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# Studies on the role of 5-$HT_3$ receptors in the mediation of the ethanol interoceptive cue

Roman Stefanski [a], Przemyslaw Bienkowski [a,b], Wojciech Kostowski [a,b,*]

[a] *Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Al. Sobieskiego 1 / 9, PL-02957 Warsaw, Poland*

[b] *Department of Experimental and Clinical Pharmacology, Medical High School, PL-00527 Warsaw, Poland*

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**Abstract**

The drug discrimination test was used to evaluate the role of 5-$HT_3$ receptors in the mediation of the stimulus properties of ethanol in rats trained to discriminate between ethanol (1.0 g/kg, 10% v/v, i.p.) and saline vehicle. Rats trained to discriminate between a lower dose of ethanol (0.5 g/kg i.p.) failed to attain discrimination criteria after 20 weeks (100 sessions) of training. None of the doses of 5-$HT_3$ receptor antagonists (0.001, 0.01, 0.1, 1.0, 10.0 mg/kg of tropisetron or ondansetron) administered i.p. 30 min before ethanol, antagonized the discriminative stimulus properties of ethanol. Furthermore, none of the centrally (1, 10, 35 μg per rat) or i.p. (0.1, 1.0, 2.5, 5.0, 10.0 mg/kg) administered doses of 5-$HT_3$ receptor agonist, 1-(*m*-chlorophenyl)-biguanide, could replace the ethanol discriminative cue. These results suggest that 5-$HT_3$ receptors are not primarily involved in the mediation of the stimulus properties of ethanol.

*Keywords:* Discriminative stimulus; Ethanol; 5-$HT_3$ receptor agonist; 5-$HT_3$ receptor antagonist

## 1. Introduction

Both theory and empirical data support the belief that a drug discrimination procedure in animals is the closest available experimental model for assessing the subjective effects of drugs. This procedure is especially useful for identifying potential receptors that mediate the stimulus effects of drugs. With this task animals learn a particular drug-induced interoceptive stimulus which can be tested for generalization to other drugs or antagonized by still other compounds (Colpaert, 1986; Holtzman, 1990).

Brain serotonin (5-hydroxytryptamine, 5-HT) is believed to regulate behavioral effects of ethanol including development of tolerance and dependence (Ollat et al., 1988; McBride et al., 1989; Little, 1991; Sellers et al., 1992; Le Marquand et al., 1994). The specific serotonergic receptor subtypes that may be involved in these effects of ethanol have not been precisely defined, although some findings are indicative of at least certain binding sites, particularly the 5-$HT_3$ receptor subtype. Thus, ethanol has been reported to enhance the 5-$HT_3$ receptor-mediated ion current (Lovinger, 1991; Lovinger and Zhou, 1993). A number of behavioral studies found that various 5-$HT_3$ receptor antagonists reduced ethanol consumption and preference in rats and marmosets (Oakley et al., 1988; Fadda et al., 1991; Higgins et al., 1992; Knapp and Pohorecky, 1992; Kostowski et al., 1993, 1994; Tomkins et al., 1995). Previous data from this laboratory have shown that 5-$HT_3$ receptor antagonists are capable of reducing the intensity of withdrawal seizures in rats withdrawn from ethanol (Kostowski et al., 1993, 1994; but see also Grant et al., 1994) and ethanol-induced hyperlocomotion in mice (Kostowski et al., 1995).

Ethanol is believed to function as a mixed discriminative stimulus, with component stimuli that are mediated at least partially through the 5-$HT_{1B}$ receptor subtype (Signs and Schechter, 1988; Grant and Colombo, 1993b), $GABA_A$/benzodiazepine receptor complex, *N*-methyl-D-aspartate (NMDA) receptors and L-type $Ca^{2+}$ channels (Grant et al., 1991; Grant and Colombo, 1993a; Sanger, 1993; Shelton and Balster, 1994; Colombo et al., 1994, 1995). Interestingly, 5-$HT_3$ receptor antagonists were reported to block the discriminative stimulus effect of

* Corresponding author. Tel./fax: +48 22 427644; fax: +48 22 6425357.


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ethanol, suggesting a role of 5-$HT_3$ receptors in mediating the interoceptive cue of ethanol (Grant and Barrett, 1991, Grant and Colombo, 1993c). If 5-$HT_3$ receptor antagonists can modify the central effects of ethanol including its discriminative properties, one would expect that agonists of these receptors may exhibit some efficacy to mimic the interoceptive cue of ethanol.

The purpose of the present study was twofold. First, we aimed to examine the effect of a 5-$HT_3$ receptor agonist, 1-(*m*-chlorophenyl)-biguanide (mCPBG) in rats trained to discriminate ethanol from saline. mCPBG was tested for its ability to replace ethanol using intraperitoneal (i.p.) and intracerebroventricular (i.c.v.) routes of administration in order to examine the role of peripheral and central 5-$HT_3$ mechanisms in ethanol cueing as this substance poorly penetrates the blood-brain barrier (Middlemiss and Tricklebank, 1992). The second purpose of this study was to evaluate the ability of two 5-$HT_3$ receptor antagonists, tropisetron (formerly ICS 205-930) and ondansetron, to block the ethanol discriminative cue. Since ethanol has a dose-effect relation for many behaviors, including aversive effects and interoceptive cueing (Froehlich et al., 1988; Grant and Colombo, 1993a,b) two training doses of 0.5 g/kg and 1.0 g/kg were studied.

## 2. Materials and methods

### 2.1. Subjects

Male Wistar rats (300–330 g at the beginning of the study) were housed individually in standard plastic cages, in a temperature-controlled (20–21°C) vivarium with 12-h light-dark cycle (lights on at 07:00 h). The rats were maintained at 85% of their free-feeding body weight by restriction of their daily food ration. Tap water was available ad libitum.

### 2.2. Apparatus

Four standard two-lever operant conditioning chambers (model E10-10RF; Coulbourn Instruments, Allentown, PA, USA) consisted of modular test cages enclosed in sound-attenuated cubicles with fans for ventilation and background white noise. A white house light was centered near the top of the front panel of the cage, which was also equipped with two response levers, separated by a liquid dipper (Coulbourn model E14-05, module size 1/2), all positioned 4.0 cm above the floor. The liquid dipper presented sweetened milk in a 0.01 ml portion for 5 s during each operation. Experimental sessions and data recording made use of the L91-04 HABITEST Universal Linc interface and the D91-12 L2T2 Control and Data Acquisition Software package (Coulbourn Instruments) running on an IBM-PC compatible via the L18-16 IBM/Linc Bus Interface Card (Coulbourn Instruments).

For i.c.v. injections of mCPBG the 28-gauge internal cannula (C313I; Plastics One, Roanoke, VA, USA) was linked to the connector (C313C-100cm; Plastics One) to the 10.0 μl syringe (Hamilton Bonaduz, Bonaduz, Switzerland) connected with a microdrive pump (CMA/100; Microdialysis, Stockholm, Sweden).

### 2.3. Training procedure

The procedure was similar to the fixed-ratio 10 (FR10) drug discrimination procedure described by Colpaert (1986). The animals were initially trained to press both levers under a fixed-ratio 1 (FR 1) schedule of sweetened milk delivery. Drug discrimination training began only after all the animals responded reliably on both levers under FR 1 conditions. Rats were trained to press one lever following ethanol injections (0.5 or 1.0 g/kg; 6.5 and 13.0 ml/kg, respectively, i.p.) and to press the other lever following saline vehicle (0.9% NaCl) injections under the FR 10 schedule of sweetened milk reinforcement. Injections occurred 15 min prior to the start of 15-min sessions. The lever corresponding with ethanol and saline pretreatments remained fixed for the duration of the study for a given animal and was counterbalanced across the group of rats. Sessions were conducted Monday through Friday under the alternating drug sequence used by Colpaert (1986): drug-vehicle-vehicle-drug-drug and vehicle-drug-drug-vehicle-vehicle. To avoid the possibility that the correct lever for rats previously tested in the chambers could serve as an olfactory cue, the sequence of treatments on training days was alternated for successive groups (i.e. half the animals received vehicle and half received ethanol). Besides, the levers were carefully cleaned with 50% ethanol solution after each session. Responses emitted on the incorrect lever were recorded but did not result in sweetened milk delivery. The animals continued to be trained under these conditions until they exhibited the acquisition criteria, which were defined as both correct first-lever selection ($\geq$ 80%) and greater than 90% correct-lever responding for 9 out of 10 consecutive sessions. In addition, the animals were also required to maintain response rates greater than 0.45 responses/s throughout the 10-session period (Colpaert, 1986).

### 2.4. Testing procedure

After the animals had reached the criteria, dose-response, antagonism and substitution tests were initiated. Test sessions were conducted once or twice times per week with training sessions intervening during the remaining days. In order to be tested, rats had to have reached the acquisition criteria for at least 5 days before the consecutive test. In dose-response sessions, rats were tested after the administration of various doses of ethanol (0.0–1.25 g/kg i.p.; 10% v/v) 15 min before start of the session. Dose-response tests were performed three times: before

antagonism and i.p. substitution tests, after surgery and after i.c.v. substitution tests. In antagonism tests, rats were injected with 5-$HT_3$ receptor antagonist (0.001, 0.01, 0.1, 1.0 mg/kg of ondansetron or tropisetron, i.p.) or saline 30 min before ethanol administration (1.0 g/kg i.p.) and 45 min before the start of the session. Since the highest dose of both tropisetron and ondansetron did not result in any response rate effect, a separate series of experiments, served to study higher doses of the drugs (10.0 and 50.0 mg/kg).

In i.p. substitution tests, mCPBG (0.1, 1.0, 2.5, 5.0, 10.0 mg/kg) or saline was administered i.p. 15 min before the session. In i.c.v. substitution tests, mCPBG (1, 10, 35 μg per rat) or saline was administered i.c.v. in a volume of 5 μl (1 μl/min), and after an additional 60 s the animals were placed in the chambers.

### 2.5. Surgery

The rats were anesthetized with ketamine (75 mg/kg i.p.; Gedeon Richter, Budapest, Hungary) and placed in a Stoelting stereotaxic apparatus with the incisor bar 3.3 mm below the horizontal plane. Stainless steel guide cannulae (22 gauge, C313G; Plastics One) were implanted to terminate 2.0 mm above the final place of microinjection. Cannulae were attached to the skull with dental cement and stainless steel cranial screws (# 0-80, 1.6 mm; Plastics One). The guide cannulae were sealed with removable dummy cannulae (C313DC; Plastics One). The stereotaxic coordinates used for the lateral ventricle were: P = −0.8 mm from bregma, L = 1.5 mm lateral to the midline and V = 1.5 mm from the skull surface (Paxinos and Watson, 1986).

### 2.6. Histology

Upon completion of the experiment the rats were deeply anesthetized with ketamine and injected i.c.v. with blue dye solution. The brains were removed and sectioned on a freezing microtome. Placement of cannula tips, internal cannula traces and location of the dye were assessed using a magnifying glass. Only the animals with correct cannula placement and distribution of the dye in the lateral ventricle were included in the statistical analysis of the results.

### 2.7. Drugs

A 10% v/v ethanol solution was prepared daily from a 95% stock solution. In dose-response tests, ethanol was administered in appropriate volumes to obtain the desired dose. Tropisetron hydrochloride (Sandoz, Basel, Switzerland), ondansetron hydrochloride (Glaxo, Greenford, UK) and 1-(*m*-chlorophenyl)-biguanide hydrochloride (RBI, Natick, MA) were dissolved in 0.9% NaCl. Tropisetron and ondansetron were given i.p. in a volume of 1.0 ml/kg. In i.p. substitution tests, mCPBG was injected in a volume

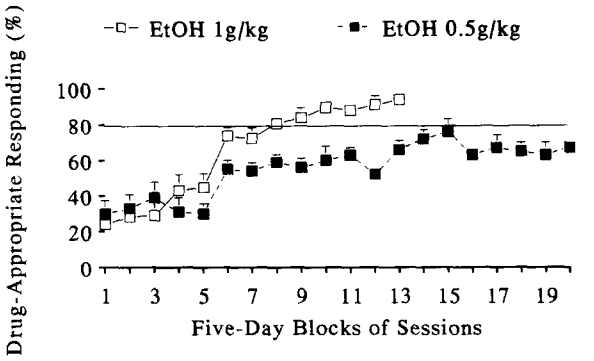


Fig. 1. Discrimination between ethanol and saline in 1.0 g/kg and 0.5 g/kg ethanol-treated groups. The data represent mean (±S.E.M) percentage accuracy (ethanol- or saline-appropriate responding prior to the completion of the first reinforcer) for consecutive 5-day blocks of training sessions. Two-way analysis of variance (see Results) was performed for weeks 1–13, i.e. for the first 65 training sessions during which ethanol-saline discrimination was established in the 1.0 g/kg ethanol-treated group. The 0.5 g/kg ethanol-treated group did not reach the discrimination criteria (see Materials and methods) within 20 weeks of training (100 sessions). Horizontal line represents the minimal accuracy accepted by discrimination criteria; $n = 12$ rats for 1.0 g/kg ethanol-treated group, $n = 8$ rats for 0.5 g/kg ethanol-treated group.

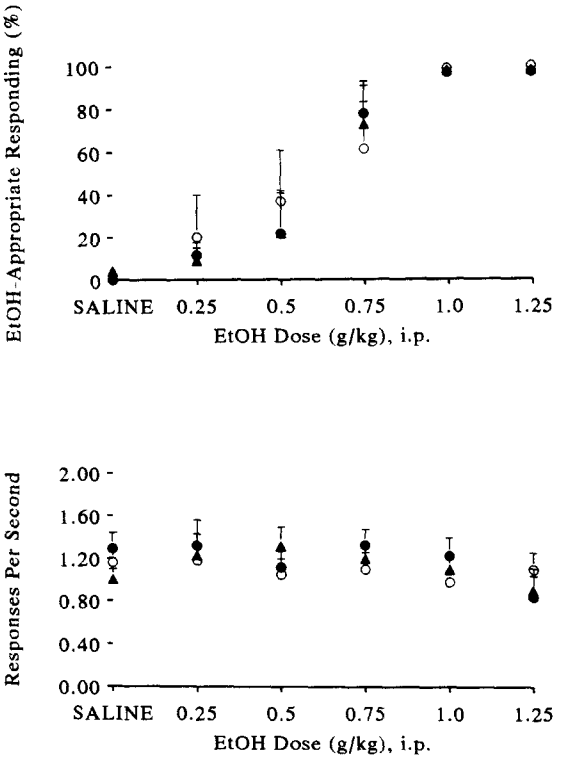


Fig. 2. Dose-response for the stimulus properties of ethanol. Mean (±S.E.M) percentage of ethanol-appropriate responding prior to the completion of the first reinforcement (upper panel) and the mean (±S.E.M) response rate (lower panel) during dose-response tests performed at different stages of the study in rats trained to discriminate 1.0 g/kg of ethanol from saline. Open circle, dose-response test at the beginning of the study; closed circle, dose-response test after the surgery; closed triangle, dose-response test at the end of the study; $n = 5–6$ rats (see Materials and methods for details).

of 13.0 ml/kg. All solutions were prepared immediately prior to use and the doses refer to the salt where appropriate.

### 2.8. Statistics

For training sessions, accuracy (%) was defined as the percentage of ethanol- or saline-appropriate lever responses with, as 100%, the total responses prior to completion of the first reinforcer (first FR10) on drug-appropriate lever. During test sessions accuracy was defined as the percentage of ethanol-appropriate lever responses with total responses prior to completion of the first reinforcer on either lever as 100%. The response rates were calculated as the total number of responses (on both levers) during the session divided by the session time in seconds. Two-way analysis of variance (Group × Week) was used to compare mean accuracy (%) of discrimination between 0.5 g/kg and 1.0 g/kg ethanol-treated group during first 13 5-day blocks of training (i.e. until discrimination between ethanol and saline was established for all rats from the 1.0 g/kg ethanol-treated group). $ED_{50}$ (and 95% confidence limit) was calculated for each dose-response test. Student's *t*-test two-tailed was used for comparing response rates from all test sessions.

## 3. Results

All animals ($n = 12$) from the 1.0 g/kg ethanol-treated group acquired the ethanol-saline discrimination (range 37–65 training sessions). None of the animals ($n = 8$) from the 0.5 g/kg ethanol-treated group attained discrimination criteria (Fig. 1). Two-way analysis of variance showed significant effect of Group – $F(1,18) = 8.12$, $P = 0.01$; Week – $F(12,21) = 21.22$, $P < 0.001$, and significant Group × Week interaction – $F(12,21) = 18.14$, $P < 0.001$. $ED_{50}$ values calculated for consecutive dose-response tests were: 0.45 g/kg (CL 0.25–0.76), 0.56 g/kg (CL 0.40–0.79) and 0.54 g/kg (CL 0.37–0.78), respectively (Fig. 2). None of the 5-$HT_3$ receptor antagonists (0.001–1.0 mg/kg), antagonized the discriminative stimulus of 1.0 g/kg of ethanol (Fig. 3). In the separate series of experiments, 10.0 mg/kg dose of tropisetron and ondansetron failed to affect either the discriminative stimulus of 1.0 g/kg of ethanol or the mean response rate ($n = 4$ rats; data not shown). The highest doses of tropisetron and ondansetron (50.0 mg/kg) completely abolished responding ($n = 3$ rats). Two rats treated with 50 mg/kg of ondansetron died 6–12 h after the test session.

None of the i.p. or i.c.v. administered doses of mCPBG were able to replace the discriminative stimulus effect of

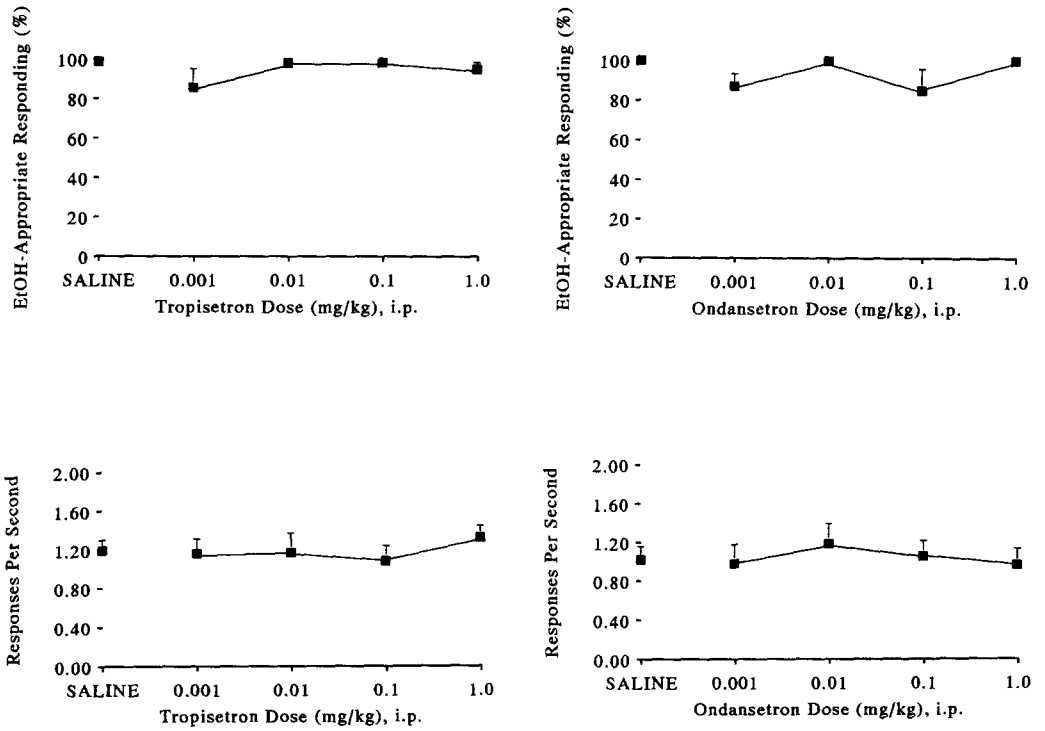


Fig. 3. Effect of increasing doses of tropisetron and ondansetron on the mean percentage (±S.E.M) of ethanol-appropriate responding prior to the completion of the first reinforcer (upper panel) and on the mean (±S.E.M) response rate (lower panel) in rats trained to discriminate 1.0 g/kg of ethanol from saline; $n = 5$–6 rats.

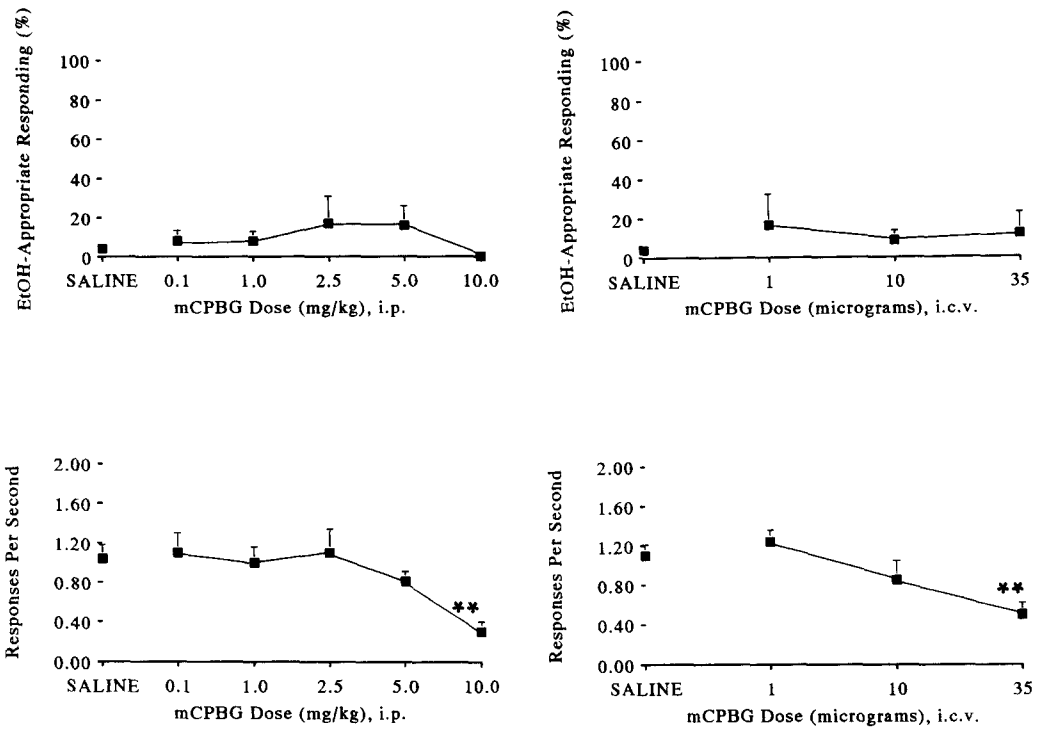


Fig. 4. Mean (±S.E.M) percentage of ethanol-appropriate responding prior to the completion of the first reinforcement (upper panel) and mean (±S.E.M) response rate (lower panel) following different doses of i.p. or i.c.v. administered mCPBG in rats trained to discriminate 1.0 g/kg of ethanol from saline; $n = 5–6$ rats. ** $P < 0.01$.

ethanol (Fig. 4). The highest doses of mCPBG (10.0 mg/kg i.p.; 35 μg per rat i.c.v.) caused a significant reduction in the response rate.

## 4. Discussion

In order to function as a reinforcer, ethanol should have discriminative stimulus properties, i.e. it should produce subjective effects that the animal can identify. Our results indicate that ethanol at the dose of 0.5 g/kg does not serve as a sufficient discriminative stimulus in rats while at the higher dose (1.0 g/kg) it produces a clear-cut discriminative stimulus effect. It is worthy of note that a number of studies using self-administration and place conditioning procedures failed to reveal a primary reinforcing effect for low doses of ethanol (Grupp, 1981; Van der Kooy et al., 1983; Numan et al., 1984; Asin et al., 1985). The present investigation confirms previous reports that the 1.0 g/kg dose of ethanol is capable of controlling discriminative responding in rats (Grant and Colombo, 1993a,b; Sanger, 1993). As ethanol has a variety of behavioral effects at this dose (or higher doses), it is postulated that a complex, multiple set of cues is responsible for the ethanol interoceptive cue (Grant et al., 1991; Sanger, 1993). Preliminary pharmacological studies support this notion and suggest that the contribution of each component of the ethanol cue is a function of the training dose of ethanol (Grant and Colombo, 1993a,b; Colombo et al., 1995).

The major discriminative cues resulting from ethanol are those that relate to its sedative and anesthetic actions because a variety of analgesics and tranquilizers can be substituted for the ethanol cue (Sanger, 1993; Shelton and Balster, 1994). Notably, low doses of ethanol (0.1–0.5 g/kg) did not yield a conditioned taste and place aversion in rats while higher doses, e.g. 1.0 g/kg and more, were capable of inducing aversive effects (Van der Kooy et al., 1983; Asin et al., 1985; Cannon and Carrell, 1987; Froehlich et al., 1988). Interestingly, ethanol-induced conditioned taste aversion in rats was also, like its discriminative stimulus effect, unaffected by the 5-$HT_3$ receptor antagonist, tropisetron (Bienkowski et al., unpublished observation).

The purpose of our study was to assess whether or not 5-$HT_3$ receptors play a role in an ethanol discriminative stimulus. Evidence to support this possibility is based (see Introduction) on the findings that ethanol influences 5-HT action on 5-$HT_3$ receptors and that 5-$HT_3$ receptor antagonists may attenuate voluntary ethanol consumption and certain central effects of ethanol, such as ethanol-induced dopamine release and ethanol-induced hyperlocomotion (Carboni et al., 1989a; Fadda et al., 1991; Lovinger, 1991; Kostowski et al., 1995). The present results, however, demonstrate that neither 5-$HT_3$ receptor antagonists nor a 5-$HT_3$ receptor agonist influences the ethanol interoceptive cue. Notably, a 5-$HT_3$ receptor agonist, mCPBG, cannot be substituted for ethanol at any dose and route of administration tested (i.p. and i.c.v.). This could suggest that the

capacity of ethanol to serve as a discriminative cue is unrelated to its interaction with peripheral and central 5-$HT_3$ receptors. In compliance with this notion is our finding that 5-$HT_3$ receptor antagonists, tropisetron and ondansetron, were unable to block the 1.0 g/kg ethanol discriminative stimulus. Taken together these results suggest that the interoceptive cue of ethanol is unrelated to the 5-$HT_3$ receptor and depends on other mechanisms. Recently, Joharchi et al. (1993) reported that 5-$HT_3$ receptor antagonists failed to reduce the discriminative stimulus properties of morphine. Together with results of the studies showing an inability of 5-$HT_3$ receptor antagonists to reduce the discriminative stimulus effects of cocaine and amphetamine (Paris and Cunningham, 1991; Lane et al., 1992; Moser, 1992), all these observations suggest that 5-$HT_3$ receptor mechanisms are not involved in the formation of the discriminative cues produced by major drugs of abuse, or at least, are not related to important aspects of these cues. On the other hand, it remains intriguing that the 5-$HT_3$ receptor seems to be involved in at least certain aspects of the positive reinforcing effects of many substances of abuse including morphine and ethanol (Carboni et al., 1989a,b; Hui et al., 1993; Kostowski et al., 1993; Tomkins et al., 1995).

Contrary to the present results, block of the discriminative stimulus of ethanol with 5-$HT_3$ receptor antagonists has been reported in rats and pigeons (Grant and Barrett, 1991; Grant and Colombo, 1993c). There are several possible reasons for the differences between the present results and those reported by Grant and Colombo (1993c) for rats. The first concerns the doses of 5-$HT_3$ receptor antagonists tested. Recent reports suggest that tropisetron and 3-tropanyl-3,5-dichlorobenzoate (MDL 72222) may interact with other receptor systems to offset their effect at the 5-$HT_3$ receptor site. In fact, high concentrations of tropisetron and MDL 72222 may reduce the function of the $GABA_A$ receptor complex (Klein et al., 1993, 1994). Interestingly, MDL 72222 dose dependently exacerbates the severity of ethanol withdrawal seizures in mice (Grant et al., 1994). Tropisetron, unlike MDL 72222, granisetron and ondansetron, exhibits a surmountable blocking activity at 5-$HT_4$ receptors (Dumuis et al., 1989; Bockaert et al., 1990). Thus, the effects mentioned above might contribute to the partial block of the discriminative stimulus effect of ethanol by high doses of tropisetron (3.0–17.0 mg/kg). Although MDL 72222 was not tested in the present study, it remains speculative that 5-$HT_3$ receptor antagonism alone accounts for its ability to block the interoceptive cue produced by ethanol. The blockade found with MDL 72222 pretreatment occurred only when ethanol was administered intragastrically (i.g.) rather than by i.p. injection. Furthermore, MDL 72222 treatment resulted in a lower blood ethanol concentration when ethanol was given by the i.g., but not by the i.p. route. Therefore, it is likely that the reported inhibitory effect of MDL 72222 on discriminative properties of intragastrically delivered ethanol due to impaired absorption of ethanol from the gut (Grant and Colombo, 1993c).

Finally, recent evidence suggests that the extent to which drugs acting at $GABA_A$, 5-$HT_1$ and NMDA receptors can be substituted for ethanol may depend on the training dose of ethanol (Grant and Colombo, 1993a,b; Colombo et al., 1995). As the dose of ethanol used by Grant and Colombo was 1.5 times higher than that in the present work, the training dose of ethanol may be another factor of possible importance in comparisons of the present study with earlier reports.

In summary, the present results showed that 5-$HT_3$ receptor antagonists, tropisetron and ondansetron, as well as the 5-$HT_3$ receptor agonist, mCPBG, do not modify the discriminative cue of ethanol in rats. As mentioned previously, since several different 5-$HT_3$ receptor antagonists also fail to alter the discriminative stimulus effects of cocaine, amphetamine and morphine, the major conclusion that can be drawn from this and previous studies is that the 5-$HT_3$ receptors are not primarily responsible for mediation of the discriminative stimulus properties of drugs of abuse.

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## References

Asin, K.E., D. Wirtshafter and B. Tabakoff, 1985, Failure to establish a conditioned place preference with ethanol in rats, Pharmacol. Biochem. Behav. 22, 169.

Bockaert, J., M. Sabben and A. Dumuis, 1990, Pharmacological characterization of brain 5-$HT_4$ receptors positively coupled to adenylate cyclase in adult guinea pig hippocampal membranes, Mol. Pharmacol. 37, 408.

Cannon, D.S. and L.E. Carrell, 1987, Rat strain differences in ethanol self-administration and taste aversion learning, Pharmacol. Biochem. Behav. 28, 57.

Carboni, E., F. Acquas, R. Frau and G. Di Chiara, 1989a, Differential inhibitory effects of 5-$HT_3$ antagonists on drug-induced dopamine release, Eur. J. Pharmacol. 164, 515.

Carboni, E., F. Acquas, P. Leone and G. Di Chiara, 1989b, 5-$HT_3$ receptor antagonists block morphine- and nicotine- but not amphetamine-induced reward, Psychopharmacology 97, 175.

Colombo, G., R. Agabio, C. Lobina, R. Reali, F. Fadda and G.L. Gessa, 1994, Blockade of ethanol discrimination by isradipine, Eur. J. Pharmacol. 265, 167.

Colombo, G., R. Agabio, N. Balaklievskaia, F. Fadda, G. Gatto, G.L. Gessa, C. Lobina, R. Reali and K.A. Grant, 1995, Drug discrimination procedures to investigate the neurochemical basis of the subjective effects of ethanol, Alcohol Alcohol. 30, 493.

Colpaert, F., 1986, Drug discrimination: behavioral, pharmacological and molecular mechanisms of discriminative drug effects, in: Behavioral Analysis of Drug Dependence, eds. S.R. Goldberg and I.P. Stolerman (Academic Press, New York) p. 161.

Dumuis, A., M. Sebben and J. Bockaert, 1989, The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-$HT_4$) positively coupled to adenylate cyclase in neurons, Naunyn Schmiedebergs Arch. Pharmacol. 340, 403.

Fadda, F., B. Garau, F. Marchei, G. Colombo and G.L. Gessa, 1991, MDL 72222, a selective 5-$HT_3$ receptor antagonist, suppresses voluntary ethanol consumption in alcohol-preferring rats, Alcohol 26, 107.

Froehlich, J.C., J.H. Harts, L. Lumeng and T-K. Li, 1988, Differences in response to the aversive properties of ethanol in rats selectively bred for oral ethanol preference, Pharmacol. Biochem. Behav. 31, 215.

Grant, K.A. and J. Barrett, 1991, Blockade of the discriminative stimulus properties of ethanol with 5-$HT_3$ receptor antagonists, Psychopharmacology 104, 451.

Grant, K.A. and G. Colombo, 1993a, Discriminative stimulus effects of ethanol: effect of training dose on the substitution of *N*-methyl-D-aspartate antagonists, J. Pharmacol. Exp. Ther. 264, 1241.

Grant, K.A. and G. Colombo, 1993b, Substitution of 5-$HT_1$ agonist trifluoromethylphenylpiperazine (TFMPP) for the discriminative stimulus effects of ethanol: effect of training dose, Psychopharmacology 113, 26.

Grant, K.A. and G. Colombo, 1993c, The ability of 5-$HT_3$ antagonists to block ethanol discrimination: effect of route of ethanol administration, Alcohol. Clin. Exp. Res. 17, 497.

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

Grant, K.A., K. Hellevuo and B. Tabakoff, 1994, The 5-$HT_3$ receptor antagonist MDL-72222 exacerbates ethanol withdrawal seizures in mice, Alcohol. Clin. Exp. Res. 18, 410.

Grupp, L.A., 1981, An investigation of intravenous ethanol self-administration in rats using a fixed ratio schedule of reinforcement, Physiol. Psychol. 9, 359.

Higgins, G.A., D.M. Tomkins, P.J. Fletcher and E.M. Sellers, 1992, Effect of drugs influencing 5-HT function on ethanol drinking and feeding behaviour in rats: studies using a drinkometer system, Neurosci. Biobehav. Rev. 16, 535.

Holtzman, S.G., 1990, Discriminative stimulus effects of drugs: relationship to potential for abuse, in: Modern Methods in Pharmacology, Vol. 6, Testing and Evaluation of Drugs of Abuse, eds. M.W. Alder and A. Cowan (Wiley-Liss, New York) p. 193.

Hui, S-C.G., E.L. Sevilla and C.W. Ogle, 1993, 5-$HT_3$ antagonists reduce morphine self-administration in rats, Br. J. Pharmacol. 110, 1341.

Joharchi, N., E.M. Sellers and G.A. Higgins, 1993, Effects of 5-$HT_3$ receptor antagonists on the discriminative stimulus properties of morphine in rats, Psychopharmacology 112, 111.

Klein, R.L., E. Sauna, S.J. McQuilkin, J.M. Sikela, P.J. Whiting and R.A. Harris, 1993, 5-$HT_3$ antagonists and $GABA_A$ receptors: a functional study, Soc. Neurosci. Abstr. 19, 1140.

Klein, R.L., E. Sanna, S.J. McQuilkin, P.J. Whiting and R.A. Harris, 1994, Effects of 5-$HT_3$ receptor antagonists on binding and function of mouse and human $GABA_A$ receptors, Eur. J. Pharmacol. 268, 237.

Knapp, D.J. and L.A. Pohorecky, 1992, Zacopride, a 5-$HT_3$ receptor antagonist, reduces voluntary ethanol consumption in rats, Pharmacol. Biochem. Behav. 41, 847.

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

Kostowski, W., A. Bisaga, E. Jankowska and P. Krzascik, 1994, Studies on the effects of certain 5-$HT_3$ receptor antagonists on ethanol preference and withdrawal seizures in the rat, Pol. J. Pharmacol. 46, 133.

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

Lane, J.D., C. Pickering, M. Hooper, K. Fagan, M.B. Tyers and M.W. Emmett-Oglesby, 1992, Failure of ondansetron to block discriminative or reinforcing stimulus effects of cocaine in the rat, Drug Alcohol Depend. 30, 151.

Le Marquand, D., O. Pihl and Ch. Benkelfat, 1994, Serotonin and alcohol intake, abuse and dependence: finding of animal studies, Biol. Psychiatry 36, 395.

Little, H.J., 1991, Mechanisms that may underlie the behavioral effects of ethanol, Prog. Neurobiol. 3, 171.

Lovinger, D.M., 1991, Ethanol potentiation of the 5-$HT_3$ receptor-mediated ion current in NCB-20 neuroblastoma cells, Neurosci. Lett. 122, 57.

Lovinger, D.M. and Q. Zhou, 1993, Alcohols potentate ion current mediated by recombinant 5-$HT_3$ RA receptors expressed in a mammalian cell line, Neuropharmacology 33, 1567.

Middlemiss, D.N. and M.D. Tricklebank, 1992, Centrally active 5-HT receptor agonists and antagonists, Neurosci. Biobehav. Rev. 16, 75.

McBride, W.J., J.M. Murphy, L. Lumeng and T-K. Li, 1989, Serotonin and ethanol preference, in: Recent Dev. Alcohol., ed. M. Galanter (Plenum, New York) p. 187.

Moser, P.C., 1992, The effects of 5-$HT_3$ receptor antagonists on the discriminative stimulus properties of amphetamine, Eur. J. Pharmacol. 212, 271.

Numan, R., A.M. Naparzewska and C.M. Adler, 1984, Absence of reinforcement with low dose intravenous ethanol self-administration in rats, Pharmacol. Biochem. Behav. 21, 609.

Oakley, N.R., B.J. Jones, M.B. Tyers, B. Costall and A.M. Domeney, 1988, The effect of GR38032F on alcohol consumption in the marmoset, Br. J. Pharmacol. 95, 870P.

Ollat, H., H. Parvez and S. Parvez, 1988, Alcohol and central neurotransmission, Neurochem. Int. 13, 275.

Paris, J. M. and K.A. Cunningham, 1991, Serotonin 5-$HT_3$ antagonists do not alter the discriminative stimulus properties of cocaine, Psychopharmacology 104, 475.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates (Academic Press, New York).

Sanger, D.J., 1993, Substitution by NMDA antagonists and other drugs in rats trained to discriminate ethanol, Behav. Pharmacol. 4, 523.

Shelton, K.L. and R.L. Balster, 1994, Ethanol drug discrimination in rats. Substitution with GABA agonists and NMDA antagonists, Behav. Pharmacol. 5, 441.

Sellers, E.M., G.A. Higgins and M.B. Sobel, 1992, 5-HT and alcohol abuse, Trends Pharmacol. Sci. 12, 69.

Signs, S.A. and M.D. Schechter, 1988, The role of dopamine and serotonin receptors in the mediation of the ethanol interoceptive cue, Pharmacol. Biochem. Behav. 30, 55.

Tomkins, D.M., A.D. Le and E.M. Sellers, 1995, Effects of the 5-$HT_3$ receptor antagonist ondansetron on voluntary ethanol intake in rats and mice maintained on a limited access procedure, Psychopharmacology 117, 479.

Van der Kooy, D., M. O'Shaughnessy, R.F. Mucha and H. Kalant, 1983, Motivational properties of ethanol in naive rats as studied by place conditioning, Pharmacol. Biochem. Behav. 19, 441.