antagonism testing was begun using various dopaminergic and serontonergic receptor agonists/antagonists. Discriminative testing of the agonists was performed 30 min after administration of the drug, a time-course found to be adequate for behavioral activity for each drug tested. This time-course was chosen to allow each test drug to reach a central site of action and to reliably produce a strong, salient interoceptive cue. The antagonists were employed in an attempt to attenuate the cue produced by an injection of ethanol. Drugs used for this purpose were given 30 min prior to ethanol or vehicle, and the animals were tested 10 min after the second injection. Again, the rats were allowed to lever press, without reinforcement, until one of the two levers was "selected" (i.e., first lever to receive 10 presses).

Measurements and Statistical Analysis

The percentage of rats "selecting" the lever appropriate for the training drug (ethanol) was the quantal measurement of discrimination. Quantal data are presented as percentage of rats making the correct first-choice selection on the ethanol-correct lever (all-or-none). The dose-response quantal data were subjected to analysis by the procedure of Litchfield and Wilcoxon [33] that employs log-dose vs. probit measurements. A computer-generated formulation of the Litchfield-Wilcoxon analysis [54] yielded an ED_{50} for the dose-response curves of ethanol and for each of the agonist test drugs. This analysis also allows for tests of parallelism between dose-response curves, as well as for potency differences between drugs.

Ethanol-trained animals were required to maintain a minimum of 80% ethanol-appropriate lever selections (quantal) during ethanol maintenance sessions and were permitted a maximum of 20% ethanol-appropriate lever selections after vehicle injection for any 10 consecutive maintenance sessions. This established that an animal needed only to recognize the ethanol- or vehicle-cue correctly 80% of the time as previously required to attain performance criterion (above). Therefore, it was determined that, in a substitution test, the test drug needed only to produce equal to or greater than 80% ethanol-appropriate quantal as the criterion for generalization or transfer of ethanol-trained rats to the test drug. Likewise, when a putatively antagonistic test drug was administered in combination with ethanol, antagonism of the cue was considered to occur if responses on the ethanol-appropriate lever were reduced to less than or equal to 20%.

Drugs

Drug doses and time-courses were chosen so as to fall within behaviorally-active ranges as determined from the scientific literature. All drugs were administered IP unless otherwise indicated. Drugs used in this study and their source include: LY 171555 HCl, Eli Lilly Co., Indianapolis, IN; SKF 38393 HCl, Smith Kline and French Co., Philadelphia, PA; SCH 23390, Schering Pharmaceutical Co., Bloomfield, NJ; haloperidol, McNeil Pharmaceutical Co., Fort Washington, PA; quipazine maleate, TFMPP (1-(3-trifluoromethylphenyl)piperazine), 5-MeODMT (5-methoxy dimethyltryptamine) oxalate, 8-OH-DPAT (8-hydroxy-2-(dipropylamino)tetralin) HBr, Research Biochemicals, Inc., Wayland, MA; buspirone HCl, Bristol-Myers, Evansville, IN; pizotifen, Sandoz Pharmaceuticals, East Hanover, NJ; fenfluramine HCl, A. H. Robins, Richmond, VA; pirenperone HCl, Janssen Pharmaceuticals, Beerse, Belgium; and (−)propranolol, Ayerst, New York, NY. Each drug was prepared as its free base in distilled water to yield a constant injection volume of 1 ml/kg.

TABLE 1

ETHANOL DOSE-RESPONSE EFFECTS, ED_{50} AND 95% CONFIDENCE INTERVAL IN RATS (n=14) TRAINED TO DISCRIMINATE ETHANOL (600 mg/kg, IP) FROM VEHICLE

Treatment	Dose (mg/kg)	No. Trials	Quantal*
	Maintenance Sessions		
Ethanol	600	5	87.5
Vehicle	—	5	8.0
	Dose-Response		
Ethanol	900	2	92.9
	450	2	64.3
	300	2	35.7
	150	2	21.4
ED_{50}			322.9 mg/kg
95% confidence interval (mg/kg)			203.2–513.3

*Quantal data are presented as percentage of rats making the correct first-choice selection on the ethanol-correct lever.

RESULTS

Training and Dose-Response

The rats used in this study were trained until all rats achieved the criterion for discriminative performance prior to any rat being used in dose-response and generalization testing. Thus, as a group, the animals required (mean±SE, range) 24.1±2.9 (9–30) training sessions to reach the first criterion or STC_1 and a mean of 36.7±2.9 (25–48) training sessions to reach the second criterion or STC_2. Thus, all rats were considered able to accurately discriminate 600 mg/kg ethanol from its vehicle by 60 sessions; 30 sessions with ethanol and 30 sessions with vehicle.

The dose-response data from these animals are presented in Table 1. Decreasing doses of ethanol (900, 450, 300 and 150 mg/kg) produced decreasing ethanol-appropriate lever selections (quantal). The quantal measurements for 600 mg/kg ethanol and vehicle were obtained from the maintenance sessions interspersed between test sessions. The ED_{50}, as calculated using the method of Litchfield-Wilcoxon, equaled 322.9 mg/kg with a 95% confidence interval of 203.2–513.3 mg/kg.

Dopaminergic Agonists

SKF 38393 was administered at doses of 10–30 mg/kg and lever selection was similar to that found after vehicle injection (Table 2). The peak quantal score (33.3%) was observed after testing 15 mg/kg of the drug. The SKF 38393 dose-

TABLE 2

THE EFFECT OF VARIOUS DOPAMINE RECEPTOR AGONISTS AND ANTAGONISTS ON THE DISCRIMINATIVE PERFORMANCE OF ANIMALS (n=14) TRAINED TO RECOGNIZE THE STIMULUS PROPERTIES OF 600 mg/kg IP ETHANOL

Maintenance Sessions

Treatment	Dose (mg/kg)	No. Trials	Quantal
Ethanol	600	14	95.1
Vehicle	—	14	13.8

D_1 Receptor Ligands

Treatment	Dose (mg/kg)	Treatment	Dose (mg/kg)	No. Trials	Quantal	Average* Behavioral Disruption min (range)
		SKF 38393	10	2	11.1	—
		SKF 38393	15	2	33.3	—
		SKF 38393	20	2	32.0	110 (0–300)
		SKF 38393	30	2	27.7	81 (0–300)
SCH 23390	0.01	vehicle	—	2	5.5	—
SCH 23390	0.01	ethanol	600	2	84.0	—
SCH 23390	0.02	ethanol	600	2	100.0	—

D_2 Receptor Ligands

		LY 171555	0.5	2	33.3	—
		LY 171555	1.0	2	55.5	11 (0–60)
		LY 171555	1.5	2	16.6	3 (0–30)
Haloperidol	0.1	vehicle	—	2	16.6	—
Haloperidol	0.1	ethanol	600	2	91.6	—

*Behavioral disruption is defined as the lack of lever pressing behavior by rats and is expressed as average min elapsed before lever selection.

response curve in relation to that produced by ethanol is illustrated in Fig. 1. Doses greater than 15 mg/kg produced decreasing ethanol-appropriate quantal responding. This trend was correlated with behavioral disruption which is defined as the lack of lever pressing behavior by rats and is expressed as min elapsed before lever selection. Behavioral disruption was not observed with any vehicle or ethanol treatment, however, doses of 20 and 30 mg/kg SKF 38393 caused interruption of operant performance as reflected by an average latency of 110 and 81 min, respectively (Table 2).

LY 171555 was injected 30 min prior to testing at doses of 0.5, 1.0 and 1.5 mg/kg. The log-dose vs. ethanol-appropriate lever selection curve depicts an inverted U-type dose-response curve for LY 171555 (Fig. 1). LY 171555 produced peak quantal responding (55.5%) that corresponded with ethanol-appropriate lever responding that would be expected after intermediate doses of ethanol (i.e., 150–450 mg/kg). Increasing the dose of LY 171555 to 1.5 mg/kg produced a dramatic fall in ethanol-appropriate responding. As with the previous dopamine agonist tested, administration of higher doses of LY 171555 produced behavioral disruption (Table 2).

Dopaminergic Antagonists

SCH 23390 (0.01 and 0.02 mg/kg) administered in combination with vehicle produced vehicle-like responding (Table 2). SCH 23390 had no effect upon ethanol-appropriate responding when administered with the training dose of ethanol as evidence by quantal responses equivalent to those found after ethanol maintenance sessions (Table 2).

The results of pretreatment of animals with haloperidol (0.1 mg/kg) prior to administration of the training dose of ethanol or vehicle are also shown in Table 2. Haloperidol, in combination with vehicle, produced quantal results equivalent to vehicle maintenance sessions. In addition, haloperidol pretreatment in animals injected with 600 mg/kg ethanol yielded quantal scores (91.6%) which were not different from ethanol responding alone. No behavioral disruption was noted for either dopamine receptor antagonist.

Serotonergic Agonists

The results of administering several serotonergic drugs upon discriminative performance in animals trained to discriminate a 600 mg/kg dose of ethanol from vehicle are presented in Table 3. Fenfluramine was administered to ethanol-trained rats at doses of 2, 3 and 4 mg/kg. Fenfluramine produced an intermediate ethanol-appropriate quantal responding (61.1%) at 3 mg/kg and the rats displayed behavioral disruption at this dose (Table 3). Increasing the dose to 4 mg/kg produced less ethanol-appropriate lever selection (44.4%) and increased disruption of operant performance.

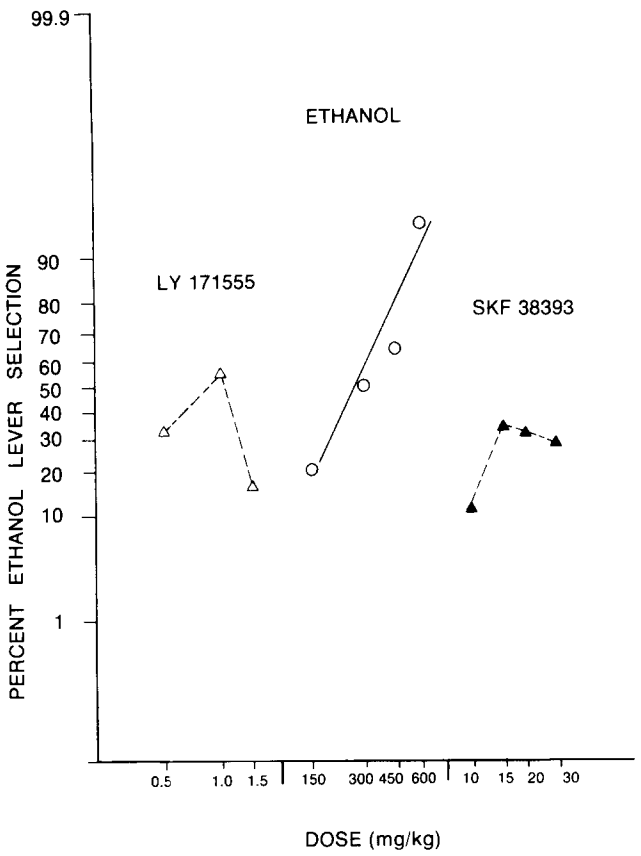

FIG. 1. Dose response effects of LY 171555, ethanol and SKF 38393. Ordinate: Percentage of rats selecting the ethanol-appropriate lever on a probit scale. Abscissa: Dose (mg/kg) of drug on a log scale.

TABLE 3

THE EFFECT OF VARIOUS SEROTONERGIC AGONISTS ON THE DISCRIMINATIVE PERFORMANCE OF RATS TRAINED TO RECOGNIZE THE INTEROCEPTIVE CUE PRODUCED BY 600 mg/kg IP ETHANOL

Treatment	Dose (mg/kg)	No. Trials	Quantal	Average Behavioral Disruption min (range)
Maintenance Sessions				
Ethanol	600	18	93.0	—
Vehicle	—	18	5.9	—
Substitution Tests With Serotonergic Agonists				
Fenfluramine	2.0	2	22.2	—
	3.0	2	61.1	7 (0–30)
	4.0	2	44.4	13 (0–30)
Quipazine	0.5	2	30.0	—
(n=5)	1.0	2	50.0	—
	2.0	2	60.0	18 (0–30)
5MeODMT	1.0	2	40.0	—
(n=5)	2.0	2	50.0	—
	3.0	2	60.0	9 (0–20)
Buspirone	0.75	2	20.0	—
	1.5	2	50.0	—
	2.0	2	25.0	—
8-OH DPAT	0.10	2	21.1	—
	0.25	2	35.0	6 (0–60)
	0.38	2	61.1	7 (0–60)
TFMPP	0.50	2	50.0	—
(n=5)	0.75	2	50.0	3 (0–10)
	1.0	2	90.0	3 (0–15)

All experiments were performed on 7 animals except where noted.

The dose effect curve for fenfluramine with respect to ethanol is presented in Fig. 2.

Quipazine, at doses of 0.5–2.0 mg/kg, produced increasing ethanol-appropriate lever selection (Table 3) with the highest dose resulting in a quantal score of 60%. These values correspond to intermediate ethanol-like effects. The quipazine dose-response curve demonstrated excellent linearity through the highest dose (Fig. 2) yet was not statistically parallel to the ethanol dose-response curve. Administration of higher doses of quipazine was precluded by behavioral disruption. At doses of 1.0, 2.0 and 3.0 mg/kg, 5-MeODMT produced linear quantal dose-responsiveness (Fig. 3) that was not parallel to the ethanol dose-response curve. The maximal quantal ethanol-appropriate lever selection was 60.0%. The highest dose of 5-MeODMT was accompanied by behavioral disruption (Table 3).

Buspirone (0.75–2.0 mg/kg) produced an inverted U-type dose-response curve with peak quantal ethanol-appropriate responding of 50% (Fig. 3). Ethanol-appropriate quantal responding was reduced at the highest dose tested (2.0 mg/kg) that was equivalent to vehicle-like discrimination (Table 3). 8-OH-DPAT was tested at doses of 0.1–0.38 mg/kg in ethanol-trained rats and produced a linear dose-effect curve on a quantal scale that was not statistically parallel to the ethanol dose-response curve (Fig. 4). Increasing doses of 8-OH-DPAT produced increasing ethanol-appropriate quantal values. 8-OH-DPAT produced a peak ethanol-appropriate quantal lever selection percentage (61.1%) corresponding to that produced by intermediate doses of ethanol (Table 3). Behavioral disruption delayed operant performance at the two highest doses of 8-OH-DPAT and, thus, precluded testing higher doses.

In contrast to other 5-HT agonists, TFMPP produced strong quantal ethanol-like responding at all doses tested (Table 3). At 1.0 mg/kg, TFMPP produced 90% ethanol-appropriate lever selection which exceeded the 80% criteria for generalization and, thus, produced an interoceptive cue equivalent to that of the training dose of ethanol. Other than a slight degree of delayed lever pressing, no behavioral changes were observed at this doses. Lesser amounts of TFMPP (i.e., 0.5 and 0.75 mg/kg) produced intermediate ethanol-like results with the quantal effects not reaching the criteria for generalization. A statistical comparison of the slopes of ethanol vs. TFMPP dose-response curves (Fig. 4) revealed that the two curves were parallel within 95% confidence limits (calculated $t=2.039 <$ critical $t=2.776$).

Serotonergic Antagonists

Pirenperone was administered at a dose of 0.16 mg/kg, 30

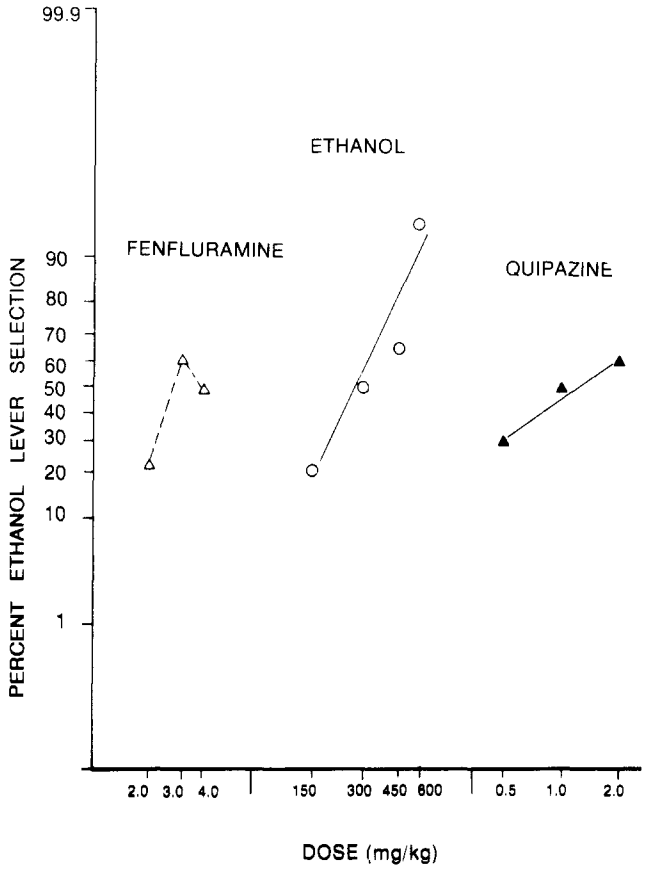

FIG. 2. Dose-response effects of fenfluramine, ethanol and quipazine. Ordinate: Percentage of rats selecting the ethanol-appropriate lever on a probit scale. Abscissa: Dose (mg/kg) of drug on a log scale.

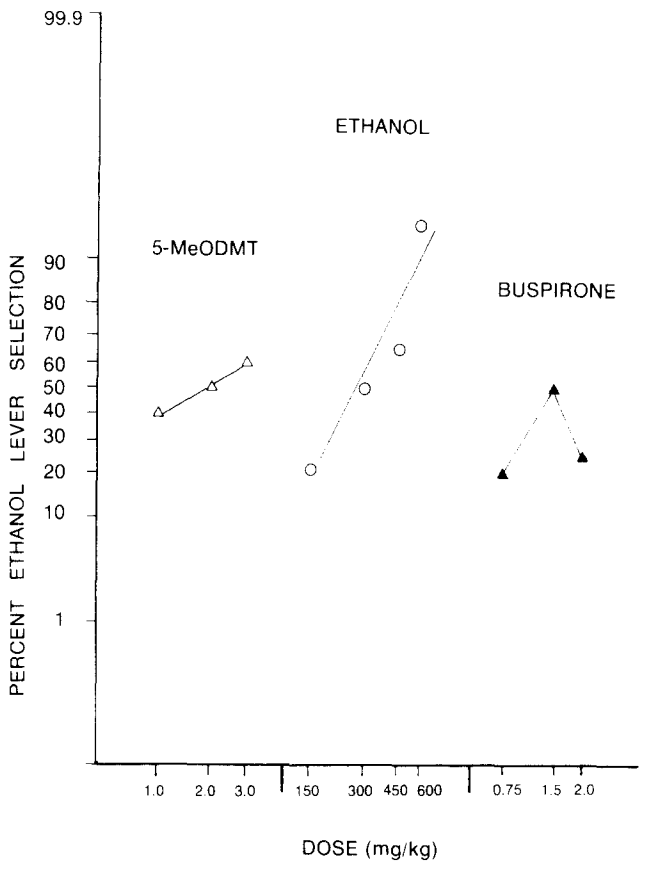

FIG. 3. Dose-response effects of 5-MeODMT, ethanol and buspirone. Ordinate: Percentage of rats selecting the ethanol-appropriate lever on a probit scale. Abscissa: Dose (mg/kg) of drug on a log scale.

min prior to ethanol (600 mg/kg) or vehicle injection (i.e., 40 min before discriminative testing). In combination with vehicle, pirenperone produced 5% ethanol-appropriate lever selections (Table 4). Rats pretreated with 0.16 mg/kg pirenperone, and given the training dose of ethanol, generated a quantal score equivalent to that found with ethanol treatment alone.

The results of pretreatment with 3.0 mg/kg pizotifen 40 min prior to lever pressing are also present in Table 4. Pizotifen, in combination with vehicle, produced quantal responding (29.4%) that exceeded the 20% criteria for vehicle responding alone and, thus, reflected low ethanol-like effects itself. When pretreated with 3.0 mg/kg pizotifen, rats injected with the training dose of ethanol produced strong ethanol-appropriate lever selection that corresponded well with that of ethanol alone.

The results of pretreatment of rats with (−)propranolol are presented in Table 4. A dose of 20 mg/kg (−)propranolol administered 30 min prior to ethanol (600 mg/kg), resulted in 100% of the animals selecting the ethanol-appropriate lever in one trial. The small supply of (−)propranolol available precluded continued trials.

DISCUSSION

The present investigation confirms previous studies [3, 47, 52, 57] that a low dose of ethanol (i.e., 600 mg/kg, IP) is capable of controlling discriminative responding in rats. Furthermore, the sessions required to attain criterion for adequate discrimination (10–48 sessions) were similar to those previously described. In the present study, the ethanol-trained rats demonstrated a linear dose-response curve for ethanol and the ED_{50} (322.9 mg/kg) derived by analysis of the curve was comparable to those previously described for a similar dose and time-course (i.e., ED_{50}=300 and 372 mg/kg) [47,48].

Recently, it has been reported that pretreatment of rats with the direct dopamine agonist apomorphine shifted the dose-response curve to the left in rats trained to recognize the stimulus properties of 600 mg/kg ethanol [49]. Furthermore, studies in humans showing that apomorphine enhanced the intoxicating effects of ethanol [2] supports a correlation between central dopaminergic systems and subjective responses to ethanol. Few studies have sought to mimic or attenuate the ethanol discriminative stimulus at the level of the neurotransmitter receptor. In light of the demonstrated mechanistic similarities of the behavioral properties of dopamine agonists and ethanol, the rats trained to discriminate ethanol in this study were first tested for generalization to the DA_1 receptor agonist SKF 38393 and the DA_2 receptor agonist LY 171555. The affinity and functional activity of these compounds for their specific receptor subtypes has been previously reported (i.e., LY 171555 [17, 56]; SKF 38393 [39]). In the present study, LY 171555 generated only partial (intermediate) ethanol-like responding, whereas the

TABLE 4

THE EFFECT OF VARIOUS SEROTONERGIC AGONISTS ON THE ABILITY OF RATS (n=7) TRAINED TO DISCRIMINATE 600 mg/kg IP ETHANOL FROM VEHICLE TO RECOGNIZE THE INTEROCEPTIVE CUE PRODUCED BY THE TRAINING DOSE OF ETHANOL

Pretreatment	Dose (mg/kg)	Treatment	Dose (mg/kg)	No. Trials	Quantal
Pirenperone	0.16	vehicle	—	2	5.0
	0.16	ethanol	600	2	85.0
Pizotifen	3.0	vehicle	—	2	29.4
	3.0	ethanol	600	2	94.4
(−) Propranolol	20.0	ethanol	600	1	100

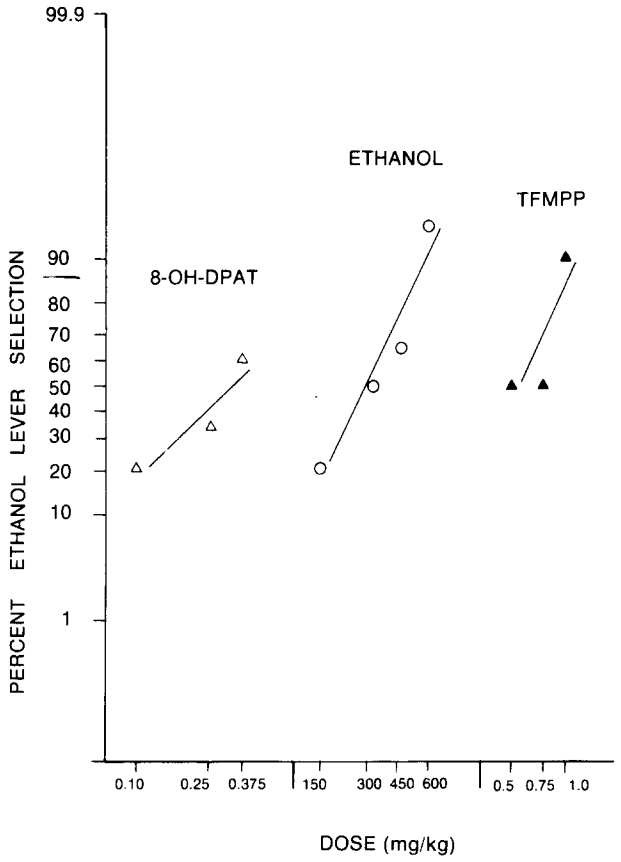

FIG. 4. Dose-response effects of 8-OH-DPAT, ethanol and TFMPP. Ordinate: Percentage of rats selecting the ethanol-appropriate lever on a probit scale. Abscissa: Dose (mg/kg) of drug on a log scale.

SKF 38393 did not substitute for ethanol at any dose tested.

The specific dopamine receptor antagonists SCH 23390 (DA_1) [28,30] and haloperidol (DA_2) [4,9] were individually coadministered with ethanol in an attempt to evaluate the role of DA receptor blockade on the rats' recognition of the ethanol cue. The results of the present experiments indicate that neither of these antagonists was capable of attenuating the interoceptive cue produced by ethanol.

Indirect evidence points to a possible role for serotonergic mediation of the stimulus properties of ethanol. In an early study, Schechter [46] demonstrated that pretreatment of rats with the CNS serotonin depleter, para-chlorophenylalanine (pCPA) was able to completely block ethanol discrimination. This effect was considered to be specific for the ethanol cue since similar treatment with pCPA did not disrupt discriminative performance in rats trained to amphetamine vs. saline [50]. These results provide reasonable evidence that serotonergic pathways may mediate ethanol discrimination, possibly at the receptor level. Indeed, Winter [57] pretreated ethanol-trained rats with cinanserin, later found to be a $5\text{-}HT_2$ receptor antagonist, and did not find an effect upon ethanol discrimination. These results were at odds with those observed after pCPA pretreatment ([46]; cited above). Present knowledge would indicate that, whereas cinanserin was capable of (at best) only a limited ($5\text{-}HT_2$ selective) blockade of serotonergic function, pCPA's effect would be upon all serotonergic neurons.

There appear to be multiple serotonin receptors in the rat brain. At least two distinct 5-HT receptors can be differentiated using binding techniques. Peroutka and Snyder [45] have designated those serotonin receptors labeled by (^{3}H)5-HT as $5\text{-}HT_1$ sites and those labeled by (^{3}H)spiperone as $5\text{-}HT_2$. The $5\text{-}HT_1$ recognition sites were further subdivided into subtypes characterized by high affinity ($5\text{-}HT_{1A}$) and low affinity ($5\text{-}HT_{1B}$) displacement of (^{3}H)5-HT by spiperone [43,51]. More recently, two additional $5\text{-}HT_1$ subtypes have been reported. The $5\text{-}HT_{1C}$ receptor, characterized by high affinity for (^{3}H)5-HT and (^{3}H)mesulergine with low affinity for selective $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$ and $5\text{-}HT_2$ ligands, is highly concentrated in rat choroid plexus [42,57] Furthermore, Heuring and Peroutka [26] have reported a distinct serotonin receptor subtype, $5\text{-}HT_{1D}$, with unique affinities for a number of different ligands and present predominantly throughout bovine brain. Since the discovery of these receptor subpopulations, specific agonists and antagonist have been developed for central $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$ and $5\text{-}HT_2$ sites.

A number of serotonergic drugs tested failed to produce interoceptive stimuli perceived as similar to that of a low dose of ethanol. Fenfluramine, a drug which releases intraneuronal serotonin stores [11,55], elicited only intermediate ethanol-appropriate responding. These results suggest that the stimulus properties of ethanol may not be the result of indirect stimulation of receptors by fenfluramine-induced release of serotonin from nerve terminals. Similarly, the direct $5\text{-}HT_2$ agonist quipazine [10, 14, 35] also failed to produce generalization to ethanol, suggest-

ing that the 5-HT_2 receptor may not be primarily responsible for mediation of the stimulus properties of ethanol. The 5-HT_2 receptor blockers pirenperone [24] and pizotifen [21] were tested for their ability to attenuate the stimulus properties of the training dose of ethanol. The two antagonists were unable to affect ethanol discrimination, supporting the contention that the stimulus properties of ethanol are not primarily mediated via the 5-HT_2 receptor.

A series of 5-HT_{1A} receptor agonists were also tested for their ability to mimic the ethanol interoceptive cue. Buspirone, a nonbenzodiazepine anxiolytic agent that has affinity for the 5-HT_{1A} receptor subtype [44], failed to transfer in ethanol-trained rats. The specific 5-HT_{1A} receptor agonist 8-OH-DPAT [25,38] produced only intermediate ethanol-like lever selection. The failure of 5-MeODMT, a nonselective serotonergic receptor agonist with action at both 5-HT_{1A} and 5-HT_2 receptors [22,23], to produce ethanol-like responding supports the previous findings with other, more specific ligands. The specific 5-HT_{1A} receptor blocker (−)propranolol [37] failed to attenuate the stimulus properties produced by the training dose of ethanol. Thus, the 5-HT_{1A} receptor does not appear to be the primary site for central mediation of the ethanol cue.

Of the serotonergic drugs used, only TFMPP produced ethanol-correct lever selection (90%) greater than that of the training dose of ethanol; this constitutes complete generalization. TFMPP is a serotonergic drug which has specific affinity for, and agonist activity at, the central 5-HT_{1B} receptor binding site [5,36]. An examination of the in vitro and in vivo properties of TFMPP suggest that, though selective for central 5-HT_{1B} binding sites, it may have antagonist action on peripheral 5-HT_2 receptors [15]. However, it is well established that drug discrimination relies upon central cue recognition [6] and, thus, is the foundation for using TFMPP to study central 5-HT_{1B} receptor sites. The TFMPP dose (1 mg/kg) at which generalization occurs in the present investigation is significantly lower than that reported to reduce locomotor activity (2.5 mg/kg) [34] or to produce "serotonergic syndrome" (20 mg/kg) [18]. However, the dose of TFMPP used in the present report does correspond well with recent drug discrimination studies correlating TFMPP to other putative 5HT_{1B} receptor agonists [10, 20, 36]. Our results would therefore suggest that ethanol's stimulus properties may be associated with stimulation of the central 5-HT_{1B} receptor. Examination of the TFMPP dose-response curve indicated that it was parallel to that produced by varying doses of ethanol. This data would further suggest that TFMPP is acting via a mechanism, or at a site of action, that is similar to that responsible for producing the interoceptive cue of ethanol [32]. The question that arises is, what is the "common" mechanism of action? It is possible that administration of TFMPP to ethanol-trained rats mimics the stimulus properties of ethanol by reducing serotonergic activity in the brain. Results from this laboratory indicate that an acute dose of ethanol (600 mg/kg, IP) rapidly reduces the release of serotonin in the striatum of unanesthetized, freely moving rats as measured in vivo voltammetry [53]. In addition, TFMPP has been shown to lower 5-HIAA in rat brain and this effect may be due to activation of serotonin autoreceptors that regulate serotonin synthesis and release [16]. Recently, it has been shown that the serotonin autoreceptor in rat brain is associated with 5-HT_{1B} binding sites [12]. It is conceivable that TFMPP is able to mimic the ethanol cue by stimulating 5-HT_{1B} autoreceptors, affecting a reduction in central serotonin activity, resulting in the recognition of an ethanol-like interoceptive cue.

The major conclusion that can be drawn from this study is that DA_1, DA_2, 5-HT_{1A} and 5-HT_2 receptor subtypes have an equivocal effect upon discrimination of a low dose of ethanol in rats. However, the putative 5-HT_{1B} receptor agonist TFMPP elicits a potent ethanol-like interoceptive cue; the exact mechanism by which this occurs is unclear.

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