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European Journal of Pharmacology 491 (2004) 149–156
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5-HT_3 antagonist ICS 205–930 enhances naltrexone's effects on ethanol intake

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Received 12 December 2003; received in revised form 26 February 2004; accepted 3 March 2004

Available online 26 April 2004

Abstract

Opioid receptor antagonist naltrexone has shown some efficacy in decreasing ethanol consumption in humans. However, naltrexone treatment is not always efficacious and produces several aversive effects such as nausea, anxiety and weight loss. Serotonin-3 (5-HT_3) receptor antagonists also modulate some of the behavioral effects of alcohol and may decrease alcohol consumption. We examined the effects of the combination of 5-HT_3 receptor antagonist ICS 205–930 ((3-tropanyl-indole-1-carboxylate, tropisetron) and naltrexone on ethanol and food intake in Sprague–Dawley rats. Both naltrexone (0.56–10 mg/kg) and ICS 205–930 (5.6 mg/kg), when administered intraperitoneally 30 min before the scheduled 3-h access to ethanol, significantly suppressed ethanol intake. Naltrexone (1 mg/kg) when given in combination with ICS 205–930 (5.6 mg/kg) was significantly more efficacious in suppressing ethanol intake in comparison with naltrexone (1 mg/kg) administered alone. The drug combination did not affect the food intake. These data suggest that 5-HT_3 receptor antagonist ICS 205–930 may be used as an effective adjunct for pharmacotherapy of alcoholism.

Keywords: Alcoholism; Naltrexone; Opioid receptor; 5-HT_3 receptor

1. Introduction

Identification of the neurotransmitter systems that are involved in regulating alcohol consumption is the key to the development of therapeutic interventions for alcohol abuse and dependence. Pharmacological treatment with an opioid antagonist naltrexone has been found to improve abstinence rates among treatment-seeking alcoholics (Anton et al., 1999; O'Malley et al., 1992; Volpicelli et al., 1992). However, naltrexone treatment is not effective in all alcoholics (reviewed in Litten and Allen, 1998). Therefore, there is a need to search for additional pharmacological adjuncts, which will improve naltrexone's efficacy.

The findings from several studies suggest involvement of various neurotransmitter systems such as serotonin, opiate, dopamine, $GABA_A$ and NMDA receptors in excessive alcohol drinking behavior (reviewed in Koob, 1992). Researchers have also suggested an interaction between a number of 5-HT_3 receptor antagonists and the effects of ethanol using electrophysiological (Lovinger and White, 1991), biochemical (Campbell and McBride, 1995; Carboni et al., 1989), and behavioral techniques (Grant and Barrett, 1991; Mhatre et al., 2001). 5-HT_3 receptor antagonists have also been found to selectively attenuate ethanol-induced increase in dopamine levels (Carboni et al., 1989), which led to the suggestion that alcohol produces its rewarding effects by increasing dopamine release in the nucleus accumbens through activation of 5-HT_3 receptors (Campbell and McBride, 1995). Consistent with these interactions between ethanol and 5-HT_3-receptors, several laboratories have reported the effectiveness of 5-HT_3 receptor antagonists in reducing ethanol consumption in various rodent models (Fadda et al., 1991; Hodge et al., 1993, 1995; Kostowski et al., 1993; McKinzie et al., 1998; Tomkins et al., 1995) and alcohol dependent individuals (Ait-Daoud et al., 2001; Johnson et al., 2000; Sellers et al., 1994).

5-HT_3 receptor antagonist ICS 205–930 ((3-tropanyl-indole-1-carboxylate, tropisetron) has high affinity (Middlemiss and Tricklebank, 1992) and shows selectivity for 5-HT_3 receptors at a low concentration. It is rapidly

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doi:10.1016/j.ejphar.2004.03.011

absorbed and penetrates the blood–brain barrier easily. The plasma terminal elimination half-life of this compound is found to be quite long in human beings, i.e. about 8 h in efficient metabolisers and about 30–40 h in poor metabolisers (Simpson et al., 2000). ICS 205–930 is anxiolytic and suppresses nausea. It has been found to suppress ethanol withdrawal seizures (Kostowski et al., 1993). It also has a low toxicity profile. 5-HT_3 receptor antagonists do not modify any aspect of normal behavior in animals or induce remarkable changes in physiological functions in healthy subjects. These characteristics of ICS 205–930 and the suggestion of a synergistic interaction between the 5-HT_3 receptor and endogenous opioids in the mesocorticolimbic dopamine reward system (Hodge et al., 1995) led to our hypothesis that a 5-HT_3 receptor antagonist ICS 205–930 will be an ideal adjunct for the pharmacotherapy of alcoholism.

The goal of the present study was to extend previous findings regarding 5-HT_3 and opioid receptor antagonists and to assess whether a 5-HT_3 receptor antagonist ICS 205–930 enhances naltrexone-induced suppression of ethanol consumption using a rat model.

2. Methods

2.1. Animals

Male, 60–90-day-old Sprague–Dawley rats (Harlan, Indianapolis, IN), weighing 300.6 ± 6.8 g, were group-housed in a temperature and humidity-controlled vivarium with lights on a 12-hour light/dark cycle (lights on at 6 AM) and were given ad libitum access to food and water during the first week after their arrival. After a week of habituation, they were single housed. Tests to determine ethanol and water intake were conducted between 12 and 3 PM. All the experiments were approved by the "Institutional animal care and use committee" of the University of Oklahoma Health Sciences Center.

2.2. Drugs

Ethanol solution was made up to 10% w/v from 95% ethanol. ICS 205–930 was purchased from Research Biochemicals, Natick, MA. Naltrexone (Sigma, St. Louis, MO) (0.56–10 mg/kg) and ICS 205–930 (0.001–10 mg/kg) were dissolved in saline and administered intraperitoneally in a ml/kg volume.

2.3. Procedure

After the 7-day habituation to the housing conditions, rats were trained to self-administer ethanol using Samson's sucrose-fading home-cage ethanol consumption procedure (Files et al., 1995). Initially, a home-cage two-bottle ethanol preference test was given to the rats. Following this, rats were deprived of water for 24 h and then 20% w/v sucrose solution was provided overnight. During the subsequent training and testing, food and water were provided ad lib. During the training procedure, sucrose concentration was gradually decreased, while ethanol concentration was increased. After the training, two bottle preference tests were conducted with 10% ethanol and water simultaneously. During the training and test periods, the amounts of ethanol solution and water consumed were recorded to the nearest 0.5 ml. Control measurement in an empty cage proved that the evaporation during a 3-h session was minimal. Positions of bottles were alternated daily throughout the study to control for any possible position preference. Rats, which preferred ethanol to water in the preference sessions (ratio of ethanol to water consumption greater than one), were selected for testing the effect of drugs on oral ethanol self-administration. Rats, which did not pass the criterion, were retrained, and were given a preference test every week.

2.4. Determination of the initial baseline ethanol and water intake

After the training to the criterion (i.e., baseline consumption of 10% ethanol greater than water in four consecutive sessions), initial baseline ethanol intake was determined. To determine the baseline intake, bottles of 10% ethanol solution were available for a restrictive period of 3 h between 12 and 3 PM during each day of the week. Water bottles were available for all the time except for 30 min before the ethanol access session. Saline was administered 30 min before ethanol access. Initial baseline intake for each rat was an average of the intake during these sessions. Initial baseline intake was compared with ethanol intake measured after finishing the test drug series.

2.5. Experiment 1: The effect of ICS 205–930 on voluntary ethanol intake in male Sprague–Dawley rats trained to drink ethanol

After determination of initial baseline ethanol intake, a group of rats ($n = 8$) was allocated to ICS 205–930 (0.001–10 mg/kg) treatment sessions. For the test-drug series, one-way repeated measures design was used. The antagonist was administered 30 min before ethanol access. Before the test sessions to determine the effect of ICS 205–930 on ethanol consumption, rats had saline-trials on three preceding consecutive days on which, saline was injected 30 min before ethanol access. Rats, having preference ratio for ethanol more than one in saline trials, were selected for test sessions in that week. Each of the doses of ICS 205–930 was tested in every rat during 10 separate test phases. ICS 205–930 was tested at 10 doses given in the following order: 0, 0.001, 0.01, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/kg body weight. After a washout period of 2 days, saline treatment preceded the next test phase in which a different dose of ICS 205–930 was used. The intake of 10% ethanol and water were recorded.

2.6. Experiment 2: The effect of naltrexone and a combination of ICS 205–930 and naltrexone on voluntary ethanol intake in male Sprague–Dawley rats trained to drink ethanol

The purpose of this experiment was to determine whether a combined antagonism of the 5-HT_3 and the opiate receptors is more effective in reducing ethanol intake in comparison to the antagonism of opioid receptors alone. After the determination of initial baseline ethanol intake, a group of rats ($n=12$) was allocated to naltrexone and combined naltrexone and ICS 205–930 treatment sessions. Rats sequentially received naltrexone (0.56 mg/kg) in combination with ICS 205–930 (0, 0.001, 0.32 and 5.6 mg/kg) 30 min before the test session with 10% ethanol and water as described under Experiment 1. After these series of tests, rats received naltrexone (1 mg/kg) in combination with ICS 205–930 (0, 0.001, 0.32 and 5.6 mg/kg) sequentially. In subsequent tests, rats received naltrexone at the dose of 1.8 and 10 mg/kg. We chose to use naltrexone at 0.56 and 1 mg/kg for combination treatment sessions, since these doses did not have any significant effect on food intake. The intake of 10% ethanol and water were recorded.

2.7. Experiment 3: The effect of ICS 205–930 and naltrexone on food intake in male Sprague–Dawley rats

The aim of the experiment 3 was to determine whether the antagonism of 5-HT_3 or opioid receptors results in a reduction of food intake. We examined the effects of drugs on 3- and 24-h food intake. A separate group of rats was randomly divided into two treatment groups ($n=6$ in each treatment group). These rats had continuous access to food and water throughout the experiment. Testing for food intake was conducted in individual home cages. The first group of rats received saline three times daily for 3 days to habituate them to the injection procedure and to obtain baseline values of food intake prior to testing with ICS 205–930. ICS 205–930 was administered i.p. at doses of 0.001, 0.32 and 5.6 mg/kg body weight. Each dose was administered three times daily (based on its half-life) and the weight of food consumed was measured 3 and 24 h after the first injection, to the accuracy of 0.1 g. Rats from Group 2 ($n=6$) received saline three times daily (beginning at 12 PM) for 3 days to habituate them to the injection procedure and to obtain baseline food intake. After the baseline sessions, naltrexone or the combination of naltrexone and ICS 205–930 was administered i.p. in the same sequence as in the experiment 2. Naltrexone was administered as a single dose per day (at 12 PM) because of its long half-life and ICS 205–930 was administered three times daily and the weight of food consumed was measured 24 h after the first injection, to the accuracy of 0.1 g. After every drug treatment, there was a washout period of 2 days.

2.8. Blood ethanol levels

The purpose of this analysis was to determine whether the changes in ethanol consumption are contributed by the effects of the drugs on the pharmacokinetics of ethanol. For this analysis, rats (from the experiments 1 and 2) were randomly divided into two groups ($n=10$). Group 1 received saline (i.p.) on the day 1 and ICS 205–930 (5.6 mg/kg, i.p.) on day 2. Rats from Group 2 received saline on the day 1 and naltrexone (1.0 mg/kg) on the day 2. Ethanol (0.75 g/kg) was administered 30 min after drug or saline injection to rats from both the groups. Blood samples (20 μl) were drawn from the brachial vein using a capillary, 15 min following ethanol administration and were analyzed for blood ethanol levels using a gas chromatographic head-space sampling technique. After collection, blood was immediately deposited in a vial with 1-ml of 0.02% v/v solution of 1-propanol in distilled water (internal standard). For blood ethanol analysis, blood aliquots were incubated for 20 min at 70 °C in a shaking water bath. One-milliliter 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 (Model number 5890A) equipped with a hydrogen-fueled flame ionization detector. An integrator calculated the area under the curve of all detectable peaks. The area of ethanol peak was expressed as a percentage of the internal standard peak. Blood ethanol concentrations were assessed using a seven-point linear regression analysis from ethanol standards.

2.9. Data analysis

Alcohol (g/kg) and water intake (ml/kg) were calculated for each test session. All the values were represented as the mean ± S.E.M. Repeated measures analysis of variance was performed followed by Tukey–Kramer multiple comparisons test. A probability below 0.05 was considered significant. Comparisons of the food intake after naltrexone or ICS 205–930 treatment with the food intake after saline treatment were made using a one-way analysis of variance.

3. Results

3.1. Experiment 1. 5-HT_3 antagonist ICS 205–930 alters voluntary ethanol intake in male Sprague–Dawley rats trained to drink ethanol

Fig. 1A and B represents the ethanol and water intake, respectively, after treatment with ICS 205–930. The mean baseline ethanol intake was 2.08 g/kg. There were no significant differences between ethanol intake of rats during the initial baseline session and the final baseline session. ICS 205–930, at the dose of 5.6 mg/kg, significantly

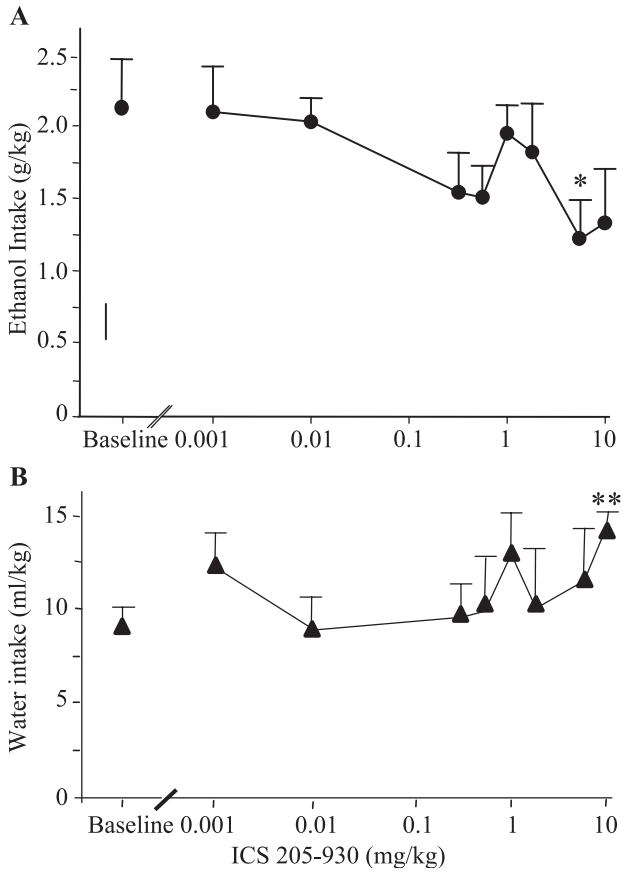

Fig. 1. Effect of ICS 205–930 (0.001–10 mg/kg, i.p.) on (A) ethanol and (B) water intake in a 3-h scheduled access period in male Sprague–Dawley rats ($n=8$). Saline or the drug was administered 30 min before the access to ethanol. Each baseline value is the average obtained from five consecutive baseline sessions. Doses were administered in the order depicted. Data represent the mean ± S.E.M. ICS 205–930 (5.6 mg/kg) significantly decreased ethanol intake (*$P<0.05$ and **$P<0.01$ using Tukey–Kramer multiple comparisons test).

decreased ethanol intake (Fig. 1A) [$F(7,42)=3.438$, using Tukey–Kramer multiple comparisons test $P<0.05$]. There was an increase in water intake after treatment with ICS 205–930 at the dose of 10 mg/kg ($P<0.01$).

3.2. Experiment 2. Naltrexone suppresses voluntary ethanol intake in male Sprague–Dawley rats trained to drink ethanol

Fig. 2A reveals that naltrexone (0.56, 1, 1.8 and 10 mg/kg) is effective in attenuating ethanol drinking in a three-hour ethanol-access period [$F(11,88)=1.94$, using Tukey–Kramer multiple comparisons as a post hoc test, $P<0.001$] at all the doses tested. Naltrexone was most efficacious at 1.8 mg/kg. Fig. 2B represents the average water intake (ml/kg) in baseline sessions and the water intake after naltrexone treatment. There was a trend of a compensatory increase in water intake at all the doses tested, which was statistically nonsignificant.

3.3. Naltrexone and ICS 205–930 in combination suppress voluntary ethanol intake in male Sprague–Dawley rats

Fig. 3A reveals that naltrexone (0.56 mg/kg) in combination with ICS 205–930 (0.001, 0.32 and 5.6 mg/kg) significantly suppressed ethanol consumption [$F(11, 88)=1.94$, Tukey–Kramer multiple comparisons as a post hoc test ($P<0.001$) using a two-factor-ANOVA between drug treatments]. The combination of naltrexone (0.56 mg/kg) with ICS 205–930 (5.6 mg/kg) showed a trend of increased suppression (37% more) in ethanol intake than naltrexone alone. There was a trend of a compensatory increase in water intake after treatment with naltrexone (0.56 mg/kg) and ICS 205–930 (0.001, 0.32, 5.6 mg/kg), whereas there

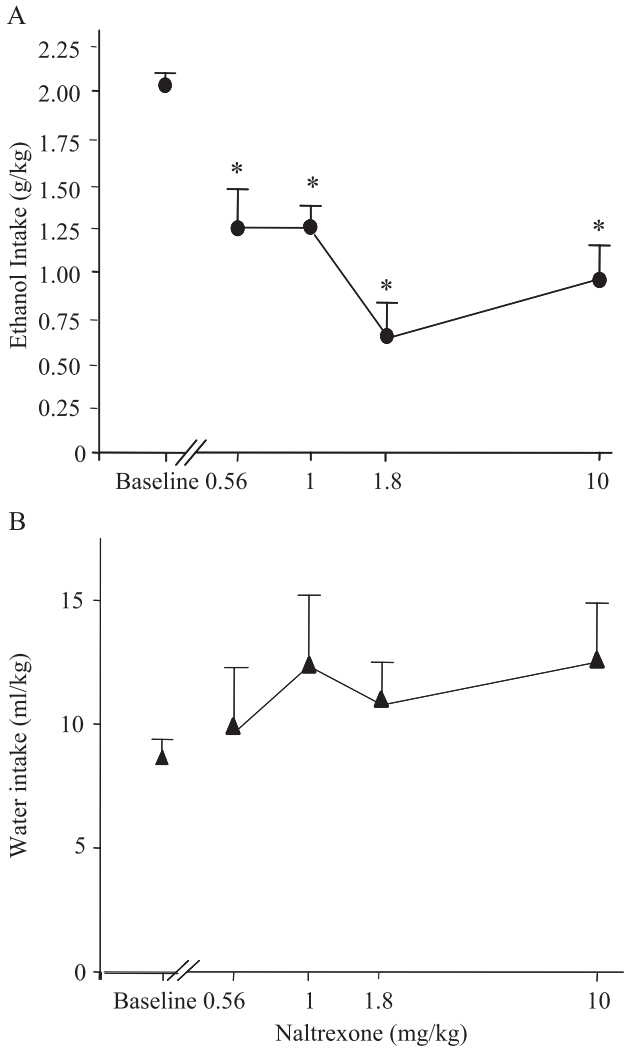

Fig. 2. Effect of naltrexone (0.56–10 mg/kg, i.p.) on (A) ethanol intake and (B) water intake in a 3-h scheduled access period in male Sprague–Dawley rats ($n=12$). Saline or the drug was administered 30 min before the access to ethanol. Each baseline value is the average obtained from five consecutive baseline sessions. Doses of naltrexone were administered in the order depicted. Data represent the means ± S.E.M. Naltrexone at all the doses administered significantly decreased ethanol intake (*$P<0.001$ using Tukey–Kramer multiple comparisons test).

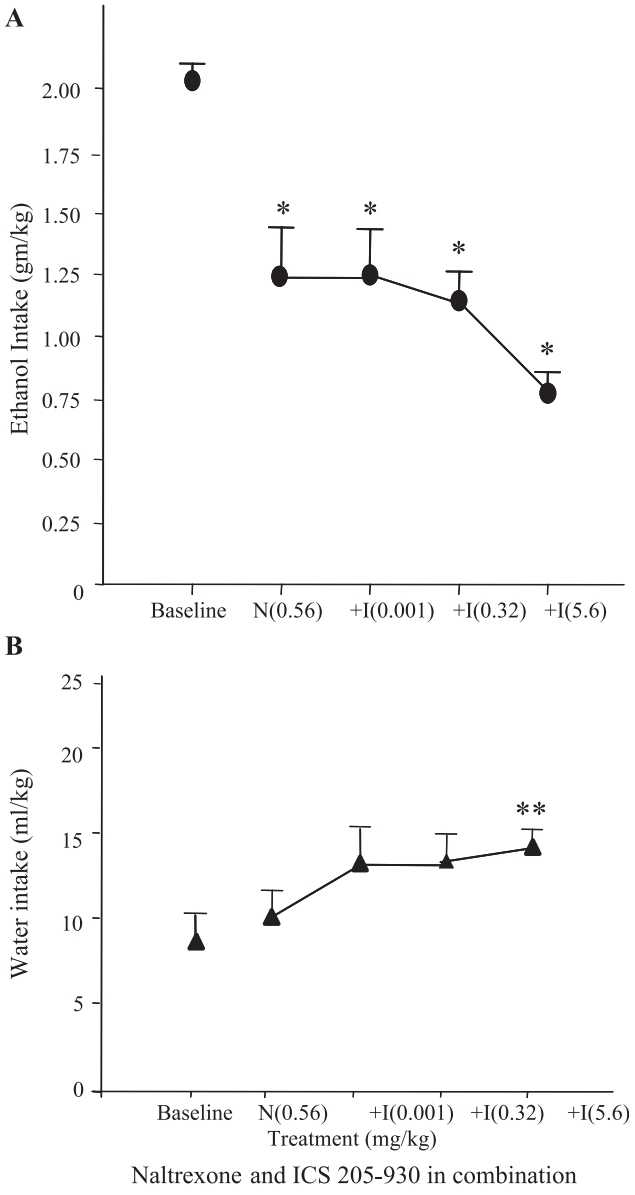

Fig. 3. Effect of naltrexone (N) (0.56 mg/kg, i.p.) in combination with ICS 205–930 (I) on (A) ethanol and (B) water intake of male Sprague–Dawley rats ($n=12$) in a 3-h scheduled access period. Treatment was as follows: (1) naltrexone (0.56 mg/kg) + vehicle, (2) naltrexone (0.56 mg/kg) + ICS 205–930 (0.001 mg/kg), (3) naltrexone (0.56 mg/kg) + ICS 205–930 (0.32 mg/kg), (4) naltrexone (0.56 mg/kg) + ICS 205–930 (5.6 mg/kg). Each baseline value is the average obtained from five consecutive baseline sessions. Saline or the drugs were administered 30 min before ethanol access. Drugs were administered in the order depicted. Data represent the mean ± S.E.M. Combined administration of naltrexone with ICS 205–930 significantly decreased ethanol intake (*$P<0.001$, **$P<0.02$ using Tukey–Kramer multiple comparisons test).

was a significant increase in water intake following treatment with naltrexone (0.56 mg/kg) and ICS 205–930 (Tukey–Kramer multiple comparisons as a post hoc test, $P<0.02$ at 5.6 mg/kg in comparison to the baseline intake).

Fig. 4A and B represents ethanol and water intake, respectively, of male Sprague–Dawley rats after treatment with naltrexone (1 mg/kg) and ICS 205–930 (0, 0.001, 0.32, 5.6 mg/kg). Naltrexone (1 mg/kg) in combination with ICS 205–930 (0.001, 0.32 or 5.6 mg/kg) significantly suppressed ethanol consumption ($P<0.001$). The combination of naltrexone (1 mg/kg) and ICS 205–930 (5.6 mg/kg)

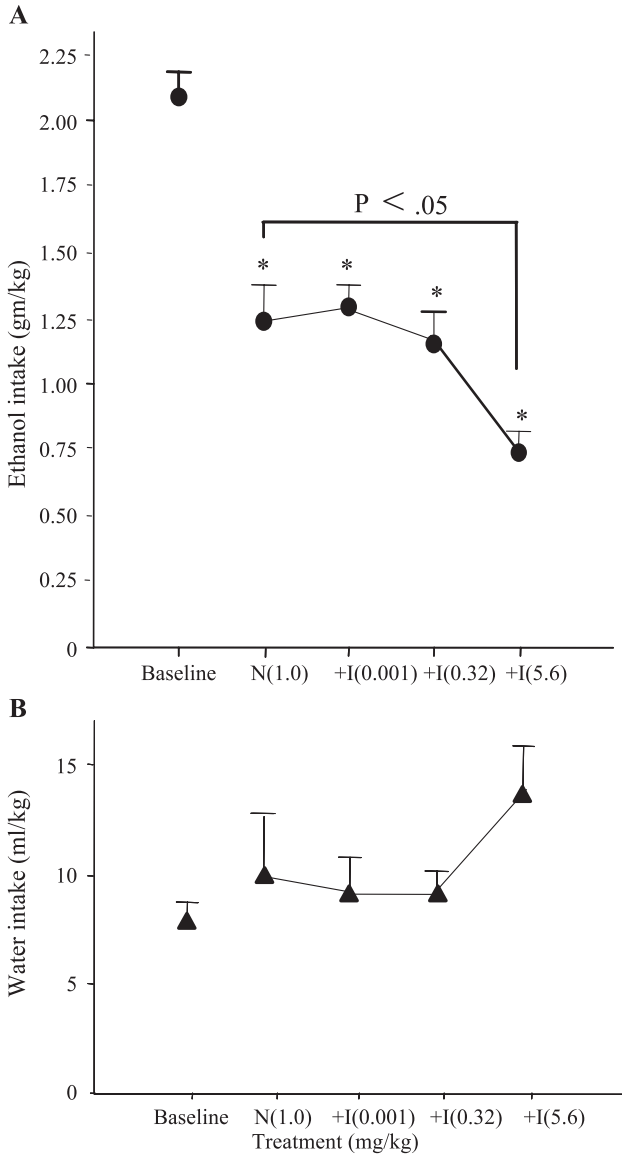

Fig. 4. Effect of naltrexone (N) (1 mg/kg, i.p.) in combination with ICS 205–930 (I) on (A) ethanol and (B) water intake in male Sprague–Dawley rats ($n=12$) over a 3-h scheduled access period. (1) Naltrexone (1 mg/kg) + saline, (2) naltrexone (1 mg/kg) + ICS 205–930 (0.001 mg/kg), (3) naltrexone (1 mg/kg) + ICS 205–930 (0.32 mg/kg), (4) naltrexone (1 mg/kg) + ICS 205–930 (5.6 mg/kg). Saline or the drugs were administered 30 min before ethanol access. Each baseline value is the average obtained from five consecutive baseline sessions. Doses were administered in the order depicted. Data represent the mean ± S.E.M. Naltrexone (1 mg/kg) in combination with ICS 205–930 significantly decreased ethanol intake (*$P<0.001$). The drug combination of naltrexone (1 mg/kg) and ICS 205–930 (5.6 mg/kg) produced significantly higher suppression in ethanol intake than naltrexone alone ($P<0.05$ using Tukey–Kramer multiple comparisons test).

was significantly more efficacious than naltrexone (1 mg/kg) alone ($P < 0.05$). It produced 38% more suppression in ethanol intake than naltrexone alone.

Three combinations producing the lowest mean intake were (1) naltrexone (1.8 mg/kg), (2) the combination of naltrexone (0.56 mg/kg) and ICS 205–930 (5.6 mg/kg), and (3) the combination of naltrexone (1 mg/kg) and ICS 205–930 (5.6 mg/kg).

3.4. Experiment 3. Effect of ICS 205–930 and naltrexone on food intake in male Sprague–Dawley rats

There was a significant variation in the baseline values of 3-h food intake. Therefore, we examined the effect of drugs on 24-h food intake. ICS 205–930 (0.001, 0.32 and 5.6 mg/kg, three times a day) did not have any significant effect on food intake (Table 1), however, naltrexone, at the dose of 1.8 mg/kg, significantly suppressed food intake in Sprague–Dawley rats ($P < 0.01$) (Table 1). Naltrexone (0.56 or 1 mg/kg) in combination with ICS 205–930 (0.001, 0.32 or 5.6 mg/kg) did not have any significant effect on food intake ($P = 0.1$, n.s.), data not shown.

3.5. Blood alcohol concentration

Blood alcohol concentration during baseline sessions ranged between 43 and 99 mg/dl. There were no significant differences in blood ethanol levels following naltrexone or ICS 205–930 treatment. Blood ethanol levels for Group 1 were as follows: Day 1 [saline and ethanol (0.75 g/kg) treatment] – 60.8 ± 7.0 mg/dl, Day 2 [ICS 205–930 (5.6 mg/kg) and ethanol (0.75 g/kg) treatment] – 62.9 ± 6.2 mg/dl. Blood ethanol levels for Group 2 were as follows: Day 1 [saline and ethanol (0.75 g/kg) treatment] – 60.8 ± 3.7 mg/dl, Day 2 [naltrexone (1 mg/kg) and ethanol (0.75 g/kg) treatment] – 66.6 ± 3.9 mg/dl.

Table 1
Effect of naltrexone or a combination of naltrexone and ICS 205–930 on 24-h food intake

Drug	Food intake (g/kg)
Saline	22.8 ± 0.5
Naltrexone (0.56 mg/kg)	22.5 ± 0.7
Naltrexone (0.56 mg/kg) + ICS 205–930 (0.001 mg/kg)	21.9 ± 1.4
Naltrexone (0.56 mg/kg) + ICS 205–930 (0.32 mg/kg)	19.9 ± 0.7
Naltrexone (0.56 mg/kg) + ICS 205–930 (5.6 mg/kg)	19.7 ± 1.1
Naltrexone (1.0 mg/kg)	19.7 ± 1.1
Naltrexone (1.0 mg/kg) + ICS 205–930 (0.001 mg/kg)	21.9 ± 0.9
Naltrexone (1.0 mg/kg) + ICS 205–930 (0.32 mg/kg)	21.2 ± 0.4
Naltrexone (1.0 mg/kg) + ICS 205–930 (5.6 mg/kg)	21.2 ± 0.6
Naltrexone (1.8 mg/kg)	19.0 ± 0.4[a]

Naltrexone was administered as a single dose per day (at 12 PM) because of its long half-life and ICS 205–930 was administered three times daily and the weight of food consumed was measured 24 h after the first injection. Values are given as the means for 6 animals ± S.E.

[a] $P < 0.01$ by the Newman–Keul's test.

4. Discussion

The observations from the present study confirm previous findings regarding the effects of naltrexone on ethanol intake in rats. This study also demonstrates that the 5-HT_3 receptor antagonist ICS 205–930 reduces ethanol intake in a limited access paradigm in a rodent model of ethanol consumption with no genetic predisposition to alcohol drinking behavior. Our observations support the findings of Tomkins et al. (1995), where they observed a significant reduction in ethanol consumption by the 5-HT_3 receptor antagonist ondansetron in Wistar rats in a 1-h limited access paradigm. These findings, consistent with previous reports, suggest the generality of the effects of 5-HT_3 receptor antagonists on ethanol intake across different animal models of ethanol consumption.

Some researchers have suggested that 5-HT_3 receptor antagonists are more efficacious in suppressing ethanol consumption under a continuous ethanol-access condition than in a limited ethanol access paradigm (Knapp and Pohorecky, 1992; McKinzie et al., 1998). McKinzie et al. (1998) observed that ICS 205–930 is not effective in reducing ethanol intake at the doses of 0.1–3.0 mg/kg in a limited access paradigm. These workers also reported that subcutaneous administration of ICS 205–930 at the doses of 3 and 5 mg/kg (at 9 AM, 1 PM, and 5 PM) significantly reduces (28–36% of total ethanol intake) 24-h ethanol intake in the same rat model. In our study also, high doses of ICS 205–930 (5.6 and 10 mg/kg) were more effective in suppressing ethanol consumption (36% to 38% of baseline ethanol intake) compared to low doses (0.32 and 0.56 mg/kg) (27% to 29% of baseline ethanol intake). ICS 205–930 is reported to have a slow elimination rate (Huang et al., 1999). Therefore, the information on the bioavailability of this compound in rats, which received ICS 205–930 every 4 h, would have been helpful to understand higher efficacy of this drug in a continuous ethanol access paradigm in McKinzie et al.'s study (1998).

It is possible that other serotonin receptors are involved in the effects of high doses of ICS 205–930 (>1 mg/kg) on ethanol consumption as previously suggested by Hodge et al. (1995). Low doses of ICS 205–930 are highly selective at 5-HT_3 receptors, but high doses of ICS 205–930 are also antagonistic at 5-HT_4 receptors (Arranz et al., 1998; Roila et al., 1997). In the study done by Costall and Naylor (1993), ICS 205–930 was anxiolytic at low doses (< 1 mg/kg) but lost its anxiolytic characteristics at a higher dose (>1 mg/kg). This property of ICS 205–930 at a high dose resembles that of 5-HT_4 receptor antagonists, which are not anxiolytic

(Costall and Naylor, 1993). Therefore, we speculate that high doses of ICS 205–930 (5.6 and 10 mg/kg) may have suppressed ethanol consumption by antagonism of 5-HT_4 receptors. Consistent with this speculation, the 5-HT_4 receptor antagonist GR113808 has been reported to reduce ethanol consumption in alcohol-preferring rats (Panocka et al., 1995). Iusco et al. (1997) have reported another intriguing observation where new synthetic 5-HT_3 antagonists that lack 5-HT_4 antagonistic activity inhibit alcohol-induced dopamine release but do not affect ethanol intake, which may explain low efficacy of low doses of ICS 205–930 (< 1 mg/kg) in reducing ethanol intake.

Naltrexone at all the doses tested significantly reduced ethanol consumption. Although, naltrexone is a nonselective antagonist at opioid receptors, it has moderately higher affinity for μ than other opioid receptors (Corbett et al., 1993). At low doses, naltrexone shows greater selectivity for μ receptors than for δ, whereas at high doses (10 mg/kg), this selectivity is lost with an increase in the antagonist potency at δ receptors (Corbett et al., 1993). Naltrexone at 10 mg/kg did not produce greater suppression in comparison to lower doses suggesting that the suppression in ethanol consumption by naltrexone may be achieved mostly through its blockade of μ receptors. The suppression of ethanol consumption by the μ_1-opioid receptor antagonist naloxonazine (Mhatre and Holloway, 2003) and by low doses of naltrexone (0.56 and 1 mg/kg) suggests that μ-opioid receptors mediate some of the reinforcing effects of ethanol. However, the involvement of delta or kappa receptors cannot be ruled out in the effects of naltrexone observed in this study. Naltrexone at the dose of 1.8 mg/kg produced higher suppression of ethanol intake in comparison to lower doses of the drug. However, this dose of naltrexone also suppressed food intake.

Naltrexone in combination with ICS 205–930 significantly suppressed ethanol intake without suppressing water intake. Concomitant treatment with ICS 205–930 (5.6 mg/kg) significantly enhanced naltrexone's (at the dose of 1 mg/kg) efficacy in reducing ethanol intake. These observations suggest a functional interaction between 5-$HT_{3/4}$ and opioid receptors (Hodge et al., 1995). It is also possible that ICS 205–930 produces aversion for ethanol. E.g. an enhancement of ethanol's intoxicating and aversive effects by a high dose of ondansetron (8 mg/kg) was observed in human volunteers (Swift et al., 1996). This increase in naltrexone-induced suppression of ethanol self-administration by ICS 205–930 is interesting and could be clinically important.

Previously, naltrexone treatment was found to be efficacious in lowering the relapse rate and increasing abstinence in alcohol dependent individuals (O'Malley et al., 1992; Volpicelli et al., 1992). However, the success of naltrexone treatment has been limited due to poor compliance and inter-individual variability in response. In the present study, 44% of rats did not exhibit a significant response (>25% suppression in ethanol intake) to naltrexone (0.56 and 1 mg/kg) treatment, whereas all 12 rats responded to the combination of naltrexone with ICS 205–930 (5.6 mg/kg). In addition, the combination therapy was almost as effective as a higher dose of naltrexone by itself, which also reduced food intake. These results support the hypothesis that due to the involvement of multiple neurotransmitter systems in the mechanism underlying excessive alcohol-drinking behavior, the combination of drugs targeting more than one neurotransmitter systems may make more effective pharmacotherapy for alcoholism with improved compliance.

In contrast to other 5-HT manipulating drugs such as serotonin reuptake inhibitors, which reduce ethanol as well as food intake (Naranjo et al., 1990), ICS 205–930 did not affect food intake, at the doses tested. This is consistent with previous findings, which revealed that treatment with 5-HT_3 receptor antagonist ondansetron (0.01–1.0 mg/kg) did not affect food consumption (Higgins et al., 1992). These observations support the proposed specificity of ICS 205–930 in attenuating ethanol consumption as opposed to a general effect on the consummatory behavior. Suppression of food intake by naltrexone (1.8 mg/kg) is consistent with previous reports showing a significant reduction in food intake in various animal models and a reduction in appetite and the body weight after naltrexone treatment in a clinical study (De Zwaan and Mitchell, 1992). Antagonism of the kappa receptor subtype is suggested to be involved in naltrexone-induced reduction in appetite and suppression of the food intake (Cooper et al., 1985). However, our observations of a reduction in food intake after treatment with the μ_1-opioid receptor antagonist naloxonazine suggest that the μ-opioid receptor subtype may also be involved in naltrexone-induced suppression of food intake (Mhatre and Holloway, 2003).

Although 5-HT_3 receptor antagonist-induced reduction in alcohol intake is suggested to be mediated through a central mechanism as described above, it is possible that such a reduction could also be mediated by an alteration in the absorption or distribution of alcohol. 5-HT_3 receptors are reported to play a functional role in the control of gut motility (Buchheit et al., 1985). However, we did not find any significant effect of ICS 205–930 and naltrexone on ethanol's pharmacokinetics, which agrees with previous reports (Grant and Barrett, 1991; Mhatre et al., 2001).

In summary, the results from the present study suggest that a 5-HT_3 receptor antagonist ICS 205–930 and an opioid receptor antagonist naltrexone are effective in suppressing ethanol intake in a limited access paradigm in a rat model with no biological predisposition to alcohol drinking behavior. ICS 205–930 at 5.6 mg/kg suppresses ethanol consumption. It shows specificity in attenuating ethanol consumption. And finally, ICS 205–930 (5.6 mg/kg) significantly enhances naltrexone's efficacy in suppressing ethanol consumption in a limited access paradigm.

Acknowledgements

This research was supported by NINDS NS10601-C1 and OCAST Contract HR99-12 to MM, OCAST Contract H97-112 to FAH and MM and NIDA TG 5T32DA07248 to FAH.

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