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Benzodiazepine receptor antagonists modulate the actions of ethanol in alcohol-preferring and -nonpreferring rats

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Abstract

The pyrazoloquinoline CGS 8216 (2-phenylpyrazolo-[4,3-*c*]-quinolin-3 (5H)-one, 0.05-2 mg/kg) and the β-carboline ZK 93426 (ethyl-5-isopropyl-4-methyl-β-carboline-3-carboxylate, 1–10 mg/kg) benzodiazepine receptor antagonists were evaluated for their capacity to modulate the behavioral actions of ethanol in alcohol preferring and -nonpreferring rats. When alcohol-preferring rats were presented with a two-bottle choice test between ethanol (10% v/v) and a saccharin (0.0125% g/v) solution, both antagonists dose-dependently reduced intake of ethanol by 35–92% of control levels on day 1 at the initial 15 min interval of the 4 h limited access. Saccharin drinking was suppressed only with the highest doses. CGS 8216 (0.25 mg/kg) and ZK 93426 (4 mg/kg) unmasked the anxiolytic effects of a hypnotic ethanol dose (1.5 g/kg ethanol) on the plus maze test in alcohol-preferring rats, but potentiated the ethanol-induced suppression in alcohol-nonpreferring rats. CGS 8216 (0.25 mg/kg) and ZK 93426 (4 mg/kg) attenuated the ethanol (0.5 and 1.5 g/kg)-induced suppression in the open field in alcohol-nonpreferring rats; however, CGS 8216 potentiated the depressant effects of the lower ethanol dose (0.5 g/kg) in alcohol-preferring rats. These findings provide evidence that benzodiazepine receptor antagonists may differentially modulate the behavioral actions of ethanol in alcohol-preferring and-nonpreferring rats. It is possible that the qualitative pharmacodynamic differences seen in the present study may be related to selective breeding for alcohol preference. The findings indicate the potential for development of receptor specific ligands devoid of toxic effects which may be useful in the treatment of alcohol abuse and alcoholism. © 1998 Elsevier Science B.V.

Keywords: Benzodiazepine receptor antagonist; CGS 8216; ZK 93426; $GABA_A$ receptor; Alcohol-preferring; Alcohol-nonpreferring; Ethanol drinking; Locomotor activity; Anxiety

1. Introduction

Over the past years, there has been much interest in the interaction of ethanol with benzodiazepine receptor ligands (for review see Harris and Lal, 1988; also Korpi, 1994), especially inverse agonists (e.g., RO15-4513 (ethyl 8-azido-5,6-dihydro-5methyl-6-oxo-4H-imidazo-[1,5-*a*][1,4]-benzodiazepine-3 carboxylate), FG 7142 (*N*-methyl-β-carboline-3-carboxamide) DMCM (*N*-methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate) (for review see Jackson and Nutt, 1995; Draski and Deitrich, 1996). A long term goal of this work has been to identify receptor specific ligands which might provide clues to the neurobiological basis of alcoholism and assist in the development of pharmacotherapies for alcoholism. Unfortunately, many of these ligands are proconvulsant which precludes their use in human subjects in their existing molecular forms.

Other types of benzodiazepine ligands that may have potential as pharmacotherapies for alcohol abuse are the benzodiazepine receptor antagonists. Unlike inverse agonists, benzodiazepine antagonists exhibit little intrinsic efficacy and do not reduce GABAergic activity at central benzodiazepine receptors (Skolnick et al., 1982; Braestrup et al., 1983a,b,c; Haefely, 1983, 1985). Benzodiazepine receptor antagonists have been reported to produce little if any toxic effects in both animals (Hunkeler et al., 1981; Bonetti et al., 1982; Jensen et al., 1984) and humans

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(Jedrychowski et al., 1986; Duka et al., 1987, 1988; Reimann et al., 1987; also for review see Duka and Dorow, 1995).

Interest in the capacity of benzodiazepine antagonists to attenuate ethanol's effects has developed from several preclinical and clinical studies. Results from our laboratory have demonstrated that an acute dose of flumazenil (16 mg/kg) attenuated ethanol drinking in outbred rats (June et al., 1994), while lower doses ($\leq$ 10 mg/kg) have no effect in fluid deprived alcohol-preferring, nonpreferring (McBride et al., 1988) and outbred rats (Beaman et al., 1984). Other investigators have evaluated the capacity of flumazenil to block the anxiolytic properties of ethanol and found no effects on ethanol's release of punished responding (Koob et al., 1986). However, Barrett et al. (1985) reported that flumazenil potentiates ethanol's rate increasing effects in punished responding in pigeons (also see Belzung et al., 1988).

A study by Lister (1988) showed that ZK 93426 and flumazenil (5.0 mg/kg) was an effective antagonist of the depressant actions of ethanol in mice, although no specific underlying mechanism to account for these effects could be identified. Clinical studies investigating the efficacy of flumazenil in antagonizing the actions of ethanol have also demonstrated equivocal results (for review see Chan et al., 1988, 1991; also File et al., 1989). Scollo-Lavizzari and Matthis (1985) reported in a nonplacebo controlled study flumazenil slightly attenuated the ethanol-induced intoxication in human subjects. Klotz et al. (1986) reported that flumazenil exerted no effects on ethanol sedation using visual analog measures and a reaction time task. Together, these data suggest that the interaction of flumazenil with ethanol is modulated by a number of factors (i.e., dose, behavioral task), and that flumazenil does not consistently antagonize ethanol effects. However, flumazenil may unmask other effects of nonbenzodiazepine compounds that do not usually occur in the absence of the antagonist (Barrett et al., 1985).

The purpose of the present study was to systematically evaluate CGS 8216 and ZK 93426 for their capacity to modulate ethanol's behavioral effects in an ethanol drinking model, a test of anxiety, and in the open-field arena. The extent to which genetic differences in ethanol preference correlate with sensitivity of the benzodiazepine antagonists in modulating ethanol's anxiolytic and sedative effects were investigated by examining the interactions of ethanol with these antagonists in the selectively bred alcohol-preferring and alcohol-nonpreferring rats.

2. Materials and methods

2.1. Animals

Female rats from the S-37th generation of the selectively bred alcohol-preferring ($N = 33$) and S-38th generation of the alcohol-nonpreferring ($N = 20$) lines were obtained from the Alcohol Research Center at Indiana University School of Medicine. Rats were approximately 4 months of age and weighed between 280 and 345 g at the beginning of the experiment. Animals in the ethanol self-administration studies were housed individually in wire-mesh stainless steel cages. The vivarium was maintained at an ambient temperature of 21°C and was on a 12:12 reversed light:dark cycle (lights off at 09:00 h). Animals in the other behavioral studies were housed similarly, except that a normal light:dark cycle was used. Alcohol-preferring rats in the ethanol self-administration experiments were given 20 h daily access to water with food (Teklad Diet #7001, Harlan Industries, Indianapolis, IN) available ad libitum. During the remaining 4 h, animals were presented with a two-bottle choice between ethanol (10% v/v) and saccharin (0.0125% g/v) as previously described (cf. Murphy et al., 1986; June et al., 1995a, 1996). Rats in the plus-maze and open field experiments had water and food available ad libitum.

2.2. Drugs and solutions

All drugs were prepared as an emulsion in 1% Tween-80 vehicle (Sigma Chemical, St. Louis) and mixed with a 0.90% sodium chloride solution to a volume of 1 ml/kg body weight. When necessary, some drugs were sonicated. ZK 93426 (ethyl-5-isopropyl-4-methyl-β-carboline-3-carboxylate), the β-carboline antagonist, was donated as a gift from Shering (Berlin) and tested at 1–10 mg/kg. CGS 8216 (2-phenylpyrazolo-[4,3-*c*]-quinolin-3(5H)-one), the pyrazoloquinoline antagonist, was donated as a gift from Ciba–Geigy (Summit, NJ) and tested alone at 0.05–2.0 mg/kg. For the ethanol self-administration study, ethanol (10% v/v) and saccharin (0.0125–0.05% g/v) solutions were prepared daily and mixed as previously described (June et al., 1996). For the studies conducted in the open field and plus maze, ethanol (10% g/v) was prepared by mixing 95% pure ethanol (U.S.P.A.) with a 0.90% sodium chloride solution. Injection volumes were administered in volumes sufficient to produce 0.5 and 1.5 g/kg doses. All drug injections were given i.p.

2.3. Apparatus

Each cage contained two 1 inch diameter openings (spaced approximately 4 inches apart) for a two-bottle presentation of the ethanol and water/saccharin solutions. A metal spring (placed on the exterior of the cage) held the 100 ml graduated drinking tubes in place. The drinking tubes protruded approximately 2.5 cm into each animal's home cage.

2.3.1. Plus-maze test

The fully automated plus-maze (Acuscan Electronics, Columbus, OH) is a test for anxiety which utilizes an apparatus with two open arms (without sides) at right angles to two closed arms (with sides), raised about four

feet from the floor. When allowed to shuttle freely among the arms, rats spend less time on the open arms than on the closed arms, presumably reflecting a fear of height and open spaces. Forced confinement to the open arms is associated with increased plasma corticosterone concentrations and treatment with anxiolytic drugs increases the time rats spend on the open arms compared with undrugged controls (Pellow et al., 1985; File, 1995). The total number of entries onto both the open and closed arms is a measure of general activity levels (see File, 1995) and provides information as to whether time spent in a given section of the maze is secondary to an increase or decrease in locomotor activity.

2.3.2. Open-field arena

Measures of locomotion in the open field were determined by two Digiscan Activity Monitors (Acuscan Electronics, Columbus, OH). Each monitor consisted of a clear plexiglas cage measuring $42 \times 42 \times 30$ cm. Movement was detected by 2 sets of four infrared perpendicular photobeams in the walls of the chamber with 16 beams along each axis. Each interruption of a photobeam constituted a count. Data were collected and analyzed by an automated Digiscan Analyzer which interfaced the two test chambers and a Macintosh LC II computer. All experiments were conducted under dim lighting (25 W) conditions. The floor of the open field was cleaned after each subject to eliminate any traces of the previous rat's path. The following activity parameters were evaluated in the open field: ambulatory counts; total distance travelled in cm; rearing; and stereotypy count.

2.4. General procedures

2.4.1. Ethanol self-administration study

2.4.1.1. Ethanol acclimation phase. Following acclimation to the colony room for several days, the rats were presented with a 4 h/day two-bottle choice test between ethanol (10% v/v) and water for two weeks using a modification of the procedures as previously described (Murphy et al., 1986). Fluid consumption was measured to the nearest 0.5 ml before and during each testing session at 15, 30, 60, 120, 180, and 240 min. Food intake was determined by weighing the powdered food before and at the conclusion of the 4 h schedule of fluid availability. Animals were weighed twice weekly before and after their fluid presentation. Following stabilization on the 10% (v/v) ethanol and water regimen, the saccharin exposure phase began.

2.4.1.2. Saccharin exposure phase. To determine an optimal control fluid to evaluate whether drug treatments would selectively alter ethanol intake during a two-bottle choice situation, alcohol-preferring rats ($N = 13$, experiment 1; $N = 11$, experiment 2) were presented with descending concentrations of saccharin (0.05%, 0.025%, 0.0125% g/v) in one bottle and 10% (v/v) ethanol solution in the other bottle for 4 h daily while food was available ad libitum. Intake of both the ethanol and the saccharin solutions were recorded at 15, 30, 60, 120, 180, and 240 min of the 4 h sessions (for a detailed discussion of the procedures see June et al., 1995a; also June et al., 1996). The optimal saccharin solution which approximated ethanol intake in ml was found to be the 0.0125% (g/v) concentration. Animals were then given 4 h daily exposure to the 10% ethanol and 0.0125% saccharin solutions throughout the remainder of the experimental protocol.

2.4.1.3. Experimental treatment phase. Following the baseline stabilization phase, animals were pretreated with CGS 8216 (0.05, 0.10, 0.25, 1, and 2 mg/kg) or ZK 93426 (1, 2, 4, 6, 8, and 10 mg/kg) 20 min prior to presenting rats their fluid regimen of ethanol and saccharin. To control for residual carryover effects, each drug pretreatment was separated by at least 4 d and subsequent pretreatments were never administered until both the ethanol and saccharin intake had returned to baseline levels.

2.4.2. Plus-maze and open-field activity studies

Separate groups of alcohol-preferring ($N = 20$) and alcohol-nonpreferring ($N = 20$) rats were used for the anxiolytic ($N = 10$/line) and open-field ($N = 10$/line) studies. All animals received their treatment condition in a randomized design to control for order and sequence effects. All antagonists (CGS 8216 or ZK 93426) were given 20 min prior to the ethanol injections (0.5 or 1.5 g/kg). Five min after the ethanol injections, animals were placed in the plus maze for a 5 min test session or in the case of the open-field apparatus, rats were given a 10 min test. The benzodiazepine antagonists were given only on day 1. However, to evaluate the development of tolerance to the sedative effects of ethanol in the open-field, identical doses of ethanol were given on days 1 and 2. Secondly, while six–seven days were allotted between experimental test sessions in the plus-maze studies, twelve–fourteen days were allotted between experimental test sessions in the open field studies. A longer time between ethanol injections was allotted for the open field studies because of the reported ability of alcohol-preferring rats to maintain tolerance up to 10 d following a single dose of ethanol (Gatto et al., 1987).

2.5. Statistical analysis

Two analyses were conducted on the data in experiment 1; the first for ethanol, and the second for saccharin intake. The analysis comprised a $2 \times 6 \times 6$ repeated measures ANOVA. The first factor represented day (day 1 and 2), the second, treatment condition (e.g., Tween-80 vehicle, 0.05, 0.1, 0.25, 1, and 2 mg/kg CGS 816); and the third, consumption interval (15, 30, 120, 180, 240, and 0–240

min). Because minimal ethanol drinking typically occurs at the 60 min interval (June et al., 1995a; June et al., 1996), data for this interval were not evaluated in the analysis. A post-hoc Newman–Keuls test was conducted for pairwise comparisons of group means for this and subsequent analyses. A two-way repeated measures ANOVA was also conducted on the day and treatment condition factors for the food intake data. Similar analyses were conducted for the ZK 93426 treatment conditions.

In experiment 2, a single factor repeated measure ANOVA was conducted to examine the effects of the treatment conditions in the plus-maze test. Separate analyses were conducted on each of the plus-maze parameters. In experiment 3, a two-way repeated measure ANOVA was conducted to examine the effects of the treatment conditions in the open field. The first factor represented day (day 1 and 2), and the second, treatment condition. Data for the alcohol-preferring and alcohol-nonpreferring rat lines were analyzed separately in all experiments.

3. Results

3.1. Effects of CGS 8216 and ZK 93426 on ethanol intake in alcohol-preferring rats

Figs. 1 and 2 show ethanol and saccharin intake on days 1 and 2, respectively, for the 15 min interval and total consumption measurement (0–240) after CGS 8216 injections (0.05–0.2 mg/kg) (the 0–15 min interval and total consumption measurement depict the most salient aspects of the data in this and all other self-administration experiments; thus, other intervals are not illustrated). A significant day × treatment condition × consumption interval in-

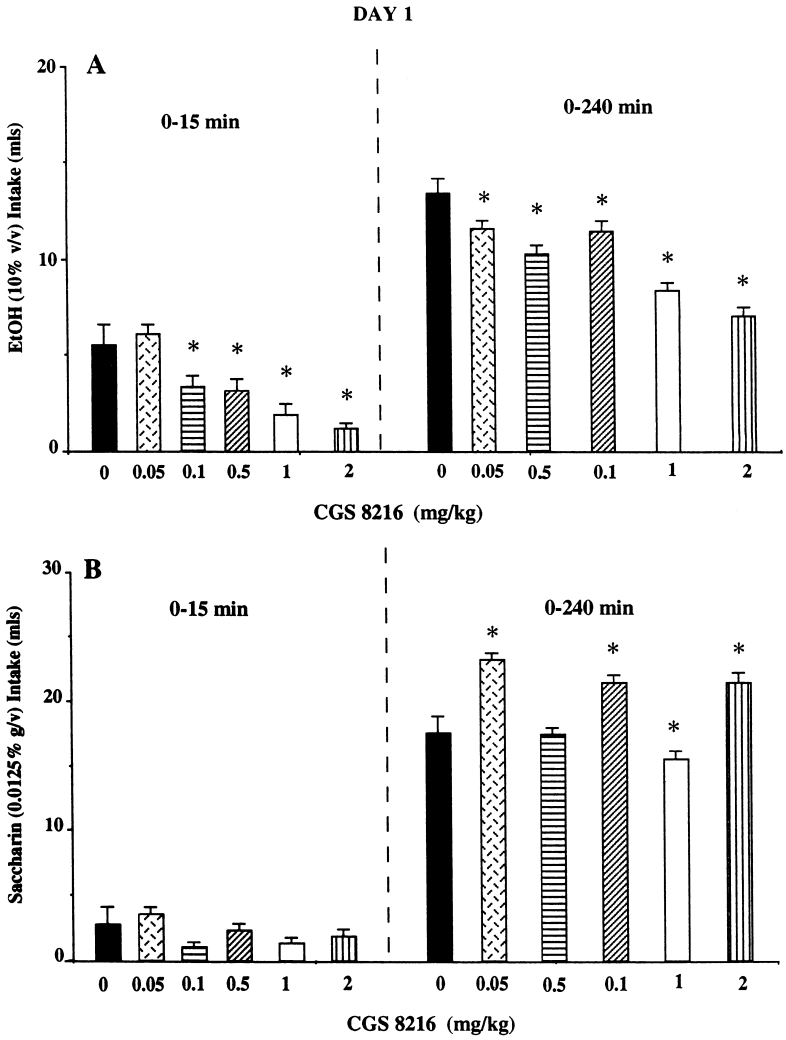

Fig. 1. Dose–response and time course of i.p. administration of CGS 8216 (Tween-80 vehicle 0.0, 0.05, 0.1, 0.5, 1, 2 mg/kg) given acutely on *day 1* on ethanol (10% v/v, A) and saccharin (0.0125% g/v, B) intake by alcohol-preferring rats during the initial (0–15 min) and total 4 hr (0–240 min) consumption period. CGS 8216 dose-dependently attenuated ethanol intake at both consumption intervals on day 1. Drug treatments were given 20 min prior to presenting rats their fluid regimen, and were separated by at least 4 days to avoid carryover effects. $^{*}P \leq 0.05$ versus control vehicle values by ANOVA and post hoc Newman–Keuls test at a corresponding time interval.

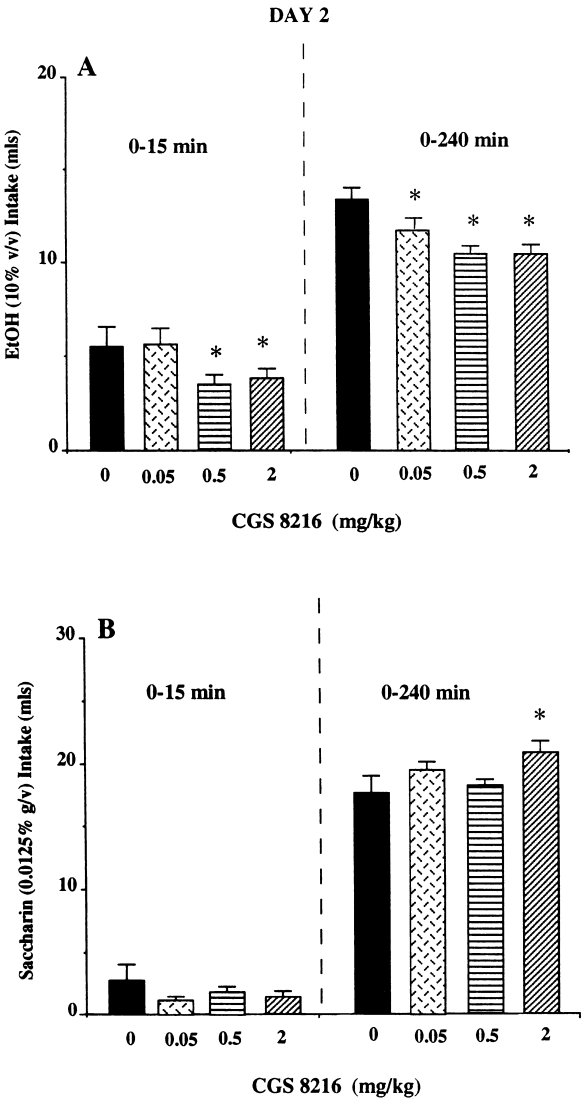

Fig. 2. Dose–response and time course of i.p. administration of CGS 8216 (Tween-80 vehicle [0.0], 0.05, 0.5, 2 mg/kg) on *day 2* on ethanol (10% v/v, A) and saccharin (0.0125% g/v, B) intake by alcohol-preferring rats during the initial (0–15 min) and total 4 h (0–240 min) consumption period. Drug treatments were given 20 min prior to presenting rats their fluid regimen only on day 1 with administration of each drug treatment separated by at least 4 days to avoid carryover effects. Only selected CGS 8216 doses attenuated ethanol intake on day 2. $^{*}P \leq 0.05$ versus control vehicle values by ANOVA and post hoc Newman–Keuls test at a corresponding time interval.

teraction emerged from these data ($F(25, 300) = 52.68$, $P < 0.001$). Post hoc analyses showed that on day 1, significant dose-dependent reductions on ethanol intake occurred following CGS 8216 injections during the initial 15 min interval and for the total measurement period (Fig. 1a, $P \leq 0.05$). Only the lowest dose (0.05 mg/kg) failed to suppress intake at the 15 min interval. On day 2 CGS 8216 treatments significantly attenuated ethanol drinking following the 0.05 and 2 mg/kg doses at the 15 min interval ($P \leq 0.05$), while the 0.05, 0.5, and 2 mg/kg doses significantly reduced drinking at the total measurement period ($P \leq 0.05$, see Fig. 2a).

3.1.1. CGS 8216 effects on saccharin intake (days 1 and 2)

Fig. 1b and Fig. 2b show saccharin intake on days 1 and 2, respectively, for the 15 min interval and total consumption measurement after CGS 8216 injections (0.05–2 mg/kg). A significant day × treatment condition × consumption interval interaction emerged for the saccharin data ($F(25, 300) = 37.32$, $P < 0.01$). Post-hoc analyses revealed that on day 1, elevations in saccharin drinking were seen for the 4 h totals with the 0.05, 0.1, and 2 mg/kg doses ($P \leq 0.05$), while a reduction was seen with the 1 mg/kg dose for the total measurement period ($P <$

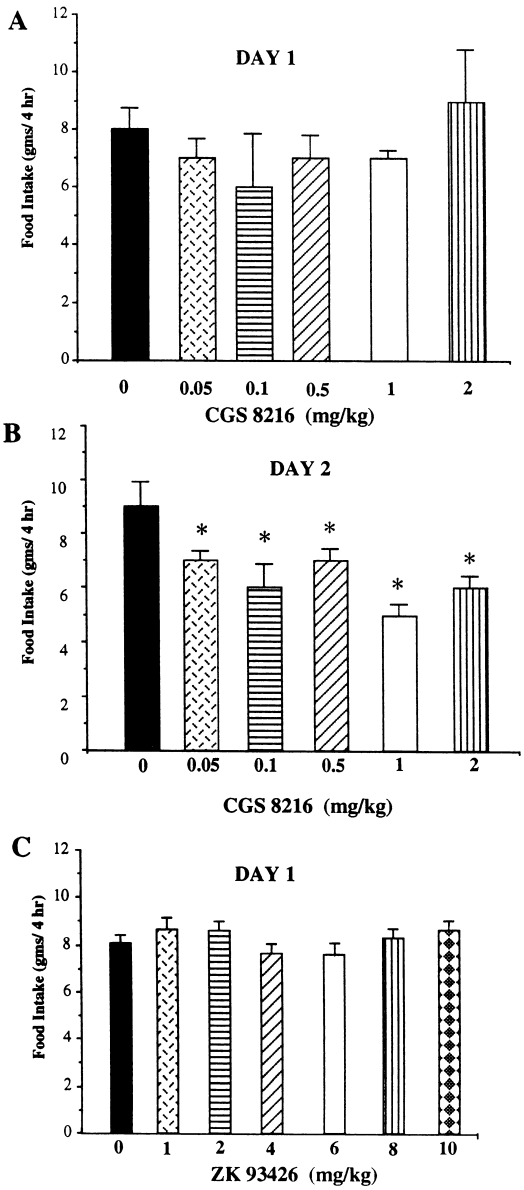

Fig. 3. Dose–response effects of i.p. administration of CGS 8216 (Tween-80 vehicle [0.0], 0.05, 0.5, 2 mg/kg, days 1 and 2; A and B) and ZK 93426 (Tween-80 vehicle [0.0], 1, 2, 4, 6, 8, 10 mg/kg, day 1, C) on food intake in grams during the 4 h (0–240 min) consumption period. CGS 8216 attenuated food intake on day 2, but did not alter intake on day 1. $^{*}P \leq 0.05$ versus control vehicle values by ANOVA and post hoc Newman–Keuls test at a corresponding time interval.

0.05, Fig. 1b). On day 2, mild elevations in saccharin drinking were seen with the 2 mg/kg dose ($P < 0.05$, Fig. 2b) over the total 4 h period.

3.1.2. CGS 8216 effects on food consumption (day 1 and 2)

A significant day × treatment interaction emerged for the food data ($F(2, 120) = 6.03$, $P < 0.01$). No effects were seen with any of the CGS 8216 doses on day 1 ($P > 0.05$, Fig. 3a); however, on day 2, all doses significantly suppressed food intake ($P \leq 0.05$, Fig. 3b).

3.1.3. ZK 93426 effects on ethanol intake

Unlike CGS 8216, except for the 10 mg/kg dose ($P < 0.01$), ZK 93426 produced little effects on ethanol intake on day 2 (data not shown). Fig. 4a shows ethanol intake on day 1 for the 15 min interval and total consumption periods, respectively, after ZK 93426 injections (1–10 mg/kg). A significant treatment condition effect emerged from these data ($F(6, 72) = 9.45$, $P < 0.001$), with ZK 93426 dose-dependently reducing intake up to the 8 mg/kg dose level. Post hoc analyses confirmed these findings for both the 0–15 min interval ($P \leq 0.05$) and total measurement period ($P \leq 0.05$).

3.1.4. ZK 93426 effects on saccharin intake

Fig. 4b shows saccharin intake for the 0–15 min interval and during the total consumption period after ZK 93426 injections (1–10 mg/kg). A significant treatment condition effect was found ($F(6, 72) = 4.45$, $P < 0.001$). Only the 2 mg/kg dose reduced saccharin consumption at the 15 min interval ($P < 0.05$). For the total consumption measurement, the 4 and 8 mg/kg doses elevated saccharin drinking ($P \leq 0.05$), while the highest dose (10 mg/kg) significantly suppressed intake ($P \leq 0.05$).

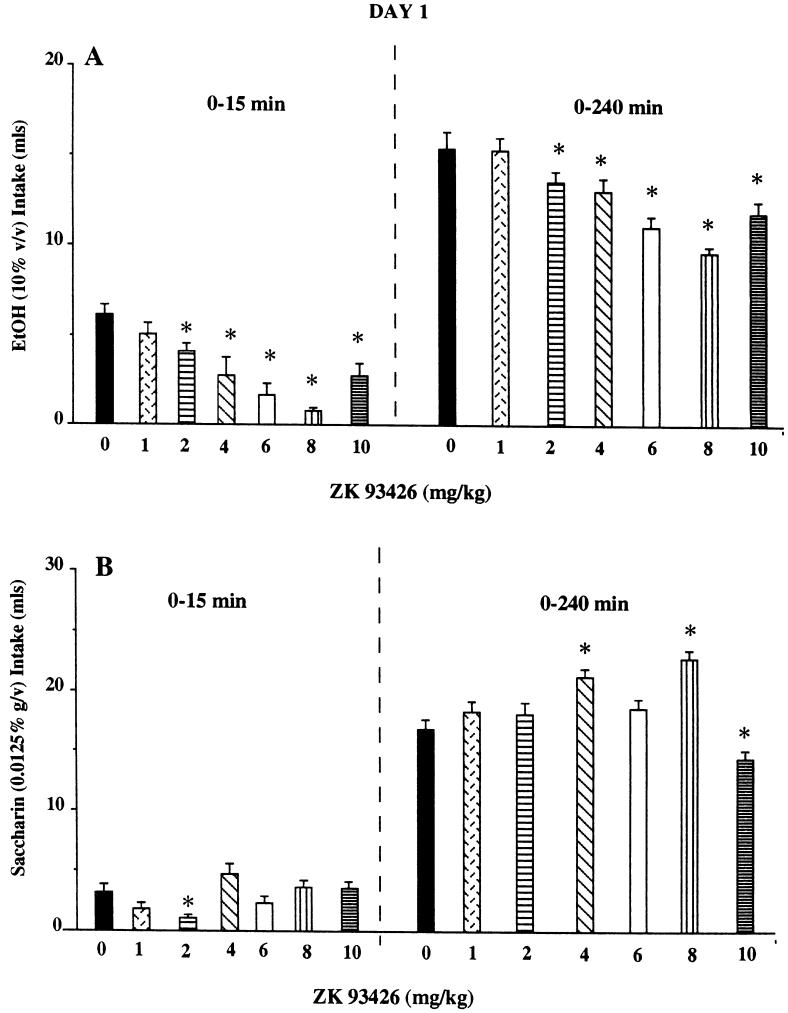

Fig. 4. Dose–response and time course of i.p. administration of ZK 93426 (Tween-80 vehicle 0.0, 1, 2, 4, 6, 8, 10 mg/kg) given acutely on day 1 on ethanol (10% v/v, A) and saccharin (0.0125% g/v, B) intake by alcohol-preferring rats during the initial (0–15 min) and total 4 h (0–240 min) consumption period. ZK 93426 dose-dependently and selectively attenuated ethanol intake at both consumption intervals up to the 8 mg/kg dose, however, nonselective effects were observed with the highest dose (10 mg/kg). Drug treatments were given 20 min prior to presenting rats' their fluid regimen, and were separated by at least 4 d to avoid carryover effects. * $P \leq 0.05$ versus control vehicle values by ANOVA and post hoc Newman–Keuls test at a corresponding time interval.

3.1.5. ZK 93426 effects on food intake

To further examine the selectivity of the ZK 93426 treatments on ingestive behavior, a single factor repeated measures ANOVA was conducted on food intake measures during the 4 h interval. None of the ZK 93426 treatments altered food intake ($F(6, 72) = 1.34$, $P > 0.05$, see Fig. 3c).

3.2. Effects of CGS 8216 and ZK 93426 on behavior of alcohol-preferring and -nonpreferring rats in the elevated plus maze

Experiment 2 investigated the extent to which ethanol preference correlated with sensitivity of CGS 8216 and ZK 93426 to modulate ethanol's actions in the plus-maze test. Effective doses of CGS 8216 (0.25 mg/kg) and ZK 93426 (4 mg/kg) which antagonized ethanol intake in experiment 1 and previous studies (unpublished observation) were evaluated.

3.2.1. Effects of CGS 8216 alone, and in combination with ethanol in alcohol-preferring and -nonpreferring rats in the plus-maze

Table 1 shows two anxiolytic parameters, time spent on open arms (top) and number of open arm entries (bottom) after treatment with ethanol alone (0.5 and 1.5 g/kg), ethanol in combination with CGS 8216 (0.25 mg/kg), and CGS 8216 alone compared with the control condition for both alcohol-preferring and alcohol-nonpreferring rat lines. In alcohol-preferring rats, significant treatment condition effects were seen on the time spent on open arms and number of open arm entries parameters ($F(5, 65) = 3.70$, $P < 0.008$, and $F(5, 65) = 3.01$, $P < 0.02$, respectively). Post hoc analysis showed that the 0.5 g/kg ethanol dose produced significant anxiolytic effects on both parameters ($P < 0.01$), while anxiolytic effects were seen with the 1.5 g/kg dose on the open arm parameter only when given in combination with the CGS 8216 treatment ($P < 0.01$). Given alone, CGS 8216 appeared to produce mild anxiolytic effects in the alcohol preferring rat, as indicated by a significant increase in the time spent on the open arms ($P < 0.01$).

Table 1 also shows data for alcohol-nonpreferring rats on the two anxiolytic parameters. Significant treatment condition effects were seen on the time spent on open arms and number of open arm entries parameters ($F(5, 65) = 3.76$, $P < 0.005$, and $F(5, 65) = 3.11$, $P < 0.02$, respectively). Post hoc analysis showed that both doses of ethanol (0.5 and 1.5 g/kg) significantly reduced time on the open arms ($P \leq 0.05$), while only the highest dose reduced the number of arm entries ($P < 0.05$). CGS 8216 attenuated the suppressant effects of the 1.5 g/kg ethanol dose on the number of open arms entries parameter ($P < 0.05$). When given alone, no significant effects were seen with CGS 8216 in alcohol-nonpreferring rats.

3.2.2. Effects of ZK 93426 alone, and in combination with ethanol in alcohol-preferring and -nonpreferring rats in the plus-maze

Table 1 shows data for alcohol-preferring and alcohol-nonpreferring rats on the two anxiolytic parameters after treatment with ethanol alone (0.5 and 1.5 g/kg), ethanol in combination with ZK 93426 (4 mg/kg), and ZK 93426 alone compared with the control condition. In alcohol-preferring rats, significant treatment condition effects were seen on the two anxiolytic parameters ($F(5, 65) = 4.23$, $P < 0.02$; $F(5, 65) = 3.83$, $P < 0.02$). Post hoc analysis showed that, similar to the CGS 8216 treatment, robust anxiolytic effects were seen with the 1.5 g/kg ethanol dose when given in combination with the ZK 93426 treatment on both anxiolytic parameters ($P \leq 0.05$). Given alone, ZK 93426 exerted no significant effects in alcohol

Table 1
Mean ± total time spent on the open arms and number of arm entries in the elevated plus-maze for alcohol-preferring (P) and alcohol-nonpreferring (NP) rats[a]

Treatment	Time spent on open arms (sec)		Number of entries in open arms	
	P	NP	P	NP
Tween-80 vehicle	8.3 ± 2.0	88.2 ± 16.5	1.6 ± 0.5	6.3 ± 0.7
0.5 g/kg EtOH	30.3 ± 8.4*	50.6 ± 10.4*	4.8 ± 1.1*	5.2 ± 0.7
1.5 g/kg EtOH	8.2 ± 1.0	51.5 ± 8.9*	1.5 ± 1.2	3.8 ± 0.7*
0.25 mg/kg CGS 8216 + 0.5 g/kg EtOH	19.8 ± 5.9	30.4 ± 8.1	3.2 ± 1.2	3.2 ± 0.5
0.25 mg/kg CGS 8216 + 1.5 g/kg EtOH	62.4 ± 12.2**	60.4 ± 17.2	3.7 ± 1.2	6.2 ± 1.1**
0.25 mg/kg CGS 8216	21.2 ± 3.4*	59.6 ± 17.4	2.5 ± 0.7	4.2 ± 1.3
4 mg/kg ZK 93426 + 0.5 g/kg EtOH	23.5 ± 5.6	22.4 ± 6.4**	2.7 ± 0.4**	2.1 ± 0.6**
4 mg/kg ZK 93426 + 1.5 g/kg EtOH	51.5 ± 9.2**	24.3 ± 7.2**	3.7 ± 0.8**	2 ± 0.3
4 mg/kg ZK 93426	14.3 ± 3.6	101 ± 12.4	2.1 ± 0.7	6.8 ± 0.5

* $P < 0.05$ compared with Tween-80 vehicle.
** $P < 0.05$ compared with the respective EtOH condition.
[a]All animals received their treatment conditions in a randomized design to control for order and sequence effects. All antagonists (CGS 8216 or ZK 93426) were given 20 min prior to the ethanol injections (0.5 or 1.5 g/kg). Five min after the ethanol injections, animals were placed in the plus maze for a 5 min test session ($N = 10$ rats/line).

preferring rats. In alcohol-nonpreferring rats, significant treatment condition effects were found for the time spent on the open arms and the number of open arm entry parameters ($F(5, 65) = 8.96$, $P < 0.001$, $F(5, 65) = 6.02$, $P < 0.001$). ZK 93426 potentiated the sedative effects of the ethanol doses (0.5 and 1.5 g/kg) on both anxiolytic parameters ($P \leq 0.05$). Similar to the findings with alcohol-preferring rats, ZK 93426 produced no effects in alcohol-nonpreferring rats when given alone.

3.2.3. Effects of CGS 8216 and ZK 93426 on the sedative effects of EtOH in alcohol-preferring and -nonpreferring rats in the open field

Experiment 2 showed that in the plus-maze test CGS 8216 and ZK 93426 were capable of producing antagonistic/agonistic effects in combination with ethanol, however the effects were dependent on both the rat line and ethanol dose. Further, no intrinsic effects were seen in the plus maze test with the selected doses of CGS 8216 or ZK 93426. Experiment 3 was conducted to determine: (1) the extent to which ethanol preference correlates with sensitivity of CGS 8216 and ZK 93426 to modulate ethanol's sedative properties, and (2) whether CGS 8216 and ZK 93426 would manifest intrinsic effects in the open field.

3.2.4. Effects of CGS 8216 alone, and in combination with ethanol in alcohol-preferring and-nonpreferring rats in the open field

Fig. 5a shows locomotor activity (total distance in cm) for alcohol-preferring rats on days 1 and 2 after ethanol was given alone (0.5 and 1.5 g/kg), and in combination

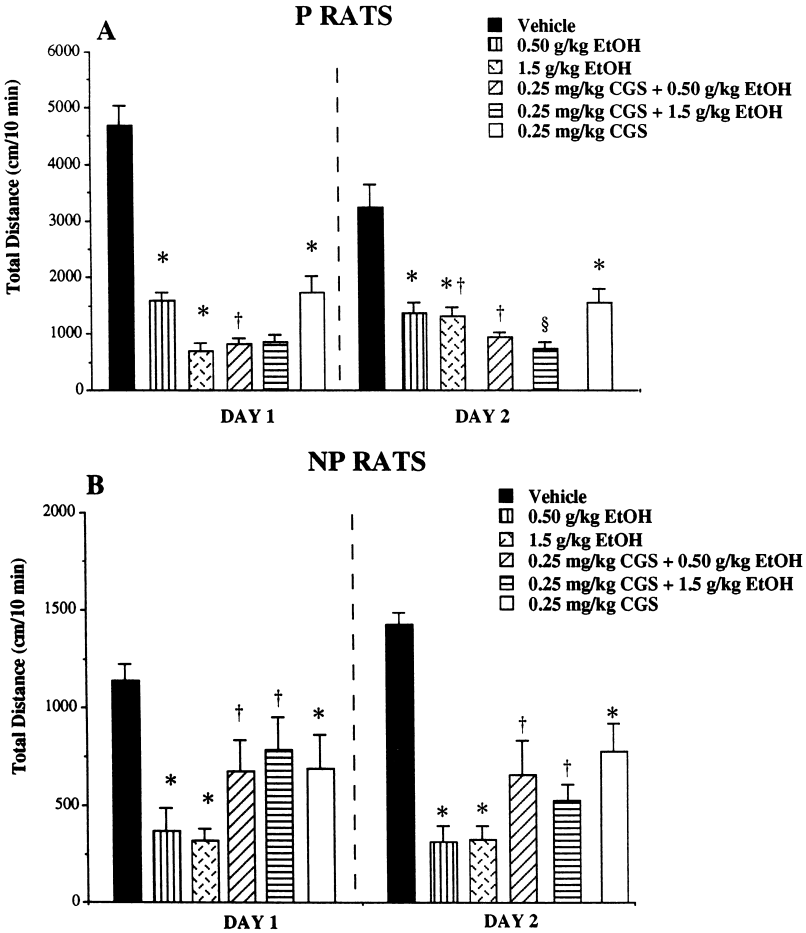

Fig. 5. Effects of ethanol alone (0.5 and 1.5 g/kg), and in combination with a CGS 8216 (0.25 mg/kg) on locomotor activity (total distance) compared with the control condition (Tween-80 vehicle) in the open field for alcohol-preferring (A) and alcohol-nonpreferring rats (B) on days 1 and 2. CGS 8216 was administered 20 min prior to the 10 min activity session only on day 1. Marked differences were observed in the magnitude of activity in the alcohol-preferring compared with the alcohol-nonpreferring rat. CGS 8216 exhibited potent intrinsic effects (i.e., suppression of activity) on day 1 and 24 h after its administration (day 2) in both rat lines. Differential effects, however, were observed with CGS 8216 and ethanol in alcohol-preferring and alcohol-nonpreferring rats. For example, potentiation was seen in alcohol-preferring rats, while antagonism was seen with alcohol-nonpreferring rats. $^{*}P \leq 0.05$ indicates significant suppression compared with the control condition in both rat lines. $^{\dagger}P \leq 0.05$ indicates significant potentiation of the low dose ethanol (0.5 g/kg) suppression by CGS 8216 (0.25 mg/kg) in alcohol-preferring rats, or significant antagonism in alcohol-nonpreferring rats. $^{*\dagger}P \leq 0.05$ indicates tolerance to the locomotor suppressant effects (i.e., increased locomotion) induced by the 1.5 g/kg ethanol condition on day 2 compared with day 1 in alcohol-preferring rats. $^{\S}P \leq 0.05$ indicates blockade of tolerance (i.e., reduced locomotor activation) by CGS 8216 (0.25 mg/kg) + ethanol (1.5 g/kg) on day 2 compared with ethanol (1.5 g/kg) alone on day 2. All post-hoc tests were based on the Newman–Keuls test following the ANOVA as indicated in Section 2.

with CGS 8216 (0.25 mg/kg) for comparison with the CGS 8216 alone and control conditions. A significant treatment condition × day interaction emerged from these data ($F(5, 65) = 7.01$, $P < 0.001$). Post hoc analyses revealed that all treatment conditions markedly suppressed activity compared with the control condition on days 1 and 2 ($P \leq 0.01$). Moreover, the 0.25 mg/kg CGS 8216 dose enhanced the sedative effects of the 0.5 g/kg dose of ethanol on day 1 and 24 h post drug administration ($P \leq 0.05$). As expected (Deumler et al., 1994), alcohol-preferring rats showed tolerance to the depressant effects of the 1.5 g/kg ethanol dose on day 2 (i.e. activity counts during the 10 min trial increased from 524 cm on day 1 to 1167 cm on day 2); the tolerance effect, however, was antagonized by the CGS 8216 treatment given on day 1 ($P < 0.05$). When given alone, the 0.25 g/kg CGS 8216 treatment produced potent intrinsic effects (i.e. reduction of activity) on day 1 and 24 h post-drug administration ($P \leq 0.01$).

Fig. 5b shows total distance for alcohol-nonpreferring rats following administration of the various drug treatment conditions on days 1 and 2, respectively. A significant treatment condition × day interaction was observed ($F(5, 65) = 7.01$, $P < 0.001$). Post hoc analysis showed that unlike the findings in alcohol-preferring rats, on day 1 CGS 8126 significantly attenuated the sedative effects of the 0.5 and 1.5 g/kg ethanol doses in alcohol-nonpreferring rats ($P \leq 0.05$). Moreover, attenuation of the sedative effects persisted 24 h post-drug administration, although only a marginal attenuation was seen with the 1.5 g/kg ethanol dose on day 2 ($P < 0.054$). When given alone to alcohol-nonpreferring rats, CGS 8216 exerted potent intrinsic effects (i.e. reduced activity), with the reduction persisting 24 h post drug administration ($P < 0.01$). Unlike the findings with alcohol preferring rats, no tolerance effects were seen in alcohol-nonpreferring rats on day 2 with either ethanol dose.

3.2.5. Effects of ZK 93426 alone, and in combination with ethanol in alcohol-preferring and alcohol-nonpreferring rats in the open field

Fig. 6a shows activity data for alcohol-preferring rats following ZK 93426 alone, and in combination with the ethanol treatments (0.5 and 1.5 g/kg) compared with the control and ethanol data reiterated from Fig. 5a. A significant treatment condition effect emerged from these data ($F(5, 65) = 25.08$, $P < 0.001$). ZK 93426 did not alter the suppressant effects of ethanol in alcohol-preferring rats ($P > 0.05$). Given alone, ZK 93426 exerted no observable intrinsic effects in alcohol-preferring rats.

Fig. 6b shows locomotor activity data for alcohol-nonpreferring rats, with the data for the control and ethanol treatments reiterated from Fig. 5b, together with the new data for the ethanol in combination with ZK 93426 (4 mg/kg), and ZK 93426 alone conditions. Significant treatment condition effects were seen on the total distance

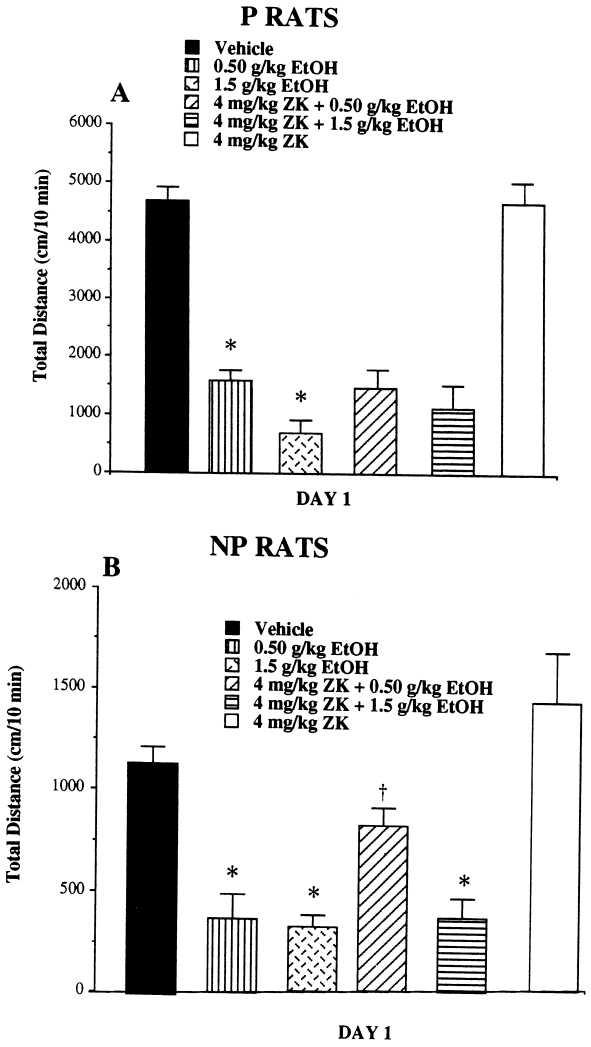

Fig. 6. Effects of ethanol alone (0.5 and 1.5 g/kg) reiterated from Fig. 5a–b in combination with a ZK 93426 (4 mg/kg) on locomotor activity (total distance) compared with the control condition (Tween-80 vehicle) in the open field for alcohol-preferring (A) and alcohol-nonpreferring rats (B) on day 1. Unlike CGS 8216 no effects of ZK 93426 were seen on day 2. ZK 93426 was administered 20 min prior to the 10 min activity session. ZK 93426 exhibited no intrinsic effects alone in the open field. Differential effects were observed with ZK 93426 and ethanol in alcohol-preferring and alcohol-nonpreferring rats. For example, no effects were seen in alcohol-preferring rats, while antagonism was seen with alcohol-nonpreferring rats at the 0.5 mg/kg dose level. $^{*}P \leq 0.05$ indicates significant suppression compared with the control condition in both rat lines. $^{\dagger}P \leq 0.05$ indicates significant antagonism of the low dose ethanol (0.5 g/kg) suppression by ZK 93426 (4 mg/kg) in alcohol-nonpreferring rats. All post-hoc tests were based on the Newman–Keuls test following the ANOVA as indicated in Section 2.

measure ($F(5, 65) = 9.90$, $P < 0.001$). ZK 93426 markedly attenuated the sedative effects of the 0.5 g/kg ethanol dose ($P < 0.01$), but was without effect on the 1.5 g/kg ethanol dose ($P > 0.05$). As with alcohol-preferring rats, ZK 93426 alone exerted no observable intrinsic effects compared with the control condition in alcohol-nonpreferring rats.

4. Discussion

CGS 8216 and ZK 93426 produced clear dose-dependent suppression of ethanol drinking behavior. These findings are in agreement with our previous research (June et al., 1994) showing that the imidazobenzodiazepine receptor antagonist, flumazenil (16 mg/kg) reduces ethanol intake in outbred rats. The data of the present study are also in agreement with a recent report from our laboratory demonstrating that ZK 93426 and CGS 8216 attenuated ethanol-maintained responding in alcohol-preferring rats (June et al., submitted). In the current and the previous study, CGS 8216 produced prolonged suppression of ethanol self-administration 24 h post-drug administration.

CGS 8216 and ZK 93426 generally produced no effect, or increased saccharin drinking over the 4 h period when ethanol drinking decreased on day 1 (Figs. 1 and 4). However, all CGS 8216 doses reduced feeding on day 2. The reduction in food intake on day 2 by CGS 8216 may be related to ethanol's reported capacity to increase the inverse agonist properties of negative GABAergic modulators following repeated ethanol exposure (Harris et al., 1988; Buck and Harris, 1990). CGS 8216 has also been reported to reduce food and saccharin intake in outbred rats (for review see Cooper et al., 1987, 1989; also see Higgs and Cooper, 1995). Unfortunately, in the previous studies (Cooper and Moores, 1985; Cooper et al., 1989; also see Higgs and Cooper, 1995) outbred rats did not concurrently consume ethanol along with palatable foods (i.e. condensed milk, saccharin solution, Cooper et al., 1989); hence, these previous studies may not be directly comparable with the present study, and previous studies of p rats (June et al., 1995a, June et al., 1996). Nevertheless, because CGS 8216 nonselectively reduced ethanol intake on day 2, antagonism of ethanol intake via its inverse agonist properties (File et al., 1982; File, 1983; File and Lister, 1983) cannot be ruled out.

As previously reported (Stewart et al., 1993), alcohol-preferring rats, were innately anxious (i.e., spent less time on the open arms) compared with alcohol-nonpreferring rats (Table 1). ZK 93426 (4 mg/kg) and CGS 8216 (0.25 mg/kg) did not attenuate the potent anxiolytic effects of the 0.5 g/kg ethanol dose (Stewart et al., 1993) in alcohol-preferring rats. However, ZK 93426 and CGS 8216 'unmasked/potentiated' the anxiolytic effects of the 1.5 g/kg ethanol dose above and beyond the 0.50 g/kg dose condition. Given alone, ZK 93426 and CGS 8216 appeared to have weak anxiolytic effects in the alcohol-preferring rats.

A different profile of effects emerged for the interaction of ethanol with CGS 8216 and ZK 93426 in alcohol-nonpreferring rats compared with alcohol-preferring rats in the plus-maze test (Table 1). ZK 93426 markedly potentiated the suppressant effect of both ethanol doses in alcohol-nonpreferring rats, while CGS 8216 potentiated the suppression of the 0.5 g/kg ethanol dose. In contrast, CGS 8216 attenuated the suppression of the 1.5 g/kg ethanol dose. That ZK 93426 augmented the suppression of a low (0.5 g/kg) and a moderate (1.5 g/kg) ethanol dose on two anxiolytic parameters in alcohol-nonpreferring rats is consistent with previous findings that ZK 93426 produces weak agonist effects in rodents (Jensen et al., 1984) and humans (Duka et al., 1987; Duka et al., 1988; Duka and Dorow, 1995). In contrast, CGS 8216 has been classified as a weak inverse agonist (File et al., 1982; File, 1983; File and Lister, 1983; Jensen et al., 1984; Jensen et al., 1986; Cooper et al., 1987), however, early neurochemical studies examining the intrinsic actions of CGS 8216 produced equivocal results (Czernik et al., 1982; Skolnick et al., 1982; Morelli et al., 1982; Brown and Martin, 1984; Wood et al., 1984).

Consistent with previous reports (Waller et al., 1986; June and Lewis, 1991), selected alcohol-preferring rats were substantially more active in the open field compared with alcohol-nonpreferring rats (distance travelled $= 4500$ versus 1100 cm/10 min). On day 1, CGS 8216 potentiated the sedative effects of the 0.5 g/kg ethanol dose in alcohol-preferring rats, but did not affect the 1.5 g/kg dose. In contrast, CGS 8216 antagonized the sedative effects of both ethanol doses in alcohol-nonpreferring rats on day 1. Moreover, in both rat lines the agonist and antagonist effects persisted 24 h post-drug administration (see Fig. 5a and b). Further, CGS 8216 prevented tolerance development to the sedative effects of the 1.5 g/kg dose in alcohol-preferring rats. This latter finding is in agreement with a previous report showing that the potent benzodiazepine inverse agonist RO19-4603 prevents tolerance development to the ethanol suppressant effects 24 h post-drug administration in outbred rats (June et al., 1995b). The findings of the present study in alcohol-nonpreferring rats are also consistent with those recently reported by Kotlinska and Langwinski (1995). These researchers demonstrated that CGS 8216 (2.5–10 mg/kg) reversed the hypnotic effects (sleep time) and locomotor depressant effects in mice and rats. In either case, no intrinsic effects of CGS-8216 were observed. Thus, given our previous data in outbred rats, and the recent work by Kotlinska and Langwinski (1995), high and low ethanol consuming rats appear highly sensitive to the intrinsic actions of negative GABAergic modulators in the open field.

CGS 8216's capacity to produce prolonged attenuation of ethanol's actions may be due to both pharmacodynamic and pharmacokinetic differences. Compared with ZK 93426, CGS 8216 has a slightly longer half life (30 min versus 1 h), and dissociates much slower from central benzodiazepine receptors (Czernik et al., 1982; Jensen et al., 1984; also see Lister et al., 1984; Jedrychowski et al., 1986). β-carbolines such as ZK 93426 are rapidly metabolized by esterase enzymes (Oakley and Jones, 1980). Thus, based on the reported half life for CGS 8216, approximately $< 1\%$ of the initial dose might remain in plasma/brain 24 h post-drug administration. Thus, is pos-

sible that these minute levels of CGS 8216 could result in sustained occupancy of BDZ receptors to produce prolonged antagonism of ethanol's behavioral effects.

Finally, it is possible that the differential effects seen with CGS-8216, and ZK 93426 may be due to differential interactions of these compounds at the diazepam insensitive receptor (Turner et al., 1991; Wong and Skolnick, 1992; Korpi et al., 1992; Yang et al., 1995; Gunnersen et al., 1996). Several reports have suggested that the $\alpha 4$ and $\alpha 6$ containing $GABA_A$ receptors might play a role in the behavioral actions of ethanol (Turner et al., 1991; Korpi et al., 1993; Kotlinska and Langwinski, 1995; Gunnersen et al., 1996; June et al., 1997).

In summary, the present study demonstrated that the benzodiazepine receptor antagonists ZK 93426 and CGS 8216, are capable of modulating the behavioral actions of ethanol, however, CGS 8216 produces more potent and long-lasting effects on ethanol's actions. The type of modulatory interaction with ethanol (i.e. agonist or antagonist), however, was highly dependent on the behavioral parameter as well as the rat line under investigation. That benzodiazepine antagonists differentially modify the anxiolytic and sedative properties of ethanol depending on the rat line could suggest the possibility that innate GABAergic differences exist between rats selectively bred for low and high ethanol preference. The degree to which CGS 8216 antagonized the actions of ethanol via inverse agonist properties is not entirely clear in the present study. Nevertheless, the findings may indicate the potential for development of 'less toxic benzodiazepine receptor ligands' with high affinity and selectively for central benzodiazepine receptors which are capable of decreasing alcohol abuse and alcoholism in humans.

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