Blockade of the discriminative stimulus effects of ethanol with 5-HT₃ receptor antagonists

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Abstract. The ability of selective 5-HT₃ receptor antagonists to block the discriminative stimulus effects of ethanol was investigated in pigeons trained with food reinforcement to discriminate ethanol (1.5 g/kg; IG) from water. The 5-HT₃ receptor antagonists that are substituted tropines, ICS 205-930 (0.1-0.56 mg/kg) and MDL 72222 (3.0-17.0 mg/kg), blocked ethanol-appropriate responding, in a dose-dependent manner, suggesting that some of the discriminative stimulus effects of ethanol are mediated via the 5-HT₃ receptor. The blockade of the discriminative stimulus effects of ethanol occurred in the presence of approximately 25-40 mM blood ethanol levels. Furthermore, the ethanol dose-effect function was shifted to the right by increasing doses of MDL 72222, suggesting a surmountable antagonism of the discriminative stimulus effects of ethanol. However, the benzamide zacopride (0.56-1.7 mg/kg), which is also a 5-HT₃ receptor antagonist, did not block the discriminative stimulus effects of ethanol. In addition, the dopaminergic antagonist haloperidol and the 5-HT₃ receptor antagonist ketanserin also failed to block the ethanol discrimination. The results suggest that 5-HT₃ mediated neurotransmission is an important component of ethanol's discriminative stimulus effects, but that the structural characteristics of the selective 5-HT₃ receptor antagonists influence their ability to block this action of ethanol. Furthermore, these findings implicate a significant role of 5-HT₃ activity in the behavioral effects of ethanol that may provide a pharmacological means for therapeutic intervention of alcohol abuse.

Key words: Ethanol – Serotonin receptors – Drug discrimination – Serotonin antagonists – Ethanol antagonist – 5-HT₃

The mechanisms through which ethanol exerts its effects on behavior are not well defined. Historically, investigations of ethanol's effect have focused on the nonspecific disruption of neuronal membrane lipids (see Deitrich et al. 1989). However, evidence gathered over the past decade has shown specific transmembrane protein complexes are particularly sensitive to ethanol (Tabakoff and Hoffman 1987). Two major examples are the GABA_A ionophore complex, where ethanol potentiates chloride influx, and the NMDA subtype of glutamate receptor, where ethanol antagonizes cation conductance (Deitrich et al. 1989). In addition to these well-characterized receptor systems, recent data indicate that ethanol potentiates the action of serotonin at the 5-HT₃/ionophore complex to increase cation conductance in cells derived from either a neuroblastoma cell line or the nodose ganglion (Lovinger 1990). Some of these findings have suggested that it may be possible to antagonize biochemical and behavioral actions of ethanol but, in general, results with compounds such as the partial benzodiazepine inverse agonist RO15-4513 have been inconsistent (Harris and Lal 1988).

Based on receptor binding studies and functional assays, four major types of serotonin receptors have been classified (Schmidt and Peroutka 1989; Bockaert et al. 1990). The 5-HT₃ receptor subtype is linked to a cation channel, which conducts primarily sodium and potassium ions (Yankel and Jackson 1988; Derkach et al. 1989; Peters and Lambert 1989). Studies of the distribution of 5-HT₃ receptors in the CNS, as measured by membrane binding and autoradiography techniques, show that these receptors are located primarily in the area postrema, cortical areas, amygdala, hippocampus and nucleus accumbens (Kilpatrick et al. 1987; Waerbel et al. 1989). Antagonists of the 5-HT₃ receptor have anxiolytic-like effects in some animal models of anxiety (Costall et al. 1988b; Glenn and Green 1989), as well as antiemetic (Cohen et al. 1989) and anti-dopaminergic (Costall et al. 1988a) effects. In addition, 5-HT₃ antagonists decrease dopamine release in the nucleus accumbens induced by the relatively specific 5-HT₃ agonist 2-methylserotonin (Jiang et al. 1990), or by psychoactive drugs such as nicotine, morphine and ethanol (Carboni et al. 1989).

If ethanol's potentiation of 5-HT₃ receptor-mediated responses is an important component of its behavioral actions, then specific 5-HT₃ receptor antagonists should...
attenuate those behavioral effects of ethanol. In the following study, this hypothesis was investigated using a drug discrimination procedure, which can be used to evaluate whether some of the stimulus effects of ethanol are mediated through specific receptor systems (Colpaert 1986).

Materials and methods

Subjects. Six male white Carneau pigeons (Palmetto Pigeon Plant, Sunter, S.C.) were maintained at approximately 85% of their free-feeding body weights. Three of the pigeons were used in a previous study in which ethanol was trained as the discriminative stimulus and various noncompetitive N-methyl-D-aspartate antagonists were examined (Grant and Barrett 1989). Three pigeons were naive at the outset of these experiments. The pigeons were housed individually in stainless steel cages with water and crushed oyster shells always available. The temperature of the room was maintained at 23°C and illuminated 14 h/day (0600–2000 hours).

Apparatus. Operant chambers (30 x 30 x 30 cm) within sound attenuating and ventilated cubicles equipped with white noise were used. The front panel of each chamber contained two Plexiglass response keys (R. Gerbrands Co., Arlington, MA) mounted 22 cm above the floor and 5.7 cm from the center of the panel. Each peck on the key with a force greater than 0.15 N was recorded as a response. An opening in the center of the front panel, 5 cm above the floor, provided access to grain when the hopper was elevated into position by solenoid activation. Reinforcement consisted of 3-s access to grain, during which time the keylight was darkened and a white light associated with the feeder was illuminated. Schedule requirements and data collection were controlled by equipment located in an adjoining room.

Discrimination training. Subjects were trained to peck the illuminated key under a fixed-ratio 30 (FR 30) schedule of food reinforcement. When the pigeons reliably responded on either key, both keys were illuminated and one key was designated as the ethanol key and one key as the water key. Immediately following the administration of ethanol, 1.5 g/kg 15% (w/v), or an equivalent volume of water via the intragastric (IG) route, the bird was placed in the chamber. The chamber remained darkened for 20 min prior to the start of the session. The session began with the illumination of both key lights and ended after 30 reinforcers were obtained or 30 min had elapsed. Thirty consecutive responses on the appropriate key resulted in reinforcement, while incorrect responses reset the ratio requirement on the correct key. Responding was considered to be under stimulus control when 27 of the first 30 responses, and 90% of the total responses emitted during the session occurred on the appropriate key over three consecutive training sessions.

Antagonist tests. Test sessions were conducted exactly the same as training sessions except that 30 consecutive responses on either key resulted in reinforcement. The rationale of this method of testing, together with data supporting the use of this procedure have been discussed by Ator (1990) and Holtzman (1990). Generally, test sessions occurred on Tuesdays and Fridays, with training sessions given on the intervening days. Test sessions were not conducted if performance on the previous day did not meet the above criterion. Prior to treatment, naive birds (n = 6) were tested with various doses of ethanol (0.5–2.5 g/kg) and water to determine the ethanol dose-effect curve. Following the characterization of the ethanol dose-response relation, the 5-HT3 antagonists ICS 205–930 (0.1–0.3 mg/kg; n = 3), MDL 72222 (5.6–17.0 mg/kg; n = 6), and zacopride (0.1–1.7 mg/kg; n = 3) were administered intramuscularly, on separate sessions, either 20 min (MDL 72222 and zacopride) or 10 min (ICS 205–930) prior to ethanol treatment.

To explore the characteristics of the antagonism of the discriminative stimulus effects of ethanol, the ethanol dose-effect function was then redetermined following the pretreatment of either 5.6 or 10.0 mg/kg MDL 72222 (n = 6). For this determination, the MDL dose was administered 20 min prior to a single dose of ethanol (1.0, 1.5, or 2.0 g/kg ethanol), and the test session conducted as described above. Immediately following selected sessions in which both MDL 72222 and ethanol were given in combination, blood samples (20 µl) were drawn from the brachial vein for blood ethanol analysis by headspace gas chromatography (Tabakoff et al. 1976).

The specificity of 5-HT3 antagonists in blocking ethanol's discriminative stimulus effects was tested by using haloperidol, primarily a D2 receptor antagonist (0.1–3.0 mg/kg; n = 3), and ketanserin, a 5-HT1 receptor antagonist (17.0–30.0 mg/kg; n = 3), administered in the breast muscle on separate test sessions, 20 min prior to ethanol. Under most conditions, each dose of a drug was tested twice, once following a training session in which water had been administered and once following an ethanol training session.

Data presentation. The percentage of responses made on the ethanol-appropriate key was calculated by dividing the number of responses made on the ethanol key by the total number of responses made on either key during a session. Response rates were expressed as the total number of responses made on either key divided by the session length (in seconds). There were no systematic differences between the pigeons in response to the antagonist challenges, regardless of their experimental history, therefore group averages were calculated expressed as the mean ± SEM. Data were not included if response rates were less than 0.1 responses/s, or if a single FR requirement had not been completed during the test session. Antagonism was defined as greater than 80% of total responses on the water-appropriate key following treatment with the combination of antagonist and ethanol.

Drugs. ICS 205–930 (3-tropanyl-indole-3-carboxylate), MDL 72222 (3-tropanyl-3,5-dichlorobenzate) and (±)zacopride (4-amino-N-1(azabicyclo[2.2.2]oct-3-yl)-5-chloro-2-methoxybenzamide maleate) were purchased from Research Biochemicals Inc., Natick, MA. Haloperidol was obtained in liquid form from McNeil laboratories, NJ. Ketanserin (3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]-ethyl]-2,4-[1H,3H]-quinazolinedione) was obtained from Janssen Pharmaceuticals, Beerse, Belgium. All drugs were dissolved in water and doses calculated as the salts.

Results

The number of training sessions necessary to reach criterion for demonstrating discriminative stimulus control over responding ranged from 20 to 60 sessions (mean = 40). Control rates of responding during training sessions following ethanol administration were not different from control rates following water (ethanol: 1.9 ± 0.1 responses per s; water: 1.8 ± 0.1 responses per s).

During the test sessions in which water or 0.5 g/kg ethanol was administered, the pigeons (n = 6) responded almost exclusively on the water-appropriate key. Doses of ethanol ranging from 1.0 to 2.5 g/kg resulted in a shift in responding from the water to the ethanol key, such that an average of over 89% of the responses were made on the ethanol key under these conditions (Fig. 1, upper panel). Both the 2.0 and 2.5 g/kg doses of ethanol resulted in rate-decreasing effects, with the highest ethanol dose yielding response rates of 0.9 ± 0.2 responses per s (Fig. 1, bottom panel).

The results of the 5-HT3 antagonist pretreatments given in combination with 1.5 g/kg ethanol are shown in Fig. 2. Pretreatment with the substituted tropines ICS