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Chronic Ethanol Produces a Decreased Sensitivity to the Response-Disruptive Effects of GABA Receptor Complex Antagonists

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RASSNICK, S., J. KRECHMAN, AND G. F. KOOB. *Chronic ethanol produces a decreased sensitivity to the response-disruptive effects of GABA receptor complex antagonists.* PHARMACOL BIOCHEM BEHAV **44**(4) 943–950, 1993.—Disruption of responding for food reinforcement may reflect the motivational state subsequent to the onset of an aversive event and has previously been shown to be sensitive to spontaneous withdrawal from ethanol and precipitated opiate withdrawal. The purpose of this study was to attempt to precipitate ethanol withdrawal with bicuculline methiodide, a competitive $GABA_A$ receptor antagonist, and Ro 15-4513, a benzodiazepine inverse agonist. A quantitative operant measure of food-motivated behavior was used to evaluate the reactivity of the $GABA_A$-benzodiazepine receptor complex during chronic ethanol treatment in rats. In the present study, rats were trained to lever-press for food reinforcement on a fixed ratio 15 schedule and then maintained for 2 weeks on a liquid diet containing 35% ethanol-derived calories or a control liquid diet that was prepared isocalorically with sucrose. Chronic ethanol treatment attenuated the disruptive effects on operant responding that were produced by bicuculline methiodide (100 ng ICV) and Ro 15-4513 (3 and 6 mg/kg). The inability of these drugs to "precipitate" EtOH withdrawal may reflect the noncompetitive interaction of ethanol with the GABA-benzodiazepine-ionophore receptor complex. These data are consistent with recent biochemical studies indicating that chronic ethanol treatment modulates the $GABA_A$-benzodiazepine-ionophore receptor complex by altering the expression of specific molecular components and inhibiting the activity of the receptor complex.

Bicuculline methiodide Ro 15-4513 Ethanol Ethanol dependence GABA Rat

THE neurochemical basis of ethanol (EtOH) dependence and the neural components that mediate the intoxicating, sedative/hypnotic properties of chronic EtOH are still unclear. Biochemical and behavioral research indicates that while EtOH affects several neuronal systems (21) the $GABA_A$-benzodiazepine (BDZ) receptor complex is a possible pharmacological site on which EtOH acts to produce the neural consequences of dependence (6,53). GABA is an inhibitory neurotransmitter and is a receptor ligand for a multiple receptor ionophore complex (42) that contains an integral Cl- ion channel (43) and recognition sites for BDZs (51) and convulsants such as picrotoxin (50). A binding site for barbiturates presumably also exists on the receptor complex, although this site has not yet been characterized molecularly (49). These binding sites interact with each other in an allosteric manner and regulate the gating properties of the Cl- ion channel, which upon activation produces an increase in membrane hyperpolarization and a decrease in neuronal excitability (33).

Although EtOH does not seem to bind with a specific receptor molecule per se, biochemical and behavioral assays indicate that many of the effects of EtOH may be mediated via the $GABA_A$-BDZ receptor complex. Ligand-binding studies investigating the effects of chronic EtOH indicate that chronic EtOH decreases the density of BDZ or low-affinity $GABA_A$ receptor sites and reduces GABA enhancement of BDZ binding (9,20). However, several studies also report that chronic EtOH does not produce significant alterations in BDZ receptor density (9,18,19,25), or high-affinity GABA receptor density (6,18,20,22), or modify the binding of convulsants to the receptor complex (30,47,52,54). It is possible that the lack of consistency across studies may reflect methodological differences such as blood EtOH levels or the membrane preparation used to study GABA or BDZ binding. In some studies, membranes are solubilized with the detergent Triton X-100 or extensively washed with buffer to remove endogenous inhibitors that may interfere with binding capacity of GABA or BDZ to the receptor complex (34,55,56).

While ligand-binding studies have not convincingly demonstrated that chronic EtOH produces alterations of GABA or BDZ receptor affinity or density, an alternative approach has

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been to examine the specific cellular events that are produced by receptor activation. Biochemical studies indicated that chronic EtOH may affect GABAergic transmission at the molecular level producing a transcriptional change in the type of $GABA_A$ receptor that is expressed (39). Chronic EtOH treatment decreased the expression of the α_1 subunit mRNA transcript of the $GABA_A$ receptor in the cerebral cortex (38–40) and abolished EtOH potentiation of GABA receptor-mediated Cl- ion activation (2,6,41).

Further support for some sort of adaptation of GABAergic systems to chronic EtOH treatment is based upon behavioral studies where pharmacological treatments that interact with the GABA, picrotoxin, or BDZ sites can modify the expression of CNS hyperexcitability associated with EtOH withdrawal. For example, the severity of EtOH withdrawal seizures in rodents is reduced by pharmacological treatments that increase GABA activity (7,13,16,17) and exacerbated by treatments that decrease GABA activity (7,16).

In opiate dependence, removal of the drug after chronic administration is also manifested behaviorally by a spontaneous withdrawal syndrome. However, this syndrome can be "precipitated" by acute administration of a competitive opioid antagonist while the subject is still dependent with high circulating blood levels of opiates. The precipitated withdrawal syndrome in opiate-dependent rats is characterized by physical signs such as ptosis and wet-dog shakes (57), but is also characterized by disruptions of motivated behavior that are much more sensitive than the measures of physical signs (15,27). Rats trained to lever press on a fixed ratio 15 (FR 15) schedule (where 15 lever presses are required for each reinforcer) show dose-dependent decreases in responding at doses of opiate antagonists that fail to precipitate major physical signs of withdrawal (27).

To examine if chronic EtOH treatment alters the sensitivity of the $GABA_A$-BDZ receptor complex that can be manifested as a precipitated withdrawal, the present study was designed to precipitate EtOH withdrawal with antagonists of the GABA-BDZ-ionophore complex during periods of EtOH intoxication using this same highly sensitive operant task. The purpose of this study was to test whether antagonism of endogenous GABA receptor activity with either bicuculline methiodide, a competitive $GABA_A$ receptor antagonist, or Ro 15-4513, a benzodiazepine inverse receptor agonist, would precipitate signs of EtOH withdrawal. Evidence of precipitated withdrawal was defined for current purposes as increased sensitivity during dependence to the effects of these drugs to decrease responding on an FR 15 schedule of reinforcement.

In the present study, an EtOH liquid diet method was used to induce EtOH dependence because this method meets most of the criteria suggested by Lester and Freed (29) and Freund (12) for an animal model of EtOH dependence and is a simple, reproducible method that allows for short durations of chronic EtOH self-administration to induce repeated episodes of intoxication that eventually lead to tolerance (10) and physical dependence upon EtOH (23,28). In addition, many studies show that shortly after the termination of unlimited access to a chronic EtOH liquid diet behavioral signs of EtOH withdrawal are displayed in rats maintained on this exact diet (3,45,46) or a similar EtOH liquid diet (11,23,28). [For actual data demonstrating behavioral and physical withdrawal signs from studies using exactly the same procedure and strain of rats, see (3,46)].

In the present study, responding for food reinforcement on an FR 15 schedule was used as the dependent measure. Schedule-controlled operant tests for food reinforcement have been previously used to evaluate CNS reactivity to EtOH withdrawal (8). Enhanced sensitivity or resistance to the response-suppressive effects of bicuculline methiodide or Ro 15-4513 may provide clues to the functional state of the $GABA_A$-BDZ receptor complex during chronic EtOH treatment.

METHOD

Experimental Design

To evaluate the reactivity of the GABA-BDZ receptor complex to GABA antagonists during chronic EtOH treatment, first animals (n = 65) were trained to respond for food reinforcements on an FR 15 task, then implanted with chronic indwelling cannulae aimed at the lateral ventricle. After a 3-day postoperative recovery, animals were randomly assigned to one of two liquid diet treatment groups: One group received EtOH liquid diet and the other group was pair fed with respect to the experimental group and maintained on a liquid diet in which EtOH-derived calories were replaced equicalorically with sucrose. All animals were maintained on liquid diet and rebaselined on the FR 15 task during the first 14-day period of liquid diet treatment. Each liquid diet treatment group was further divided into subgroups, where each rat then received one drug injection of each test drug. The first drug experiment tested the effects of ICV administration of bicuculline methiodide in doses of 0, 50, 100, or 200 ng. For this experiment, the various doses of bicuculline methiodide were tested after 14 days of chronic liquid diet treatment using 8–11 EtOH-treated rats/dose and 6–8 control-treated rats/dose. Test sessions and two baseline sessions just prior to the drug test sessions were conducted in the evening between 10:00–12:00 p.m. because this time period corresponded to the time at which maximal blood EtOH concentrations were achieved (see the Results section for further details).

The subsequent drug experiment examined the effects of Ro 15-4513 and was conducted with five to eight EtOH-treated rats/dose and four to seven control-treated rats/dose. At least 7 days elapsed between the first and second drug experiments, where all animals were maintained on a liquid diet and rebaselined on the FR 15 task. In all aspects of this study, the pair-fed control group was handled and tested identically to the EtOH group.

Subjects

Naive, male Wistar rats (Charles River Laboratory) weighing 200–220 g at the beginning of the experiment served as subjects. Animals were initially deprived of food for 24 h before the first day of operant training. Thereafter, animals were food deprived to approximately 85% of free-feeding body weight and maintained on 15 g/day of standard rat chow in addition to the 45-mg Noyes food pellets earned in the operant session. Ad lib water was provided in the home cage until chronic EtOH treatment began, when animals were maintained on a nutritionally balanced liquid diet with supplemental food pellets available during operant sessions. Animals were group housed (three/cage) in plastic cages in a temperature- and light-controlled environment on a 12 L : 12 D cycle (light on at 5:00 a.m.).

Liquid Diet Method

EtOH liquid diet was prepared to be 35% EtOH-derived calories [as previously described by Lochry and Riley (31)]. It

consisted of chocolate Sustacal, a nutritionally complete liquid food (Mead Johnson, Inc., Evansville, IN), vitamin and mineral diet fortification mixtures (ICN Nutritional Biochemicals), and EtOH (200 proof ethyl alcohol). Control liquid diet was prepared with sucrose instead of EtOH to make the control diet isocaloric (1.3 kcal/ml). A pair-feeding procedure was used to keep caloric intake and operant responding in the food reinforcement test equal between control and dependent animals; rats receiving EtOH diet had unlimited access, while the control diet was given in restricted amounts each day. Diets were prepared every other day and changed daily between 2:00 and 3:00 p.m.

Behavioral Testing Apparatus and Procedure

Behavioral testing was conducted in operant chambers (Coulbourn Instruments) that were located in sound-attenuated cubicles equipped with exhaust fans. Each chamber contained an operant lever where responses delivered 45-mg Noyes food pellets into a food hopper. On the first day of training, food was automatically released in the food hopper to acclimate rats to the operant chambers. Rats were then trained to lever-press for food pellets on a continuous reinforcement schedule 1 (FR 1). Once stable responding on the FR 1 schedule was achieved, the schedule of reinforcement was then increased to a fixed ratio 5 schedule (FR 5), where five lever presses were required to obtain each reinforcement. The schedule contingency was then increased to an FR 10 and then to an FR 15 schedule of reinforcement. This training period averaged 11 days. Once stable responding on the FR 15 schedule was established, animals were implanted surgically with ICV cannulae, allowed to recover, and then reintroduced in the FR 15 food reinforcement task.

Surgical Procedure for ICV Cannulation

Following the establishment of stable responding on the FR 15 task, rats were anesthetized with halothane and stereotaxically (Kopf Instruments, Topanga, CA) implanted with a single unilateral 23-ga, 7-mm stainless steel intracranial cannula aimed 1 mm above the lateral ventricle. Stereotaxic coordinates, based upon the atlas of Pellegrino, Pellegrino, and Cushman (44), were with the incisor bar 5.0 mm above ear bar zero: 0.6 mm posterior from bregma; ±2.0 lateral to midline; and −3.2 mm ventral from the skull surface. Placement of cannulae were equally distributed between the left and right ventricles. Cannulae were fastened to the skull with dental cement and secured with three stainless steel screws. A 7-mm stainless steel stylet wire was inserted into each guide cannula to retain patency. Animals were allowed a minimum of 3 days recovery before being placed on liquid diets and then reintroduced into the operant task.

Drug Administration

Bicuculline methiodide (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% sodium chloride (saline), sonicated, and prepared immediately before administration. Bicuculline methiodide was administered ICV in doses of 0, 50, 100, and 200 ng in a volume of 2 μl. ICV injections were administered by gravity through 30-ga injectors inserted to extend 1 mm beyond the ventral tip of the cannulae using a microinjection technique with pieces of calibrated polyethylene tubing that were connected to 10-μl Hamilton syringes (Hamilton, Reno, NV). To habituate animals to the injection technique, all rats were first injected with saline vehicle ICV by gravity in the home cage environment. Ro 15-4513 was prepared as a suspension (0.05% emulfer, 0.05% of 10% EtOH and 0.95% saline) and administered by IP injection. Prior to drug administration in the test session, all animals received an IP injection of vehicle in the home cage.

Data Analysis

For data analysis, the number of reinforcements obtained was the dependent measure and each drug injection was treated as an independent observation because each drug experiment included a group that received injection of vehicle. For each animal, a baseline measure was obtained prior to drug injection where baseline responding (i.e., preinjection responding) for each rat was calculated as an average number of reinforcements obtained per 5-min interval during the first 10 min of the session. The number of reinforcements obtained during each of the four subsequent 5-min postinjection periods were then analyzed using a 2 × 4 × 4 factorial analysis of variance (ANOVA) with a between-subjects factor for liquid diet (EtOH or control), a between-subjects factor for dose, and a within-subjects repeated measure of time. Student's *t*-test analysis was used to compare the effects of liquid diet treatment after injection of each dose of GABA antagonist during each postinjection time interval.

EtOH Concentrations

To verify blood EtOH content at the time of operant testing, blood samples from all animals were obtained immediately after the completion of baseline sessions conducted in an early morning session (6:30 a.m.) and then immediately after the completion of baseline sessions conducted in an evening session (10:30 p.m.) 2 days before injection of bicuculline methiodide (Experiment 1). Samples of mixed arterial and venous blood from EtOH- and control-treated rats were obtained by the tail bleed method. Samples were assayed for blood EtOH content using the NAD-ADH enzyme spectrophotometric method (Sigma Chemical Co.).

Cannulae Placement Verification

After completion of these experiments, cannulae placements for injection were verified in 10 randomly selected animals by gravity infusion of blue dye into the cannulae and tissue slicing to visualize spread of blue dye within the lateral ventricle.

RESULTS

Time of Testing

Animals maintained with unlimited access to 8.7% EtOH diet failed to have high blood EtOH levels at the onset of the light cycle. Blood EtOH concentrations determined from samples obtained after a morning session averaged (±SEM) 42.8 ± 9.6 mg%. Therefore, the time of behavioral testing for the drug studies was set at 10:30 p.m. for all tests so that intoxicating amounts of EtOH would reliably be present at the time of testing. When blood EtOH samples were obtained after an evening session, EtOH concentrations were considerably higher, averaging 173.4 ± 9.2 mg%. The difference in morning and evening EtOH concentrations reflects a circadian variation of EtOH liquid diet intake in group-housed animals maintained on a 12 L : 12 D cycle.

Average (±SEM) body weight for animals in the EtOH liquid diet group was 216.5 ± 2.2 g upon arrival in the labora-

tory; 273.5 ± 4.9 g after 3 days of EtOH liquid diet administration; 274.0 ± 2.5 g after 12 days of EtOH diet; and 328.2 ± 7.5 after 21 days of EtOH liquid diet administration. The average (± SEM) body weight for animals in the control liquid diet group was 227.2 ± 3.0 g upon arrival in the laboratory; 287.9 ± 4.4 g after 3 days of control liquid diet administration; 284.2 ± 4.0 g after 12 days of control diet; and 384.2 ± 7.6 g after 21 days of control liquid diet administration.

Behavioral Effects of Bicuculline Methiodide

Bicuculline methiodide administered ICV disrupted lever pressing for food reinforcement in a dose-dependent manner (see Figs. 1 and 2). ANOVA on the number of reinforcements obtained during the postinjection intervals revealed that there was a significant difference between the EtOH and control liquid diet groups, $F(1, 58) = 9.79$, $p < 0.05$, a significant effect of dose, $F(3, 58) = 5.58$, $p < 0.05$, but the effect of time on the number of reinforcements obtained across the liquid diet treatment groups, $F(3, 174) = 0.22$, $p > 0.10$, was not statistically significant. The group × dose interaction, $F(3, 58) = 1.81$, $p > 0.10$, and group × dose × time interaction, $F(9, 174) = 0.63$, $p > 0.10$, were not statistically significant.

When the number of reinforcements obtained after administration of each dose of bicuculline methiodide at each postinjection time interval were analyzed by Student's t-test analysis, significant differences between EtOH and control-treated groups were noted: ICV administration of 50 ng bicuculline methiodide produced a greater suppression of responding in control-treated rats during the 15-min postinjection interval, $t(15) = 2.75$, $p < 0.05$, and ICV administration of 100 ng bicuculline methiodide produced a greater suppression of responding in control-treated rats during the 10-, $t(13) = 2.33$, $p < 0.05$, 15-, $t(13) = 2.99$, $p < 0.05$, and 20-, $t(13) = 3.34$, $p < 0.01$, min postinjection intervals. Administration of 50 and 100 ng bicuculline methiodide produced a greater suppression in responding in the control-treated group than in the EtOH-treated group during the 5-min postinjection interval, but these differences were not statistically significant at the $p < 0.05$ level, $t(14) = 1.90$, $p = 0.077$; $t(13) = 2.02$, $p = 0.064$, respectively. There were no significant differences between the EtOH- and control-treated groups in the number of reinforcements obtained during each postinjection interval after ICV administration of vehicle or 200 ng bicuculline methiodide (all comparisons $t < 2.0$).

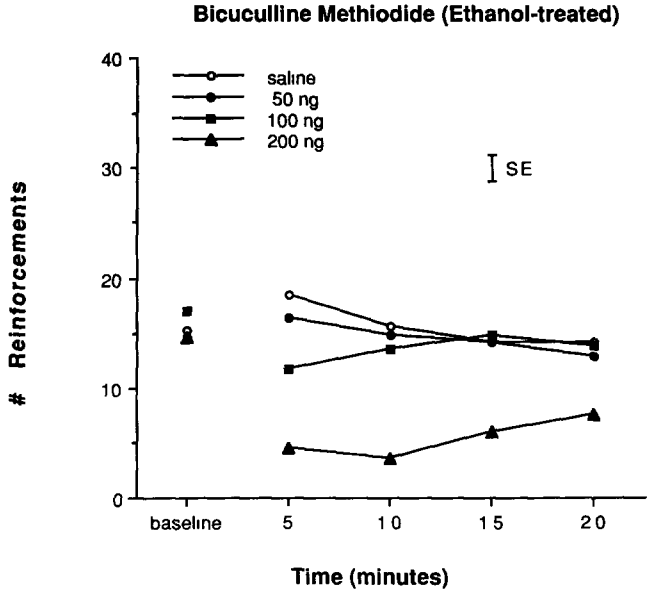

FIG. 1. Effects of bicuculline methiodide injected ICV in rats chronically treated with EtOH on the number of food reinforcements obtained while responding on a fixed ratio 15 schedule (SE = average SE).

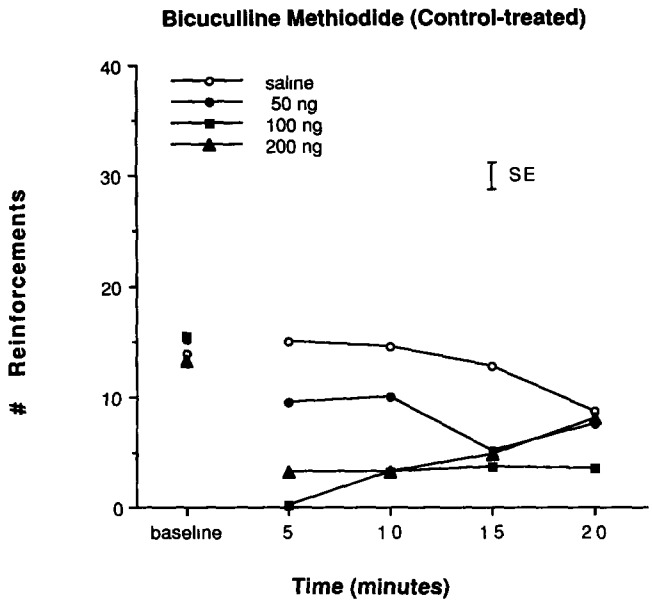

FIG. 2. Effects of bicuculline methiodide injected ICV in pair-fed control rats on the number of food reinforcements obtained while responding on a fixed ratio 15 schedule (SE = average SE).

Behavioral Effects of Ro 15-4513

Systemic administration of Ro 15-4513 in the EtOH-treated group did not produce much disruption in lever pressing (see Fig. 3) and administration of Ro 15-4513 in the control-treated group disrupted lever pressing in a dose-related manner (see Fig. 4). ANOVA on the number of reinforcements obtained during the postinjection intervals revealed that there was a significant difference in responding between the EtOH and control liquid diet groups, $F(1, 37) = 8.89$, $p < 0.05$, and a significant effect of dose, $F(3, 37) = 2.78$, $p = 0.05$. The effect of time on the number of reinforcements obtained across the liquid diet treatment groups was not statistically significant, $F(3, 111) = 0.92$, $p > 0.10$. The group × dose interaction was not statistically significant, $F(3, 37) = 0.87$, $p > 0.10$, and dose × group × time interaction, $F(9, 111) = 0.43$, $p > 0.10$, was not statistically significant.

Comparison of the effects of the various doses of Ro 15-4513 on responding in EtOH- and control-treated animals with Student's t-test indicated that administration of 3.0 mg/kg Ro 15-4513 produced a greater suppression in responding in the control-treated group than in the EtOH-treated group during the 15-min postinjection interval, but this difference was not statistically significant, $t(9) = 2.05$, $p = 0.071$. Ad-

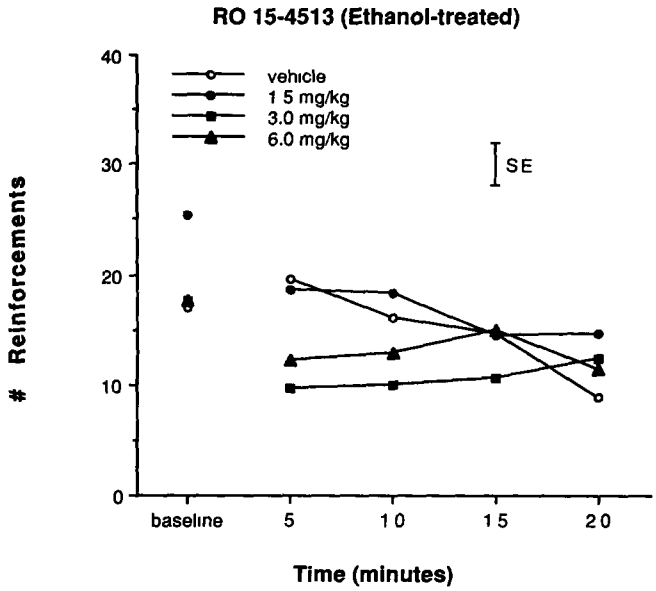

FIG. 3. Effects of Ro 15-4513 injected IP in rats chronically treated with EtOH on the number of food reinforcements obtained while responding on a fixed ratio 15 schedule (SE = average SE).

ministration of 6.0 mg/kg Ro 15-4513 also produced a greater suppression of responding in control-treated rats during the 10- and 15-min postinjection intervals, $t(10) = 2.23, 2.24$, respectively, $p < 0.05$, and a marginally significant suppression of responding in control-treated rats during the 20-min postinjection interval, $t(10) = 2.04$, $p = 0.069$. The number of reinforcements obtained after injection of vehicle or 1.5 mg/kg Ro 15-4513 were not significantly different during any of the postinjection intervals (all comparisons $t < 2.0$).

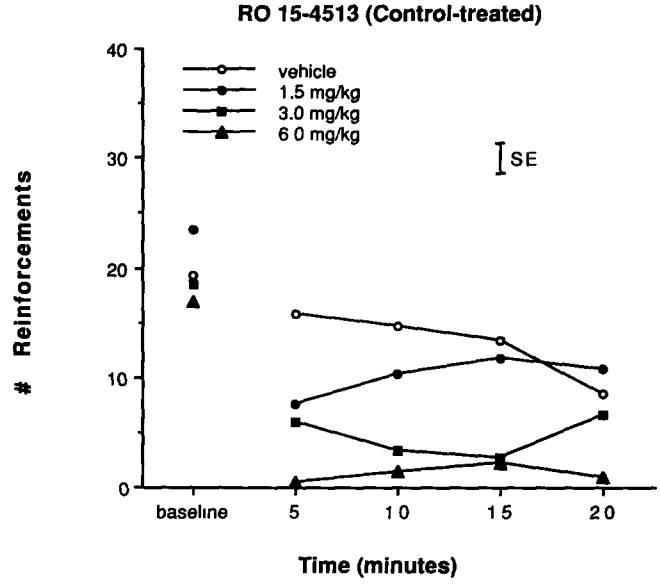

FIG. 4. Effects of Ro 15-4513 injected IP in pair-fed control rats on the number of food reinforcements obtained while responding on a fixed ratio 15 schedule (SE = average SE).

Upon completion of the study, cannulae placement was verified to be located within the lateral ventricle by using the blue dye gravity infusion/tissue slicing method.

DISCUSSION

It had been hypothesized that if the mechanisms underlying EtOH dependence were similar to those of the classic opiate dependence model pharmacological precipitation of EtOH withdrawal would produce an immediate disruption in operant responding, analogous to naloxone- and methylnaloxonium-precipitated opiate withdrawal (27). Response suppression as a sign of precipitated EtOH withdrawal was also anticipated to resemble the suppression of operant responding that occurs with spontaneous EtOH withdrawal when testing was conducted on a mixed schedule of food reinforcement containing a fixed ratio 30 schedule at times when maximal behavioral signs of EtOH withdrawal occur (8).

Surprisingly, the present results indicate that animals maintained on chronic EtOH liquid diet were, if anything, *less* sensitive to the effects of antagonism of the $GABA_A$-BDZ-ionophore complex. Chronic EtOH-exposed rats showed less response disruption to 100 ng bicuculline methiodide and 3 and 6 mg/kg Ro 15-4513. In contrast, identical doses of these drugs produced a strong suppression in responding for food reinforcement in control subjects. In the present study, responding in the control liquid diet treatment group tended to decline over the 20-min period postinjection, in particular during the last 5-min interval (see Figs. 2 and 4). The gradual decline in the number of reinforcements obtained over time observed here has been reported in previous studies employing the same schedule contingency (27) and probably reflects response fatigue because animals were responding at reasonably high rates.

In the event that EtOH withdrawal had been precipitated by bicuculline methiodide or Ro 15-4513, a noticeable decline in the number of reinforcements was expected to occur much earlier in the test session (at the 5- or 10-min postinjection interval) in EtOH-treated animals. Presumably, this decline would have been directly related to injections of these drugs. At the very least, if EtOH withdrawal could be precipitated an increased sensitivity to the disruptive effects of these drugs would be expected and would probably appear as a shift to the left of the dose-effect curve, where EtOH-treated animals would be disrupted by doses that did not alter responding in control animals. There was no evidence of an increased sensitivity to the response-disruptive effects of either bicuculline methiodide or Ro 15-4513 injections in rats that were chronically treated with EtOH.

In fact, it appeared that chronic EtOH treatment attenuated the response-disruptive effects of bicuculline methiodide and Ro 15-4513 in the food reinforcement task, and signs of "precipitated" EtOH withdrawal were not evident. It is possible that the suppressive effects (floor effects) of 100 ng bicuculline methiodide or 3.0 and 6.0 mg/kg Ro 15-4513 in the control group could decrease the statistical power for detecting a drug-induced decrease in responding in EtOH group. However, following injection of these doses of GABA antagonists the average number of reinforcements obtained in the EtOH group actually was significantly greater than the average number of reinforcements obtained in the control group during certain postinjection intervals. The present results do not support the hypothesis that antagonism of GABA receptor

activity during chronic EtOH treatment results in a precipitated EtOH withdrawal syndrome but instead indicate adaptation of GABAergic systems during periods of chronic EtOH treatment.

It is possible that the effects of chronic EtOH treatment plus bicuculline methiodide (or chronic EtOH plus Ro 15-4513) may simply reflect the additive effects of acute EtOH as a GABA indirect agonist and a GABA antagonist, as is seen with the acute effects of ethanol (26). Although the experimental design used in the present study did not distinguish between acute and chronic effects of EtOH with GABA complex antagonists on operant responding, previous research shows that the response-disruptive effects of acute EtOH in an operant reaction time task (26) are attenuated by doses of Ro 15-4513 that do not alter responding when administered alone (26), again suggesting that each drug can cancel out the effects of the other at certain dose ranges. However, in the present study Ro 15-4513 did produce a disruption in responding in control animals, and in this case the chronic EtOH attenuated the response-disruptive effects of Ro 15-4513. These results suggest that the response requirement of the FR 15 task may be more sensitive than the reaction time task to the motor incapacitating effects of Ro 15-4513 and that the interaction of Ro 15-4513 with both acute or chronic EtOH may be mediated by activity at the GABA-BDZ receptor complex.

Previous research has demonstrated the ability of chronic EtOH to affect GABA neurotransmission by examining the cellular events associated with activation of the $GABA_A$-BDZ receptor complex. It is known that the recognition sites of the complex interact with each other in an allosteric manner and regulate the gating properties of the Cl- ion channel, which upon activation produces an increase in membrane hyperpolarization and a decrease in neuronal excitability (33). In chronic EtOH experiments where EtOH concentrations are greater than 150 mg % at the time of sacrifice, a decrease in muscimol, a GABA agonist, and pentobarbital-stimulated Cl- ion flux is observed in rat cerebral cortex synaptoneurosome preparations (41). When concentrations of EtOH at the time of sacrifice were less than 150 mg %, [^{3}H]muscimol-stimulated Cl- ion flux was unchanged by chronic EtOH treatment (6,41), suggesting that the degree of EtOH exposure, or at least the maximal concentrations of EtOH obtained before sacrifice, is critical for revealing any change in GABA-stimulated Cl- ion activation.

The present results demonstrating that bicuculline methiodide does not precipitate signs of EtOH withdrawal are consistent with a decrease in functioning of the $GABA_A$-BDZ receptor complex during chronic EtOH treatment. The observed reduced sensitivity to the response-disruptive effects of a GABA antagonist is also supported by molecular studies that indicate that chronic EtOH administration changes the molecular composition of $GABA_A$ receptors, possibly giving rise to a population of structurally distinct or desensitized $GABA_A$ receptors (39).

The inability of Ro 15-4513 to produce signs of precipitated EtOH withdrawal in the present study is in agreement with the results of Becker and Anton (4), who showed that Ro 15-4513, administered in the presence of intoxicating concentrations of EtOH in chronically treated EtOH mice, did not precipitate signs of withdrawal. In fact, administration of Ro 15-4513 was described as producing less withdrawal-like behavior (ambulatory behavior, handling- and sound-induced seizure activity) in those mice that were chronically exposed to EtOH by inhalation, as compared to control subjects (4). This observation is consistent with resistance to the response-disruptive effects of Ro 15-4513 in EtOH-treated animals in the present study.

In contrast to the reports of decreases in GABA activity that occur with chronic EtOH administration, some ligand binding studies and functional biochemical studies suggest that the neuronal mechanisms that mediate the actions of Ro 15-4513 may be upregulated by chronic EtOH treatment. For example, chronic EtOH exposure during chronic intoxication and withdrawal increases the number of Ro 15-4513 binding sites in the rat cerebral cortex and cerebellum (35) and in cultured spinal cord cells (36). However, Buck and Harris (6) showed that chronic EtOH treatment does not change Ro 15-4513 receptor density or affinity in mouse whole-brain samples but does result in an increased sensitivity of the receptor complex to Ro 15-4513 during chronic intoxication that is further enhanced during withdrawal. This inverse BDZ receptor agonist inhibits muscimol-activated Cl- uptake in microsacs prepared from cerebral cortices of mice, and chronic EtOH treatment produces a greater degree of inhibition (6), suggesting that the receptor complex becomes more sensitive to the actions of BDZ inverse agonists with chronic EtOH treatment (6). Therefore, it appears that chronic EtOH treatment may differentially effect the functioning of the specific recognition sites of the receptor complex because the reactivity of the receptor complex to Ro 15-4513 increases while GABA and BDZ reactivity decrease with chronic EtOH treatment.

Chronic administration of benzodiazepines and barbiturates may alter the $GABA_A$-BDZ receptor complex in a comparable way to that which has been described for chronic EtOH (37,39,48). Specific experiments conducted with a focus on the functioning of the $GABA_A$-BDZ receptor complex show that chronic treatment of lorazepam, a benzodiazepine, in mice produces a decrease in BDZ receptor density and a decrease in muscimol stimulated Cl- uptake (37) and that chronic pentobarbital administration causes a decrease in the affinity of cortical binding of BDZ (flunitrazepam) and muscimol to their recognition sites (48). Similar to the decrease in receptor-mediated Cl- activation that is observed with chronic EtOH, chronic pentobarbital treatment produces a decrease in muscimol- and pentobarbital-stimulated Cl- uptake in rat cerebral cortical synaptoneurosomes (39). Therefore, the effects of chronic administration of BDZ and barbiturate on GABA neurotransmission resemble some of the biochemical alterations that are reported to occur with chronic EtOH treatment.

Barbiturates, BDZ, and EtOH administration have a common ability to act as "anxiolytics" (14), anticonvulsants, and muscle relaxants, and cross-tolerance between these drugs occurs (24). However, BDZ withdrawal (32) and not EtOH withdrawal (1) can be precipitated by a benzodiazepine receptor antagonist, RO 15-1788, suggesting that the neural processes underlying dependence produced by BDZ and EtOH do not appear to be parallel. Whether mechanisms of EtOH dependence are more similar to those of barbiturate dependence is not clear because precipitation of barbiturate withdrawal has not yet been demonstrated to our knowledge. Therefore, further research is necessary for a conceptual understanding of the neurochemical mechanisms that trigger signs of EtOH withdrawal during periods of intoxication. Moreover, the identification of a neuropharmacological tool to precipitate EtOH withdrawal may someday have clinical importance to assess dependence risk in humans, inasmuch the same way that R0 15-1788-precipitated BDZ withdrawal is used to evalu-

ate dependence risk in persons who use diazepam to alleviate symptoms related to panic disorder (5).

In summary, the present results show that chronic ethanol treatment may produce a decreased sensitivity to GABA antagonists and BDZ inverse agonists. These effects may be mediated by neuroadaptive changes in the CNS such as a functional decrease in the sensitivity of $GABA_A$ receptor recognition sites to GABA. Consequently, chronic EtOH may attenuate the maximal effectiveness of cooperative receptor interactions that are mediated by the allosteric regulatory sites of the $GABA_A$-BDZ receptor complex, which, under normal conditions, interact in a modulatory manner to facilitate receptor binding and Cl- ion activation. The reduced sensitivity to the disruptive effects of a GABA antagonist or a benzodiazepine inverse agonist in EtOH-treated rats on a quantitative measure of motivation for food reinforcement may offer a behavioral correlate to support the cellular downregulation of neural activity of the $GABA_A$-BDZ-ionophore receptor complex that occurs during chronic EtOH treatment.

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