High-Affinity Benzodiazepine Antagonists Reduce Responding Maintained by Ethanol Presentation in Ethanol-Preferring Rats

HARRY L. JUNE, DANA ZUCCARELLI, LUCAS TORRES, KAREN S. CRAIG, JANA DELONG, ANGELA ALLEN, MISTY R. BRAUN, CHARITY R. CASON and JAMES M. MURPHY

Department of Psychology (H.L.J., D.Z., L.T., K.S.C, J.D., A.A., M.R.B., C.R.C, J.M.M), Purdue School of Science, and Institute of Psychiatric Research and Program in Medical Neurobiology (H.L.J, J.M.M), Department of Psychiatry, Indiana University School of Medicine, Indiana University-Purdue University, Indianapolis, Indianapolis, Indiana

Accepted for publication November 28, 1997 This paper is available online at http://www.jpet.org

ABSTRACT

In the present study, we examined two high-affinity and selective benzodiazepine (BDZ) receptor antagonists (ZK 93426, CGS 8216) in ethanol (EtOH)-preferring rats whose responding (i.e., lever pressing) was maintained by the presentation of EtOH. The in vivo actions of CGS 8216 (1–30 mg/kg) and ZK 93426 (5–75 mg/kg) were examined after intraperitoneal or oral administration. Flumazenil (10–40 mg/kg) was used as a reference BDZ antagonist. EtOH (10% v/v) and saccharin (0.05 g/v) solutions were concurrently available for 30 min each day under a two-lever fixed-ratio schedule in which four responses on one lever produced the EtOH solution and four responses on the other lever produced the saccharin solution. A 40 mg/kg intraperitoneal injection of flumazenil given on the first injection day (day 1) nonsignificantly reduced control levels of responding maintained by EtOH by 36%. No effects were observed 24 hr after drug administration (day 2). Oral administration of flumazenil was without effect. On day 1, intraperitoneal administration of CGS 8216 (1–20 mg/kg) and ZK 93426 (1–50 mg/kg) reduced control levels of responding maintained by EtOH by 44% to 73%; on day 2, EtOH maintained responding continued to be suppressed with the highest doses (>20 mg/kg) suppressing control levels of responding by as much as 82%. Oral administration of higher doses of CGS 8216 (5–30 mg/kg) and ZK 93426 (10–75 mg/kg) reduced responding maintained by EtOH by as much as 54% to 84% of controls; however, no effects were seen on day 2. Only the highest intraperitoneal dose of ZK 93426 (50 mg/kg) suppressed responding maintained by saccharin. These findings demonstrate that some BDZ antagonists decrease responding maintained by EtOH. The findings suggest that certain BDZ antagonists may have potential as pharmacotherapies to prevent or decrease EtOH abuse in humans.

EtOH shares many properties with the BDZs and barbiturates, including sedation, ataxia and antianxiety. At the neurochemical level, EtOH, BDZs and barbiturates potentiate GABA-stimulated Cl⁻ flux (Morrow et al., 1991). Therefore, it has been postulated that the action of EtOH at the level of the GABA-coupled Cl⁻ ion channel may underlie many of the behavioral properties of EtOH (Suzdak et al., 1986; Harris et al., 1988; Mehta and Ticku, 1988). More recently, several groups used molecular biological approaches to study the binding domains for BDZs at the GABAₐ receptor and identified important structural features for ligand recognition and modulatory action that may have important implications in the elucidation of the neuromechanism or neuromechanisms mediating some of the behavioral actions of EtOH (Morrow et al., 1991; Turner et al., 1991; Korpi, 1994; Mihindukulasuriya and Harris, 1996).

There also is evidence from our laboratory (June et al., 1994, 1995, 1996a, in press a) as well as from others (Rassnick et al., 1993; Hyytia and Koob, 1995; Hodge et al., 1995, 1996) to suggest that the GABAₐ-BDZ receptor complex plays a prominent role in the reinforcing properties of EtOH. Specifically, it has been demonstrated that BDZ inverse agonists (e.g., RO15–4513, RO19–4603) (for a review, see Wegelius et al., 1994; June et al., 1995, 1996b, in press a) and GABAₐ antagonists (e.g., bicuculline, SR 95531) (Hyytia and Koob, 1995; Hodge et al., 1995) are potent blockers of EtOH intake and operant responding maintained by EtOH. Unfortunately, many of these agents exhibit a proconvulsant-convulsant profile, and this may preclude their use in

ABBREVIATIONS: EtOH, ethanol; BDZ, benzodiazepine; GABA, γ-aminobutyric acid; GABAₐ, γ-aminobutyric acid type A; P, ethanol-preferring rats; FR, fixed-ratio; ANOVA, analysis of variance; i.p., intraperitoneal; BAC, blood alcohol concentration.
human subjects in their existing molecular forms. Other types of BDZ ligands that may have potential as pharmacotherapies for alcohol abuse are the BDZ antagonists. In general, BDZ antagonists exhibit little if any toxic effects in both animals (Bonetti et al., 1981; Czernik et al., 1982; Jensen et al., 1984, Gardner, 1992) and humans (Dutton et al., 1988; Duka et al., 1987, 1988; Duka and Dorow, 1995; Reimann et al., 1987).

Several studies have examined the BDZ antagonists in combination with EtOH. For example, a 16 mg/kg dose of flumazenil has been shown to attenuate EtOH drinking in outbred rats (June et al., 1994), whereas lower doses (<10 mg/kg) did not attenuate drinking in fluid-deprived EtOH-prefering (P), nonpreferring (NP) (McBride et al., 1988) or outbred rats (Beamann et al., 1984). Flumazenil does not block the effects of EtOH on punished responding in some studies (Koob et al., 1986), whereas it potentiates the rate-increasing effects of EtOH on punished responding in others (Barrett et al., 1985). Moreover, ZK 93426, CGS 8216 and flumazenil have been shown to antagonize the locomotor effects of EtOH in rodents (Lister, 1988; Kotlinska and Langwinski, 1995).

Studies in human subjects indicate that flumazenil has amnestic effects in EtOH-intoxicated subjects (Scollo-Lavizzari and Matthys, 1985); however, another study reported that flumazenil did not alter EtOH sedation using visual-analog measures and a reaction time task (Klotz et al., 1986).

The objective of the present study was to examine two high-affinity and selective BDZ receptor antagonists (ZK 93426, CGS 8216) in rats whose responding was maintained by the presentation of EtOH or saccharin. The relative potency of the antagonists was assessed by generating dose-response curves over a broad range of doses, and the duration of effect was examined over a 2-day period. To examine the specificity of the agents on ingestive behaviors, a palatable saccharin reinforcer (0.05% g/v) was presented along with the EtOH solution in both the operant chamber and the rat’s home cage. Food intake also was measured in the home cage. The prototypical BDZ antagonist RO15–1788 (flumazenil) was used as a reference compound. Second, the contribution of route of drug administration was examined. The test agents were administered intraperitoneally or orally (gavage). All of the antagonists used in the present study have been examined in humans (for a review, see Haefely, 1983; Reimann et al., 1987; Duka and Dorow, 1995) and little if any untoward effects have been reported.

Materials and Methods

Animals

Male selectively bred, EtOH-prefering (P) rats (n = 20) from the S38 and S39 generations (Lumeng et al., 1995) were used in the present study. The rats were approximately 4 to 5 months old and weighed 287 to 390 g at the beginning of the experiment. Animals were individually housed in wire-mesh stainless steel cages at an ambient temperature of 21°C on a normal light cycle. All rats were provided ad libitum access to food and water, except for the conditions noted below in the training phase.

Drugs and Solutions

The EtOH (10% v/v) and saccharin (0.05% g/v) solutions were prepared as previously described (June et al., 1995, 1996b). All intraperitoneally and orally (gavaged) administered drugs were prepared as an emulsion in 1% Tween-80 vehicle (Sigma Chemical, St. Louis, MO). When given i.p., drugs were mixed with a 0.90% sodium chloride solution to a fixed volume (for more details, see June et al., 1996b). When given orally, drugs were dissolved in deionized water. All of the drugs were sonicated. RO15–1788 (flumazenil) was donated from Hoffman-La Roche (Basel, Switzerland). The BDZ antagonists ZK 93426 (Schering, Berlin, FRG) and CGS 8216 (Ciba-Geigy, Summit, NJ) were also donated. All compounds were examined across a broad dose range: flumazenil, 10 to 40 mg/kg; ZK 93426, 5 to 75 mg/kg; and CGS 8216, 1 to 30 mg/kg. The doses were based on a series of two-bottle EtOH preference studies in our laboratory (for an extensive review, see June et al., 1996b).

Experiment 1: Effects of Flumazenil, CGS 8216 and ZK 93426 on EtOH and Saccharin Intake

The effects of low to moderate doses (0.05–10 mg/kg) of flumazenil, CGS 8216 and ZK 93426 on EtOH intake in P rats have been examined previously (June et al., 1996b, in press b). Therefore, experiment 1 examines the effects of a higher dose (40 mg/kg) of flumazenil, CGS 8216 and ZK 93426 on EtOH intake in P rats given concurrent availability to EtOH and saccharin solutions. In addition, experiment 1 examines the effects of flumazenil, CGS 8216 and ZK 93426 on concurrent food consumption.

EtOH and saccharin acclimation phase. To equalize the intake of saccharin and EtOH (10% v/v), descending concentrations of saccharin (0.05%, 0.025%, 0.0125%) were made available along with an EtOH solution (10% v/v) for 4 hr daily until approximately equal EtOH and saccharin intakes were obtained. Fluid consumption was measured to the nearest 0.5 ml at 15, 30, 60, 120, 180 and 240 min of each 4-hr session. Animals were provided only water during the remaining 20 hr. After determining the saccharin concentration (e.g., 0.0125% w/v) that yielded intake levels approximately equal to that of EtOH intake, rats remained on this two-bottle daily 4-hr limited access to EtOH and saccharin throughout the remainder of the experimental protocol (see June et al., 1996b).

Data analysis. Data were analyzed by repeated measures ANOVA with drug treatment, consumption day (days 1 and 2) and consumption interval (15, 30, 60, 120, 180 and 240) as the independent factors. Similar three-way analyses were also performed for the total measurement (0–240 min) period. For all experiments, data for EtOH and saccharin intakes were analyzed separately. Post-hoc comparisons between individual drug treatments were made using the Newman-Keuls test. Although data were analyzed for the six consumption intervals, only the initial 15-min and total measurement periods for EtOH and saccharin are presented below.

Experiment 2: Effects of Flumazenil, CGS 8216 and ZK93426 on EtOH or Saccharin Maintained Responding

Behavioral training. Rats were trained to lever press for EtOH using a modification of the sucrose fading technique (Samson, 1986, 1987); the only exception was that saccharin was used instead of sucrose. To facilitate shaping, animals were water-deprived for 16 hr/day for the first 2 days of the training period. Rats were initially trained to lever press under an FR1 schedule, then subsequently an FR4 schedule. Under the FR1 schedule, each lever press produced a 0.1 ml solution of saccharin. Under the FR4 schedule, every fourth lever press produced a 0.1 ml solution of saccharin. A fading procedure was used to introduce EtOH into the solution. At the beginning of the fading procedure, responding on either of the two levers produced a 0.0125% (g/v) saccharin solution on an FR1 schedule. On day 6, rats were trained to respond on one of two levers for an EtOH (2% v/v) plus saccharin (0.1% g/v) cocktail. For example, a 100-ml solution of the cocktail comprised 20 ml of the EtOH (2% v/v) solution plus 80 ml of saccharin (0.1% g/v) solution. Responses on the other lever produced the same quantity of water.
The EtOH concentration was then gradually increased stepwise to 10% (v/v) over the next 5 days (i.e., 5%, 7%, 9%, 10%, 10%), whereas saccharin decreased (0.1%, 0.075%, 0.05%, 0.0125%, 0.0%). Responding was maintained under the FR1 schedule for 3 weeks, and then the concurrent schedule was introduced. Under the concurrent schedule, responding was maintained under an FR4-FR4 schedule of EtOH (10% v/v) presentation on one lever and a saccharin (0.05% g/v) presentation on the second. Responding was considered stable when responses were ≥20% of the average responses for 5 consecutive days. To acclimate the animals to the injection schedule, intermittent injections of saline (0.9%) and Tween-80 vehicle (1%) were given during this period. After stabilisation on the FR4-FR4 schedule for 3 weeks, the drug treatment phase began. Tail blood (100 ml) was taken after the session and evaluated using previously published procedures (Lumeng et al., 1982). BACs were determined on days when no drug treatments were administered.

Treatment phase. Experiment 2a determined dose-response and time course (days 1 and 2) effects of i.p. flumazenil, CGS 8216 and ZK 93426 on EtOH (10% v/v) and saccharin (0.05% g/v) maintained responding. In the first set of investigations, rats received either vehicle or one of the three antagonists 20 min before the experimental session. After a 2-week period in which no injections were given, flumazenil, CGS 8216 and ZK 93426 were examined again via the oral route (experiment 2b). Under these conditions, flumazenil, CGS 8216 and ZK 93426 were administered 30 min before the operant sessions via a stainless-steel feeding tube (i.e., gavaged). Antagonists were administered only on day 1 in experiments 2a and 2b. To control for carryover effects, subsequent pretreatments were not administered until both EtOH and saccharin responding had returned to their predrug baseline levels for at least 4 days, with a minimum of 4 days between all drug treatments.

Data analysis. In experiments 2a and 2b, data were analyzed by repeated measures ANOVA with drug treatment (dose) and EtOH/saccharin as the independent factors. Days 1 and 2 were analyzed for at least 4 days, with a minimum of 4 days between all drug treatments.

Results

BAC Determination

Body weights of the 10 rats used for BACs ranged from 514 to 700 g, and rates of responding maintained by 10% (v/v) EtOH ranged from 140 to 424 responses/30 min. BACs ranged from 0 to 43.8 mg/kg%. The responding for EtOH yielded intakes of 0.41 to 1.5 g of absolute EtOH/kg. The BACs correlated significantly with EtOH responding (r = .64, P < .001) and EtOH intake (g/kg) (r = .66, P < .001).

Experiment 1: i.p. Administration of CGS 8216, ZK 93426 and Flumazenil

EtOH intake. After Tween-80 vehicle injections, animals averaged 5.5 ± 0.4 and 12.6 ± 0.9 ml of the 10% (v/v) EtOH solution during the initial 15-min and total 4-hr measurement periods, respectively (fig. 1). Analysis of the six consumption intervals yielded a significant treatment condition × day × consumption interval interaction [F(15, 135) = 2.89, P < .006]. Pairwise comparisons indicated that EtOH intake was significantly attenuated at the initial 15-min interval by all three BDZ antagonists on days 1 and 2 (P ≤ .05) (fig. 1). A significant treatment condition × day × consumption interval interaction also emerged for the EtOH data at the total measurement period [F(18, 162) = 3.10, P < .001]. On day 1, ZK 93426 and CGS 8216 significantly reduced EtOH drinking, with the suppressant effects persisting 24 hr after drug administration for the CSG 8216 condition.

Saccharin intake. Animals averaged 2.1 ± 0.2 and 15.4 ± 4.0 ml of the saccharin solution during the 15-min and 4-hr access periods, respectively. Analysis of the data across the six consumption intervals yielded a significant treatment condition × day × consumption interval interaction [F(15, 135) = 4.2, P < .018]. At the 0- to 15-min intervals, only nonsignificant increases were observed for the drug treatments (fig. 2). In contrast, for the total measurement period (0-240 min) a significant treatment condition × day × consumption interaction was found [F(18, 162) = 8.54, P < .001]. Pairwise comparisons showed that on day 1, flumazenil and ZK 93426 markedly elevated saccharin drinking (P < .01). On day 2, CGS 8216 also enhanced saccharin drinking; however, the effects did not reach statistical significance (fig. 2) (P < .05).

Food intake. The 40-mg/kg dose of flumazenil, CGS 8216 and ZK 93426 given 1 hr before the drinking session did not alter food intake [F(3, 30) = 1.12, P > .378] (table 1).
Experiment 2a: i.p. Administration of CGS 8216, ZK 93426 and Flumazenil

Effects of CGS 8216 on responding maintained by EtOH and saccharin (days 1 and 2). Figure 3 shows rates of responding on days 1 and 2, respectively, after i.p. injections of CGS 8216 (1–20 mg/kg). A significant treatment condition × consumption type interaction emerged from the data for days 1 and 2 [F(4, 36) = 3.58, P < .012; F(4, 36) = 2.60, P < .05], respectively. Post-hoc analyses showed that on day 1, CGS 8216 (5–20 mg/kg) significantly attenuated responding maintained by EtOH (P ≤ .05); however, the effects were not dose related. The 20-mg/kg dose reduced responding by as much as 76% of control. On day 2, the 20-mg/kg dose continued to suppress responding maintained by EtOH, with the magnitude of suppression being similar to that of day 1. Responding maintained by saccharin was not significantly altered after CGS 8216 administration on day 1 or 2, except for a significant elevation after the 1-mg/kg dose on day 1 (P < .05).

Effects of ZK 93426 on responding maintained by EtOH and saccharin (days 1 and 2). Figure 4 shows rates of responding maintained by EtOH and saccharin on days 1 and 2, respectively, after i.p. injections of ZK 93426 (5–50 mg/kg). A significant treatment condition × consumption type interaction emerged from the data for days 1 and 2 [F(4, 36) = 3.58, P < .012; F(4, 36) = 2.60, P < .05], respectively. Post-hoc analyses showed that on day 1, ZK 93426 (5–20 mg/kg) significantly attenuated responding maintained by EtOH; however, significant effects were seen only with the 30- and 50-mg/kg doses (P ≤ .05). On day 2, the 50-mg/kg dose significantly attenuated responding maintained by EtOH. As shown in figure 4a, the 50-mg/kg dose of ZK 93426 suppressed responding maintained by both EtOH and saccharin on day 1 (P < .05). On day 2, the 15-mg/kg dose significantly elevated responding maintained by saccharin (P < .05).

TABLE 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mg/kg</th>
<th>Food consumption (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.0</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>Flumazenil</td>
<td>40</td>
<td>7.0 ± 1.2</td>
</tr>
<tr>
<td>CGS 8216</td>
<td>40</td>
<td>6.0 ± 0.4</td>
</tr>
<tr>
<td>ZK 93426</td>
<td>40</td>
<td>5.8 ± 0.6</td>
</tr>
</tbody>
</table>

Fig. 2. Effects of i.p. administration of 40 mg/kg of RO15–1788 (flumazenil), ZK 93426 and CGS 8216 given acutely on day 1 and 24 hr after drug administration (day 2) on intake of saccharin (0.0125% g/v) by P rats during the initial 15-min and total 4-hr consumption period. All antagonists were administered 1 hr before the 4-hr test session on day 1 only. *P < .01 vs. control vehicle values by ANOVA and post-hoc Newman-Keuls testing at a corresponding time interval (n = 10).
Effects of flumazenil on responding maintained by EtOH and saccharin (days 1 and 2).

Figure 5 shows rates of EtOH- and saccharin-maintained responding after i.p. injections of flumazenil (10–40 mg/kg). No statistically significant effects were observed after any of the doses examined. At 20 mg/kg, flumazenil elevated responding maintained by EtOH, but these increases were not significant. Flumazenil did not alter responding on day 2 (data not shown).

Experiment 2b: Oral Administration of CGS 8216, ZK 93426 and Flumazenil

Effects of CGS 8216 on responding maintained by EtOH and saccharin (day 1 only). Figure 6 shows rates of EtOH- and saccharin-maintained responding after oral administration of CGS 8216 (1–30 mg/kg). Doses of CGS 8216 markedly suppressed EtOH-maintained responding, whereas the 10-mg/kg doses produced small, nonsignificant decreases in responding. A significant drug treatment × response type interaction emerged \[F(4, 36) = 5.976, P < .001\]. Newman-Keuls post-hoc testing confirmed that the 25- to 75-mg/kg doses significantly suppressed responding maintained by EtOH (P ≤ .01), but responding maintained by saccharin was not significantly altered, even at the 75-mg/kg dose level (P > .05).

Similar to the 20-mg/kg dose of flumazenil given i.p., oral administration of flumazenil (20–40 mg/kg) did not alter operant responding for reinforcement by EtOH and saccharin (data not shown).

Cumulative Response Profiles

Figure 7 shows the cumulative EtOH and saccharin response profiles for the control condition compared with the CGS 8216 and ZK 93426 conditions after i.p. administration. Under control conditions (fig. 7, top), ≈50% of responding maintained by EtOH occurred during the initial 10-min interval, whereas most of the remaining 50% occurred during the second 10-min interval (11–20 min). Little EtOH-maintained responding occurred during the third 10-min period (21–30 min). Analysis of the saccharin data after Tween-80 vehicle injections (fig. 7, bottom) shows that 75% of responding maintained by saccharin occurred during the initial 10-min interval, whereas most of the remaining 25% occurred during the second 10-min interval (11–20 min).

Intraperitoneal CGS 8216 treatments (1–20 mg/kg) suppressed operant responding for EtOH primarily during the first few minutes of responding, with little additional suppression occurring during the latter 20-min interval (fig. 7, top left). Compared with the control condition, only mild suppression on responding maintained by saccharin was observed with the 5- to 20-mg/kg doses across the 30-min operant session (fig. 7, bottom left). With the 1-mg/kg dose, a marked elevation in responding by saccharin was seen be-
Newman-Keuls testing at a corresponding day (n = responding maintained by saccharin (fig. 8, bottom left). A similar profile occurred for interval and continued dose-dependently throughout the 30-min session (fig. 8, top left). Little if any suppression of operant responding remained suppressed thereafter (fig. 7, top right). The 5- to 30-mg/kg doses (fig. 7, bottom right). The 5- to 30-mg/kg doses (fig. 7, bottom right). The elevation persisted with flumazenil to produce prolonged attenuation of responding compared with the control condition (fig. 8, bottom right).

Discussion

The present study demonstrates that responding maintained by EtOH can be attenuated by two different types of benzodiazepine antagonists: the pyrazoloquinoline CGS 8216 and the β-carboline ZK 93426. Specifically, intraperitoneal administration of both CGS 8216 and ZK 93426 reduced EtOH-maintained responding on the day they were administered (day 1) with some reduction still apparent 24 hr after drug administration. Oral administration of CGS 8216 and ZK 93426 also reduced responding maintained by EtOH; however, these effects were not apparent on day 2. These findings are in keeping with previous reports that both antagonists are highly effective via the oral as well as the intraperitoneal route (Bernard et al., 1981; Jensen et al., 1984; Reimann et al., 1987).

An analysis of the time course of these effects revealed that CGS 8216 reduced responding maintained by EtOH throughout the experimental session. The time course was similar for the intraperitoneal and oral routes, suggesting that bioavailability at active central nervous system BDZ sites were comparable for the two routes of drug administration. An analysis of the time course of the effects of ZK 93426 revealed that the highest intraperitoneal dose of ZK 93426 (50 mg/kg) nonselectively decreased responding maintained by EtOH and saccharin. In contrast, an oral dose of 75 mg/kg of ZK 93426 suppressed responding maintained by EtOH without decreasing responding maintained by saccharin. These data suggest that the bioavailability for ZK 93426 is greater when given by the intraperitoneal route.

The findings of the present study are in agreement with a recent report from our laboratory demonstrating that ZK 93426 and CGS 8216 attenuate home cage EtOH intake (i.e., two-bottle choice) and can modulate the anxiolytic and sedative properties of EtOH (June et al., in press b). In both studies, the higher doses of CGS 8216 and ZK 93426 produced prolonged effects 24-hr after drug administration. However, the capacity of CGS 8216 and ZK 93426 compared with flumazenil to produce prolonged attenuation of responding maintained by EtOH may be related to both pharmacodynamic and pharmacokinetic differences. For example, the half-lives for CGS 8216 and ZK 93426 in rats are in the range of 30 min to 1 hr (Szczepaniak et al., 1982; Jensen et al., 1984; also see Lister et al., 1984a; Jedrychowski et al., 1986). In contrast, the half-life for flumazenil in rats is ~15 to 30 min (Lister et al., 1984b). Thus, based on the reported half-lives for CGS 8216 and ZK 93426, ~1% of the initial dose might remain in plasma/brain 24 hr after drug administration. It is possible that the remaining minute levels of the compounds could result in sustained occupancy of BDZ receptors to pro-

![Fig. 6. Dose-response of gavage (oral) administration of CGS 8216 and ZK 93426 in doses of 0 (Tween-80), 1, 5, and 30 mg/kg and 0 (Tween-80), 10, 25, 50 and 75 mg/kg, respectively, on responding maintained by EtOH (10% v/v) and saccharin (0.05% g/v) (FR4-FR4) on day 1. Oral infusions were given 30 min before the operant sessions. Neither agent altered responding maintained by EtOH and saccharin on day 2 (data not shown). **P < .01 vs. control vehicle values by ANOVA and post-hoc Newman-Keuls testing at a corresponding day (n = 10).](attachment:image.png)
duce prolonged reductions in responding maintained by EtOH.

CGS 8216 has been shown to antagonize the effects of diazepam in protecting rats against metrazole-induced seizures for 6 to 8 hr. In contrast, flumazenil antagonizes the effects of diazepam for 15 min. Although it is likely that the convulsant properties of negative GABAergic modulators and the reinforcing effects of EtOH are mediated via different neurobiological mechanisms, these data suggest that CGS 8216 can produce a very long antagonism of some GABA-mediated effects. It is important to note that the shorter time courses for both flumazenil and ZK 93426 are consistent with...
their rapid metabolism by esterase enzymes (Jackson and Nutt, 1995). Previous research (Czernik et al., 1982) also shows that CGS 8216 dissociates much slower from BDZ receptor binding sites than flumazenil (t1/2 = 53 min vs. 15 min at 0°C, respectively). This slow dissociation from central BDZ receptors is consistent with the recent demonstration of the subnanomolar affinity of CGS-8216 (e.g., 0.05–0.25 nM) at all GABA_A containing diazepam-sensitive recombinant receptors (e.g., alpha-1–3, alpha-5) (Lui et al., 1997).

It also is possible that the differential effects seen with flumazenil, CGS-8216 and ZK 93426 may be due to differential interactions of these compounds at the diazepam-insensitive receptor (Turner et al., 1991; Wisden et al., 1991; Korpi and Uusi-Oukari, 1992, Yang et al., 1995; Gunnersen et al., 1996). Several reports have suggested that the alpha-4 containing GABA_A receptors in the nucleus accumbens (see Wisden et al., 1992) might play a role in the reinforcing properties of EtOH (Cason et al., 1996; June et al., 1997, in press a).

The results of the present study with CGS 8216 on operant responding contrast with findings of Galizio et al. (1986) in an avoidance learning situation. Specifically, Galizio et al. reported that CGS 8216 (5 mg/kg) did not alter the dose-dependent decreases in avoidance responding produced by EtOH (0.5–2.0 g/kg) in rats; however, CGS 8216 alone disrupted both avoidance and time out responding. Shannon and Davis (1984) (also see Shannon and Herling, 1983) found that CGS 8216 failed to antagonize the effects of diazepam in rats responding under a schedule of food presentation. Sudzak et al. (1988) also reported that CGS 8216 (≤20 mg/kg) failed to block the intoxicating effects of high doses of EtOH. Taken together, these studies suggest that CGS 8216 does not antagonize all of the behavioral effects of EtOH, nor do flumazenil or CGS 8216 reverse all of the behavioral effects of the BDZs.

Because ZK 93426 and CGS 8216 have a safe, nontoxic profile in human subjects (for a review, see Duka and Dorow, 1995), it is possible these compounds have potential as treatments for alcohol abuse. For example, in a randomized placebo-controlled double-blind study, Reimann et al. (1987) demonstrated that a single oral dose of 650 mg/kg of CGS 8216 and subchronic doses up to 100 mg/kg/day for 7 days are well tolerated by healthy young adult volunteers. Virtually none of the subjects (n = 46) reported any anxiogenic-like effects. Further, unlike flumazenil and CGS 8216, ZK 93426 does not precipitate withdrawal in BDZ-dependent cats (Giorgi et al., 1988; Thiobet et al., 1988), suggesting a potential use for ZK 93426 and other neutral beta-carboline antagonists (e.g., BCCt) (Cox et al., 1995) in EtOH detoxification.

The half-lives for CGS 8216 and ZK 93426 in humans have been estimated to be in the range of 2 to 4 hr (Reimann et al., 1987) and 50 min to 1 hr (Duka et al., 1987), respectively. The half-life for flumazenil in humans is ≈30 min (Lister et al., 1984b). Thus, as suggested for the rats (see above), the half-lives for CGS 8216 and ZK 93426 in humans, it would be predicted that ≈<1% to 2% of the initial doses of CGS 8216 and ZK 93426 might remain in plasma/brain 24 hr after drug administration; hence, sustained occupancy of BDZ receptors might result in prolonged suppression of responding maintained by EtOH. However, it is important to note that none of the clinical studies reported here were conducted with “alcohol-dependent” subjects; furthermore, although the rats in the present study were engaging in alcohol-seeking behavior, they could hardly be considered “alcohol dependent.” Thus, the degree to which the findings of the present study might be generalized to an alcohol-dependent population is not known. It is known that subjects undergoing alcohol withdrawal will evidence heightened central nervous system excitability (for a recent review, see Metten and Crabbe, 1996). It is possible that BDZ antagonists might be an appropriate pharmacotheraphy in decreasing the subjective/euphoric properties of alcohol in alcoholics who habitually abuse but are not physically dependent on alcohol. This in turn might lead to a reduction in motivated behavior for alcohol. In contrast, subjects who are physically dependent on alcohol or manifest a hyperresponse to BDZ antagonists (e.g., increased vigilance) obviously would not be appropriate candidates for such treatments. It should be noted that BDZ antagonists also consistently produce agonist-like effects in animals and humans depending on the dose and experimental paradigm (see Duka and Dorow, 1995). Finally, it is important to note that the highest dose of CGS 8216 and ZK 93426 tested in the present study did not exceed 30 and 75 mg/kg, respectively. Therefore, it remains to be determined whether higher doses of the two antagonists would selectively antagonize EtOH maintained responding.

In summary, these findings demonstrate that some BDZ antagonists can antagonize the reinforcing properties of EtOH and suggest a direct role for the BDZ component of the GABA_A-BDZ receptor complex in EtOH-seeking behavior. The findings further indicate the potential for development of certain BDZ antagonists as pharmacotherapies to attenuate alcohol consumption in humans and provide impetus for the synthesis and development of novel BDZ antagonists (Cox et al., 1995; Zhang et al., 1995) as a possible therapeutic approach to reduce alcohol consumption associated with alcoholism.

Acknowledgments

The authors thank Drs. T.-K. Li and L. Lumeng and the Alcohol Research Center at Indiana University School of Medicine for providing the P rats. We also express our gratitude to Drs. Jerry Sepinwall and Peter Sorber (Hoffman-La Roche, Nutley, NJ/Basel, Switzerland) for their generous supply of RO15–1788 (flumazenil). Their donations and prompt efforts in providing this compound are greatly appreciated. The authors also thank Dr. David Stephens and Schering AG (Berlin, Germany) for their generous contribution of ZK 93426. Finally, we also express gratitude to Dr. Richard Lovell and the Ciba-Geigy Corporation (Summit, NJ) for the samples of CGS 8216.

References


Send reprint requests to: Harry L. June, Ph.D., Department of Psychology, LD 124, IUPUI, 402 North Blackford Street, Indianapolis, IN 46202-3275. E-mail: hjune@iupui.edu

GABAergic Synaptic Transmission