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5-HT_3 receptor antagonist ICS 205-930 alters the discriminative effects of ethanol

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Abstract

The ability of a selective 5-hydroxytryptamine (5-HT_3) receptor antagonist, ICS 205-930 (3-tropanyl-indole-1-carboxylate, tropisetron), to block the discriminative stimulus effects of ethanol was investigated in rats that were trained to discriminate ethanol (1.25 g/kg ip) from saline with food as the reinforcement. Prior administration of ICS 205-930, at the dose of 0.01 mg/kg, significantly decreased ethanol's discriminative stimulus effect at ED_{75} dose of ethanol, while higher doses of ICS 205-930 (10 and 17 mg/kg) showed enhancement of ethanol's discriminative effects at ED_0, ED_{25}, and ED_{50} doses of ethanol. Under conditions where ICS 205-930 (10, 17 mg/kg) was tested alone, rats responded exclusively on the saline-appropriate lever. These effects occurred without significantly altering response rates or blood ethanol concentrations. The results suggest that the 5-HT_3 antagonist ICS 205-930 at lower concentration decreases, and at higher concentration enhances the discriminative stimulus effects associated with a lower to moderate dose of ethanol.

Keywords: Ethanol; Serotonin receptors; Drug discrimination; Serotonin antagonists; 5-HT_3 receptors; ICS 205-930

1. Introduction

Ethanol appears to produce its behavioral effects through a number of distinct mechanisms involving various neurobiological components (Grant and Colombo, 1993a; Kostowski and Bienkowski, 1993). It is believed to function as a compound discriminative stimulus, with component stimuli that are mediated partly through the $GABA_A$/benzodiazepine receptor complex, *N*-methyl-D-aspartate (NMDA) receptors and L-type calcium channels (Grant and Colombo, 1993b; Grant et al., 1991, 1993; Sanger, 1993; Shelton and Balster, 1994). In addition to these neurotransmitter receptor systems, several studies have suggested the involvement of serotonin (5-hydroxytryptamine, 5-HT) receptors in ethanol's behavioral effects (Grant and Colombo, 1993c,d; Grant et al., 1997; Maurel et al., 1997; Schechter, 1974; Signs and Schechter, 1986). Studies in laboratory animals have shown that drugs, which increase synaptic availability of serotonin (5-HT), such as reuptake inhibitors (e.g., fluoxetine, paroxetine) and releasers (fenfluramine), substitute for ethanol discrimination (Maurel et al., 1997; Schechter, 1974). Studies have also shown substitution of ethanol's discriminative stimulus by the 5-HT_{1B} receptor agonists (Grant and Colombo, 1993c). Contrary to these findings where enhancement of central serotoninergic tone has substituted for ethanol's discriminative stimulus, 5-HT_3 receptor antagonists (e.g., MDL72222 and ICS 205-930 [3-tropanyl-indole-1-carboxylate, tropisetron]) have been shown to antagonize ethanol's discriminative effects (Grant and Barrett, 1991; Grant and Colombo, 1993d).

Several studies have demonstrated an interaction between 5-HT_3 antagonists and some of ethanol's effects using electrophysiological (Lovinger and White, 1991), biochemical (Carboni et al., 1989; Wozniak et al., 1990), and behavioral techniques (Grant and Barrett, 1991; Grant and Colombo, 1993d; Johnson et al., 1993; McKenzie et al., 1998; Tomkins et al., 1995). The 5-HT_3 receptor subtype is a ligand-gated cation channel, which conducts primarily sodium and potassium ions (Derkach et al., 1989; Peters and

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Lambart, 1989; Yankel and Jackson, 1988). Direct evidence of ethanol's interaction with 5-HT_3 receptors is also provided by the electrophysiological finding showing ethanol's potentiation of the actions of 5-HT at the 5-HT_3 receptor to increase cation conductance in the cells derived from a neuroblastoma cell line or the nodose ganglion (Lovinger and White, 1991). A specific 5-HT_3 antagonist, ICS 205-930 (Lovinger and White, 1991) blocked this potentiating effect of ethanol.

Ondansetron, at doses of 1–100 μg/kg ip, and other 5-HT_3 antagonists have been reported to reduce the behavioral and neurochemical consequences of stimulation of the mesolimbic dopaminergic system in rats (Costall et al., 1987). Ethanol induces a release of dopamine within the nucleus accumbens (Di Chira and Imperato, 1988; Gessa et al., 1985; Imperato and Di Chira, 1986; Wozniak et al., 1990; Yoshimoto et al., 1991), and this has been postulated to play a role in the reinforcing properties of ethanol. There is some evidence that suggests that brain serotonin modulates this ethanol-induced dopamine release by interacting with 5-HT_3 receptors (Carboni et al., 1989; Imperato and Angelucci, 1989; Jiang et al., 1990; Kostowski et al., 1995). To support this, antagonists of 5-HT_3 receptors have been found to decrease dopamine release in the nucleus accumbens induced by psychoactive drugs such as ethanol, morphine, and nicotine (Carboni et al., 1989).

The possible involvement of the mesolimbic dopaminergic system in regulating alcohol drinking and the attenuation of alcohol-stimulated dopamine release in the nucleus accumbens has resulted in the efforts to examine the effects of 5-HT_3 antagonists on alcohol drinking in rats. In agreement with the hypothesis relating dopaminergic involvement in ethanol's reinforcing effects, a variety of 5-HT_3 receptor antagonists have been shown to reduce alcohol consumption in rodents (Grant, 1995; Hodge et al., 1993; Knapp and Pohorecky, 1992; Kostowski et al., 1993; McKenzie et al., 1998; Sellers et al., 1992, 1994; Tomkins et al., 1995; Toneatto et al., 1991), with no effect on the food intake. In a clinical trial by Sellers et al. (1992, 1994), ondansetron was also found to reduce the alcohol intake in human alcohol-dependent subjects.

Although the effectiveness of 5-HT_3 antagonists in reducing alcohol consumption has been demonstrated in a number of animal studies (Grant, 1995; Hodge et al., 1993; Knapp and Pohorecky, 1992; Kostowski et al., 1993; McKenzie et al., 1998; Sellers et al., 1992, 1994; Tomkins et al., 1995; Toneatto et al., 1991), as well as in human subjects (Sellers et al., 1994; Toneatto et al., 1991), the behavioral mechanism underlying this effect is still unclear. One possible mechanism could be that 5-HT_3 antagonists antagonize certain behavioral effects of ethanol, which contribute to the reinforcing effects of ethanol. In support of this, specific antagonists of the 5-HT_3 receptor, like MDL72222 (bemesetron) and ICS 205-930 (tropisetron), have been shown previously to block the discriminative stimulus effects of ethanol in a dose-dependent manner in pigeons and rats (Grant and Barrett, 1991; Grant and Colombo, 1993d). On the other hand, studies have failed to demonstrate any effect of tropisetron, ondansetron, and MDL72222 on ethanol's discriminative stimulus in Wistar rats and C57BL/6 mice, respectively (Middaugh et al., 1998; Stefanski et al., 1996). This suggests a possibility of differences in the actions of various 5-HT_3 antagonists across species, procedures, and doses (Grant and Colombo, 1993d). In view of these conflicting findings, the present study was undertaken to examine in more detail the experimental conditions that influence the effects of the 5-HT_3 antagonist ICS 205-930 on ethanol's discriminative stimulus using a drug-discrimination procedure.

2. Materials and methods

2.1. Subjects

Fifteen male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN, USA) weighing 275–300 g were housed individually in suspended cages and were given ad libitum access to food and water during the first week after their arrival. Before the beginning of the study, rats were reduced to approximately 85% of their free feeding body weights, and during the study, they were allowed to gain 10 g/month to allow for normal growth and development. The animal colony room was maintained on a 12-h light/dark cycle (lights on at 06:00 h), temperature of 20–22°C, and relative humidity of 60%. The experiments were performed in accordance with the guidelines of the University of Oklahoma Health Sciences Center Institutional Animal Care and Use Committee and the National Institute of Health for the care and use of animals in research.

2.2. Apparatus

Experimental sessions were conducted in standard rat operant chambers (Lafayette Instruments, Lafayette, IN) equipped with two response levers, two stimulus lamps on the top of levers, a house lamp, and a pellet dispenser, housed within a sound-attenuating cubicle. Experimental contingencies and data collection were controlled by a Commodore 64C-microcomputer system interfaced with the operant chambers (American Neuroscience Research Foundation, Yukon, OK).

2.3. Drug discrimination training

Subjects were initially trained to the location and operation of the pellet dispenser and operation of both levers by the method of a successive approximation. The illumination of the stimulus and house lamps signaled the beginning of experimental sessions. Initially, each response on either lever was reinforced (one 45-mg food pellet; P.J. Noyes, Lancaster, NH). Once rats were trained to press the lever for

food, they received saline or 1.25 g/kg ip ethanol 15 min prior to the session and were trained to discriminate between ethanol and saline. The appropriate lever to obtain food was determined by the drug (ethanol or saline) administered before the session. The number of responses required for reinforcement for food delivery was raised in successive training sessions to 10 consecutive responses (FR10). Responses on the inappropriate lever reset the FR requirements on the injection-appropriate lever. For "Drug Discrimination training," the exposure to ethanol and saline was kept comparable by alternating ethanol and saline sessions throughout training. Training was conducted 5 days/week and continued until each rat emitted (a) fewer than 20 responses before the delivery of the first food pellet, (b) greater than 90% of the total responses, within a trial, on the correct injection-appropriate lever, and (c) earned at least 50 reinforcers within each trial. Responding was considered to be under stimulus control when 90% of the total responses emitted during the session occurred on the appropriate lever and the number of responses required for the first reinforcement were less than 20, for 8 out of 10 consecutive training sessions.

2.4. Drugs

The injections of ethanol and saline during training and test sessions were administered intraperitoneally. Each rat was tested with five doses of ethanol (ranging between 0.25 and 1.25 g/kg).

The experimental drug ICS 205-930 was purchased from Research Biochemicals, Natic, MA. ICS 205-930 (0.001–17 mg/kg) was dissolved in saline and administered intraperitoneally 15 min before ethanol administration. The ethanol stock solution was a 10% (w/v) mixture of ethanol and normal saline.

2.5. Discrimination test sessions

The test sessions were conducted in the same manner as the training sessions except that in the test sessions, 10 consecutive responses on either lever resulted in food. Test sessions were conducted following two consecutive training sessions in which the training criteria were met. Conditioning and test trials were carried out at the same time every day.

2.6. Ethanol discrimination test sessions

Once stimulus control was established in the discrimination task, dose–effect curves for ethanol were generated in every animal using five doses of ethanol ranging from 0.25 to 1.25 g/kg. During a test session, saline or a dose of ethanol was administered 15 min prior to the start of the session. During test sessions, 10 consecutive responses on either lever produced food. Training sessions and ethanol dose–response test sessions were alternated. If a rat fails to reach a required criterion during a training session, further testing was postponed until two sessions of criterion performance were achieved. To insure the stability of the dose–response functions for the stimulus and rate-reducing effects of ethanol, ethanol dose–effect curves were determined at regular intervals between a set of test conditions with ICS 205-930. From the data collected from dose–response test sessions, ED_{25}, ED_{50}, and ED_{75} dose values were calculated for each rat using a regression analysis.

2.7. Experiment 1. Effects of ICS 205-930 on the discriminative stimulus effects of the ED_{100} dose of ethanol

The purpose of this experiment was to determine whether the pretreatment with ICS 205-930 results in antagonism of the discriminative stimulus of ethanol. To achieve this, following the characterization of the initial ethanol dose–response curve, ICS 205-930 (0.1–17 mg/kg ip) was administered 15 min prior to ethanol (the ED_{100} dose) or saline treatment in a group of rats ($n=7$) randomly selected from the original group of rats.

2.8. Experiment 2. Effects of ICS 205-930 on the discriminative stimulus effects of the ED_{75} dose of ethanol

The purpose of this experiment was to determine the effect of the pretreatment with ICS 205-930 on the discriminative effects of the ED_{75} dose of ethanol. To achieve this, ICS 205-930 (0.001–17 mg/kg ip) was administered 15 min prior to ethanol (the ED_{75} dose) and 30 min prior to the beginning of the ethanol–saline discrimination test session in the same group of rats. Each rat was administered its own predetermined ED_{75} dose of ethanol.

2.9. Experiment 3. Effects of ICS 205-930 (10 mg/kg) on the dose–response curve of ethanol

The purpose of this experiment was to determine the effect of ICS 205-930 (10 mg/kg) on the ethanol dose–response curve. To achieve this, following the characterization of the initial ethanol dose–response curve, ICS 205-930 (10 mg/kg ip) was administered 15 min prior to ethanol (the ED_0, ED_{25}, ED_{50}, ED_{75}, and ED_{100} doses) administration and 30 min prior to the test session in a group of 10 rats randomly selected from the original group of rats. Each rat was given its predetermined ED_0, ED_{25}, ED_{50}, ED_{75}, and ED_{100} doses of ethanol.

2.10. Blood ethanol levels

Blood samples (20 μl) were drawn from the brachial vein following ICS 205-930 (0.32 mg/kg) and ethanol (0.75 and 1.0 g/kg), as well as following ICS 205-930 (10 mg/kg) and ethanol (0.75 and 1.0 g/kg) administration and were immediately deposited in a vial with 1 ml of 0.02% (v/v) solution of 1-propanol in distilled water

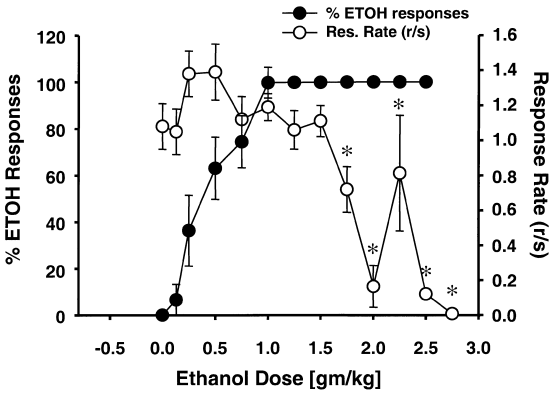

Fig. 1. Effect of increasing dose of ethanol on the percentage ethanol lever responses (●) and the rate of responding (○). $P < .05$.

(internal standard). These samples were collected using the same time course (30 min following ICS 205-930 and 15 min following ethanol administration) that was used in the discrimination test sessions and were analyzed for blood ethanol levels using gas chromatographic headspace sampling technique as previously described (Pohorecky and Brick, 1982). In brief, the blood aliquots were incubated for 20 min at 70°C in a shaking water bath. A 1-ml air sample was drawn from each sample bottle using a gas-tight syringe and immediately injected into the entry port of a Hewlett-Packard Gas chromatograph equipped with a hydrogen-fueled flame ionization detector. A Hewlett-Packard integrator calculated the area under the curve of all detectable peaks. For each set of 20 samples, a complete set of ethanol standards of known concentrations was quantified on the chromatograph. Expressing the area of the ethyl alcohol peak as a percentage of the internal standard peak normalized the area of each ethanol-associated peak. Blood ethanol concentrations were assessed using a 7-point linear regression analysis from the ethanol standards.

2.11. Data analysis

The percentage of responses made on the ethanol-appropriate lever was calculated by dividing the number of responses made on the ethanol-appropriate lever by the total number of responses made on either lever during a session. Response rates were expressed as the total number of responses made on either lever divided by session length (in seconds). Group averages were calculated and expressed as the mean ± S.E.M. Total antagonism was defined as 20% or less of total session responding on the ethanol-appropriate lever. Differences in the percentage drug lever-responding and response rates on the saline or ethanol lever following pretreatment with vehicle and drug were tested for significance using repeated-measures ANOVA. The individual ED_{25}s, ED_{50}s, and ED_{75}s were calculated by a linear regression analysis.

3. Results

The number of training sessions required, reaching the criterion for demonstrating discriminative stimulus control over responding, ranged from 50 to 70 sessions (mean = 61 ± 5). Control rates of responding during training sessions following ethanol administration were not different from control rates following saline administration: (ethanol: 1.08 ± 0.06 responses/s, saline: 1.06 ± 0.06 responses/s).

During the test sessions in which saline or 0.125 g/kg ethanol was administered, the rats ($n = 15$) responded almost exclusively on the saline-appropriate lever. Doses of ethanol ranging from 0.5 gm/kg resulted in a shift in responding from the saline to the ethanol lever. The mean ED_{25}, ED_{50}, ED_{75}, and ED_{100} values were 0.25 ± 0.05, 0.47 ± 0.09, 0.77 ± 0.09, and 1.0 ± 0.09 g/kg, respectively. Administration of 1.75 g/kg and higher doses of ethanol had a rate-decreasing effect (Fig. 1).

3.1. Experiment 1. Effect of ICS 205-930 pretreatment on the discriminative effect produced by ED_{100} dose of ethanol

The results of ICS 205-930 pretreatment (0.1–17 mg/kg) given 15 min prior to the ED_{100} dose of ethanol (for each rat) are shown in Fig. 2. Pretreatment with ICS 205-930 (0.56–17 mg/kg) did not have any significant effect on ethanol-appropriate responding as shown in Fig. 2. There is a trend of a decrease in the number of ethanol lever responses following pretreatment with ICS 205-930 (0.1 and 0.32 mg/kg). ICS 205-930 did not have any significant effect on the response rate.

3.2. Experiment 2. Effect of ICS 205-930 pretreatment on the discriminative effect produced by the ED_{75} dose of ethanol

The results of ICS 205-930 pretreatment (0.1–17 mg/kg) given 15 min prior to the ED_{75} dose of ethanol (for

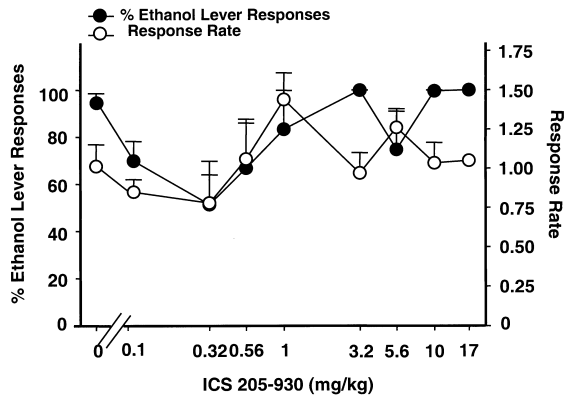

Fig. 2. Effect of ICS 205-930 (0.1–10 mg/kg) on the percentage ethanol lever responses (●) and the rate of responding (○) following ethanol (ED_{100} dose) administration.

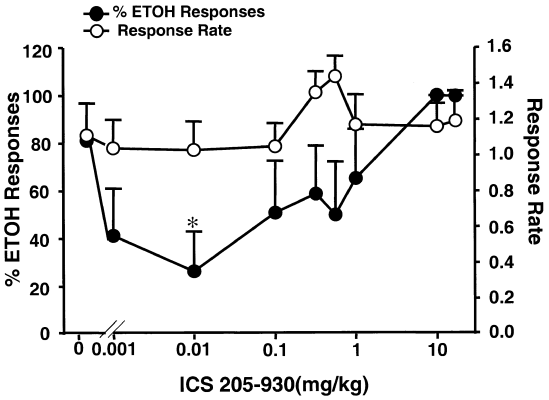

Fig. 3. Effect of ICS 205-930 (0.001–17 mg/kg) on the percentage ethanol lever responses (●) and the rate of responding (○) following ethanol (ED_{75} dose) administration. * $P < .05$.

each rat) are shown in Fig. 3. Each data point is the mean percentage ethanol lever responses or the mean rate of responding corresponding to the ED_{75} dose of ethanol for a group of seven rats selected randomly for this study. Though the pretreatment with all lower doses of ICS 205-930 (0.001–0.56 mg/kg) decreased the percentage ethanol-appropriate responding, only a significant reduction was observed following pretreatment with ICS (0.01 mg/kg). On the other hand, following pretreatment with higher doses of ICS 205-930 (10 and 17 mg/kg) and the ED_{75} dose of ethanol, rats responded exclusively on the ethanol lever (Fig. 3).

3.3. Experiment 3. Effect of ICS 205-930 (10 mg/kg) pretreatment on the ethanol dose–response curve

The combination of ICS 205-930 (10 mg/kg) and ED_0, ED_{25}, ED_{50}, and ED_{75} doses of ethanol resulted in a shift of the ethanol dose–effect curve to the left, suggesting partial substitution of the discriminative stimulus effects of ethanol

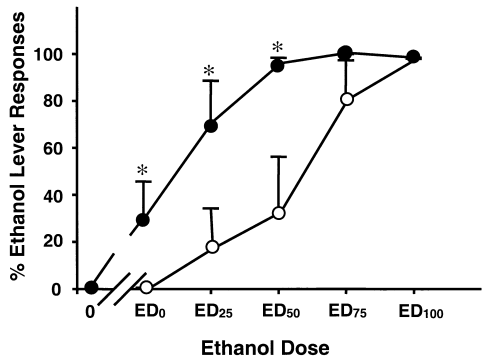

Fig. 4. Effect of ICS 205-930 (10 mg/kg) on the percentage ethanol lever responses made following ethanol (ED_0, ED_{25}, ED_{50}, ED_{75}, and ED_{100} doses) administration. (●) Percentage ethanol lever responses following ICS 205-930 administration. (○) Percentage ethanol lever responses following vehicle administration. * $P < .05$.

Table 1
Blood ethanol levels following ICS 205-930 administration

Pretreatment	Ethanol (0.75 g/kg)	Ethanol (1 g/kg)
Vehicle	60.82 ± 3.7[a] mg/dl	102 ± 11 mg/dl
ICS 205-930 (0.32 mg/kg)	63.88 ± 5.2 mg/dl	103 ± 18 mg/dl
ICS 205-930 (10 mg/kg)	65.26 ± 3.8 mg/dl	102 ± 9 mg/dl

$N = 15$.
[a] S.E.M.

by higher concentration of this compound (Fig. 4). ICS 205-930 (10 mg/kg) significantly increased ethanol lever responses following ED_0, ED_{25}, and ED_{50} doses of ethanol ($P < .05$). Under conditions where ICS 205-930 (10, 17 mg/kg) was tested with saline treatment, rats responded exclusively on the saline-appropriate lever.

3.4. Effect of ICS 205-930 pretreatment on the blood ethanol levels produced by the ED_{75} and the ED_{100} dose of ethanol

To determine whether ICS 205-930 and ethanol have any pharmacokinetic interaction, blood ethanol levels following ICS 205-930 vs. vehicle pretreatment were compared. Blood ethanol concentrations were not different between rats treated with vehicle and ethanol (0.75 and 1 g/kg) and a combination of ICS 205-930 (0.32 or 10 mg/kg pretreatment) and ethanol (0.75 and 1 g/kg; Table 1).

4. Discussion

In the present study, ICS 205-930 at the concentration of 0.01 mg/kg partially antagonized ethanol discrimination in a drug discrimination paradigm in rats receiving the ED_{75} dose of ethanol, without having any effect on the rate of responding. Also, there was a trend of a decrease in percentage ethanol-appropriate responding following the pretreatment with the low doses of ICS 205-930 in animals receiving the ED_{75} and ED_{100} dose of ethanol.

These observations are consistent with the previous studies where 5-HT_3 receptor antagonists have been reported to block the discriminative stimulus properties of ethanol in pigeons and rats (Grant and Colombo, 1993a,d). In agreement with these findings in rats, in a study done on human subjects, pretreatment with ondansetron (4 mg) was found to attenuate certain pleasurable mood states induced by alcohol (Johnson et al., 1993). These observations suggest that 5-HT_3 receptors mediate some of the subjective effects of ethanol, and the antagonism of 5-HT_3 receptors reduces these behavioral effects of ethanol. Because of the compound nature of ethanol's discriminative stimulus and the involvement of multiple neurotransmitters in ethanol's discriminative stimulus, the effects of 5-HT_3 antagonists are not highly significant and have varied depending on the dose of ethanol or the strain specificity. This may explain the observations of Stefanski et al. (1996), where they did

not find any antagonism of ethanol discrimination by ICS 205-930 (0.001–1 mg/kg).

The partial antagonism of ICS 205-930 of ethanol's discriminative effects may be mediated by the ability of ICS to decrease ethanol-mediated dopamine release in the nucleus accumbens (Wozniak et al., 1990). 5-HT_3 receptor antagonists have also been found to act as $GABA_A$ receptor antagonists (Squires and Saederup, 1999). However, the doses of ICS 205-930, observed to be effective in this study, are too low to produce CNS concentrations (in the range of 10–20 μM) needed to have an effect at the $GABA_A$ receptor (Klein et al., 1994). On the other hand, very low concentrations of a 5-HT_3 antagonist (e.g., ondansetron at doses of 1–100 μg/kg ip) have been able to inhibit the hyperactivity induced by infusion of dopamine into the nucleus accumbens in rats (Costall et al., 1987). In a clinical study, ondansetron was found to reduce the stimulating effects of ethanol without affecting the anxiolytic effects of ethanol, which suggests dopaminergic involvement in the effects of ondansetron (Johnson et al., 1993). Therefore, it is possible that a decrease in ethanol's discrimination by lower doses of ICS 205-930 is probably mediated via a dopaminergic mechanism.

In the present study, higher doses of ICS 205-930 did not antagonize ethanol's discriminative effect but rather enhanced the discriminative effects produced by lower doses of ethanol. ICS 205-930 (10 and 17 mg/kg), by itself, did not completely substitute for ethanol. These observations suggest that via some unknown mechanism, ICS 205-930 at higher doses enhances certain behavioral effects of ethanol but does not completely substitute for ethanol's discriminative stimulus. A similar dose–response profile has been reported for ondansetron's effects on ethanol-induced behavior (Costall et al., 1987; Swift et al., 1996). Like the enhancement of ethanol's discriminative effects by ICS 205-930, observed in the present study, the enhancement of ethanol's certain stimulant, sedative, and intoxicating effects by ondansetron (8 mg) has been observed in men and women (Swift et al., 1996). Ondansetron was also found to increase alcohol-induced impairment without affecting psychomotor performance or alcohol pharmacokinetics (Swift et al., 1996). At present, the mechanism underlying such enhancement of ethanol's behavioral effects by 5-HT_3 antagonists has not been identified. It is possible that higher concentrations of 5-HT_3 antagonists are not as effective in attenuating dopamine release, since the studies reported so far have only examined the effect of lower doses of 5-HT_3 antagonists on dopamine release. It is also possible that higher doses of these compounds may interact with other systems to offset their action at the 5-HT_3 receptor site and enhance some of the subjective effects of ethanol.

The biphasic effect of ICS 205-930 observed in the present study suggests differential dose-dependent involvement of ICS 205-930 in ethanol discrimination. The differences in the findings of Swift et al. (1996) and the ones reported by Johnson et al. (1993) regarding the effect of ondansetron on ethanol-induced behavior may also be due to differences in the concentration of ondansetron and alcohol used in these two studies. From the results of the present study and the observations from these two clinical studies, it can be stated that the ability of 5-HT_3 antagonists to affect the ethanol-induced subjective effects depends on the dose of a 5-HT_3 antagonist and ethanol used in the given experiment. Ethanol is suggested to function as a mixed or a compound discriminative stimulus (Grant and Colombo, 1993a; Kostowski and Bienkowski, 1993). Various behavioral effects of ethanol (e.g., the stimulating, anxiolytic, sedative, ataxic, and myorelaxant effects) play a critical role in the formation of its discriminative stimulus. Therefore, it is possible that same drugs can interact with these distinct behavioral stimuli in different ways at different concentrations and can reduce or enhance ethanol discrimination.

The 5-HT_3 antagonists, ondansetron, bemesetron zacopride, and ICS 205-930 have been consistently shown to reduce alcohol consumption in rats and humans (Fadda et al., 1991; Hodge et al., 1993; Litten and Allen, 1991; Sellers et al., 1994). The decrease in alcohol consumption observed in men treated with ondansetron (0.25 mg/kg) was suggested to be due to the drug's capacity to block some of the reinforcing properties of ethanol (Sellers et al., 1994). In support of this hypothesis, pretreatment with ondansetron in healthy volunteers and social drinkers was found to reduce alcohol's stimulant, sedative, and pleasurable effect (Johnson et al., 1993). In our study, ICS 205-930 at lower concentration, which decreases ethanol discrimination, and at the concentration of 10 mg/kg, which exhibits partial substitution, significantly decreased ethanol intake in Sprague–Dawley rats, which were trained to drink ethanol [Mhatre and Holloway, unpublished findings]. The reduction in alcohol consumption observed at 0.01 and 10 mg/kg may be mediated by a decrease in ethanol's reinforcing effects and an increase in the aversive or intoxicating effects of ethanol, respectively (Johnson et al., 1993; Swift et al., 1996). These results suggest the clinical significance of these biphasic effects of ICS 205-930 on ethanol's subjective effects.

Pretreatment with ICS 205-930 did not have any effect on the response rate in any of the test conditions. This suggests that the partial antagonism or partial generalization of the discriminative stimulus effects of ethanol by this compound occurred independent of any effect on the psychomotor performance of these rats. Grant and Barrett (1991) saw some rate-suppressing effects of ICS 205-930 (0.3 mg/kg) in combination with 1.5 g/kg ethanol in pigeons. In our study, ethanol doses used were much lower than the dose used in the study done by Grant and Barrett. Our study demonstrates that the partial blockade of the discriminative stimulus effects of ethanol by lower doses of 5-HT_3 antagonists occurs independent of any nonspecific effects on motor performance.

It was previously suggested by Grant et al. (1991) that pharmacokinetic factors rather than a direct interaction at the receptor level were responsible for a blockade of the ethanol cue by a 5-HT_3 antagonist, bemesetron (MDL72222), in rats

(Grant and Colombo, 1993d). In the previous studies, the blockade of ethanol discrimination occurred only when ethanol was given intragastrically rather than intraperitoneally, which suggests that the inhibitory effect of 5-HT_3 antagonists is due to impaired absorption of ethanol from the gut (Grant and Colombo, 1993d). In our studies, ICS 205-930 partially antagonized ethanol discrimination when ethanol was administered intraperitoneally. Also, pretreatment with ICS 205-930 did not have any significant effect on the blood ethanol level produced by ED_{75} and ED_{100} doses of ethanol. Therefore, the partial antagonism and partial generalization of ethanol discrimination following pretreatment with ICS 205-930 does not appear to be contributed by any effects on ethanol's pharmacokinetics.

In conclusion, prior administration of ICS 205-930 at low doses causes partial antagonism and at high doses (≥ 10 mg/kg) causes enhancement of discriminative effects of a low dose of ethanol. The results from the present study suggest that some of ethanol's effects are mediated by 5-HT_3 receptors. Also, the dose of 5-HT_3 antagonist and ethanol used can have a significant impact on ethanol discrimination. Although the neurobiological mechanisms that could account for the biphasic effect of ICS 205-930 on ethanol's discrimination are currently unknown, the results from the present study indicate that ICS 205-930, a 5-HT_3 antagonist, modifies ethanol discrimination in rats.

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References

Carboni E, Acquas E, Frau R, Di Chira G. Differential effects of 5-HT_3 antagonists on drug induced stimulation of dopamine release. Eur J Pharmacol 1989;164:515–9.

Costall B, Domency AM, Naylor RJ, Tyers MB. Effects of the 5-HT_3 receptor antagonist GR38-032F, on raised dopaminergic activity in the mesolimbic system of the rat. Br J Pharmacol 1987;92:881–94.

Derkach V, Supernant A, North RA. 5-HT_3 receptors are membrane ion channels. Nature 1989;339:706–9.

Di Chira G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA 1988;85:5274–8.

Fadda F, Garau B, Marchei F, Colombo G, Gessa GL. MDL 72222, a selective 5-HT_3 antagonist, suppresses voluntary ethanol consumption in alcohol preferring rats. Alcohol Alcohol 1991;26:107–10.

Gessa GL, Muntoni F, Collu M, Vargiu L, Meeru G. Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Res 1985;348:201–3.

Grant K. The role of 5-HT_3 receptors in drug dependence. Drug Alcohol Depend 1995;38:155–71.

Grant KA, Barrett JE. Blockade of the discriminative stimulus effects of ethanol with 5-HT_3 receptor antagonists. Psychopharmacology 1991;104:451–6.

Grant KA, Colombo G. Pharmacological analysis of the mixed discriminative stimulus effects of ethanol. Alcohol Alcohol 1993a; 2(Suppl):445–9.

Grant KA, Colombo G. Discriminative stimulus effects of ethanol: effect of training dose on the substitution of *N*-methyl-D-aspartate antagonists. J Pharmacol Exp Ther 1993b;264:1241–7.

Grant KA, Colombo G. Substitution of the 5-HT_1 agonist trifluoromethylphenylpiperazine (TFMPP) for the discriminative stimulus effects of ethanol: effect of training dose. Psychopharmacology 1993c;113(1): 26–30.

Grant KA, Colombo G. The ability of 5-HT_3 antagonists to block ethanol discrimination: effect of route of ethanol administration. Alcohol: Clin Exp Res 1993d;17:497.

Grant KA, Colombo G, Tabakoff B. Competitive and non-competitive antagonists of the NMDA receptor complex have ethanol-like discriminative stimulus effects in rats. Alcohol: Clin Exp Res 1991;15:321–4.

Grant KA, Purdy RH, Paul SM, Griffiths RR. Drug discrimination analysis of endogenous neuroactive steroids in rats. Eur J Pharmacol 1993;241: 237–43.

Grant KA, Colombo G, Gatto GJ. Characterization of the ethanol-like discriminative stimulus effects of 5-HT receptor agonists as a function of ethanol training dose. Psychopharmacology 1997;133(2):133–41.

Hodge CW, Samson HH, Lewis RS, Erickson HL. Specific decreases in ethanol- but not in water-reinforced responding produced by the 5-HT_3 antagonist ICS 205-930. Alcohol 1993;10:191–6.

Imperato A, Angelucci L. 5-HT_3 receptors control dopamine release in the nucleus accumbens of freely moving rats. Neurosci Lett 1989;101:214–7.

Imperato A, Di Chira G. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J Pharmacol Exp Ther 1986;239:219–28.

Jiang LH, Ashby CR, Kasser RJ, Wang RY. The effect of intraventricular administration of the 5-HT_3 receptor agonist 2-methyl serotonin on the release of dopamine in the nucleus accumbens: an in vivo chronocoulometric 1 study. Brain Res 1990;513(1):156–60.

Johnson BA, Campling GM, Griffiths P, Cowen PJ. Attenuation of some alcohol-induced mood changes and the desire to drink by 5-HT_3 receptor blockade: a preliminary study in healthy male volunteers. Psychopharmacology 1993;112:142–4.

Klein RL, Sanna E, McQuilkin SJ, Whiting PJ, Harris RA. Effects of 5-HT_3 receptor antagonists on binding and function of mouse and human $GABA_A$ receptors. Eur J Pharmacol 1994;268:237–46.

Knapp DJ, Pohorecky LA. Zacopride, a 5-HT_3 receptor antagonist, reduces voluntary ethanol consumption in rats. Pharmacol, Biochem Behav 1992;41:847–50.

Kostowski W, Bienkowski P. Discriminative stimulus effects of ethanol: neuropharmacological characterization (review). Alcohol 1993; 10:369–73.

Kostowski W, Dyr W, Krzascik P. The abilities of 5-HT_3 receptor antagonist ICS 205-930 to inhibit alcohol preference and withdrawal seizures in rats. Alcohol 1993;10:369–73.

Kostowski W, Sikora J, Bisaga A, Rosnowska E. Effects of 5-HT_3 receptor antagonists on ethanol-induced hyper locomotor activity in mice. Pol J Pharmacol 1995;46:133.

Litten RZ, Allen JP. Pharmacotherapies for alcoholism: promising agents and clinical issues. Alcohol: Clin Exp Res 1991;15:620–33.

Lovinger DM, White G. Ethanol potentiation of 5-hydroxytryptamine-3 receptor-mediated ion current in neuroblastoma cells and isolated neurons. Mol Pharmacol 1991;40:263–70.

Maurel S, Schreiber R, De Vry J. Substitution of the selective serotonin reuptake inhibitors fluoxetine and paroxetine for the discriminative stimulus effects of ethanol in rats. Psychopharmacology 1997;130: 404–6.

McKenzie DL, Eha R, Cox R, Stewart RB, Dyr W, Murphy JM, McBride WJ, Lumeng L, Li T-K. $Serotonin_3$ receptor antagonism of alcohol intake: effects of drinking conditions. Alcohol 1998;15(4):291–8.

Middaugh LD, Kelly BM, Cuison ER, Groseclose CH. Effects of naltrin-

dole and MDL72222 on ethanol reward and discrimination in C57BL/6 mice. Alcohol: Clin Exp Res 1998;22(284):51A.

Peters JA, Lambart JJ. Electrophysiology of 5-HT in neuronal cell lines. Trends Pharmacol Sci 1989;10(5):172–5.

Pohorecky LA, Brick J. A new method for the determination of blood ethanol levels in rodents. Pharmacol, Biochem Behav 1982;16:693–6.

Sanger DJ. Substitution by NMDA antagonists and other drugs in rats trained to discriminate ethanol. Behav Pharmacol 1993;4:441–50.

Schechter MD. Ethanol as a discriminative cue: reduction following depletion of brain serotonin. Eur J Pharmacol 1974;29:52–7.

Sellers EM, Higgins GA, Sobell MB. 5-HT and alcohol abuse. Trends Pharmacol Sci 1992;13:69–75.

Sellers EM, Toneato T, Romach MK, Somer GR, Sobell LC, Sobell MB. Clinical efficacy of the 5-HT_3 antagonist ondansetron in alcohol abuse and dependence. Alcohol: Clin Exp Res 1994;18:879–85.

Shelton KL, Balster RL. Ethanol drug discrimination in rats: substitution with GABA agonists and NMDA antagonists. Behav Pharmacol 1994;5:441–50.

Signs SA, Schechter MD. The role of dopamine and serotonin receptors in the mediation of the ethanol interoceptive cue. Pharmacol, Biochem Behav 1986;24:769–71.

Squires RF, Saederup E. Clozapine's antipsychotic effects do not depend on blockade of 5-HT_3 receptors. Neurochem Res 1999;24(5):659–67.

Stefanski R, Bienkowski P, Kostowski W. Studies on the role of 5-HT_3 receptors in the mediation of the ethanol interoceptive cue. Eur J Pharmacol 1996;309:141–7.

Swift RM, Davidson D, Whelihan W, Kuznetsov O. Ondansetron alters human alcohol intoxication. Biol Psychiatry 1996;40:514–21.

Tomkins DM, Le AD, Sellers EM. Effect of the 5-HT_3-antagonist ondansetron on voluntary ethanol intake in rats and mice maintained on a limited access procedure. Psychopharmacology 1995;117:479–85.

Toneatto T, Romach MK, Sobell MK. Ondansetron: a $5HT_3$ antagonist reduces alcohol consumption in ethanol abusers. Alcohol: Clin Exp Res 1991;15:382.

Wozniak KM, Pert A, Linnoila M. Antagonism of 5-HT_3 receptors attenuates the effects of ethanol on extracellular dopamine. Eur J Pharmacol 1990;187:287–9.

Yankel JL, Jackson MB. 5-HT_3 receptors mediate responses in cultured hippocampus and a clonal cell line. Neuron 1988;1:615–21.

Yoshimoto K, McBride WJ, Lumeng L, Li T-K. Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. Alcohol 1991;9:17–21.