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A 5-HT$_{1A}$ agonist and a 5-HT$_{2c}$ antagonist reduce social interaction deficit induced by multiple ethanol withdrawals in rats

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Abstract

Rationale—Repeated withdrawals from chronic forced ethanol exposure sensitize animals to withdrawal-induced deficits in social interaction behavior. The deficits in social interaction behavior following withdrawal from continuous ethanol exposure can be reduced following acute treatments with 5-HT$_{2C}$ antagonists or 5-HT$_{1A}$ agonists.

Objectives—The present study investigated whether prior treatment with these serotonergic agents during early withdrawals in rats subjected to repeated withdrawals from ethanol exposure would ameliorate the social interaction deficits observed following the final withdrawal.

Methods—Sprague-Dawley rats were exposed to three cycles of 5 days forced ethanol (7%, w/v), with 2 days of control diet after the first and second cycles. Drugs were administered IP 4 h after removal of ethanol on the first and second cycles but not the third in one group and 4.5 h after removal of ethanol on the third cycle in another. The social interaction test was performed 5 h after removal of ethanol on the third cycle. Drugs tested included SB-242084, a 5-HT$_{2C}$ antagonist; buspirone, a 5-HT$_{1A}$ partial agonist; WAY-100635, a 5-HT$_{1A}$ antagonist; ketanserin, a 5-HT$_{2A}$ antagonist; ritanserin, a mixed 5-HT$_{2A/2C}$ antagonist; and Ro-601075, a 5-HT$_{2C}$ agonist.

Results—Both SB-242084 and buspirone reduced ethanol withdrawal-induced deficits in social interaction when given either acutely 30 min before the test or at 4 h after withdrawal from the first and second cycles. WAY-100635 and ketanserin were completely ineffective regardless of mode of treatment. In contrast, the 5-HT$_{2C}$ agonist, Ro-601075, accentuated the withdrawal-induced deficit in social interaction behavior in rats exposed to either 4.5 or 7% ethanol diet.

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Conclusions—These results support the utility of 5-HT_{1A} agonists and 5-HT_{2C} antagonists in reducing anxiety-like behavior induced by ethanol withdrawal and reducing the adaptive changes associated with repeated withdrawals.

Keywords

Anxiety-like behavior; Forced ethanol exposure; Repeated ethanol withdrawals; Social interaction test; 5-HT_{1A}; 5-HT_{2C}; 5-HT_{2A} receptors

Introduction

Animals exposed to forced ethanol in repeated cycles show sensitization of withdrawal-induced anxiety-like behavior (Overstreet et al. 2002). In particular, rats exposed to a low concentration of ethanol in three cycles of 5 days exhibit reduced social interaction behavior following the third cycle, whereas rats exposed to the same amount of ethanol continuously do not (Overstreet et al. 2002). This sensitization of anxiety-like behavior provides support for the postulate of Ballenger and Post (1973) that repeated withdrawals from chronic ethanol exposure contribute to a worsening of symptoms and seizures during a lifetime of alcoholism (see also McCown and Breese 1990).

There is increasing evidence for the involvement of serotonergic mechanisms in anxiety associated with ethanol and diazepam withdrawal (Andrews and File 1993; File et al. 1993). Moreover, several studies have reported changes in serotonin (5-HT) receptor subtypes in rats following chronic treatment with alcohol (e.g. Ulrichsen 1991; Pandey et al. 1992; Overstreet et al. 1994; Pandey and Pandey 1996). It has also been suggested that long-term excessive alcohol consumption is toxic to serotonergic neurons because the low prolactin response to fenfluramine, a serotonin (5-HT) releaser, is correlated with the amount of alcohol consumption (Berggren et al. 2002; Soloff et al. 2000).

It has been previously determined that buspirone, a 5-HT_{1A} receptor partial agonist, will reduce the anxiety-like symptoms associated with withdrawal from ethanol (Dougherty and Gates 1990; Lal et al. 1991; File et al. 1993), but its success as a treatment in alcoholics has been mixed (Bruno 1989; Kranzler and Meyer 1992; Malcolm et al. 1992; Tollefson et al. 1992; Malec et al. 1996; Fawcett et al. 1999; George et al. 1999). Recently, a selective 5-HT_{2C} receptor antagonist, but not 5-HT_{1A} or 5-HT_3 receptor antagonists, blocked the reduction in social interaction following withdrawal from chronic ethanol exposure (Knapp et al. 2000). Thus, both the stimulation of 5-HT_{1A} receptors and the blockade of 5-HT_{2C} receptors can ameliorate anxiety symptoms associated with ethanol withdrawal.

The present communication provides novel support for the involvement of 5-HT_{1A} and 5-HT_{2C} receptors in the social interaction deficit displayed by rats following withdrawal from forced exposure to ethanol by employing a multiple withdrawal protocol (Overstreet et al. 2002). Given that 5-HT_{1A} agonists and 5-HT_{2C} antagonists are able to reduce anxiety-like behavior related to chronic ethanol exposure when given acutely before the behavioral tasks, we tested whether these compounds would also be effective in counteracting the reduced social interaction if given during the withdrawal periods after the first and second cycles of a three-cycle multiple withdrawal protocol. A preliminary report of some of these results has been given (Knapp et al. 2001).

Materials and methods

Animals

Sprague-Dawley rats (Charles-River, Raleigh, N.C., USA) were obtained at 160–180 g. The animal rooms were maintained at 22°C and 40% humidity with a 12:12 light:dark cycle (lights on from 0900 to 2100 hours). After adaptation to the laboratory for 5 days, they were individually housed and placed on a nutritionally complete lactalbumin-dextrose diet for 3 days (Frye et al. 1983; Moy et al. 1997). The experimental rats were exposed to a diet containing ethanol (ED; 7%, w/v), while control rats remained on the control diet in which the dextrose replaced the ethanol isocalorically. The control diet rats were pair fed based on the average volume of ED consumed.

Drugs

Buspirone hydrochloride (RBI-Sigma, St Louis, Mo., USA) was the 5-HT_{1A} partial agonist (Stahl 1997) used in this study. The acute dose of 0.2 mg/kg and pretreatment dose of 0.6 mg/kg were selected from previously published reports (Lal et al. 1991; File et al. 1993). SB-242084 (gift of Glaxo-Smith-Kline, Harrow, United Kingdom), the 5-HT_{2C} antagonist (Kennett et al. 1997), and WAY-100635 (RBI-Sigma), a 5-HT_{1A} antagonist (Forster et al. 1995), were given acutely at a dose of 1 mg/kg or at a pretreatment dose of 3 mg/kg; doses were based on recently published studies (Bristow et al. 2000; Bagdy et al. 2001). In a subsequent dose-response study rats were pretreated with 0.3, 1.0 and 3.0 mg/kg doses of SB-242084. Ketanserin (RBI-Sigma), a 5-HT_{2A} antagonist (Boess and Martin 1994; Bonhaus et al. 1997), and ritanserin (RBI-Sigma), a mixed 5-$HT_{2A/2C}$ antagonist (Boess and Martin 1994; Bonhaus et al. 1997), were given acutely at a dose of 1 mg/kg and at a pretreatment dose of 3 mg/kg to correspond with the doses of SB-242084. Ketanserin also has some affinity for the 5-HT_{2C} receptor, but it is much less than that of ritanserin (Boess and Martin 1994; Bonhaus et al. 1997). The 5-HT_{2C} receptor agonist Ro-601075 (gift of Roche, Basel, Switzerland), highly selective for the 5-HT_{2C} receptor (Kennett et al. 2000), was given at a pretreatment dose of 0.3 mg/kg. Buspirone was dissolved in isotonic saline vehicle, while the other drugs were suspended in 0.5% carboxymethylcellulose (CMC).

Protocol

The experiments described in this section conformed to the Laboratory Guide for the Care of Animals and were approved by the UNC Institutional Animal Care and Use Committee.

The study consisted of five experiments that tested the efficacy of drugs that interact with 5-HT receptor subtypes on social interaction deficits induced by ethanol withdrawal, a sixth experiment on whether the 5-HT_{2C} agonist would potentiate withdrawal-induced deficits in social interaction, and a seventh experiment to determine whether the drugs and treatment protocols influenced the social interaction behavior of rats maintained on control diet. Each of the first five experiments consisted of at least eight rats maintained on control diet and 32 rats maintained on ethanol diet (ED) for a total of 15 days. Those rats on ED were withdrawn from ethanol and placed on control diet for 2 days after the fifth and tenth exposures to ethanol. Rats maintained on ED were injected with the drugs of interest (see above) either acutely 30 min before the social interaction test at 4.5 h after removal of the 15th ethanol exposure, or pretreated at 4 h after removal of the fifth and tenth ethanol exposures. All the rats that did not receive an active drug at 4.5 h after removal of the 15th ethanol exposure were injected with the vehicle, CMC (0.5%). At approximately 30 min after injection, rats (matched for weight and experimental treatment) were placed as pairs in the social interaction test for a 5-min session.

The design was altered to determine if the 5-HT_{2C} receptor agonist would exacerbate the effects of ethanol withdrawal on social behavior. Three groups (n=8) were maintained on control diet for 13 days, while two groups (n=8) were maintained continuously on 4.5% ethanol diet (ED4.5). One group from each of these two dietary exposures was given 0.3 mg/kg Ro-601075 IP on days 8 and 13 (days 5 and 10 of exposure to ethanol) of exposure to the diet. Immediately after day 13 on the diet, two groups of control diet rats were exposed to 7% ethanol for 5 days, a treatment that does not produce a deficit in social interaction behavior (Overstreet et al. 2002). After day 18 of exposure to the diet (5 or 15 days of exposure to ethanol), ethanol was removed from the diet and the social interaction test was carried out between 5 and 6 h later. This 5-h time point was selected on the basis of extensive previous experience (e.g. Knapp et al. 1998; Moy et al. 2000; Overstreet et al. 2002). A previous study found a similar time course of withdrawal-induced anxiety in rats repeatedly withdrawn or withdrawn only once (Overstreet et al. 2002).

In the seventh experiment, rats were maintained on control diet throughout. After at least 7 days on the diet, the rats were subjected to either a pretreatment protocol or an acute protocol. In the pretreatment protocol, rats were injected with the test agent twice 5 and 10 days before being tested in the social interaction test. In the acute protocol the rats were tested in the social interaction test 30 min after the administration of the test compound.

Social interaction test

The social interaction test was used in these studies because it can detect the anxiolytic and anxiogenic effects of serotonergic agents (e.g. Gonzalez et al. 1998; Bristow et al. 2000; Bagdy et al. 2001). In the present studies, pairs of rats with the same treatment were placed in the arena and the social interactions initiated by each member of the pair were recorded during the 5-min session. The sums of the individual scores were analyzed, as recommended by File (1980).

The social interaction test was carried out in a square open field (60×60 cm) with 16 squares marked out on the floor. The experienced observers were blind to the treatments given the rats. To generate an intermediate level of social interaction behavior, the rats were unfamiliar with the open field and the lighting conditions were low. Alcohol intakes, body weights, and treatment conditions were used to match up the rat pairs. During the 5-min session, line crosses (by two forepaws) and time spent in social interaction (grooming, sniffing, following, crawling over or under) were scored individually for each rat (Kampov-Polevoy et al. 2000; Overstreet et al. 2002).

Data analysis

Statistical analyses were carried out using the GBStat software package. The data were initially analyzed by one-way ANOVA. If the main effects were statistically significant, post hoc analyses were performed using Tukey's protected t-tests. Analyses were conducted separately for time spent in social interaction behavior and number of line crosses. Because of some concern about the most appropriate method of analysis (see Overstreet et al. 2002), the time spent in social interaction was analyzed both for individual animals and for pairs. Although the result of both analyses will be presented, the graphs and tables will be based on the scores of the rat pairs.

Results

5-HT_{2A} and 5-$HT_{2A/2C}$ antagonists have mixed effects on multiple withdrawal induced anxiety

As illustrated in Fig. 1, animals that were repeatedly withdrawn from chronic ethanol and treated with vehicle showed a marked decrease in time spent in social interaction [F(5,18)

=21.74, $P<0.0001$]. Groups with different letter superscripts are significantly different, according to Tukey's protected *t*-test. Only ritanserin, a mixed 5-$HT_{2A/2C}$ receptor antagonist, significantly attenuated this deficit in social interaction, but only when given during the first and second withdrawal periods (the pretreatment regimen; Fig. 1, upper panel). When the data on individual rats were analyzed, a significant ANOVA was also obtained [$F(5,42)=34.21$, $P<0.0001$].

Although there were significant group differences for line crossings [$F(5,18)=9.92$, $P<0.0001$ for pairs and $F(5,42)= 6.61$, $P<0.001$ for individual rats], none of the drug treatments counteracted the reduced line crossings of the ethanol-withdrawn rats. Indeed, both ritanserin and ketanserin reduced activity even further when given acutely during the final withdrawal (Fig. 1, lower panel).

The acute effects of ketanserin and ritanserin on social interaction behavior in rats maintained on control diet, summarized in Table 1, provide an explanation for their lack of counteraction of the deficit induced by ethanol withdrawal. Both agents had suppressing effects on time spent in social interaction and ketanserin also suppressed line crossings (Table 1). Because ketanserin was not effective in counteracting the deficit in social interaction in the pretreatment protocol, it was not tested in rats maintained on control diet. Ritanserin had no effect on social interaction behavior when given to control rats 5 and 10 days prior to the social interaction test (58.5±7.1 s for four pairs compared to 54.5±4.3 s for four pairs of rats given vehicle, $P>0.05$).

A 5-HT_{2C} antagonist counteracts multiple withdrawal induced anxiety

The fact that ritanserin has high affinity for both 5-HT_{2A} and 5-HT_{2C} receptors (Boess and Martin 1994) suggested that a compound more selective for the 5-HT_{2C} receptor might be effective in counteracting anxiety when given during prior withdrawals. Indeed, when the 5-HT_{2C} antagonist, SB-242084, was administered either 4.5 h after the final withdrawal or 4 h into the first and second withdrawals of the multiple withdrawal protocol, it significantly increased time spent in social interaction [$F(3,16)=24.05$, $P<0.0001$; Fig. 2, upper panel]. Analysis of the individual data also revealed a highly significant ANOVA [$F(3,30)=14.18$, $P<0.0001$]. Groups with different letter superscripts are significantly different, according to Tukey's protected *t*-test.

Even though SB-242084 reduced the withdrawal-induced deficit in social interaction, this 5-HT_{2C} antagonist did not significantly increase the reduced line crossings, with all groups exposed to ED being significantly less active than rats exposed to control diet [$F(3,12)=20.07$, $P<0.0001$ for pairs and $F(3,30)=14.37$, $P<0.001$ for individual rats; Fig. 2, lower panel]. Therefore, the 5-HT_{2C} receptor antagonist had a selective effect on time spent in social interaction.

Two pretreatments with SB-242084 5 and 10 days before the test did not alter social interaction behavior in rats maintained continuously on control diet or ethanol diet (58±11 s for control, 56±9.4 s for control diet given SB 24084; 60±2.4 s for ethanol diet given SB-242084; $P>0.05$). Neither did acute treatment with SB-242084 significantly alter time spent in social interaction or line crosses when given to rats maintained on control diet (Table 1). Thus, under the conditions used in these experiments SB-242084, a 5-HT_{2C} antagonist, does not have anxiolytic effects in control rats.

A 5-HT_{2C} antagonist has dose-dependent effects on multiple withdrawal-induced anxiety

As illustrated in Fig. 3, upper panel, pretreatment with SB-242084 exhibited a dose-dependent counteraction of the deficit in social interaction [$F(4,17)=16.41$, $P<0.0001$ for rat pairs and $F(4,42)=28.03$, $P<0.0001$ for individual rats]. Only the high dose of SB-242084 significantly

reduced the deficit in social interaction, replicating the result above (Fig. 2). Groups with different letter superscripts are significantly different, according to Tukey's protected *t*-test. As before, the high dose of SB-242084 did not counteract the deficit in line crossings (Fig. 3, lower panel). The significant ANOVA [$F(4,17)= 24.4$, $P<0.0001$ for pairs and $F(4,42)=17.02$, $P<0.0001$ for individual rats] resulted because all of the ethanol-exposed rats were less active than the rats maintained on control diet (Fig. 3, lower panel).

A 5-HT$_{1A}$ antagonist does not counteract multiple withdrawal induced anxiety

Testing of the effects of the 5-HT$_{1A}$ receptor antagonist, WAY-100635, on social interaction behavior of multiply ethanol-withdrawn rats is illustrated in Fig. 4. A group of rats pretreated with the 5-HT$_{2C}$ receptor antagonist SB-242084 is included for comparison. There were highly significant group differences for time spent in social interaction [$F(4,18)=25.23$, $P<0.0001$ for pairs and $F(4,42)=24.75$, $P<0.0001$ for individual rats]. Different letter superscripts indicate which groups are significantly different, according to Tukey's protected *t*-test. Only pretreatment with the 5-HT$_{2C}$ receptor antagonist during the first two withdrawals induced a significant inhibition of the withdrawal-induced deficit in social interaction during the third withdrawal (Fig. 4, upper panel). The reduced line crossings in the rats withdrawn from ethanol were not affected by any of the drug treatments (Fig. 4, lower panel), although there were statistically significant differences among the groups [$F(4,18)=17.04$, $P<0.0001$ for pairs and $F(4,42)=5.81$, $P=0.001$, for individual rats].

A 5-HT$_{1A}$ partial agonist counteracts multiple withdrawal-induced anxiety

Buspirone, the 5-HT$_{1A}$ partial receptor agonist, influenced social interaction behavior in a manner similar to that of SB-242084, the 5-HT$_{2C}$ receptor antagonist. As illustrated in Fig. 5, buspirone, whether administered acutely during the final withdrawal or during the first and second withdrawals only, significantly decreased the withdrawal-induced deficit in social interaction [$F(3,11)=13.53$, $P=0.0005$ for pairs and $F(3,26)=7.20$, $P=0.013$ for individual rats]. Groups with different letter superscripts are significantly different, according to Tukey's protected *t*-test. Neither of the buspirone-treated groups was significantly different from the rats maintained on control diet, a finding that suggests a complete normalization of the behavior (Fig. 5, upper panel). Despite this normalization of social interaction behavior, buspirone did not significantly alter the reduced line crossings of the ethanol-withdrawn rats (Fig. 5, lower panel). The significant group differences [$F(3,11)=6.87$, $P=0.007$ for pairs and $F(3,26)=6.00$, $P=0.0033$, for individual rats] were consequences of all ethanol-withdrawn rats being less active than the rats maintained on control diet.

Two pretreatments with buspirone 5 and 10 days before the test did not alter social interaction behavior in rats maintained continuously on control diet or ethanol diet (58±11 s for control; 53±6.5 s for control diet given buspirone; 57±9.4 for ethanol diet given buspirone; $P>0.05$). It was also found that buspirone given acutely did not alter either time spent in social interaction or line crossings (Table 1). Thus, under conditions used for these experiments, buspirone did not have either anxiolytic or sedative effects in the control rats.

A 5-HT$_{2C}$ agonist sensitizes withdrawal induced anxiety

Since the 5-HT$_{2C}$ receptor antagonist blocked the social interaction deficit induced by multiple withdrawals, it was tested whether prior treatment with a 5-HT$_{2C}$ receptor agonist would affect social interaction if given instead of the initial two withdrawals of the multiple exposure protocol. When rats received two prior treatments of Ro-601075, a 5-HT$_{2C}$ agonist, before a single 5-day exposure to ethanol and subsequent withdrawal, they exhibited significant reductions in social interaction (Fig. 6 upper panel), like that seen with multiple withdrawals (Fig. 1). The difference among the control diets was highly significant, both for rat pairs [$F(4,15)=9.01$, $P=0.0006$] and for individual rats [$F(4,35)=16.52$, $P<0.001$]. Groups with

different letter superscripts are significantly different, according to Tukey's protected *t*-test. While there were also group differences in line crossings [$F(4,15)= 3.88$, $P=0.02$ for rat pairs and $F(4,35)=4.08$, $P=0.016$ for individual rats], only the rats exposed to 7% ethanol were significantly different from the group maintained on control diet (Fig. 6, lower panel).

Body weight and ethanol intake—There were no significant differences in the body weights and alcohol intakes of the different rats that were maintained on control diet or 7% ED, as summarized in Table 2. Rats exposed to 4.5% ED drank less ethanol and gained more weight than rats exposed to 7% ED (data not shown). Therefore, any differential behavioral effects of the treatments cannot be explained by differences in these measures.

Discussion

Work from our laboratory demonstrated that withdrawal from chronic ethanol exposure induces a deficit in social interaction (Knapp et al. 2000; Overstreet et al. 2002). Both buspirone, a 5-HT_{1A} partial receptor agonist, and SB-242084, a 5-HT_{2C} receptor antagonist, given during the final withdrawal of the multiple withdrawal protocol, attenuated the deficit in social interaction induced by the ethanol withdrawal. This finding is consistent with previous reports on withdrawal from continuous ethanol exposure (Lal et al. 1991; File et al. 1993; Knapp et al. 2000). The fact that neither buspirone nor SB-242084 altered social interaction behavior in rats maintained on control diet suggests that their effects in the ethanol-withdrawn rats may result from a counteraction of serotonergic abnormalities occurring during ethanol withdrawal.

The role of serotonin in the behavioral deficit in social interaction induced by withdrawal from ethanol can be said to resemble its role in the anxiety-like behavior following acute fluoxetine treatment in that both are blocked by the 5-HT_{2C} antagonist (Fig. 2; Bagdy et al. 2001). The increased anxiogenic effect of m-chlorophenylpiperazine during ethanol withdrawal (Wallis et al. 1993) is consistent with the involvement of the 5-HT_{2C} receptor during chronic ethanol treatment. WAY-100635, a 5-HT_{1A} antagonist, ketanserin, a 5-HT_{2A} antagonist, and ritanserin, a mixed 5-$HT_{2A/2C}$ antagonist, did not alter the social interaction deficit during the final withdrawal. The report (Lal et al. 1993) of anxiolytic effects of mianserin, a mixed 5-$HT_{2A/2C}$ receptor antagonist, in ethanol-withdrawn rats contrasts with the present findings. Regardless, the present results indicate selectivity in the 5-HT receptor subtypes that modulate social interaction behavior following ethanol withdrawal.

Because serotonin was implicated in anxiety-like behavior induced by withdrawal from a single long exposure to ethanol (e.g. File et al. 1993; Knapp et al. 2000) and after the third withdrawal of the multiple withdrawal protocol, the next goal of the present investigation was to determine whether sensitization of the deficit in social interaction associated with multiple withdrawals was mediated by the serotonergic system. In this case, compounds were injected during the initial two withdrawals and the social interaction testing was performed during the final withdrawal. Pretreatment with either the 5-HT_{2C} receptor antagonist, SB-242084, or the 5-HT_{1A} partial receptor agonist, buspirone, significantly reduced the deficit in social interaction observed after the third withdrawal of the multiple withdrawal protocol. In contrast, pretreatment with either the 5-HT_{1A} receptor antagonist, WAY-100635, or the 5-HT_{2A} receptor antagonist, ketanserin, was effective in reducing the sensitization of the deficit in social interaction with repeated withdrawals. When the drugs were administered to rats maintained on control diet using the same protocol, no effects on social interaction behavior were observed. Thus, the present findings clearly support the hypothesis that adaptive changes in 5-HT_{2C} and 5-HT_{1A} receptors and/or an increased serotonin release during these early withdrawal periods contribute to the sensitized deficit in social interaction following the final withdrawal (Overstreet et al. 2002).

The beneficial effect of buspirone may be due to its interaction with 5-HT_{1A} autoreceptors (e.g. Yocca 1990), rather than to an action on postsynaptic 5-HT_{1A} receptors, thereby counteracting the exaggerated release of 5-HT that accompanies ethanol withdrawal (File et al. 1993). The fact that the 5-HT_{1A} receptor antagonist, WAY-100635, did not counteract the reduced social interaction in ethanol-withdrawn rats is consistent with this view. A parallel study (data not shown) was conducted with 1-(2-pyrimidinyl)-piperazine (1-PP), an active metabolite of buspirone that may mediate some of its actions (e.g. Amano et al. 1993; Cao and Rodgers 1997), but this compound did not modify social interaction in ethanol-withdrawn rats. This finding supports the interpretation that buspirone's anxiolytic-like effects are mediated by its interaction with the 5-HT_{1A} autoreceptor, while the 5-HT_{2C} receptor antagonist acts post-synaptically to induce its anxiolytic-like effects in ethanol-withdrawn rats.

A key role of 5-HT_{2C} receptor activation in the sensitized deficit in social interaction associated with repeated ethanol withdrawals is supported not only by the antagonism of the 5-HT_{2C} receptor but also by two findings with the 5-HT_{2C} receptor agonist, Ro-601075. Rats that were pretreated with Ro-601075 while on control or ethanol diets exhibited a reduction in social interaction when subsequently withdrawn from ethanol (Fig. 6). Consequently, the multiple activation of 5-HT_{2C} receptors with Ro-601075 in the presence and absence of ethanol produced the same adaptive change as repeated withdrawals from ethanol (Overstreet et al. 2002).

The beneficial effects of a 5-HT_{1A} receptor agonist and a 5-HT_{2C} receptor antagonist on the social interaction deficits in alcohol-withdrawn rats are consistent with some of the literature on adaptive changes in 5-HT receptor subtypes following chronic ethanol treatment. Changes in the 5-HT_{2C}, 5-HT_{2A} or 5-HT_{1A} receptors have been reported by some investigators (Ulrichsen 1991; Pandey et al. 1992; Pandy and Pandey 1996). However, it is difficult to relate these findings directly to the present pharmacological observations due to differences in method and timing of ethanol treatments. Nonetheless, because of the key role the 5-HT_{2C} and 5-HT_{1A} receptors are playing in the multiple withdrawal adaptation to sensitize anxiety-like behavior, changes in the levels of these serotonin receptor subtypes might be expected in the rats with a sensitized deficit in social interaction. Future data are needed to test this view in brain regions most likely related to the sensitization of anxiety-like behavior seen with repeated withdrawals.

Despite buspirone and SB-242084 pretreatment during the initial two withdrawals reducing the withdrawal-induced deficit in social interaction during the third withdrawal, neither of these agents nor any other drug studied in this protocol was able to ameliorate the reduction in line crossings induced by withdrawal from ethanol (Figs 1, 2, 3, 4, 5, 6). The independent control over line crossings and social interaction behavior due to experimental manipulations (File 1980;Overstreet et al. 2002) and pharmacological challenges (this study) suggest that independent neural mechanisms may be involved in the affective and physical withdrawal signs in alcoholics (De Soto et al. 1989;Miller and Harris 2000). Because the drugs used in this study preferentially counteracted the affective symptom (anxiety-like behavior), and because affective symptoms are important risks to relapse in alcoholics (Curran et al. 2000;Strowig 2000;Driessen et al. 2001), it is possible that the agents that counteracted the social interaction deficits in the rats may reduce relapse in alcoholics.

The present findings suggest that blockade of the 5-HT_{2C} receptor or stimulation of the 5-HT_{1A} autoreceptor may offer some potential as therapeutic approaches in the treatment of acute anxiety-like symptoms during withdrawal in the alcoholic. Use of such drugs to reduce these symptoms during detoxification of alcoholics might prevent subsequent anxiety-like symptoms during withdrawal from becoming worse. Presently, there are no known reports of a selective 5-HT_{2C} receptor antagonist being used to treat alcoholics, but there have been several negative

or mixed clinical studies on ritanserin, a mixed 5-$HT_{2A/2C}$ antagonist (Meert 1994; Naranjo et al. 1995; Wiesbeck et al. 1999, 2000).

Although early clinical studies used buspirone during acute detoxification (Dougherty and Gates 1990; Tollefson et al. 1992), more recent studies with this drug have used chronic regimens in an attempt to prevent alcoholics from relapsing (Malec et al. 1996; Fawcett et al. 1999; George et al. 1999). The failure of these latter studies could have been predicted from the failure of a recent preclinical study that employed chronic as well as acute buspirone treatment (Hedlund and Wahlstrom 1999). Buspirone might be best used as an adjunct to other drugs used in detoxification of alcoholics.

In summary, our findings demonstrate that both a 5-HT_{1A} partial receptor agonist and a 5-HT_{2C} receptor antagonist can ameliorate deficits in social interaction observed following the third withdrawal from ethanol when given during the first and second withdrawals of a multiple withdrawal regimen. Clinical trials of these agents as anxiolytics during acute abstinence in alcoholics seem warranted.

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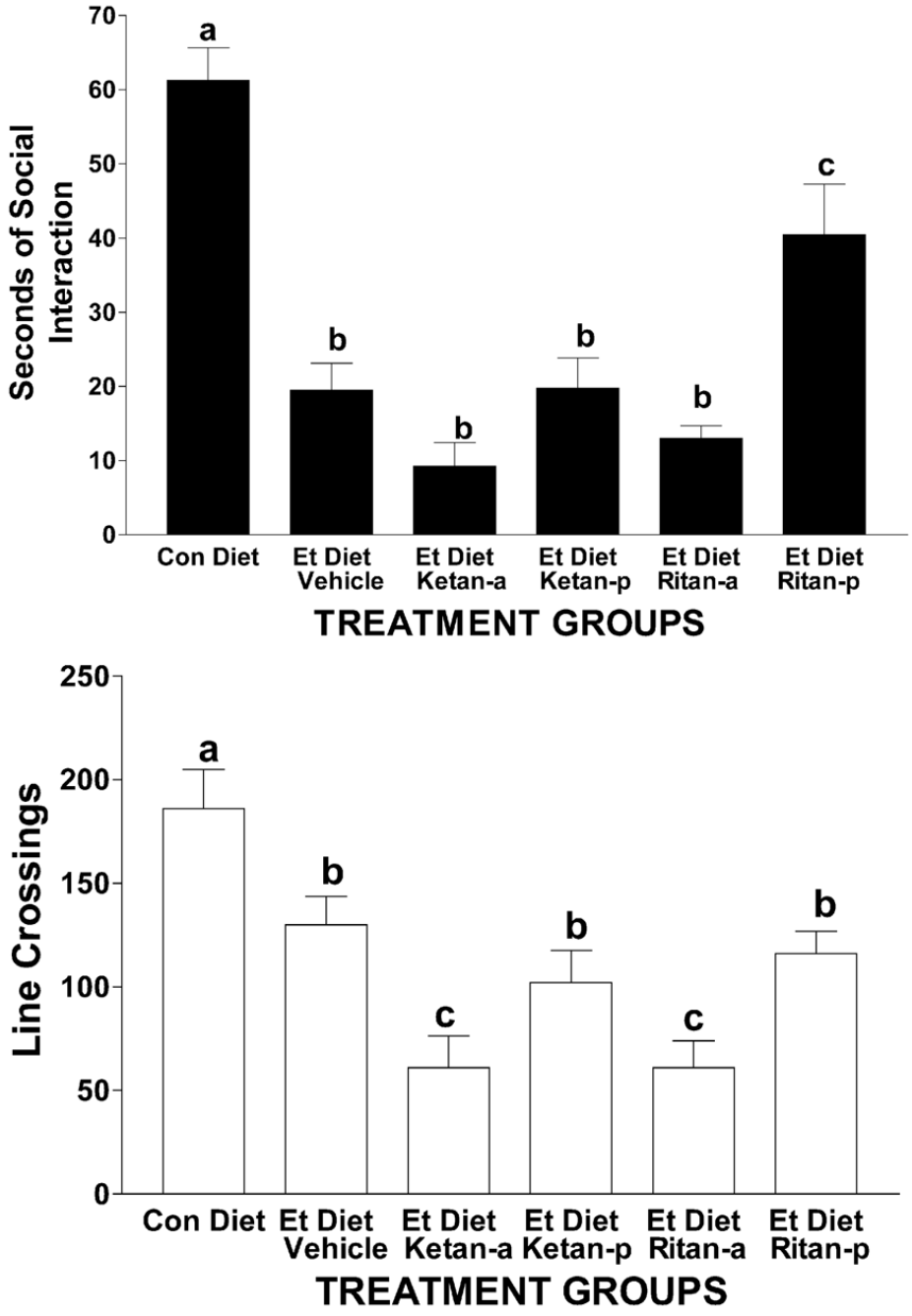

Fig. 1.

Effects of 5-HT_{2A} antagonist ketanserin and mixed 5-$HT_{2A/2C}$ antagonist ritanserin on social interaction behavior of rats repeatedly withdrawn from ethanol. The values represent the mean ±SEM seconds spent in social interaction (*upper panel*) or line crossings (*lower panel*) for four pairs per group. *a* acute treatment, *p* pretreatment

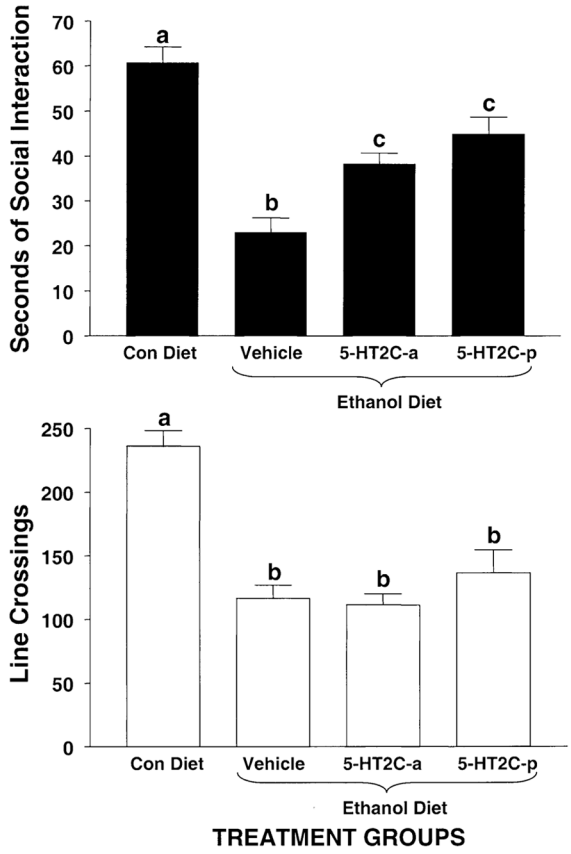

Fig. 2.

Effects of 5-HT_{2C} antagonist SB-242084 on social interaction behavior of rats repeatedly withdrawn from ethanol. The values represent the mean±SEM seconds spent in social interaction (*upper panel*) or line crossings (*lower panel*) for four to six pairs per group. *a* acute treatment, *p* pretreatment

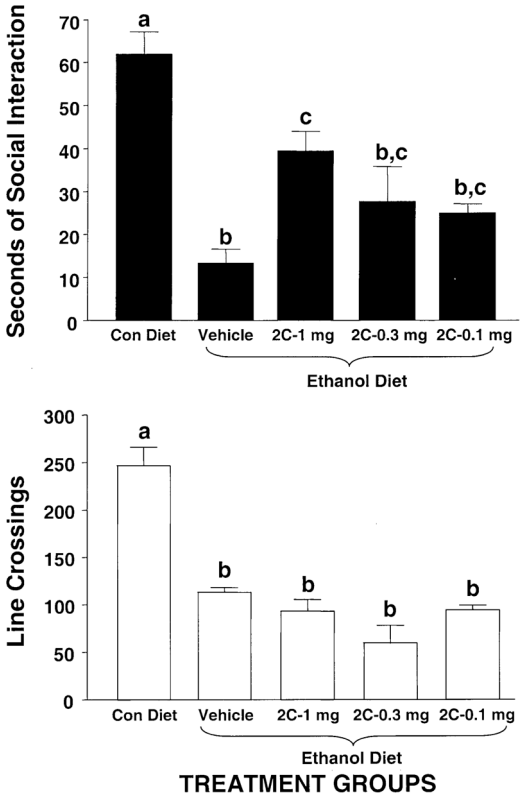

Fig. 3.

Dose-dependent effects of 5-HT_{2C} antagonist SB-242084 on social interaction behavior of rats repeatedly withdrawn from ethanol. The values represent the mean±SEM seconds spent in social interaction (*upper panel*) or line crossings (*lower panel*) for three to five pairs per group. *a* acute treatment, *p* pretreatment

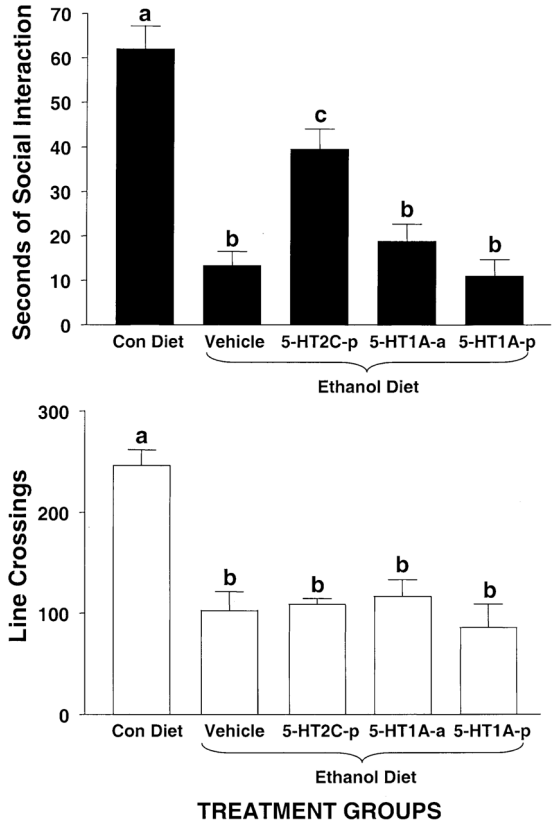

Fig. 4.

Effects of 5-HT_{1A} antagonist WAY-100635 on social interaction behavior of rats repeatedly withdrawn from ethanol. The values represent the mean±SEM seconds spent in social interaction (*upper panel*) or line crossings (*lower panel*) for four pairs per group. *a* acute treatment, *p* pretreatment

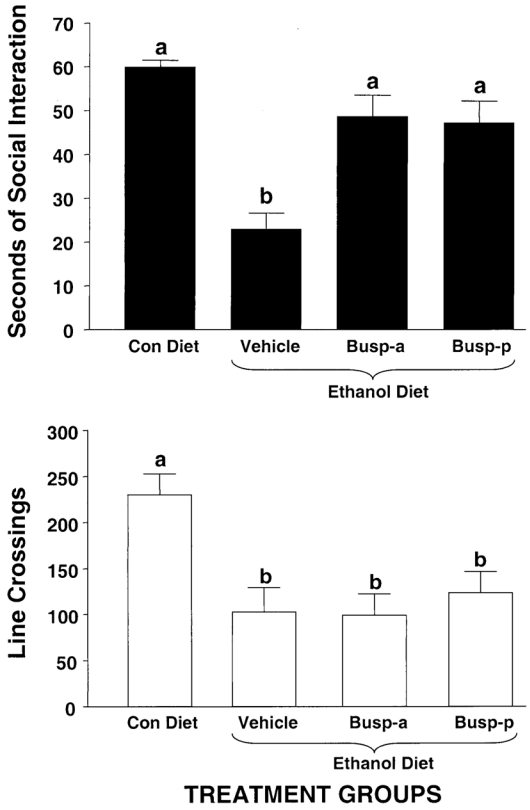

Fig. 5.

Effects of 5-HT_{1A} partial agonist buspirone on social interaction behavior of rats repeatedly withdrawn from ethanol. The values represent the mean±SEM seconds spent in social interaction (*upper panel*) or line crossings (*lower panel*) for four pairs per group. *a* acute treatment, *p* pretreatment

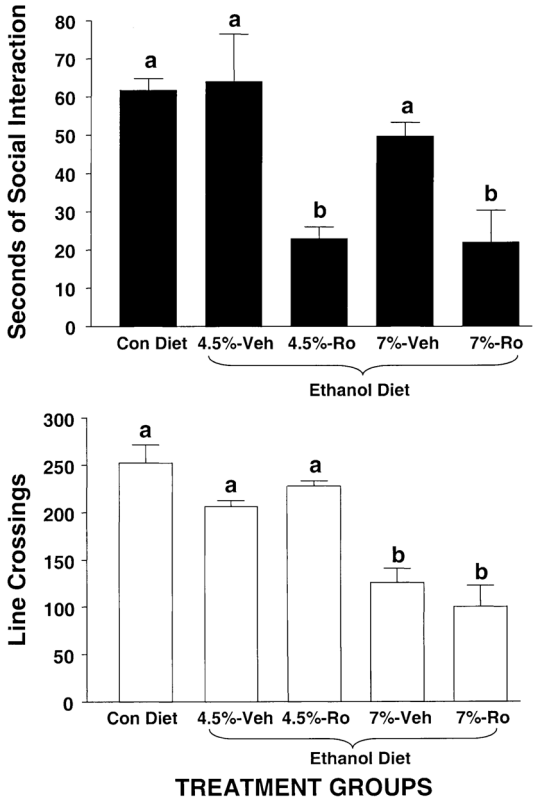

Fig. 6.

Effects of 5-HT_{2C} agonist Ro-601075 on social interaction behavior of rats exposed to control or 4.5% ethanol diets. The values represent the mean±SEM seconds spent in social interaction (*upper panel*) or line crossings (*lower panel*) for four pairs per group. *a* acute treatment, *p* pretreatment

Table 1

Effects of acute treatment of ketanserin, ritanserin, SB-242084 and buspirone on social interaction behavior in rats maintained on control diet. The values represent the mean±SEM for four pairs of rats. $F(4,18)=7.88$, $P<0.01$, for social interaction. $F(4,15)=4.37$, $P=0.015$, for line crossings. Groups with different letters are significantly different, $P<0.05$, according to Tukey's test

Treatment group	Measure	
	Time spent in social interaction	Line crossings
Vehicle	63.0±6.2[a]	232±30[a]
Ketanserin (1 mg/kg)	22.3±10.0[b]	94±35[b]
Ritanserin (1 mg/kg)	29.5±3.9[b]	174±11[a,b]
SB 242084 (1 mg/kg)	74.3±11.4[a]	258±49[a]
Buspirone (0.2 mg/kg)	52.5±3.9[a]	154±14[a,b]

Table 2

Body weights and terminal ethanol intake of rats subjected to repeated withdrawals and treated with drugs interacting with 5-HT receptor subtypes. *A* acute, *P* pretreatment, *N/A* not applicable

Treatment condition	Initial weight (g)	Final weight (g)	Ethanol intake[a] (g/kg per day)
Control diet	198.5±2.6	296.8±3.3	N/A
Ethanol diet	197.5±2.1	294.3±4.9	11.3±0.9
ED±SB-242084 (A)	196.5±2.8	301.0±6.9	11.3±0.4
ED±SB-242084 (P)	196.7±2.7	298.6±6.8	11.2±0.7
ED±WAY-100635 (A)	199.0±4.3	304.3±10.4	10.9±1.1
ED±WAY-100635 (P)	195.4±3.1	288.3±4.6	11.4±1.4
ED±ritanserin (A)	191.4±5.1	293.8±5.7	10.8±1.5
ED±ritanserin (P)	188.4±1.8	293.8±5.7	10.8±0.5
ED±ketanserin (A)	193.2±4.1	290.3±9.2	11.4±1.4
ED±ketanserin (P)	199.7±3.0	287.1±5.1	10.8±1.5
ED±buspirone (A)	204.3±3.1	303.5±6.4	11.2±1.5
ED±buspirone (P)	203.8±2.2	311.1±5.2	12.1±0.9
F-values	1.57, NS	1.11, NS	1.00, NS

[a] Values represent the means for the last 3 days of access to the ethanol diet. There were no differences in alcohol intake at other time points