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Benzodiazepine Receptor Ligands with Different Intrinsic Efficacies Alter Ethanol Intake in Alcohol-Nonpreferring (NP) Rats

Harry L. June, Ph.D., James M. Murphy, Ph.D., Robert L. Hewitt, M.S., Terri L. Greene, B.S., Margaret Lin, B.S., Jackie J. Mellor-Burke, B.S., Lawrence Lumeng, M.D., and Ting-Kai Li, M.D.

Benzodiazepine (BDZ) receptor ligands with varying intrinsic efficacies [RO19-4603, 0.02–0.15 mg/kg; FG 7142 1–16 mg/kg; DMCM, 1–8 mg/kg; RO16–6028 (bretazenil), 8–32 mg/kg] in modulating GABAergic activity were examined for the ability to alter palatability-induced ethanol (EtOH) intake in the alcohol-nonpreferring (NP) line of rats. NP rats on a 22-hour fluid-deprivation schedule were given 2-hour daily access to a 10% (v/v) EtOH/ 3% (g/v) polycose solution and water. Average EtOH intake was 2.1 ± 0.2 g/kg/2 hours, and water intake was 17.1 ± 0.9 ml/2 hours. During the initial 15 minutes of the 2-hour session, RO19-4603, the imidazothienodiazepine partial inverse agonist reduced EtOH intake to 19% of control values at 0.04 mg/kg and completely suppressed drinking of the EtOH solution at 0.15 mg/kg. Twenty-four-hour postdrug administration, the 0.08-mg/kg dose of RO19-4603 completely suppressed drinking of the EtOH solution at the 60-minute interval, and the 0.15-mg/kg dose reduced intake to 20% of control levels at the 15-minute interval. FG 7142, the partial β-carboline inverse agonist reduced EtOH drinking at the 60-minute interval with the 1-mg/kg dose, and the 16-mg/kg dose reduced water intake at the 15-minute interval. DMCM, the full β-carboline inverse agonist, significantly reduced water intake at 15 minutes (4 and 8 mg/kg), and the same doses caused a substantial increase in EtOH drinking at the 120-minute interval. The anxiolytic agent bretazenil (16 and 32 mg/kg) increased EtOH consumption during the initial 15 minutes to 270% to 425% of control levels, and water intake increased by the end of the 2-hour session to as much as 210% of control following administration of the 32-mg/kg dose. These findings support existing evidence suggesting that BDZ receptor ligands may modify neuronal processes that mediate some reinforcing and/or aversive properties of alcohol. They further demonstrate a potential importance of the $GABA_A$-BDZ receptor complex in mediating palatability- (environmentally) induced EtOH drinking even in rats selectively bred for low alcohol preference.
[Neuropsychopharmacology 14:55–66, 1996]

KEY WORDS: *Benzodiazepine inverse agonist; Ethanol; Alcohol-nonpreferring (NP) rats; $GABA_A$-BDZ*

Identifying both the environmental and biological variables that promote and maintain high alcohol-seeking or alcohol self-administration behavior is key to understanding the disorder of alcohol abuse and alcoholism (Li et al. 1991). Selective breeding methodology has demonstrated convincingly that there is genetic influence on alcohol-seeking behavior (Li et al. 1991). Currently there are four pairs (high and low) of rat lines that differ in alcohol preference (Ericksson and Rusi 1981; Mardones and Segozia-Riquelene 1983; Li et al.

From the Department of Psychology (HLJ; JMM; RLH; TLG; JJM-B), Purdue School of Science, and Program in Medical Neurobiology (HLJ; JMM; ML), Departments of Psychiatry, Medicine (LL; T-KL), and Biochemistry (T-KL), Indiana University School of Medicine and VA Medical Center (LL), Indiana University-Purdue University, Indianapolis, IN.

Address correspondence to: Harry L. June, Ph.D., Assistant Professor of Psychology and Medical Neurobiology, Department of Psychology, LD 3124, IUPUI, 402 North Blackford Street, Indianapolis, IN 46202-3275.

Received February 18, 1994; revised October 6, 1994; accepted December 7, 1994.

Terri L. Greene is the recipient of the 1994 IUPUI Student Research Award in Psychology for excellence in psychopharmacological research.

Published 1996 by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/96/$15.00
SSDI 0893-133X(94)00128-M

1991). These pharmacogenetically different animal lines provide useful tools for investigating alcohol's neurochemical and neuropharmacological effects (McBride et al. 1992). The alcohol-preferring (P) and -nonpreferring (NP) rats are one pair of rats that have been developed through selective breeding from a Wistar stock for their divergent preference for EtOH (Lumeng et al. 1977; Li et al. 1987). With food and water ad libitum, P rats voluntarily consume at least 5 g/kg body weight/day of an unflavored EtOH solution (10% v/v), whereas NP rats consistently consume less than 1 g/kg body weight/day. Response-contingent EtOH intake without the use of sweeteners has also been demonstrated in P rats with concentrations as high as 30% (v/v) (Penn et al. 1978; Waller et al. 1984; Murphy et al. 1989); however, NP rats respond very little for alcohol concentrations exceeding 5% (v/v) (Murphy et al. 1989), unless EtOH initiation procedures are employed (Samson et al. 1989a). Thus, rats of the NP line may be used as a model for studying environmental variables that mediate alcohol consumption.

Some information is available on the environmental variables that can initiate EtOH self-administration in the NP rat. Samson and his colleagues (1989b), as well as others (Rassnick et al. 1993), have successfully employed the sucrose-fading technique to initiate EtOH drinking to pharmacologically relevant levels in NP rats. Blood alcohol concentrations (BACs) have been reported in the range of 30 to 110 mg/dl following a 30-minute operant session (Samson et al. 1989b; also see Rassnick et al. 1993). Previous research from our laboratory has employed a modification of the palatability-induced polydipsia method (Kulkosky 1978) to initiate 24-hour continuous EtOH consumption in NP rats (Gatto et al. 1990). This procedure consists of presenting animals a two-bottle choice of water and a "cocktail" comprised of 3% (w/v) polycose, 0.5% (w/v) NaCl, and 0.125% (w/v) saccharin, with EtOH added in 2% increments every third day until a concentration of 10% (v/v) is obtained. Although the amount of the cocktail consumed declines as EtOH concentration increases, EtOH intake levels stabilize and remain measurable. BACs were approximately 31 and 61 mg % at 30 and 60 minutes, respectively, into the dark cycle (see Methods), indicating that pharmacologically relevant EtOH levels were obtained. Gatto and colleagues (1990) further indicated that this modified palatability-induced polydipsia method was capable of maintaining high EtOH drinking in NP rats for several months.

EtOH self-administration in rodents can be altered by a variety of neuropharmacological agents that affect different neurotransmitter systems (Weiss and Koob 1991; McBride et al. 1992), suggesting that several neurotransmitter systems may interact to mediate EtOH reinforcement. Considerable research implicates the $GABA_A$-BDZ receptor complex as one neuroreceptor that plays an important role in the reinforcing actions of EtOH (for review, see June et al. 1994a). Previous research with P and NP rats has shown that RO15-4513, a partial BDZ inverse agonist, selectively attenuates EtOH intake without affecting water consumption during limited fluid availability (McBride et al. 1988). Recently, we (June et al. 1995; also see June et al. 1993) compared the ability of several BDZ inverse agonists [RO15-3505 (sarmazenil), FG 7142, RO19-4603, DMCM] with a broad spectrum of intrinsic efficacies (Haefely 1990; Richards et al. 1991) with that of RO15-4513 in modifying the reinforcing properties of EtOH in the P rat. Besides exerting prolonged suppression of EtOH intake, RO19-4603 was approximately 10 to 20 times more potent than RO15-4513 in attenuating EtOH intake. The results with DMCM, a full negative allosteric modulator of GABAergic activity (Richards et al. 1991), showed that, although it was capable of decreasing EtOH intake, the reduction appeared to be part of a general overall deficit on motivated behaviors, insofar as reductions were also observed in the control fluid and food intake. Sarmazenil did not alter EtOH intake, and FG 7142 demonstrated selective antagonism only at moderate to high doses (16 and 32 mg/kg), presumably reflecting the lower affinity of these compounds at the BDZ receptor (Sieghart et al. 1987; Lister and Nutt 1988).

The objective of the present study was to determine the generalizability of our previous findings with P rats by showing that inverse agonists with different intrinsic efficacies effectively antagonize palatability-induced EtOH intake of NP rats. Specifically, the BDZ inverse agonists FG 7142, RO19-4603, and DMCM were evaluated for their ability to selectively suppress EtOH intake, following initiation using a palatability-induced polydipsia method (Kulkosky 1978; Gatto et al. 1990). In addition, to examine the ability of a BDZ receptor ligand with very weak intrinsic efficacy (Haefely 1984), but at the agonist end of the continuum, RO16-6028 (bretazenil), a BDZ partial agonist, was also investigated. Bretazenil, like many congeners of flumazenil (e.g., RO15-4513, RO19-4603, sarmazenil), exerts high affinity for BDZ receptors (Richards et al. 1991) and is currently under clinical investigation as a pharmacotherapeutic agent for the treatment of anxiety disorders in humans (Moreau et al. 1991). Hence, information on the ability of this drug to modify the reinforcing properties of EtOH would be of clinical interest.

METHODS

Subjects

Experimentally naive adult male rats ($n = 7$) of the selectively bred NP line (S-37 generation) were individually housed in wire-mesh stainless steel cages in a temperature-controlled (21°C) room on a 12:12 reversed light:dark cycle (lights off at 0900 hours). Rats were pre-

viously tested for free-choice drinking of unflavored 10% (v/v) EtOH (Lumeng et al. 1977), and EtOH intake averaged 0.63 ± 0.20 g/kg/day. Approximately 1 month elapsed before the animals received any further exposure to EtOH. Body weights at the beginning of the study ranged from 370 to 400 g.

Palatability-Induced Ethanol Intake by NP Rats

The rats were acclimated over 2 weeks to 22-hour fluid deprivation schedule, while all fluid access was restricted to the dark phase of the cycle between 1000 and 1200 hours. Food (Teklad Diet #7001, Harlan Industries, Indianapolis, IN) was available ad libitum. Animals remained on this schedule throughout the remainder of the experiment. This experimental approach was used because of the reported short half-life (Mandema et al. 1992) and short duration of action (d'Argy et al. 1987) of some of the BDZ ligands and the ability to measure drug actions on both the cocktail and water intake when food was available ad libitum. The position of the bottles was alternated daily to avoid the development of any position preferences.

During the initial deprivation phase, animals were provided water in two 100-ml graduated Richter bottles for 2 hours daily. After 2 weeks of acclimation to the fluid-deprivation schedule, a modification of the procedures of Kulkosky (1978) was implemented to initiate EtOH intake (see Gatto et al. 1990, for complete details of the procedure). Briefly, before EtOH was added, the "cocktail" consisted of 3% (w/v) polycose, 0.5% (w/v) NaCl, and 0.125% saccharin (w/v). EtOH was added to the cocktail in 2% (v/v) increments every third day until a 10% (v/v) EtOH concentration was attained. Once the drinking patterns of both the EtOH cocktail and water had stabilized, Tween-80 vehicle injections were administered prior to the drinking sessions to acclimate the animals to the injection procedure. Intakes were measured at 15-, 30-, 60-, and 120-minute intervals during the 2-hour period of fluid availability. Subjects were weighed twice per week to monitor body weight gain. Animals were given daily access to the two fluids for 1 month prior to the experimental drug treatments.

Drugs

Drugs were prepared as an emulsion by powder agitation (Scientific Mixer) in 1% Tween-80 vehicle (Sigma Chemical Co., St. Louis, MO) and mixed with a 0.90% NaCl solution to a fixed volume. RO19-4603 (tert-butyl 5, 6-dihydro-5-methyl-6oxo-4H-imidazo-[1,5-a]-thieno-[2,3-f][1,4]-diazepine-3-carboxylate) from Hoffmann-La Roche (Nutley, NJ) was tested at doses of 0.02, 0.04, 0.08, and 0.15 mg/kg. FG 7142 (N'-methyl-Beta-carboline-3-carboxamide) from Sigma Chemical Co. (St. Louis, MO) was tested at 1, 4, 8, and 16 mg/kg. RO16-6028 (bretazenil; tetracyclic imidazo [1,5-a] pyrrolo-(2,1c]1, 4-benzodiazepine) from Hoffmann-La Roche (Nutley, NJ) was tested at 8, 16, and 32 mg/kg. The full BDZ inverse agonist, DMCM (methyl 6,7-dimethoxy-r-ethyl-Beta-carboline-3-carboxylate), from Sigma Chemical Co. (St. Louis, MO) was tested at 1, 4, and 8 mg/kg. All drug solutions were prepared immediately prior to injections and administered intraperitoneally (IP) in a volume of 1 ml/kg body weight. Tween-80 vehicle was administered as the control injection for all drug conditions. Drug treatments were administered in a random order. To control for any residual drug carryover effects, pretreatments with Tween-80 vehicle were administered every 3 to 4 days, after baseline drinking patterns were reestablished. At the conclusion of the study, the EtOH was removed from the cocktail and animals were provided access to the polycose solution and water 2 hours daily for an additional 2 weeks. Drug treatments that showed selectivity in attenuating EtOH intake were evaluated for their ability selectively to alter the 3% polycose solution in the absence of EtOH and water.

Method of Analysis

Two analyses were conducted on the data; the first for EtOH, and the second for water intake. The analysis consisted of a 16 × 4 repeated-measures analysis of variance (ANOVA). The first factor represented treatment condition (Tween-80 vehicle; 0.02, 0.04, 0.08, and 0.15 mg/kg RO19-4603; 1, 4, 8, and 16 mg/kg FG 7142; 8, 16, and 32 mg/kg RO16-6028; 1, 4 and 8 mg/kg DMCM; and a combination condition comprising 16 mg/kg FG 7142 + 0.08 mg/kg RO19-4603). The second factor represented consumption interval (15, 30, 60, and 120 minutes). The combination condition was administered to evaluate whether an inverse agonist with a β-carboline structure would resemble the imidazobenzodiazepine RO15-4513 (June et al. 1993) in its ability to attenuate RO19-4603's suppressant effects on EtOH self-administration. The Newman-Keuls a posteriori test was conducted on pairwise comparisons of group means.

Previously, we (June et al. 1994b) demonstrated that in P rats maintained on 24-hour free-choice access to 10% (v/v) unflavored EtOH and water, RO19-4603 selectively suppressed EtOH intake up to 32 hours postdrug administration. Because of RO19-4603's potential therapeutic usefulness in the development of less toxic agents in reducing abnormal alcohol-seeking behavior, our previous work with P rats was replicated in the NP line. Hence, to examine the time course of RO19-4603, separate two-way ANOVAs (drug condition × time) were conducted on the EtOH and water data 24 hours postdrug administration for the four RO19-4603 dose conditions (0.02, 0.04, 0.08, and 0.15 mg/kg) compared with the vehicle control.

RESULTS

Consumption of the polycose solution decreased from approximately 50.0 ± 1.3 ml during days 1–3 (no EtOH) to approximately 23.8 ± 2.8 ml when the EtOH concentration (v/v) was 2% (days 4–6). It then decreased further to 14.3 ± 0.64 ml, when EtOH was 4% (days 7–9); only additional small decreases of intake occurred when the EtOH concentration was 6% or higher. When the solution contained 10% EtOH, intakes were 11.2 ± 1.1 ml (2.1 ± 0.20 g/kg/2 hours) prior to beginning vehicle and drug injections.

Figures 1A – 5A show EtOH intake for the five drug treatment conditions compared with the Tween-80 vehicle condition across the four consumption intervals. A significant treatment condition-by-consumption interval interaction emerged from the data [$F(45,225) = 4.44$, $p < .0001$]. As illustrated in Figure 1A, following vehicle injections, the NP rats consumed 4.4 ± 1.4, 3.1 ± 0.4, 3.1 ± 0.5, and 0.55 ± 0.2 ml of EtOH (mean ± SEM) during the 15-, 30-, 60-, and 120-minute consumption intervals, respectively. Compared with the control condition, the highest RO19-4603 dose (0.15 mg/kg) completely suppressed EtOH intake during the 15-minute interval ($p < .05$), but when the animals were given the 0.04-mg/kg dose, drinking of the EtOH solution decreased to about 19% of control values ($p < .05$). In addition, the highest dose (0.15 mg/kg) continued to suppress EtOH intake during the 30-minute interval ($p < .05$). At the 120-minute interval, RO19-4603 generally elevated EtOH consumption compared with the vehicle condition, with statistically significant effects seen only with the 0.02-, 0.04-, and 0.15-mg/kg dose conditions ($p \leq .05$).

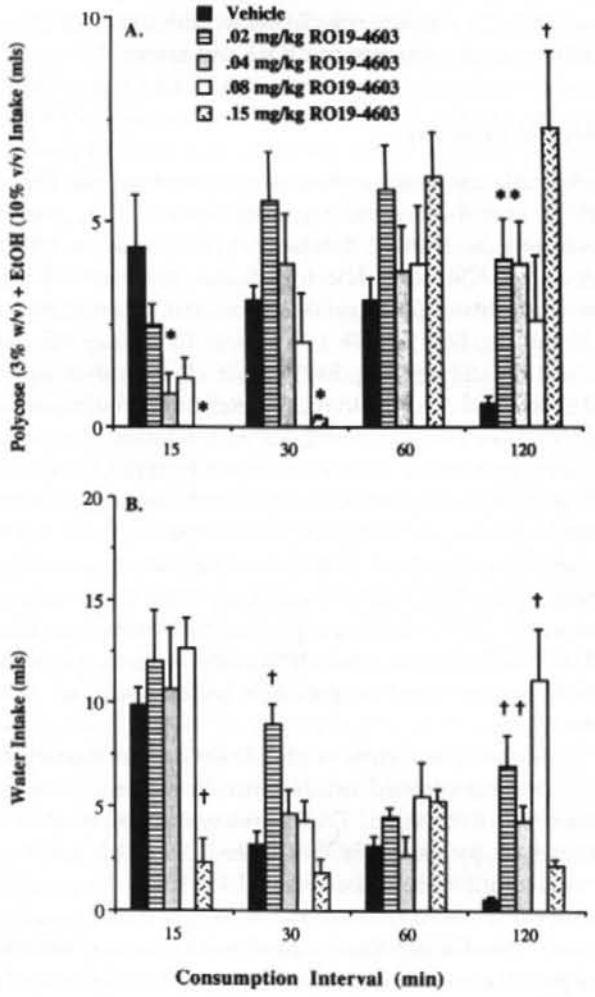

Figure 1. Effects of the IP administration of vehicle and four doses of RO19-4603 on the intake of **(A)** 3% polycose + 10% EtOH and **(B)** H_2O by NP rats across four consumption intervals. Data are the mean ± SEM ($n = 7$ rats each). †$p < .01$ and *$p < .05$ vs. control vehicle values by ANOVA and post hoc Newman-Keuls test.

Figures 1B–5B show water intake for the five drug treatments compared with the Tween-80 vehicle condition across the four consumption intervals. A significant treatment condition-by-interval interaction emerged from the data [$F(45,225) = 2.57$, $p < .0001$]. Figure 1B shows water intake for the Tween-80 vehicle control compared with the RO19-4603 treatment conditions. Following vehicle injections, the NP rats consumed approximately 9.9 ± 0.8, 3.2 ± 0.7, 3.2 ± 0.6, and 0.8 ± 0.2 ml across the 15-, 30-, 60-, and 120- minute consumption intervals, respectively. Post hoc analyses showed that a statistically significant decrease in water consumption occurred at the 15-minute interval with the 0.15-mg/kg dose ($p < .01$). Significant increases in water consumption were found for the 0.02-mg/kg dose condition at the 30- and 120-minute intervals and for the 0.04- and 0.08-mg/kg conditions at the 120-minute interval ($p < .01$).

Effects of RO19-4603 on Consumption of 3% Polycose and EtOH and Water 24 Hour Postdrug Administration

Figures 2A and 2B show EtOH and water intake for the four RO19-4603 dose conditions (0.02, 0.04, 0/08, and 0.15 mg/kg) compared with the vehicle condition 24 hours after injection. A significant treatment condition-by-consumption interval interaction was found [$F(12,60) = 3.78$, $p < .0003$]. Post-hoc analyses showed that the 0.08- and 0.15-mg/kg dose conditions significantly reduced EtOH drinking at the 60- and 15-minute intervals, respectively ($p < .05$).

Figure 2B shows water intake following the four RO19-4603 dose conditions compared with the control condition. A significant treatment condition-by-consumption interval interaction emerged [$F(12,60) = 2.64$, $p < .006$]. Post-hoc analyses indicated that statistically significant reductions in water intake were found for the 0.04- and 0.08-mg/kg dose conditions at the 15-minute interval ($p < .01$), while significant elevations were found for

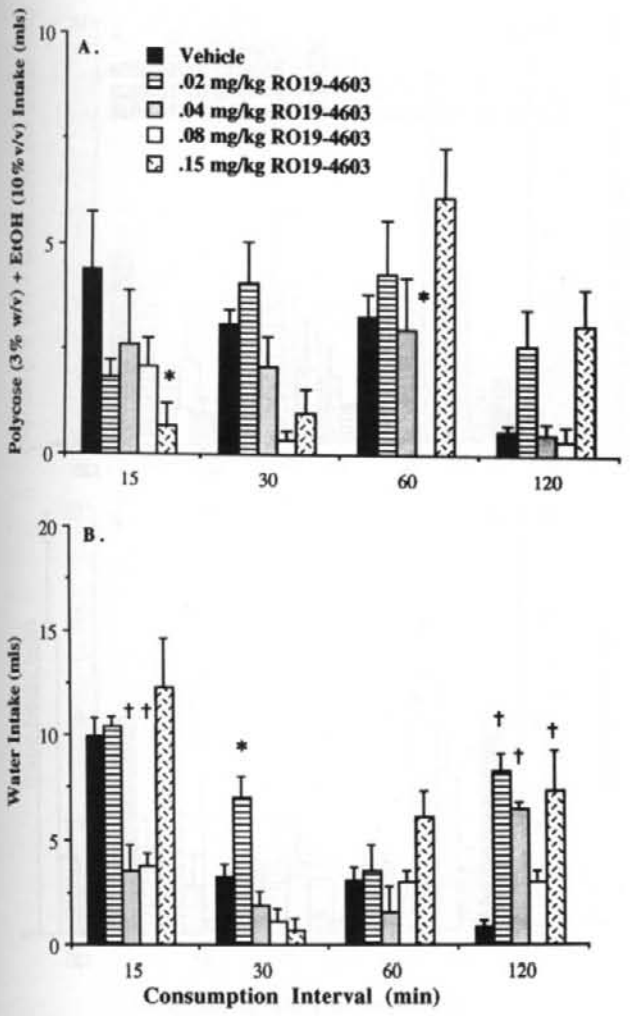

Figure 2. Effects of the IP administration of vehicle and four doses of RO19-4603 24 hours postdrug administration on the intake of **(A)** 3% polycose + 10% EtOH and **(B)** H_2O by NP rats across four consumption intervals. Data are the mean ± SEM ($n = 7$ rats each). †$p < .01$ and *$p < .05$ vs. control vehicle values by ANOVA and post hoc Newman-Keuls test.

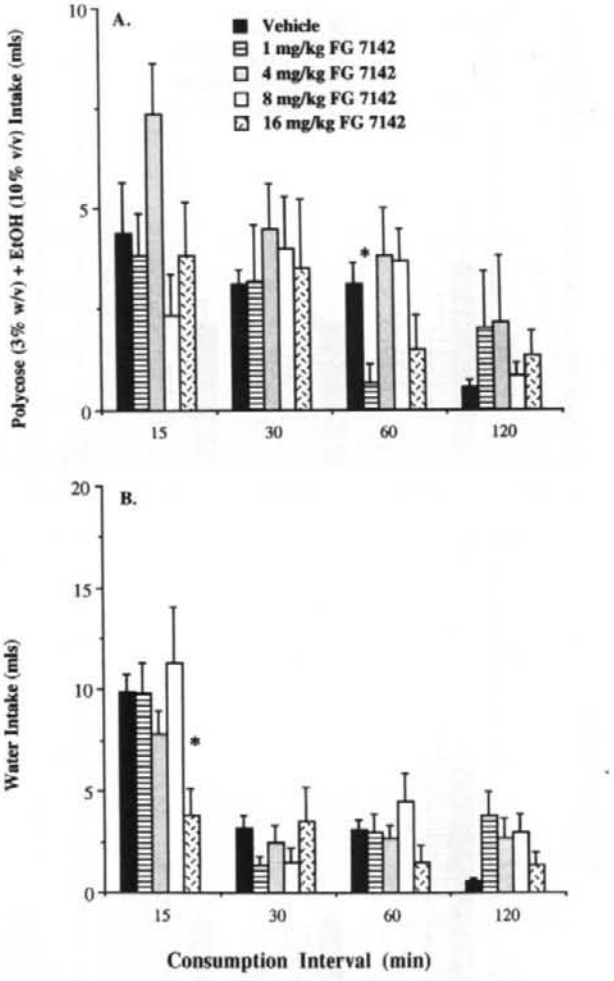

Figure 3. Effects of the IP administration of vehicle and four doses of FG 7142 on the intake of **(A)** 3% polycose + 10% EtOH and **(B)** H_2O by NP rats across four consumption intervals. Data are the mean ± SEM ($n = 7$ rats each). †$p < .01$ and *$p < .05$ vs. control vehicle values by ANOVA and post hoc Newman-Keuls test.

the 0.02-mg/kg dose condition at the 30- and 120-minute intervals ($p < .05$ and $p < .01$, respectively). Statistically significant elevations were also found at the 120-minute interval for the 0.04- and 0.15-mg/kg dose conditions ($p < .01$).

Figure 3A shows intake of the EtOH solution after vehicle injections compared with the partial β-carboline inverse agonist, FG 7142. At the 15-minute interval, the 1-, 4-, 8-, and 16-mg/kg doses did not alter EtOH intake significantly ($p > .05$). Only the 1-mg/kg dose significantly suppressed intake at the 60-minute interval ($p < .05$). Figure 3B shows that at the 15-minute interval, the 16-mg/kg dose significantly reduced water consumption ($p < .05$), whereas the 1-, 4-, and 8-mg/kg doses did not alter water intake ($p > .05$). During the 30-, 60-, and 120-minute intervals, no significant differences were observed.

Figure 4A shows consumption of the EtOH solution for vehicle injections compared with the full β-carboline inverse agonist, DMCM. Significant suppression of EtOH intake was apparent at the 60-minute interval with the 8-mg/kg dose of DMCM ($p < .05$). In contrast, marked elevations in intake were observed at the 120-minute interval following the 4- and 8-mg/kg dose conditions ($p < .01$). Figure 4B shows that both the 4- and the 8-mg/kg doses of DMCM significantly suppressed water intake at the 15-minute interval ($p < .05$). No significant effects were observed with any of the dose conditions at the 30-, 60-, or 120-minute intervals ($p < .05$).

The data in Figure 5A show intake of the EtOH solution after injection of the partial BDZ agonist, bretazenil

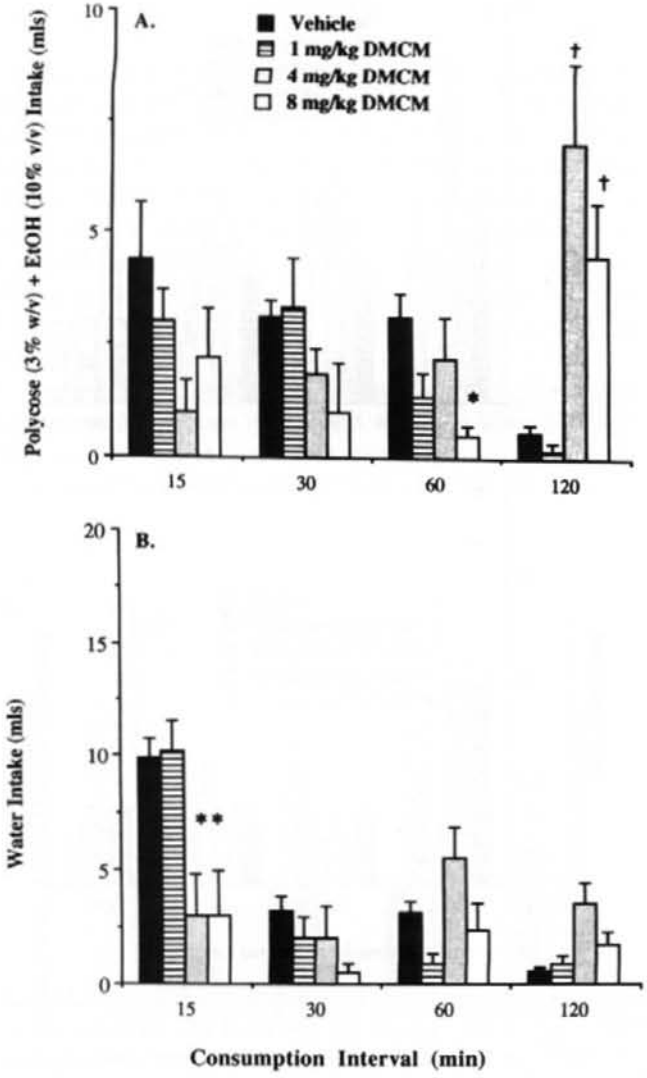

Figure 4. Effects of the IP administration of vehicle and three doses of DMCM on the intake of **(A)** 3% polycose + 10% EtOH and **(B)** H_2O by NP rats across four consumption intervals. Data are the mean ± SEM ($n = 7$ rats each). †$p <$ .01 and *$p < .05$ vs. control vehicle values by ANOVA and post hoc Newman-Keuls test.

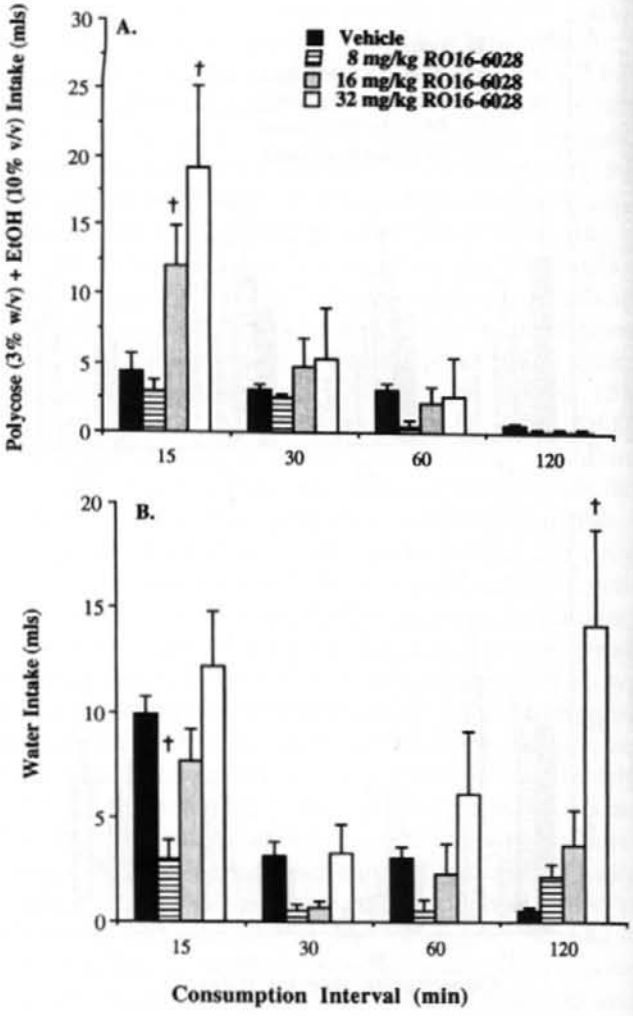

Figure 5. Effects of the IP administration of three doses of bretazenil (RO16-6028) on the intake of **(A)** 3% polycose + 10% EtOH and **(B)** H_2O by NP rats across four consumption intervals. Data are the mean ± SEM ($n = 7$ rats each). †$p <$ .01 and *$p < .05$ vs. control vehicle values by ANOVA and post hoc Newman-Keuls test.

(RO16-6028). A different profile of effects emerged for bretazenil relative to the other tested inverse agonists (above) when compared with the control condition. Specifically, the lower dose (8 mg/kg) did not alter drinking of the EtOH solution at the initial 15-minute consumption interval ($p > .05$), but the two higher doses (16 and 32 mg/kg) markedly elevated EtOH intake ($p <$ .01). As seen in Figure 5B, only the lowest dose of bretazenil (8 mg/kg) reduced water intake at the 15-minute interval ($p < .01$). The highest dose of bretazenil (32 mg/kg) significantly increased water consumption at the 120-minute interval ($p < .01$).

Figures 6A and 6B reiterate the consumption of the EtOH and water data, respectively, for the vehicle, the 16-mg/kg dose of FG 7142, and the 0.08-mg/kg dose of RO19-4603, while also showing the results of EtOH consumption for a combination of the two drug doses. Apparent in this figure is a failure of the 16-mg/kg dose of FG 7142 to reverse the suppressant actions of RO19-4603 on EtOH consumption. As shown in Figure 1A, EtOH intake was not significantly reduced ($p > .05$) with the 0.08-mg/kg dose of RO19-4603. EtOH intake for the animals under the combination condition was nearly identical to that of animals given 0.08 mg/kg RO19-4603 alone across each consumption interval ($p >$ .05). Compared with the RO19-4603 condition, the combination condition was without effect on water intake at the 15-, 30-, and 60-minute intervals. However, water intake was substantially suppressed at the 120-minute interval compared with the RO19-4603 condition ($p < .01$).

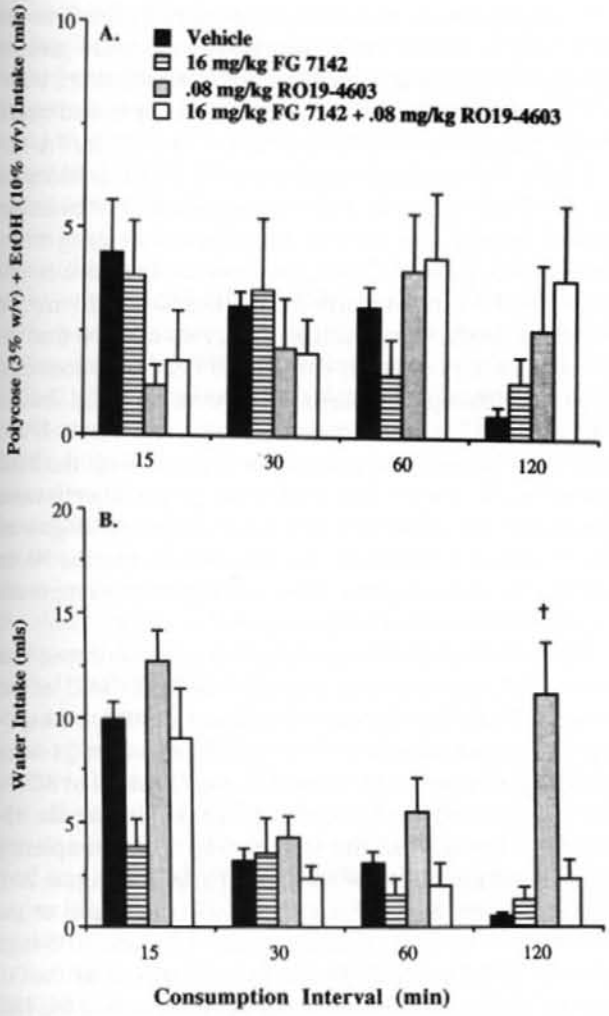

Figure 6. Effects of the IP administration of vehicle, 16 mg/kg FG7142, 0.08 mg/kg RO19-4603, and a combination dose of these two drugs on the intake of **(A)** 10% EtOH and **(B)** H_2O by NP rats across four consumption intervals. Data are the mean ± SEM (n = 7 rats each). †$p < .01$ and *$p < .05$ vs. control vehicle values by ANOVA and post hoc Newman-Keuls test.

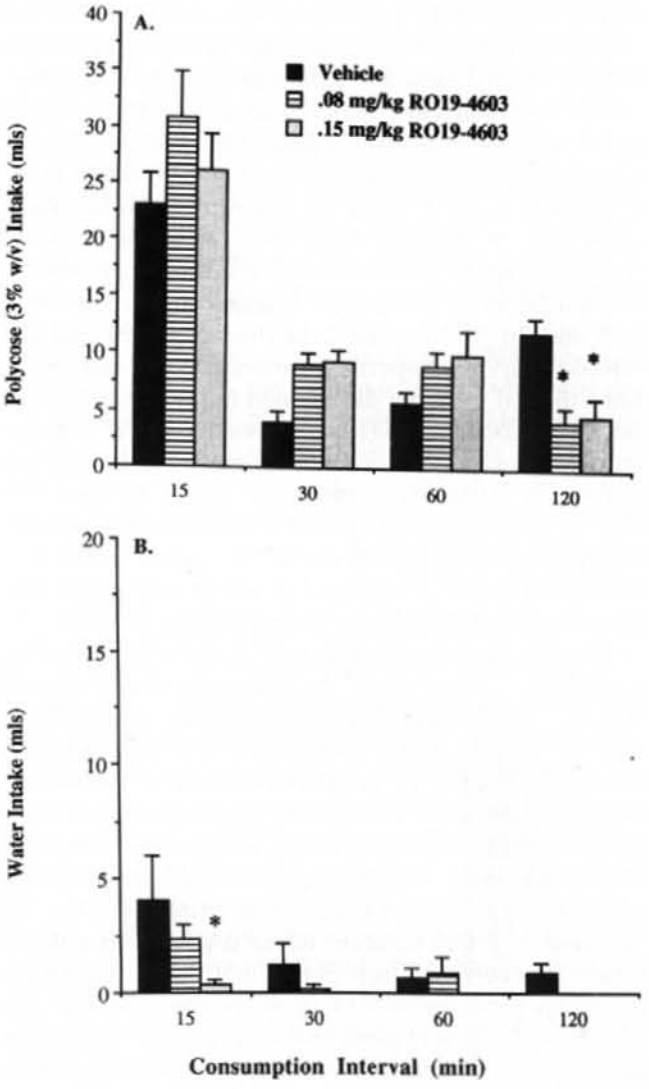

Figure 7. Effects of the IP administration of vehicle and two doses of RO19-4603 on the intake of **(A)** 3% polycose and **(B)** H_2O by NP rats across four consumption intervals. Data are the mean ± SEM (n = 7 rats each). †$p < .01$ and *$p < .05$ vs. control vehicle values by ANOVA and post hoc Newman-Keuls test.

Effects of RO19-4603 on Consumption of 3% Polycose without EtOH

Compared with the other BDZ receptor ligands, RO19-4603 was the most potent agent that blocked EtOH intake. To further elevate the degree to which RO19-4603 alters ingestive behaviors, the effects of RO19-4603 (0.08 and 0.15 mg/kg) were studied in rats provided 2 hours daily access to the cocktail solution without EtOH for 2 weeks. A single-factor ANOVA for drug condition (vehicle, 0.08, and 0.15 RO19-4603 mg/kg) was conducted on the EtOH and water data separately. As seen in Figure 7A, RO19-4603 at the 0.08- and 0.15-mg/kg doses significantly reduced consumption of the polycose solution at the 120-minute interval compared with the vehicle control ($p < .05$), resulting in a significant treatment condition-by-consumption interval interaction [$F(6,30) = 4.09$, $p < .004$]. A significant reduction in water consumption occurred only for the 0.15-mg/kg dose condition at the 15-minute interval ($p < .05$) (see Figure 7B), resulting in a significant treatment condition-by-consumption interval interaction [$F(6,30) = 4.63$, $p < .001$].

DISCUSSION

The present study investigated the ability of various BDZ receptor ligands with different intrinsic efficacies to modify palatability-induced EtOH consumption in NP rats. NP rats were used as a model to study effects

on environmentally influenced EtOH drinking. Because NP rats do not readily consume EtOH to pharmacologically relevant levels, the palatability-induced polydipsia method (Kulkosky 1978; Gatto et al. 1990) and fluid deprivation were used to initiate EtOH intake. Similar to previous studies (Samson et al. 1989b; Gatto et al. 1990; Rassnick et al. 1993), the data reported here clearly show that environmental processes can be manipulated to enhance EtOH intake in rats that normally avoid substantial EtOH consumption. Average EtOH intake was 1.95 and 2.1 ± 0.2 g/kg/2 hr during the 60 and 120 minute intervals, respectively. Blood alcohol concentrations (BACs) were not determined in the present study, however, Gatto et al. (1990) showed that NP rats attained BACs of approximately 60 mg% 1 hour into the dark cycle when given unlimited access to the Polycose/EtOH cocktail and water. These findings indicate that the initiation procedure of the current study was successful in inducing the NP rat to self-administer pharmacologically relevant concentrations of EtOH.

In agreement with a previous report (Samson et al. 1989b), the current study demonstrated that, unlike P (Murphy et al. 1986) and nonselected stock rats (Samson 1986), NP rats distributed their EtOH drinking in smaller bouts over the length of the session. Specifically, animals maintained a moderate level of intake over 60 minutes, with negligible intake occurring during the 120-minute interval (see Figure 1A). Gatto et al. (1990) suggested that NP rats reduce their drinking of the 3% Polycose + EtOH solution when BACs reach a point where the aversive effects of the EtOH begin to counter the highly palatable sweet taste of the polycose solution. This hypothesis is consistent with previous research indicating that, in contrast to P rats, the postingestional pharmacological properties of EtOH at comparatively moderate BACs are aversive the NP rat (Waller et al. 1984; Froehlich et al. 1988; Bice and Kiefer 1990).

The primary purpose of the present study, however, was to examine the ability of BDZ receptor ligands with different intrinsic efficacies selectively to alter palatability-induced EtOH intake. The major findings of the study are that some compounds acting via the GABA-BDZ receptor complex can modify drinking patterns in the NP rat line.

Consistent with our previous work in P rats under limited (June et al. 1993) and continuous 24-hour access (June et al. 1994b), the present study demonstrates that a single injection of the potent BDZ inverse agonist, RO19-4603 (0.04 and 0.15 mg/kg) given acutely, markedly suppressed EtOH intake during the initial 15 minutes of the 2-hour consumption period in NP rats. In addition, the highest dose continued to suppress intake at the 30-minute interval. Water intake showed no differences from vehicle control intakes or showed increases across two of the consumption intervals (e.g., 30 and 120 minutes), although the highest dose (0.15 mg/kg) nonselectively reduced intake of both fluids at the 15-minute interval. Moreover, when rats were given a choice between water and the polycose solution, without EtOH, only the 0.15-mg/kg dose suppressed water intake during the 15-minute interval (see Figure 7A–B).

Given the reported consistency of EtOH drinking in NP rats (McBride et al. 1988; Samson et al. 1989b) under limited access protocols in the absence of drug treatments, one possible interpretation of the results observed in the current study during the 30- to 120-minute postdrug administration time intervals may be that increases in EtOH or water drinking reflect elevations due to pharmacologically induced effects on fluid intake during the 15-minute interval (Weiss and Koob 1991). However, because the pretreatment times with the BDZ ligands were all similar, and none of the agents were given after the initial hour of fluid intake, the degree to which elevated EtOH or water intake during the 30- to 120-minute consumption intervals represent compensatory increases cannot be determined.

The results of the present study further confirm (June et al. 1993) that a single injection of RO19-4603 at the 0.15- and 0.08-mg/kg dose levels are capable of exerting prolonged attenuation of EtOH intake; at 24-hour postdrug administration, the 0.15-mg/kg dose of RO19-4603 significantly reduced EtOH intake during the 15-minute interval, and the 0.08-mg/kg dose completely suppressed intake at the 60-minute interval (Figure 2A). Water consumption was significantly decreased at the 15-minute interval 24 hours after the 0.04- and 0.08-mg/kg dose of RO19-4603, but it was increased at the 30-minute interval for the 0.02-mg/kg dose, and at the 120-minute interval for the 0.02-, 0.04-, and 0.15-mg/kg doses (Figure 2B). Recently, we demonstrated that when P rats were provided 24-hour continuous access to unflavored EtOH and water in their home cage, EtOH intake during the 8 hours after RO19-4603 (0.075–0.30 mg/kg) was substantially reduced; however, during the 9-to 24-hour interval, EtOH intake did not differ from control values. At the 24- to 32-hour post-RO19-4603 administration, significant suppression was again seen with the higher RO19-4603 dose (0.30 mg/kg). Water drinking in that study was not altered or showed increases at the 8-, 24- and 32-hour consumption intervals. The differential effects observed with RO19-4603 24 hours after administration between animals under continuous access in the previous study and limited access drinking in the present study may be related to the pattern of EtOH drinking under the two types of schedules on the one hand, and the manner in which EtOH intake is measured on the other (for review see Murphy et al. 1986; also June et al. 1994b).

Unlike the profile of effects seen with RO19-4603, the β-carboline partial inverse agonist, FG7142, did not reduce EtOH intake at any of the doses tested at the 15-minute and 30-minute intervals, although reductions

were noted for the 1-mg/kg dose at the 60-minute interval (see Figure 3A). These findings with FG7142 could be attributed to the drug's low affinity for BDZ receptor (Braestrup et al. 1983). A previous study (Lister 1987) found that FG7142 is approximately 7 to 13 times less potent than RO15-4513 in blocking the EtOH-induced depressant action (2 g/kg) on exploration. However, recent work (June et al. submitted) in our laboratory has shown that a 16- and 32-mg/kg dose of FG7142 effectively antagonized EtOH intake in P rats. The findings of the present study also contrast with previous work by Samson et al. (1989a) in outbred rats, who found that FG7142 (3–10 mg/kg) dose-dependently antagonized EtOH-reinforced lever-press responding. The discrepant findings among these studies may be attributed to several methodological differences. One of the most salient differences is the maintenance of the NP rats in the present study on the polycose + EtOH regimen while the animals in our previous work (June et al. 1995), and the study by Samson et al. (1989) were maintained on unflavored EtOH. Samson et al. (1989a) have demonstrated that EtOH-reinforced responding is considerably more sensitive to the attenuating effects of BDZ inverse agonists (RO15-4513 and FG 7142) than to sucrose-reinforced responding. Thus, because of the purported low affinity of FG 7142 for the BDZ receptor, along with the simultaneous use of the polycose polydipsia method and a fluid-deprivation schedule, it is possible that higher doses of FG 7142 may have attenuated EtOH intake.

Results with the full β-carboline inverse agonist DMCM showed that, with the exception of the lowest dose (l mg/kg), there was a generalized behavioral suppressant effect on fluid intake and level of activity. Moreover, with the highest dose, 50% of the animals exhibited seizure activity evidenced by myoclonic jerking of the head and forelimbs. These effects persisted for approximately 5 minutes. This generalized behavioral suppressant effect in NP rats can possibly be attributed to a greater reduction in GABAergic transmission caused by the full inverse agonist.

The BDZ partial agonist, bretazenil (RO16-6028), did not alter EtOH intake with the lowest dose (8 mg/kg); however, the higher doses (16 and 32 mg/kg) substantially elevated EtOH intake during the initial 15-minute consumption period. A significant reduction in water intake occurred at the 15-minute interval for the 8-mg/kg dose, and an increase occurred at the 120-minute interval for the 32-mg/kg dose (see Figures 5A and B). These findings contrast with the recent work by Rassnick and her colleagues (1993) who showed that chlordiazepoxide (2.5–10 mg/kg) did not alter EtOH- or water-reinforced responding in NP rats, although nonsignificant increases were observed for the 2.5- and 5-mg/kg doses in P rats. Others (Beaman et al. 1984), using a two-bottle choice test between a sweetened EtOH solution and water in stock rats, found that chlordiazepoxide (3–12 mg/kg) generally increased total fluid intake, but it did not alter the rats' propensity to self-administer EtOH. Samson and Grant (1985), using a concurrent schedule procedure, demonstrated that chlordiazepoxide generally reduced EtOH-reinforced responding and intake. Despite the consistent decreases of EtOH-reinforced responding and intake, no overall statistically significant compensatory increase in the alternative fluid (e.g., sucrose, water) was observed for either of these measures.

Several reports have suggested that BDZ agonists (Cooper and Francis 1982; Cooper 1983) and partial agonists (Moreau et al. 1991) are capable of increasing ingestive behaviors. Bretazenil has approximately a 10-fold higher affinity for the BDZ receptor than diazepam, and it produces anticonflict effects at much lower doses and over a wider dose range (Richards et al. 1991). The potent dipsogenic effects of bretazenil on EtOH drinking at 15 minutes and water drinking at 120 minutes with the high dose (32 mg/kg) (see Figures 5A and B) may be related to its ability to exert relatively high BDZ receptor occupancy. Hence, the increase in EtOH intake in the current study may simply be an indirect consequence of bretazenil generally enhancing ingestive behaviors. An alternative interpretation of the findings with bretazenil on EtOH intake is that bretazenil acts as an anxiolytic in NP rats by blocking some of the aversive properties associated with EtOH consumption, thereby enhancing the reinforcing effects of EtOH (Rassnick et al. 1993). As mentioned, the aversive properties of EtOH may be related to its postingestional effects in the NP rat (Bice and Kiefer 1990; Waller et al. 1984). NP rats are reported to have a lower threshold for the aversive effects of EtOH in conditioned taste aversion studies (Froehlich et al. 1988).

Unlike our previous findings with RO15-4513 (June et al. 1993), FG 7142 (16 mg/kg) did not alter the actions of RO19-4603 on EtOH intake (see Figures 6A and B). These findings and its weak potency in blocking the reinforcing actions of EtOH further confirm the weak affinity of FG 7142 at BDZ receptors compared with RO15-4513. FG 7142 also does not antagonize the intoxicating action of EtOH (Suzdak et al. 1986; Lister and Durcan 1989). A related hypothesis regarding the weak potency of FG 7142 to antagonize EtOH's actions pertains to the complete failure of FG 7142 to bind at diazepam-insensitive (DI) sites in the cerebellum (Turner et al. 1991). Although the significance of DI sites are not well understood, Turner and his colleagues (1991; also Olsen and Tobin 1990) suggested that the selective/unique efficacy of compounds in blocking EtOH's action may be related to their efficacy at different receptor subtypes (Olsen and Tobin 1990). Unlike β-carboline negative allosteric modulators (e.g., FG 7142, βCCE, βCCM), imidazobenzodiazepines show high affinity

binding at diazepamsensitive (DS) and DI sites (Turner et al. 1991; Wong and Skolnick 1992). At DI sites RO19-4603 binds at $K_i \approx 2.6$ nM and at $K_i \approx 0.2$ nM at DS sites (Wong and Skolnick 1992). RO15-4513 binds with the same affinity ($K_d \approx 4.5$ nM) to both DS and DI sites (Turner et al. 1991). Others (Wong and Skolnick 1992) have also confirmed the RO15-4513 DI/DS potency ratio of about 1. Similarly, the partial agonist bretazenil binds at DI sites with a comparable affinity ($K_i \leq 10$ nM), but classical BDZ agonists such as diazepam and midazolam possess an $IC_{50} > 10{,}000$ nM (Wong and Skolnick 1992). These findings suggest the possibility that the pharmacophore for annelated 1, 4-diazepines that possess high affinity to DI BDZ receptors is fundamentally different at the DS BDZ receptors (Wong and Skolnick 1992). The identification of high-affinity ligands at DI BDZ receptors may assist in elucidating the pharmacological importance of these sites in the behavioral actions of EtOH.

Although food intake was not determined in the present study, with the exception of bretazenil at the highest doses (16 and 32 mg/kg) and at the 8-mg/kg DMCM dose condition, none of the drug treatments have been observed to exert any significant alteration on food intake in freely feeding P rats provided 4-hour limited access to 10% EtOH and saccharin solutions (June et al. 1993). It is also worth noting that body weights for the animals in the present study were consistent with weight gain profiles for male NP rats in previous studies, suggesting that the caloric balance was maintained throughout the experiment.

In summary, the present findings confirm that environmental processes can be manipulated to initiate EtOH intake in rats of the NP line (Samson et al. 1989b; Gatto et al. 1990). The results also confirmed (June et al. 1993, 1994b) that doses of RO19-4603 (0.08 and 0.15 mg/kg), may be capable of reducing EtOH intake 24 hours after administration, although some effects on water drinking may also occur. Moreover, compared with the other anxiogenic ligands (e.g., FG 7142, DMCM) RO19-4603 was the most potent agent to block EtOH intake. However, it should be noted that a selective suppression of EtOH intake with all RO19-4603 doses was not observed in the present study with NP rats, as have been observed during ad libitum drinking situations with P rats (June et al. 1993, 1994b). The anxiolytic agent bretazenil (16 and 32 mg/kg) markedly increased EtOH consumption during the initial 15 minutes compared with control levels, although the increased consumption may result from a general dipsogenic effect on fluids. Although caution should be used in extrapolating the findings of the current study to human situations, there is a suggestion that moderate to high doses of bretazenil may potentiate EtOH intake via an indirect interaction on ingestive behaviors. It is important to note that the animals in the present study were 22-hour fluid deprived and initiated EtOH intake in the home cage. Thus, the results of the present study may not generalize to subjects in ad libitum drinking and EtOH-reinforced responding situations. Given the present findings with NP rats, and our previous work with RO15-4513 (McBride et al. 1988; June et al. 1994a), RO19-4603 (June et al. 1993, 1994b), and the β-carboline inverse agonists FG 7142 and DMCM (June et al. 1995) in P rats, it appears that the property of the ligand (ligand structure, e.g., annelated 1, 4-diazepine) may be important in modifying EtOH intake. Similarly, bretazenil, at the agonist end of the BDZ continuum, also binds to DI sites (Wong and Skolnick 1992) and increases EtOH intake to a greater degree than classical agonists (e.g., chlordiazepoxide, diazepam) that do not confer DI (Samson and Grant 1985; Rassnick et al. 1993). Thus, the data of the present study with both anxiogenic ligands and the anxiolytic bretazenil, along with previous research from our laboratory (June et al. 1993, 1994b, submitted) and others (Samson and Grant 1985; Rassnick et al. 1993) appear consistent with a qualitative (i.e., imidazobenzodiazepine-selective) as well as a quantitative (relative receptor affinity) difference in the interaction of these ligands (affinity for DI sites). Together, the present findings support existing evidence in P and outbred rats (for review, see June et al. 1994a, 1994b) suggesting that BDZ receptor ligands may modify neuronal processes that mediate some reinforcing and/or aversive properties of alcohol. They further suggest a potential importance of the $GABA_A$-BDZ receptor complex in mediating palatability- (environmentally) induced EtOH drinking even in rats selectively bred for low alcohol preference.

ACKNOWLEDGEMENTS

We would like to express our gratitude to Dr. Peter Sorter (Hoffmann-La Roche, Nutley, NJ) for kindly providing the RO19-4603 and RO16-6028. This research was supported in part by grants AA08553 and AA07611; Graduate (RLH) and SPUR (TLG, JJM-B) Awards from the Department of Psychology; and, Undergraduate Mentorship Research Fellowship (TLG) from the Office of Faculty Development.

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