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Preclinical Anxiolytic Versus Antipsychotic Profiles of the 5-HT_3 Antagonists Ondansetron, Zacopride, 3α-Tropanyl-1H-Indole-3-Carboxylic Acid Ester, and 1αH, 3α, 5αH-Tropan-3-yl-3,5-Dichlorobenzoate

Robert W. Dunn, William A. Carlezon, Jr., and Roy Corbett

Department of Biological Research, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, New Jersey

ABSTRACT

Dunn, R.W., W.A. Carlezon, Jr., and R. Corbett: Preclinical anxiolytic versus antipsychotic profiles of the 5-HT_3 antagonists ondansetron, zacopride, 3α-tropanyl-1H-indole-3-carboxylic acid ester, and 1αH, 3α, 5αH-tropan-3-yl-3,5-dichlorobenzoate. Drug Dev. Res. **23:** 289–300, 1991.

In preclinical studies, the behavioral effects of serotonin antagonists at 5-HT_3 receptor sites suggest potential efficacy in the treatment of anxiety and schizophrenia. The present study shows that 5-HT_3 antagonists were effective in disinhibiting behavior in non-conditioned rodent models which elicit a behavioral state presumed to be analogous to anxiety, but were generally not effective in conditioned rodent anxiety models or in assays that are traditionally predictive of antipsychotic agents. Ondansetron (0.01–0.1 mg/kg), zacopride (0.1–1.0 mg/kg), ICS 205-930 (3α-tropanyl-1H-indole-3-carboxylic acid ester; 0.5 and 1.0 mg/kg), and MDL 72222 (1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate; 10.0 and 20.0 mg/kg) demonstrated anxiolytic effects in the social interaction and elevated plus maze procedures, while having little or no effect in a modified Cook and Davidson conflict procedure. Ondansetron, zacopride, and ICS 205-930 had no effect in neuroleptic screening procedures, such as the apomorphine climbing mouse assay (CMA), the pole climb avoidance (PCA)

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Address reprint requests to Robert W. Dunn, Ph.D., Department of Biological Research, Hoechst-Roussel Pharmaceuticals, Inc., Route 202/206, P.O. Box 2500, Somerville, NJ 08876-1258.

procedure, and the intracranial self-stimulation of the medial forebrain bundle (ICSS-MFB) assay. MDL 72222 had no effect on CMA but dose-dependently antagonized PCA (ED_{50} = 10.9 mg/kg) and ICSS-MFB (ED_{50} = 8.8 mg/kg). The results of the present study suggest that MDL 72222 possesses a profile of activity that is different from the other 5-HT_3 antagonists tested and that, in general, 5-HT_3 antagonists may prove to be efficacious in the treatment of certain forms of anxiety in man.

Key words: serotonin, 5-HT_3 receptor, anxiety, neuroleptic, rodents

INTRODUCTION

The 5-HT_3 receptor has been characterized in the peripheral and central nervous systems [Richardson and Engel, 1986; Kilpatrick et al., 1987]. High densities of these receptors have been identified in brain areas implicated in anxiety and psychosis, such as the entorhinal cortex, frontal cortex, hippocampus, amygdala, nucleus accumbens, and olfactory tubercle [Kilpatrick et al., 1987].

The 5-HT_3 antagonists ondansetron, zacopride, and 3α-tropanyl-1H-indole-3-carboxylic acid ester (ICS 205-930) have displayed anixolytic effects in rats in non-conditioned anxiety models, such as the social interaction (SI) and the elevated plus maze (EPM) paradigms [Tyers et al., 1987; Costall et al., 1987a,b; Jones et al., 1988]. The paradigms traditionally used to evaluate anxiolytic efficacy in rodents induce a state of behavioral inhibition (presumed to be analogous to anxiety) in an animal, and then measure the ability of a test agent to disinhibit these suppressed behaviors. For example, the SI paradigm [Gardner and Guy, 1984] measures the amount of time two unfamiliar rats actively interact with each other in a familiar environment; therefore, the novelty of the partner is the only stimulus which inhibits social behavior between the rats. In the EPM assay [Pellow and File, 1986], exposure to an elevated open alley versus a closed alley creates an approach-avoidance conflict situation which results in an inhibition of exploratory behavior in the open alley. The SI and EPM assays are described as non-conditioned behavioral anxiety models, since they measure behavior in untrained animals. The Cook and Davidson model [1973] (in which low levels of foot shock elicit an inhibition of lever pressing for food reward) is a conditioned conflict procedure in which trained animals can be used to measure the anxiolytic effects of test agents.

Recent evidence has also shown that the 5-HT_3 antagonists ondansetron and ICS 205-930 have subtle effects on dopaminergic systems, suggesting possible antipsychotic activity [Costall et al., 1987a,b; Carboni et al., 1989; Sorensen et al., 1989]. For example, direct injection studies have shown that while 5-HT_3 antagonists have no effect on the basal activity of dopaminergic pathways or on behaviors induced by systemic administration of amphetamine or apomorphine [Costall et al., 1987b], these agents do attenuate hyperactivity elicited by direct injection of amphetamine or chronic dopamine (DA) infusion into the mesolimbic system [Costall et al., 1979] and indirect DA activation by neurokinin infusion [Hagan et al., 1987]. Classical neuroleptic procedures that are sensitive to an agent's propensity to elicit behavioral changes due to dopaminergic modulation include the climbing mouse assay (CMA), where dopaminergic antagonism is measured by the ability of a test agent to block apomorphine-induced stereotyped climbing behavior in mice [Costall et al., 1979; Protais et al., 1984]; the intracranial self-stimulation of the medial forebrain bundle paradigm (ICSS-MFB) in rats, a procedure in which agents that block dopaminergic transmission cause a decrease in responding for rewarding brain stimulation [Wise, 1978; Gallistel and Freyd, 1987]; and the pole climb avoidance assay (PCA), a conditioned avoidance procedure that is sensitive to disruption by antipsychotic agents, and can distinguish between the neuroleptic and sedative properties of a test agent [Cook and Wiley, 1957].

The present investigation was designed to define more completely the profile of the behavioral effects of the 5-HT_3 antagonists ondansetron, zacopride, ICS 205-930, and

1αH,3α,5αH-tropan-3-ly-3,5-dichlorobenzoate (MDL 72222) in both non-conditioned and conditioned procedures predictive of potential anxiolytic agents, as well as in behavioral screening assays classically utilized for their ability to identify potential antipsychotic agents.

MATERIALS AND METHODS

Male rodents were obtained from either Charles River Laboratories (Wistar rats and CD-1 mice) or Blue Spruce Laboratories (Long-Evans rats). All animals were housed under standard laboratory conditions as outlined in the "NIH Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publications, No. 85-23, Revised 1985), with a 12-hr light, 12-hr dark cycle, and allowed free access to food and water, except for rats used in the conflict procedure which were food deprived to 80% of their original body weight and then maintained on a restricted diet. The following drugs were used: apomorphine HCl (Sigma), diazepam (Hoffmann-LaRoche), haloperidol (McNeil), ICS 205-930 and MDL 72222 (Research Biochemicals, Inc), ondansetron (Dept. of Chemical Research, Hoechst AG), and zacopride (A.H. Robins). Compounds were either dissolved or suspended in distilled water with one drop of Tween 80, and administered intraperitoneally, except for apomorphine (subcutaneously) in a dosage volume of 1 ml/kg in rats or 10 ml/kg in mice. All test compounds were administered either 30 or 60 min prior to testing.

Anxiolytic Assays

Social interaction test. For the social interaction test [Gardner and Guy, 1984], naive male Wistar rats (250–300 g) were housed in pairs for 10 days prior to the start of the test. Groups of six pairs of rats were suitable for each experimental condition since the coefficient of variation between each pair of rats and for sensitivity to drug effects within groups of rats was less than 15%. The SI test consisted of familiarizing each pair (cagemates) of rats to the arena (50 × 50 × 30 cm) for a period of 8 min on 2 consecutive days. On the 3rd day, each rat was randomly assigned according to weight to an unfamiliar partner in groups of 12 animals (six pairs) which were subsequently administered the appropriate drug. These rats were then replaced into their home cage with their original cagemate until testing. Following the appropriate pretreatment time, each pair of unfamiliar rats was placed in the test arena and observed for SI behavior and overall motor activity for 5 min, with a summed score totaled for each parameter per pair of rats. SI time (sec) per pair of rats was measured as time spent sniffing partner, climbing over and crawling under partner, mutual grooming, genital investigation, and following and walking around partner. Aggressive behavior (biting, boxing, and pulling each other) was not considered as a SI behavior. Also passive social contact was not counted as SI; i.e., if the animals were next to each other for more than 10 sec and did not actively interact, the scoring was discontinued until movement resumed. Motor activity was measured by counting the number of rears (lifting of both front paws) and walks (of one body length) per pair of rats.

Elevated plus maze. For the EPM [Pellow and File, 1986], male Wistar rats (200–250 g) were also housed in pairs for 10 days prior to testing in the apparatus. During this time the rats were handled by the investigator on alternate days to reduce stress. Groups consisted of eight rats for each experimental condition since the coefficient of variation between rats and for sensitivity to drug effects was less than 15%. The day prior to testing in the EPM procedure, rats were familiarized to the laboratory test room for 30 min and handled by the experimenter for 5 min. On the test day, drugs were administered with a 30 min pretreatment time and the rats were placed in the center of the maze, facing one of the enclosed arms. The maze, elevated to a height of 50 cm, consisted of two open arms (50 × 10 cm) and two enclosed arms (50 × 10 × 30 cm) arranged such that the two arms of each type were opposite each other. During a 5 min test period, the following measures were taken by an observer: the number of entries into, and time spent in open and enclosed arms; and the total number of arm

entries. Both the SI and the EPM procedures were conducted in a sound-attenuated room, with observations made in an adjacent room via a remote control TV camera. Data for both SI and EPM assays were analyzed by a one-way ANOVA followed by Dunnett's test to compare group drug effects to vehicle control behavior.

Conflict test. Male Wistar rats (300–350 g) were trained for 6–8 weeks using the modified Cook and Davidson [1973] conflict procedure. Approximately 60% of animals reached the criteria established for stable colony responding i.e., 2–7 conflict responses per session and stable variable interval (VI) responding with less than 10% variation from baseline. In order to achieve stable conflict responding each rat's shock level was individually titrated to a value between 0.3 and 0.7 mA and delivered by a Coulburn scrambled shocker. As a result there was very low within-subject variability in conflict responding but a large between-subject variability in VI control responding and in drug sensitivity so that drug dosage was titrated for each individual subject. The conflict test was conducted in operant conditioning chambers consisting of a Plexiglas cubicle (25 × 25 × 25 cm) with a stainless steel grid floor and aluminum front panel. The chamber was enclosed in a sound- and light-attenuating cubicle with white noise to mask extraneous sound and equipped with a ventilating fan. The response lever was mounted on the front aluminum panel 5 cm above the grid floor and 5 cm from the Plexiglas side wall. Sweetened condensed milk (40 μl) was presented by a dipper from a well behind the midline of the front panel 3 cm above the grid floor. Recording equipment was located in an adjacent room. Sessions were conducted Monday through Friday and drugs were administered with a 30 min pretreatment time before the session on either Tuesday or Friday, if response rates were stable on the preceding control day.

The test parameters for the modified Cook and Davidson procedure were as follows: food-deprived rats were trained to lever press for milk reward during two distinct periods, six VI (VI-30 sec) no shock periods lasting 4 min each alternating with six conflict periods lasting 3 min each during which every fifth lever press (FR-5) was followed by a milk reward and simultaneously a low- level foot shock (0.3–0.7 mA) to minimize lever pressing to 2–7 shocks per control session. Statistical analysis for this procedure was by a dependent Student's t-test comparing an animal's previous day control behavior to the behavior following drug administration.

Neuroleptic Assays

Apomorphine induced climbing behavior. Male CD-1 mice (20–30 g) were assigned to groups of 8 and placed individually in wire mesh stick cages (4 × 4 × 10 in). After 1 hr habituation to the cage, animals were dosed with the test compound at appropriate pretreatment times. Apomorphine was then administered subcutaneously (s.c.) at 1.5 mg/kg, a dose which causes a stereotyped climbing behavior in all mice. For evaluation of climbing behavior, readings were taken at 10, 20, and 30 min after apomorphine administration, according to the following scale: mice with 4 paws on bottom = 0; mice with 1–2 paws on vertical wall = 1; mice with 3–4 paws on vertical wall = 2. The climbing scores were individually totaled (maximum score = 6 per animal), and the mean score of each group was compared to the mean score of the control group, which was set to equal 100%.

Pole Climb avoidance behavior. Male Long-Evans rats (250–350 g) were tested in a 25 × 25 × 40 cm experimental chamber that has a 2.5 cm-diameter, smooth stainless steel pole in the center, and a grid floor connected to a Coulburn Scrambled Shocker. This is a conditioned avoidance procedure where activation of a tone and light represents the conditioned stimulus (CS), which is presented alone for 4 sec, and then paired with an unconditioned stimulus (UCS) of electric shock (1 mA) for 26 sec. Test sessions consisted of 20 presentations of CS and UCS with an inter-trial interval (ITI) of 90 sec. An avoidance response was recorded if the rat jumped on the pole during the initial presentation of the CS alone, while an escape response was recorded if the animal jumped onto the pole during the presentation of the electric shock. An animal's avoidance and escape responses following administration of

test compounds were compared to their previous day's control scores, which was set to equal 100%.

Intracranial self-stimulation behavior. Male Wistar rats (350–400 g) were anesthesized with 50 mg/kg (i.p.) sodium pentobarbital (Nembutal, Abbott Laboratories, Chicago, IL), and their heads were placed on a level plane in a Kopf stereotaxic instrument. Using bregma as a reference point, the electrode (Plastic Products # MS303/1) was aimed at the medial forebrain bundle according to the atlas of Paxinos and Watson [1986], using the coordinates of AP = −0.8 mm, Lat = +1.8 mm, and DV = −7.2 mm below dura. The assembly was then permanently affixed to the skull using stainless steel screws and bone cement.

After a minimum of 10 days for recovery, the animals were trained to bar press for electrical stimulation on a continuous reinforcement schedule in a standard operant box outfitted with a single lever. The reward stimulus was a train of biphasic square-wave pulses generated by a Haer stimulator (Pulsar 4i). The parameters were set at a pulse duration of 0.5 msec with 2.5 msec between each pulse pair. The train of pulses varied between 16 and 30 per sec, and the intensity of the pulse that was delivered ranged from 0.1 to 0.5 mA using the lowest setting that would sustain maximal responding. After consistent baseline responding was obtained for five consecutive 30 min sessions, the animals were ready for testing with standard agents. Compounds were administered 60 min prior to testing, and the number of drug responses were compared to the number of responses made during each animal's 30 min control session on the preceding day, which was set to equal 100%. Data for CMA, PCA, and ICSS-MFB were analyzed using linear regression analysis to determine ED_{50} values and 95% confidence limits for all compounds that demonstrated dose-dependent activity.

RESULTS

Table 1 shows the effects of the 5-HT$_3$ antagonists and diazepam in the SI test. Ondansetron at 0.05 mg/kg significantly increased SI time by +52%, without affecting motor activity. Zacopride at 0.3 and 1.0 mg/kg also significantly increased SI time by +34% and +43%, respectively, while having no significant effects on motor activity. Similarly, ICS 205-930 at 1.0 mg/kg increased SI time by +26%, while being devoid of effects on motor activity. However, MDL 72222 at 20 mg/kg significantly increased SI time by +41%, but also elicited a significant (−61%) decrease in motor activity. The benzodiazepine diazepam at 1.25 and 2.5 mg/kg increased SI by +25% and +64%, respectively, with a significant (−37%) decrease in motor activity observed at the higher dose.

A similar activity profile for these compounds was observed in the EPM assay (Table 2). Ondansetron at 0.05 and 0.1 mg/kg, zacopride at 0.1 and 0.3 mg/kg, and ICS 205-930 at 0.25 and 0.5 mg/kg significantly increased open arm exploration time from +49% to +65% without significantly affecting motor activity. On the other hand, MDL 72222 at 10.0 mg/kg significantly increased time spent in the open arm by +68%, while significantly reducing motor activity by −38%. Diazepam at 1.25 and 2.5 mg/kg also significantly increased open arm exploration time by +47% and +105%, respectively, with a significant reduction (−44%) in motor activity observed at the higher dose.

In the Cook and Davidson conflict paradigm, the 5-HT$_3$ antagonists ondansetron (0.05 to 0.3 mg/kg), zacopride (1.0 to 10.0 mg/kg), ICS 205-930 (1.0 to 10.0 mg/kg), and MDL 72222 (5.0 to 20.0 mg/kg) all failed to significantly disinhibit conflict responding as measured by the mean number of conflict rewards (Table 3). Also, these compounds did not significantly affect non-conflict (VI) responding. Only diazepam at 4.0 and 20.0 mg/kg significantly disinhibited conflict responding by +300% and +510%, respectively, without affecting non-conflict (VI) responding.

Table 4 shows that the 5-HT$_3$ antagonists ondansetron (2.5 mg/kg), zacopride (10.0 mg/kg), ICS 205-930 (10.0 mg/kg), and MDL 72222 (20 mg/kg) failed to antagonize apo-

TABLE 1. The Effects of 5-HT$_3$ Receptor Antagonists and Diazepam in the Social Interaction Test†

		Social interaction		Motor activity	
Compound	Dose (mg/kg)	$\bar{X}$ ± S.E. (sec)	% change	$\bar{X}$ ± S.E. (act. units)	% change
Ondansetron	Veh.	99.3 ± 4.8	—	141.2 ± 4.0	—
	0.01	131.6 ± 7.5	+32	148.6 ± 4.9	+5
	0.05	151.1 ± 6.2*	+52	169.1 ± 6.7	+20
Zacopride	Veh.	96.0 ± 3.3	—	136.1 ± 6.9	—
	0.1	108.5 ± 2.4	+13	147.6 ± 4.2	+8
	0.3	128.1 ± 3.2*	+34	148.3 ± 3.4	+9
	1.0	137.3 ± 4.2*	+43	146.1 ± 1.7	+7
ICS 205-930	Veh.	102.8 ± 2.2	—	141.5 ± 4.8	—
	0.5	126.5 ± 3.2	+23	145.3 ± 10.5	+30
	1.0	130.3 ± 3.7*	+26	128.0 ± 3.5	−10
MDL 72222	Veh.	98.1 ± 2.5	—	132.8 ± 4.0	—
	10.0	117.5 ± 1.6	+20	101.3 ± 4.4	−24
	20.0	138.5 ± 2.7*	+41	51.6 ± 4.5*	−61
Diazepam	Veh.	100.0 ± 8.2	—	139.5 ± 8.1	—
	1.25	125.3 ± 4.2*	+25	109.1 ± 3.1	−21
	2.5	164.4 ± 9.2*	+64	87.3 ± 9.7*	−37

†Rats were administered compounds (mg/kg, i.p.) 30 min prior to testing. Social interaction behavior was measured in sec (mean ± S.E.) over 5 min. Motor activity was measured in activity units (mean ± S.E.) equal to rearing and/or movement of one body length. N = 6 pairs of rats per group.
*$P < .05$ one way ANOVA; Dunnett's test.

morphine-induced climbing behavior in mice. Only haloperidol dose-dependently blocked climbing, with an ED_{50} value of 0.13 mg/kg.

In the PCA assay (Table 5), ondansetron, zacopride, and ICS 205-930 failed to decrease either conditioned avoidance responding or escape responding. However, MDL 72222 and diazepam dose-dependently antagonized avoidance responding (ED_{50} values of 10.9 mg/kg and 13.3 mg/kg, respectively), and escapes (ED_{50} values of 16.9 mg/kg and 25.4 mg/kg, respectively). The antipsychotic agent haloperidol also decreased both avoidance responding (ED_{50} value of 0.04 mg/kg) and escape responding (ED_{50} value of 0.2 mg/kg). A similar profile for these agents was observed in the ICSS-MFB paradigm (Table 6). Ondansetron, zacopride, and ICS 205-930 failed to antagonize this behavior, while both MDL 72222 (ED_{50} = 8.8 mg/kg) and haloperidol (ED_{50} = 0.08 mg/kg) dose-dependently decreased lever pressing for electrical stimulation of the MFB.

DISCUSSION

The anxiolytic effects of the 5-HT$_3$ antagonists were first reported using the SI test in rats [Tyers et al., 1987] where ondansetron, zacopride, and ICS 205-930 all significantly increased SI time. More recently, the 5-HT$_3$ antagonists have produced anxiolytic effects in a number of non-conditioned paradigms predictive of anxiolytic activity such as light/dark transitions, social isolation of rats, and an approach-threat model in Marmoset monkeys [Costall et al., 1988; Jones et al., 1988; Cutler, 1990]. Furthermore, after direct injection into the amygdala but not the area postrema, Higgins et al. [1989] observed that ondansetron, zacopride, ICS 205-930, and MDL-72222 all significantly increased SI time in rats indicating regional specificity of the anxiolytic effects of the 5-HT$_3$ antagonists. The present study shows that the 5-HT$_3$ antagonists ondansetron, zacopride, and ICS 205-930 significantly increased

TABLE 2. The Effects of 5-HT$_3$ Receptor Antagonists and Diazepam in the Elevated Plus Maze†

		Open arm exploration		Arm crossings	
Compound	Dose (mg/kg)	$\bar{X}$ ± S.E. (sec)	% change	$\bar{X}$ ± S.E.	% change
Ondansetron	Veh.	52.0 ± 4.6	—	10.0 ± 0.5	—
	0.025	70.5 ± 3.6	+35	9.0 ± 0.6	−10
	0.05	81.0 ± 5.5*	+56	9.8 ± 0.6	−2
	0.1	77.8 ± 5.4*	+50	9.8 ± 0.7	−2
Zacopride	Veh.	52.4 ± 2.8	—	10.0 ± 0.4	—
	0.03	60.8 ± 3.7	+16	9.3 ± 0.7	−7
	0.1	80.8 ± 4.1*	+54	10.6 ± 0.4	+6
	0.3	83.3 ± 10.1*	+59	11.0 ± 0.5	+10
ICS 205-930	Veh.	46.4 ± 3.4	—	9.5 ± 0.4	—
	0.125	60.6 ± 4.5	+31	9.1 ± 0.5	−4
	0.25	76.3 ± 6.6*	+65	9.8 ± 0.6	+3
	0.5	69.0 ± 5.8*	+49	11.5 ± 1.6	+21
MDL 72222	Veh.	49.6 ± 4.6	—	11.3 ± 0.6	—
	5.0	66.6 ± 2.4	+36	10.3 ± 0.3	−9
	10.0	82.3 ± 5.0*	+68	7.0 ± 0.4*	−38
Diazepam	Veh.	49.2 ± 5.4	—	11.1 ± 2.4	—
	1.25	72.4 ± 6.1*	+47	9.3 ± 4.2	−16
	2.5	101.1 ± 7.6*	+105	6.2 ± 3.2*	−44

†Rats were administered compounds (mg/kg i.p.) 30 min prior to testing. Open arm exploration time was measured in sec (mean ± S.E.) over 5 min. Motor activity was measured by the No. of arm crossings (mean ± S.E.). N = 8 rats per group.
*$P < .05$ one way ANOVA; Dunnett's test.

both SI time and open arm exploration time, without affecting motor activity. MDL 72222 was the exception, significantly increasing both SI time and open arm exploration time, while significantly decreasing motor activity at the highest doses tested. These agents were active at dose levels previously shown in vivo to antagonize 5-HT$_3$ receptor-mediated effects [Fozard 1984; Cohen et al., 1989]. However, File and Johnston [1989] failed to observe any significant effects with the 5-HT$_3$ antagonists ondansetron and zacopride in either the SI test or the EPM assay. The discrepancies between our findings and those of File and Johnston [1989] in the analagous SI model of low light, unfamilar rats in a familar arena may be due to the following differences in methodology: rats were singly housed for 5 days prior to testing, individually acclimated to the test arena and only then exposed to another rat during testing; File and Johnston's measurement of SI behavior included aggressive behaviors (kicking, boxing) which may have masked drug effects on SI behaviors per se, since acute administration of anxiolytic agents has been shown to produce taming of aggression [Krsiak, 1979]. In addition, the 5-HT$_3$ antagonists failed to disinhibit conflict responding in the conditioned Cook and Davidson conflict paradigm. These results are in agreement with the observations of Jones et al. [1988] that the 5-HT$_3$ antagonist ondansetron failed to disinhibit conditioned behavior in the Vogel lick-shock conflict paradigm and suggest that these agents are less likely to reduce anxiety in a more stressful situation.

Previous research has suggested that the serotonin system participates in the modulation of anxiety. In particular, the septo-hippocampal system with its cells of origin in the dorsal raphe nucleus has been implicated in the control of anxiety [Gray, 1982; Glaser et al., 1985]. The 5-HT$_{1a}$ agonists as well as the benzodiazepines all suppress neuronal activity in the dorsal raphe nucleus and hippocampus following either systemic or iontophoretic administration

TABLE 3. The Effects of 5-HT$_3$ Receptor Antagonists and Diazepam in the Modified Cook and Davidson Conflict Procedure†

		VI responses		Conflict rewards	
Compound	Dose (mg/kg)	$\bar{X}$ ± S.E.	% change	$\bar{X}$ ± S.E.	% change
Ondansetron	Veh.	130.0 ± 23.2	—	2.5 ± 0.3	—
	0.05	130.7 ± 21.9	+1	2.7 ± 0.4	+10
	Veh.	206.7 ± 90.1	—	2.0 ± 0.1	—
	0.1	230.0 ± 87.9	+13	1.5 ± 0.3	−25
	Veh.	202.2 ± 99.2	—	2.2 ± 0.2	—
	0.3	145.0 ± 59.7	−28	1.1 ± 0.4	−50
Zacopride	Veh.	211.0 ± 99.2	—	3.3 ± 0.9	—
	1.0	312.5 ± 67.9	+48	8.0 ± 2.1	+142
	Veh.	194.7 ± 96.2	—	3.7 ± 1.4	—
	3.0	150.5 ± 64.3	−23	1.7 ± 0.4	−53
	Veh.	249.0 ± 27.4	—	4.5 ± 1.2	—
	10.0	272.0 ± 99.2	+9	5.5 ± 2.1	+22
ICS 205-930	Veh.	123.7 ± 21.0	—	2.5 ± 0.2	—
	1.0	105.0 ± 36.5	−15	1.5 ± 0.3	−40
	Veh.	136.5 ± 55.9	—	2.2 ± 0.1	—
	3.0	112.5 ± 54.8	−17	1.7 ± 0.2	−22
	Veh.	131.5 ± 26.5	—	2.3 ± 0.2	—
	10.0	44.5 ± 21.1	−66	0.7 ± 0.2	−67
MDL 72222	Veh.	165.2 ± 55.1	—	2.0 ± 0.1	—
	5.0	176.2 ± 80.4	+7	1.8 ± 0.3	−10
	Veh.	152.5 ± 65.0	—	2.0 ± 0.1	—
	10.0	124.7 ± 53.5	−18	2.0 ± 0.2	±0
	Veh.	116.7 ± 38.3	—	3.2 ± 0.6	—
	20.0	68.2 ± 18.0	−41	3.0 ± 1.0	−8
Diazepam	Veh.	117.2 ± 40.1	—	3.5 ± 0.9	—
	4.0	123.0 ± 42.2	+7	14.0 ± 3.1*	+300
	Veh.	116.0 ± 25.2	—	2.0 ± 0.6	—
	20.0	87.2 ± 26.2	−25	12.2 ± 1.8*	+510

†Rats were administered compounds (mg/kg i.p.) 30 min prior to testing. Conflict responding was measured as mean No. of conflict rewards received (mean ± S.E.) per session. N = 6 rats per group. *$P < .05$ dependent Student's t-test.

[Basse-Tomusk and Rebec, 1986; Thiebot, 1986; Van der Maelen et al., 1986] and disinhibit behavior in paradigms predictive of anxiolyitc activity [Dunn et al., 1989]. Kilpatrick et al. [1989] have identified high densities of 5-HT$_3$ receptors in brain areas implicated in anxiety such as entorhinal cortex, frontal cortex, hippocampus, and amygdala. Therefore, antagonism at these 5-HT$_3$ receptor sites postsynaptically may reduce 5-HT-induced excitation similar to presynaptic inhibition by the 5-HT$_{1a}$ agonists and benzodiazepines. However, recent evidence suggests that although affinity for the antagonism of the 5-HT$_3$ receptor site correlates with the antiemetic effects of these agents, there is some doubt that anxiolytic effects are due solely to antagonism at the 5-HT$_3$ receptor site [Young and Johnson, 1989; Pinkus et al., 1990].

The 5-HT$_3$ antagonists ondansetron, zacopride, and ICS 205-930 were ineffective in three preclinical assays predictive of neuroleptic activity, namely the CMA assay, the conditioned PCA procedure, and the ICSS-MFB assay at doses well above those required to produce anxiolytic activity. Generally, these behaviors are dependent on dopaminergic transmission since compounds which attenuate these behaviors act as dopaminergic antagonists. In agreement with Costall et al. [1987a], 5-HT$_3$ antagonists lack effects on behaviors induced by

TABLE 4. The Effects of 5-HT$_3$ Receptor Antagonists and Haloperidol in the Climbing Mouse Assay*

Compound	Dose (mg/kg i.p.)	Climbing behavior Control ± SEM	Treated ± SEM	% change
Ondansetron	2.5	6.0 ± 0.0	5.88 ± 0.13	−2
Zacopride	10.0	5.88 ± 0.13	6.0 ± 0.0	+2
ICS 205-930	10.0	5.88 ± 0.13	5.88 ± 0.13	±0
MDL 72222	20.0	5.88 ± 0.13	5.0 ± 0.5	−15
Haloperidol	0.05	5.63 ± 0.74	5.0 ± 0.5	−11
	0.1	5.63 ± 0.74	3.88 ± 0.61	−31
	0.2	5.63 ± 0.74	1.75 ± 0.86	−69
	0.4	5.63 ± 0.74	0.38 ± 0.26	−93
		ED_{50} (95% confidence limits) = 0.13 (0.11 and 0.15) mg/kg		

*Mice were administered compounds (mg/kg i.p.) 30 min prior to testing (except haloperidol; 60 min pretreatment). Linear regression analysis was used to determine ED_{50} values and 95% confidence limits for all compounds that demonstrated dose-dependent activity. N = 8 mice per group.

TABLE 5. The Effect of 5-HT$_3$ Receptor Antagonists, Haloperidol, and Diazepam in the Pole Climb Avoidance Procedure*

		% change compared to control responding ED_{50} and 95% confidence limits (mg/kg i.p.)	
Compound	Dose (mg/kg)	Avoidance responding	Escape responding
Ondansetron	1.0	+7%	0%
Zacopride	10.0	−1%	0%
ICS 205-930	10.0	−5%	0%
MDL 72222		10.9 (10.1–11.6)	16.9 (16.0–18.2)
Haloperidol		0.04 (0.03–0.05)	0.2 (0.19–0.23)
Diazepam		13.3 (10.0–15.8)	25.4 (21.8–29.7)

*Rats were administered compounds (mg/kg i.p.) 1 hr prior to testing. Linear regression analysis was used to determine ED_{50} values and 95% confidence limits for all compounds that demonstrated dose-dependent activity. N = 8 rats per group.

apomorphine (such as climbing behavior) or amphetamine although these agents do attenuate hyperactivity elicited by direct injection of amphetamine or chronic DA infusion directly into the mesolimbic DA system [Costall et al., 1987b] and indirect DA activation by neurokinin infusion [Hagan et al., 1987]. In addition, unlike classical dopaminergic antagonists, acute administration of 5-HT$_3$ antagonists does not affect either the metabolic [Koulu et al., 1989] or electrophysiological activity of dopaminergic pathways under resting conditions [Sorensen et al., 1989].

However, the 5-HT$_3$ antagonist ICS 205-930 has been shown to partially inhibit morphine-, nicotine-, and ethanol-induced stimulation of mesolimbic DA release while being ineffective on amphetamine-induced release [Carboni et al., 1989]. Morphine-, nicotine-, and ethanol-induced release of DA is dependent upon the ability of these agents to stimulate firing rates at DA cell bodies [Bunney et al., 1973; Mereu et al., 1984; Iwatsubo and Clouet, 1977; Gessa et al., 1985] whereas amphetamine stimulates DA release while not altering the firing rate of DA neurons [Bunney et al., 1973]. Similarly these agents were ineffective against ICSS-MFB behavior where electrical stimulation of axonal tracts in the MFB resulted in

TABLE 6. The Effects of 5-HT$_3$ Receptor Antagonists and Haloperidol on ICSS-MFB responding in rats*

Compound	Dose (mg/kg i.p.)	Mean responses: Control ± SEM	Mean responses: Treated ± SEM	% change
Ondansetron	1.25	2,757 ± 325	3,197 ± 328	+16
Zacopride	10.0	3,221 ± 643	3,029 ± 280	−06
ICS 205-930	10.0	2,745 ± 309	3,094 ± 637	+13
MDL 72222[a]	5.0	3,042 ± 406	2,602 ± 644	−14
	10.0	3,239 ± 746	1,831 ± 925	−43
	14.14	2,376 ± 551	88 ± 75	−96
Haloperidol[b]	0.05	3,250 ± 407	2,425 ± 303	−25
	0.07	3,085 ± 425	2,060 ± 462	−33
	0.1	3,189 ± 660	801 ± 202	−75

*Rats were administered compounds (mg/kg i.p.) 60 min prior to testing. Linear regression analysis was used to determine ED_{50} values and 95% confidence limits for all compounds that demonstrated dose-dependent activity. N = 4–6 rats per group.
[a]ED_{50} (95% confidence limits) = 8.8 (8.1 and 9.5) mg/kg.
[b]ED_{50} (95% confidence limits) = 0.08 (0.07 and 0.08) mg/kg.

depolarization-induced release of DA without increasing the firing rate of DA cell bodies [Rompre and Shizgal, 1986; Shizgal et al., 1989]. Also, the 5-HT$_3$ antagonists were generally ineffective in antagonizing conditioned avoidance behavior in the PCA assay. Avoidance responding in this assay has been attributed to meso-cortical dopaminergic transmission and it has been shown that the cells of origin of this system in the ventral tegemental area do not show increased firing rates prior to a conditioned behavior [Miller et al., 1981]. Therefore, since there is no increase in firing rate of DA neurons, the 5-HT$_3$ antagonists do not affect this behavior.

An unexpected finding in this study was that the 5-HT$_3$ antagonist MDL-72222 dose-dependently attenuated behavior in both the ICSS-MFB and PCA paradigms, despite the fact that it lacks D_2 antagonist activity in vitro [Fozard, 1984] and in vivo (present results in CMA). Recently, the structurally related compound MDL 73147EF was found to have no acute effect on the firing rate of DA neurons, but after chronic administration was found to elicit depolarization inactivation in both A9 and A10 cell bodies [Sorensen et al., 1989]; to date, however, the effects of MDL 72222 in DA single unit activity have not been published. However, it has recently been reported that this agent has equal affinity for both the histamine (H_1) receptor and the 5-HT$_3$ receptor [Wijngaarden et al., 1990]. These results suggest that the behavioral effects observed may not be due to direct modulation of DA transmission. In fact, the effects of MDL 72222 in the PCA assay were similar to diazepam with a less than two-fold separation between avoidance and escape responding, unlike the separation seen with neuroleptics such as haloperidol (five-fold).

Our results show that the 5-HT$_3$ antagonists resemble anxiolytic agents in the non-conditioned anxiety procedures but are unlike benzodiazepines in the conditioned conflict procedures. Also, the 5-HT$_3$ antagonists lack the traditional preclinical profile of antipsychotic agents. In summary, the 5-HT$_3$ antagonists may be efficacous in certain forms of anxiety but apparently lack antipsychotic activity.

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