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Antagonism of Ethanol-Reinforced Behavior by the Benzodiazepine Inverse Agonists Ro15-4513 and FG 7142: Relation to Sucrose Reinforcement

HERMAN H. SAMSON, MIKI HARAGUCHI, GERALD A. TOLLIVER AND KEVIN G. SADEGHI

Alcohol and Drug Abuse Institute, NL-15, University of Washington, Seattle, WA 98195

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SAMSON, H. H., M. HARAGUCHI, G. A. TOLLIVER AND K. G. SADEGHI. *Antagonism of ethanol-reinforced behavior by the benzodiazepine inverse agonists Ro15-4513 and FG 7142: Relation to sucrose reinforcement.* PHARMACOL BIOCHEM BEHAV **33**(3) 601–608, 1989.—The partial inverse benzodiazepine agonist Ro15-4513 has been shown to antagonize many of ethanol's actions, including the reduction of behavior reinforced with ethanol presentation. The studies reported here compared the effects of the Ro compound on sucrose reinforcement alone and concurrently available with ethanol reinforcement. Also, a second inverse agonist, FG 7142, was tested. The result indicated that ethanol reinforcement was more sensitive to the inverse agonists compared to sucrose reinforcement. This was seen as a graded effect upon ethanol responding at doses which failed to have any effect upon sucrose-reinforced behavior. The Ro compound was approximately three times more potent than the FG compound in suppressing ethanol-reinforced responding. Possible explanations for the greater sensitivity of ethanol reinforcement compared to sucrose reinforcement was discussed in terms of ethanol's potential actions at the benzodiazepine-GABA receptor complex.

Ethanol reinforcement Sucrose reinforcement Inverse benzodiazepine agonists
Benzodiazepine-GABA receptor complex Ethanol antagonists Ro15-4513 FG 7142

THE relation between ethanol and the GABA receptor mediated chloride ion channel has received an increasing amount of attention in the past few years (1, 10, 31, 34). The relationship of the benzodiazepine receptor with the GABA system has also provided insight into how many of ethanol's actions may be related to this benzodiazepine-GABA receptor complex (20,30). These findings help explain a variety of data from previous investigations. For example, the use of benzodiazepines during ethanol withdrawal is part of most standard alcohol detoxification processes with alcoholics (6) and cross tolerance between ethanol and the benzodiazepines has been well documented (11).

The imidazobenzodiazepine Ro15-4513 (ethyl-8-azido-5,6-dihydro-5-methyl-6-Oxo-4H-imidazo [1,5],[1,4] benzodiazepine-3-carboxylate), a partial inverse benzodiazepine agonist, has been shown by some investigators to partially antagonize several of ethanol's physiological and behavioral actions. Ethanol-induced narcosis (29, 30, 33), ethanol's anticonvulsive actions (17) and ethanol's ability to decrease activity and produce motor incoordination (2, 9, 12) have all been shown to be antagonized by Ro15-4513. However, others have failed to replicate some of these findings (7,15). The discriminative cue properties of ethanol were shown to be attenuated by Ro15-4513 (21), but other investigators have failed to replicate this finding (8). Ro15-4513 failed to reverse ethanol's hypothermic effects (9,33), nor could the lethal effects of ethanol be reversed (18). In addition, some investigators have failed to antagonize barbiturate effects with Ro15-4513, while blocking ethanol's actions (9, 29, 30). Others, however, have antagonized some of the effects of barbiturates (17). It is thus unclear as to the specificity of the compound for ethanol's actions.

It also remains unclear whether or not all inverse benzodiazepine agonists will reverse ethanol's effects, or if the Ro compound has unique properties for ethanol (13). While some investigators have failed to antagonize ethanol's effects with other inverse agonists (30), others have reported similar effects with differing efficacy among several additional inverse agonist compounds (12,34).

Because of Ro15-4513's ability to antagonize some of ethanol's effects, it has been postulated that administration of the drug could result in increased ethanol intakes, acting in much the same way that low doses of opiate antagonists increase opiate self-administration (16). To examine this question, we initially explored the effect of acute doses of Ro15-4513 on ethanol self-administration in rats (26). We found that at all doses tested, ethanol self-administration was either not altered or that intakes

were decreased in a dose-dependent manner. Under none of the various dose conditions did an increase in ethanol intake occur. This result has been replicated for other ethanol intake situations (5,14). However, the questions of specificity of this reduction for ethanol and inverse agonist specificity have not been addressed. The present work extends our initial work on ethanol by examining the effects of Ro15-4513 on sucrose self-administration alone and on sucrose and ethanol self-administration when both were concurrently available. Also, the effects of a second inverse benzodiazepine agonist, FG 7142, were tested in animals self-administering ethanol.

EXPERIMENT ONE

Because ethanol self-administration was reduced in a dose-dependent manner by Ro15-4513 (26), it was important to determine if other self-administration behaviors would also be reduced to the same extent by administration of the inverse agonist. In past research, we have employed the use of low concentration sucrose solutions to compare drug effects to ethanol (23,25). While there are difficulties in trying to match behavior with qualitatively different reinforcers, the use of low concentration sucrose solutions results in behavior patterns and choices basically similar to those observed when 5–10% ethanol is presented as the reinforcer (23). Thus, for the following study, the concentrations of sucrose presented as the reinforcer were chosen in an attempt to match the behavioral patterns observed with ethanol reinforcement from our prior Ro15-4513 work (26). Obviously, even if the behavioral patterns and fluid intakes obtained were identical, no assurance can be made that the two reinforcers have equal efficacy. However for the present study, the issue of different rates of responding (4) are held to a minimum.

METHOD

Animals

Male, Long-Evans rats (n = 4), weighing 350–400 g at the start of the experiments, were obtained from the University of Washington's Department of Psychology breeding facility. At all times during the experiments, except for one to three days during initial lever press training, the animals had food and water available ad lib in their home cages. The animals were housed individually in hanging rodent cages. Artificial lighting was on from 700 to 1900 hours daily. Temperature and humidity were maintained within the guidelines set by the National Institutes of Health.

Apparatus

The operant chambers and their enclosures have been previously described (22). Briefly, each chamber was equipped with two removable rodent levers and two dipper fluid delivery systems (Gerbrands Corporation, Model G5600 B-RH), fitted with a 0.1 ml cup. For this experiment, only one lever and dipper system were used, with the other lever removed from the chamber.

A 1-watt house light was illuminated when the session was in progress. Apple microcomputers were used to record lever presses and schedule control of dipper presentations.

Drugs

Ro15-4513 was suspended in approximately 0.15 ml to 0.20 ml of Tween 80 and diluted with 0.9% sodium chloride to a fixed volume. All solutions were made immediately prior to injection and were shaken on a mechanical shaker for one to two minutes before each injection. Doses of 3, 6, and 10 mg/kg were tested. Drugs were injected IP, 15 minutes before the 30-minute operant session.

Procedure

The animals were initially given seven days to adapt to the home cage conditions, during which time they were handled and weighed daily. To facilitate shaping the lever-press response in the operant chambers, the animals were water-deprived for 16 hours. During the initial training sessions, 20% sucrose solution (weight/volume) was available in the dipper on a continuous reinforcement (CRF) schedule. Once the animals were shaped and responding rapidly on the CRF schedule (one to three sessions), the 16-hour presession water deprivation was discontinued and the lever-press requirements gradually increased to fixed-ratio (FR) 4. From this time on, no food or water restriction was used. The concentration of the sucrose was decreased over sessions until lever-press behavior was comparable to that observed for ethanol reinforcement. Comparability was based on both amount and pattern of responding during the 30-min session. As stated above, identical response patterns are impossible to achieve but every attempt was made to have patterns as close as possible to those observed with ethanol reinforcement. The animals received one 30-min session each day in the operant chambers, Monday through Friday.

After the baseline was established, control injections of 0.9% sodium chloride were given on Wednesdays and Ro15-4513 was administered on Thursdays. On Mondays, Tuesdays and Fridays, the animals received no treatment prior to the start of their daily 30-minute sessions. All doses of the drug were administered at least twice, with some doses tested three times. In addition, a dose of 4 mg/kg was tested in two of the rats following completion of testing at the other dose levels.

RESULTS

Following training, the sucrose concentration which was presented as the reinforcing stimulus varied from 1.5% to 3% (w/v) depending upon the individual rat. The concentrations were chosen in order to try to maintain responding in a pattern and rate similar to that previously found for ethanol reinforcement. However, in some cases, while the onset and initial rates of responding were similar, total session responding was greater in the sucrose animals. This total responding was on the order of 15% to 20% more responses per session when compared to the amount of lever pressing in our previously reported work with Ro15-4513, when 10% ethanol was presented as the reinforcer (26).

Responding for sucrose was suppressed by Ro15-4513 at doses of 6 mg/kg and greater with no further increases in response suppression occurring with increases in dose (Fig. 1). The 3 mg/kg dose, which suppressed ethanol-reinforced responding by 70% in our prior work (26), was ineffective, with some animals exhibiting a 5% to 10% increase in responding over vehicle rates. In the two animals tested with a dose of 4 mg/kg, a significant 50% suppression in responding occurred at this dose as well (50% of the no injection baseline, 49% of vehicle control). This suppression at 4 mg/kg was as great as that found at 6 mg/kg. An analysis of variance using a multiple factor within-between repeated measures design found a significant effect across dose, $F(2,14) = 28.476$, $p < 0.001$. Using the Bonferroni *t*-test, the 6 mg/kg, and 10 mg/kg doses were significantly different from 3 mg/kg, but were not significantly different from each other. At no time was there a significant difference between no injection baseline responding and vehicle control injections (Fig. 1).

Examination of the cumulative records suggested that in most cases the decrease in total session responding was a function of a

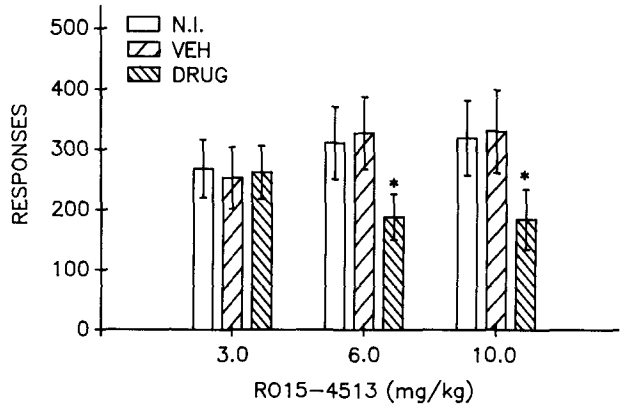

FIG. 1. The effect of Ro15-4513 upon lever press responding maintained by sucrose reinforcement. (N.I. = No Injection control: VEH = vehicle injection control: DRUG = drug injection) (Values are Means and SEM; *Significantly different from N.I.)

delay in the onset of responding until late in the 30-minute session (Fig. 2). In these cases, it appeared that responding began at a time at which most of the drug could have been metabolized. This late onset of behavior resulted in the decrease in total responding observed. However, in a few cases, the animals responding appeared to have a normal onset, but was greatly disrupted, with long breaks occurring between runs of responding. There did not appear to be any specific dose relation to these different patterns of disruption but rather they were related to individual animals.

Since the dose of 3 mg/kg which completely suppressed ethanol responding was found not to be effective in the four animals tested, an additional 3 rats were trained in the identical manner and tested with doses of 3 and 6 mg/kg. An identical result was observed in these three animals with 3 mg/kg showing no effect (Mean No Injection responding = 243, SEM = 42; Mean Drug = 264, SEM = 61) while at 6 mg/kg, a significant reduction was found (Mean No Injection = 273, SEM = 41; Mean Drug = 71, SEM = 18; paired $t(5) = 7.039$, $p < 0.001$). The effects of the 6 mg/kg dose on pattern of responding were similar to those noted for the original 4 rats at this dose.

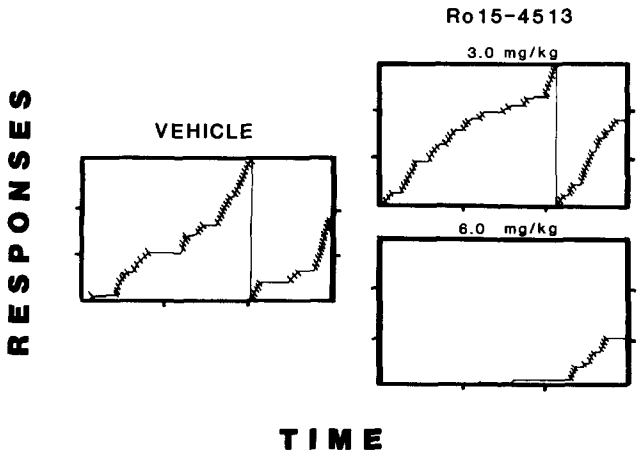

FIG. 2. Sample cumulative records from one rat for Ro15-4513 doses of 3 mg/kg and 6 mg/kg. (Responses on the Y axis = 50/division; Time on the X axis = 10 min/division)

DISCUSSION

From these results, an all or none dose-response function upon sucrose-reinforced responding was observed. With ethanol reinforcement, Ro15-4513 reduced responding at doses that had no effect upon sucrose responding (26). In the ethanol studies, there was a graded effect upon responding at the low and medium doses tested, such that the number of reinforcers presented per bout of lever activity decreased and greater pauses between lever pressing bouts occurred. As well, no effects upon response onset latency were observed until the 3 mg/kg dose. In the present study with sucrose, no effect upon responding was seen at the 3 mg/kg dose, with an apparent maximum effect occurring at 4 mg/kg. This suggests that ethanol reinforcement may be more sensitive to the effects of Ro15-4513 than sucrose reinforcement. However, before this interpretation can be accepted, the alternative hypothesis of unequal reinforcing efficacy between the ethanol and sucrose solutions employed must be ruled out. While every attempt was made to equate response patterns between animals receiving ethanol reinforcement and animals receiving sucrose reinforcement, the sucrose reinforcement group consistently maintained a slightly greater amount of responding during the 30-minute session. It is possible that this increased amount of behavior could be the result of more efficacious sucrose reinforcement than that provided by ethanol. If this is the case, the decreased sensitivity to Ro15-4513 in the sucrose reinforcement situation could be a result of increased reinforcer efficacy and not of a greater sensitivity of ethanol reinforcement. To try to determine if this latter explanation was the case, an additional experiment was performed.

EXPERIMENT TWO

It was impossible to determine from Experiment One if the failure of a 3 mg/kg dose of Ro15-4513 to suppress sucrose responding, while having a marked effect upon ethanol responding (26), was due to differences in the reinforcing efficacy of the two reinforcers or whether ethanol reinforcement is more sensitive to the Ro15-4513. One way the question of reinforcing efficacy can be examined is to use a concurrent schedule procedure, in which both ethanol and sucrose reinforcement are provided at the same time (23,25). In past work in our laboratory, the effects of the benzodiazepine agonist, chlordiazepoxide, upon concurrent responding in which ethanol and sucrose were available as reinforcers indicated that the drug's effect was dependent upon the concurrent conditions (24). The use of this procedure could assist in determining if the increased sensitivity of Ro15-4513 to suppress ethanol-reinforced responding was related to reinforcer efficacy.

METHOD

Animals

Male Long-Evans rats (n = 3) were used for this experiment. They were obtained and housed in an identical manner as the animals used in Experiment One.

Apparatus

The same chambers used in Experiment One were used for this study. However, following initiation of ethanol reinforcement, two lever and two fluid delivery systems were employed.

Drugs

The Ro15-4513 was administered as in Experiment One. Doses of 1, 3 and 6 mg/kg were tested.

Procedure

The rats were initiated to lever press using ethanol (10%) reinforcement following the sucrose-substitution procedure employed by our laboratory (23). During initiation, only the right lever was placed in the operant chamber. The sucrose-substitution procedure initially trains the animals to lever press using 20% sucrose as the reinforcing stimuli. Following the establishment of lever pressing, the sucrose concentration is reduced and ethanol is added to the reinforcing solution presented. Over a period of weeks, the sucrose is faded out and ethanol (10%, v/v) becomes the solution presented as the reinforcer [for a more complete description of the procedure, see (23)]. Once ethanol-reinforced responding was initiated and the rats were responding on a FR 4 schedule, they were given seven sessions in which the left lever and dipper system were used, rather than the right. Ethanol (10%) was still presented as the reinforcer. Following these sessions, the concurrent schedule was begun. At this time, and for the rest of the study, both levers were always in the operant chamber. Sucrose (1% w/v) and ethanol (10% v/v) were the two reinforcers concurrently available. The position of the levers and associated dippers for each reinforcer was alternated each session, i.e., ethanol was associated with the right dipper and lever on session one, and with the left lever and dipper on session two, etc. The rats received 40 sessions in the concurrent FR 4 FR 4 condition before drug administration was started.

Since there were slight lever preferences across animals, the schedule of weekly injections was changed for the concurrent study. Mondays, Wednesdays and Fridays were no injection days. On Tuesday, the vehicle was administered 15 minutes prior to the session. On Thursday, Ro15-4513 was administered 15 minutes before the session. In this way, the vehicle and drug days corresponded to days in which the reinforcers were associated with the same lever configuration. All drug doses were tested at least twice at each of the two ethanol-sucrose lever configurations.

RESULTS

All three rats were successfully initiated to lever press when 10% ethanol was presented as the reinforcer. Prior to the start of the concurrent procedure, when only ethanol reinforcement was available, the animals averaged 136 responses/session when the right lever (initiation lever) was used and 110 responses/session when the left lever was used. Two of the three animals showed a slight preference for the right lever, when right only vs. left only responding was compared.

Upon institution of the concurrent conditions, the rats initially showed some decline in overall responding, but levels recovered in three to five sessions, with the animals responding on both levers throughout the session. One rat developed a strong lever preference, and made approximately two-thirds of its daily session responses on that lever, independent of which reinforcer presentation was related to that lever. The other two animals showed little if any lever preferences, but had increased variability in responding across days compared to their single lever performance. The data were analyzed with and without consideration for the lever with which the presentation of ethanol was associated. Since the results of both analysis were identical, only the analysis independent of lever position will be presented.

At the end of the baseline concurrent period, stable and similar response levels were obtained for both sucrose and ethanol (Fig. 3—No Injection). Thus, drug effects were assessed upon similar behavioral rates of responding. At the 1.0 mg/kg dose, there was a small but nonsignificant decrease in responding for ethanol when compared to both no injection and vehicle controls. For sucrose,

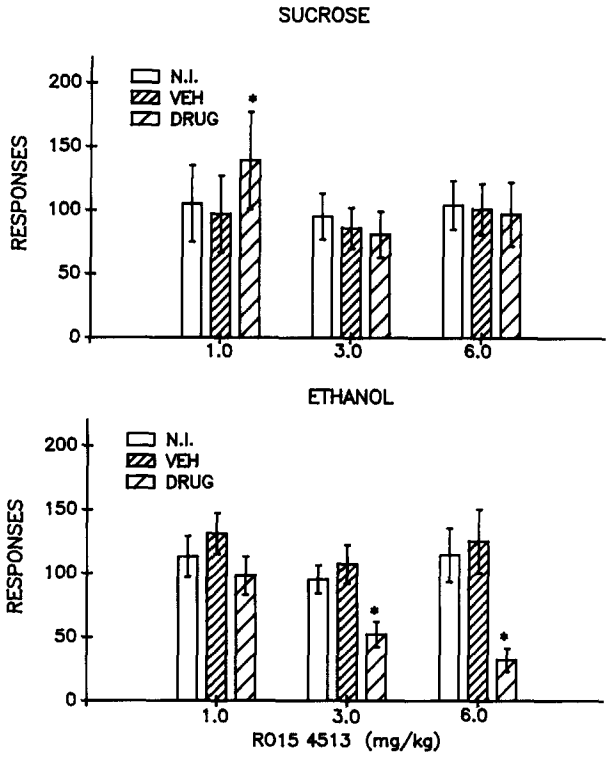

FIG. 3. The effect of Ro15-4513 on lever press responding in the concurrent situation. Sucrose-reinforced responding is in the upper panel, ethanol-reinforced responding in the lower panel. (Abbreviations and values as in Fig. 1.)

using a repeated designs ANOVA, a significant increase in responding resulted (Fig. 3), $F(2,10)=8.387$, $p<0.01$. This increase was approximately 30% above both the no injection and vehicle control response levels.

At the 3 mg/kg and the 6 mg/kg doses, ethanol responding was significantly reduced (Fig. 3), 3 mg/kg, $F(2,22)=13.349$, $p<0.01$; 6 mg/kg, $F(2,10)=24.342$, $p<0.01$. Responding at the 3 mg/kg dose was reduced by 45% compared with no injection control and by 72% at the 6 mg/kg dose. At neither dose was there any effect upon sucrose responding (Fig. 3).

Examination of the cumulative records indicated that in some cases of reduced ethanol responding, sucrose responding appeared to be delayed, but not altered in general response pattern. Unlike the effects seen with the 6 mg/kg dose in Experiment One, no long delays in the onset of responding connected with sucrose reinforcement were found in the concurrent condition. An example of the differential effects of the 3 mg/kg dose on ethanol and sucrose reinforced responding is presented in Fig. 4.

DISCUSSION

In the concurrent situation, in which overall session response rates were similar for both sucrose and ethanol reinforcement, Ro15-4513 selectively suppressed ethanol-reinforced responding but did not affect sucrose-reinforced responding. For ethanol-reinforced responding, a shift to the right in the dose effect curve occurred in the concurrent situation, compared to that observed when only ethanol reinforcement was available, i.e., a 1 mg/kg dose significantly reduced responding when ethanol was the only

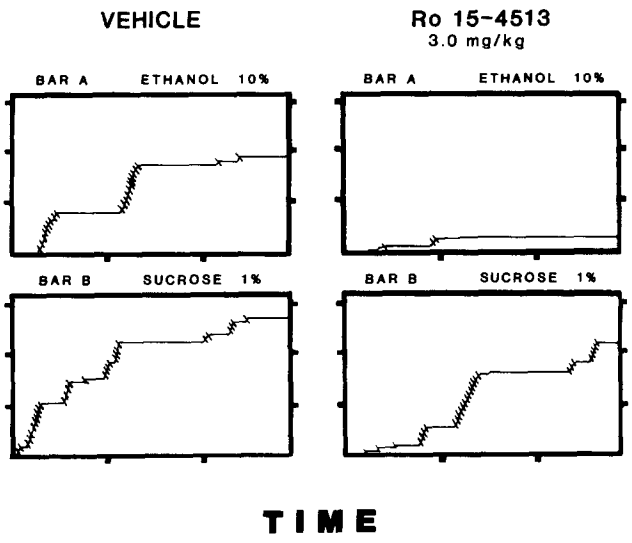

FIG. 4. Sample cumulative records from one rat during concurrent schedule presentation of ethanol and sucrose reinforcement. Top and bottom panels on the right are from a single session following vehicle injection. The panels on the left are from a single session in which 3 mg/kg of Ro15-4513 was injected 15 min prior to the session. In both sessions, ethanol presentation was associated with responses on lever A (left lever). (Axis markers are as in Fig. 2).

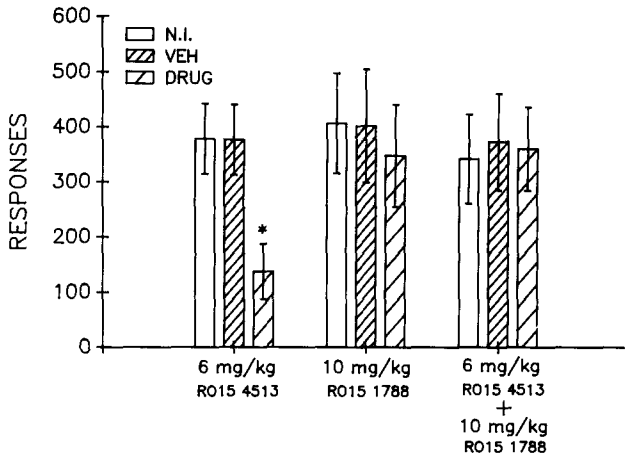

FIG. 5. The effect of Ro15-1788 alone and in combination with Ro15-4513 upon sucrose-reinforced lever pressing. (Abbreviations and values as in Fig. 1.)

reinforcer available (26). It is possible that this shift also occurred for sucrose, at the 6 mg/kg dose, which produced a decrease in sucrose-reinforced responding when only sucrose reinforcement was available, failed to alter sucrose-reinforced responding in the concurrent situation. However, higher doses were not tested, so the possible extent to which the dose effect curve may have been altered for sucrose reinforcement is unclear.

EXPERIMENT THREE

While it was assumed in Experiments One and Two that the effect of Ro15-4513 was through its action at the benzodiazepine receptor, no specific attempt was made to block its actions by use of an antagonist. The following study determined the capability of the benzodiazepine antagonist Ro15-1788 to block the Ro15-4513 effect upon sucrose-reinforced responding.

METHOD

Animals

Five animals from Experiment One were used for this experiment following completion of that study. They were chosen based upon the stability of their responding and their general health at the time of the experiment. The housing and other conditions were the same as in Experiment One.

Apparatus

The same apparatus as in Experiment One was used.

Drugs

Ro15-4513 was prepared and used as in Experiment One. The only dose employed was 6 mg/kg, given 15 minutes prior to the operant session. Ro15-1788 was suspended in the same manner as Ro15-4513 and injected IP. A dose of 10 mg/kg was employed, injected 15 minutes before the session when given alone and injected immediately after the Ro15-4513 when given in combination.

Procedure

Upon completion of Experiment One, the five rats chosen for Experiment Three were given an additional 10 sessions in which no drugs were injected. They were then given the standard weekly block of five sessions as used in Experiment One, in which they received a vehicle injection prior to the Wednesday session and a 6 mg/kg Ro15-4513 prior to the Thursday session. Following this repeated test of the 6 mg/kg Ro15-4513 dose, two blocks of five sessions each were given in which Ro15-1788 was administered on the drug day at a dose of 10 mg/kg. Following the testing with Ro15-1788 alone, two additional blocks of five sessions were performed in which both Ro15-4513 (6 mg/kg) and Ro15-1788 (10 mg/kg) were administered together on the drug session day. The same analysis procedures used in Experiment One were employed.

RESULTS

The retest of Ro15-4513 alone at the 6 mg/kg dose resulted in a 60% suppression of lever pressing behavior (Fig. 5). This was slightly greater than had been observed during Experiment One, but was not statistically different. Administration of Ro15-1788 produced a slight but not significant suppression in responding (Fig. 5). When both Ro15-4513 and Ro15-1788 were administered together, the suppressive effects of Ro15-4513 were completely blocked by Ro15-1788 (Fig. 5). Examination of the cumulative records indicated that the nature of the suppression produced by Ro15-4513 was similar to that observed in Experiment One and the addition of Ro15-1788 resulted in behavior patterns indistinguishable from no injection or vehicle control performance.

DISCUSSION

These results indicate that the suppressive effects of Ro15-4513 are associated with its actions at the benzodiazepine receptor complex, and that the effects can be antagonized by Ro15-1788.

How this receptor complex is involved in the control of sucrose-reinforced lever pressing remains to be clarified.

EXPERIMENT FOUR

From the above experiments, differences in the effect of Ro15-4513 on ethanol and sucrose-reinforced responding were apparent. Whether or not this was specific to Ro15-4513, or was an action of all inverse benzodiazepine agonists, remained to be tested. Therefore, this study was performed to examine the effects of another inverse agonist, FG 7142, on ethanol-reinforced responding.

METHOD

Animals

Three male Long-Evans rats obtained from the breeding facilities of the Department of Psychology, University of Washington, were used. The rats were 90 days old with a mean weight of 355 grams at the start of the experiment. The animals were housed as in the preceding experiments. Food and water were available as in Experiment Two.

Apparatus

The operant chambers and their enclosures were as in the preceding experiment. For this experiment, each chamber was equipped with one lever and one dipper fluid delivery system.

Drugs

FG 7142 was suspended in approximately 0.15 ml to 0.20 ml of Tween 80 and diluted with 0.9% sodium chloride to a fixed volume. All solutions were made immediately prior to injection and were shaken on a mechanical shaker for one to two minutes before each injection. Doses of 3 mg/kg, 5 mg/kg, 7 mg/kg, and 10 mg/kg were tested.

Procedure

The animals were initiated to lever press using the same ethanol initiation procedure described in Experiment Two. Following initiation, the animals were given one week of baseline self-administration with 10% ethanol available as the reinforcer prior to starting the drug injection series. The animals received a single 30-minute session in the operant chamber, Monday through Friday. Control injections of 0.9% sodium chloride were given on Wednesdays and FG 7142 was administered on Thursdays. On Mondays, Tuesdays and Fridays, the animals received no treatment prior to the start of their daily session. All injections were given intraperitoneally 15 minutes before the start of the session. Each dose was tested twice in a descending order of drug concentration.

RESULTS

The main effect in all rats was a decrease in responding for ethanol with increasing doses of FG 7142. At all doses tested, ethanol responding decreased (Fig. 6). An analysis of variance using repeated measures for dose across injection conditions found a significant effect of dose, $F(3,15)=5.427$, $p<0.01$, and injection condition, $F(2,10)=42.121$, $p<0.01$. Using a repeated measures ANOVA across injection conditions at each drug dose, significant reductions in responding on drug days compared to either no injection or vehicle control days were found for all doses (Fig. 6). No significant differences between noninjection and vehicle injection occurred. The 10 mg/kg dose reduced responding

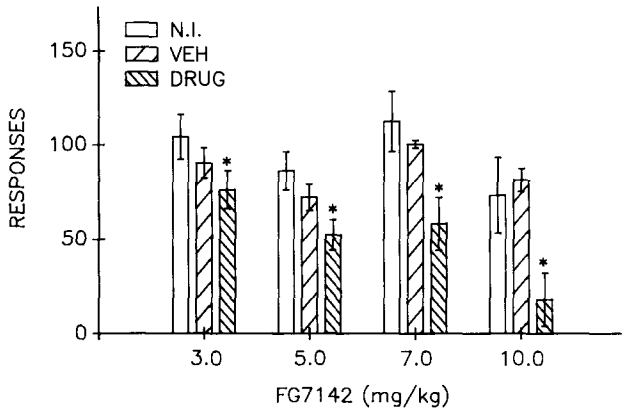

FIG. 6. The effect of FG 7142 upon ethanol-reinforced lever press responding. (Abbreviations and values as in Fig. 1.)

by an average of 89% vs. control injections. The 3 mg/kg, 5 mg/kg and 7 mg/kg dose levels reduced responding by an average of 28%, 37% and 42% respectively, compared to noninjection control data.

Examination of the cumulative records indicated that as dose increased, the maintenance of sustained responding was disrupted, such that at the higher doses of 7 mg/kg and 10 mg/kg, the rats failed to maintain responding for more than one or two reinforcements at a time. FG 7142 lengthened the pauses occurring between runs of responding, with greater pause time found as dose increased. Furthermore, latency to initiate responding in the session also was a function of dose. The time to the first reinforcement presentation was progressively longer as dose increased from 3 mg/kg to 10 mg/kg.

DISCUSSION

As with Ro15-4513, FG 7142 suppressed ethanol-reinforced responding in a dose-related manner. The nature of the suppression was similar to that found for the Ro compound, but efficacy was reduced by a factor of approximately three. These results are in general agreement with other investigators who have found that FG 7142 is less potent than Ro15-4513 in countering ethanol's actions (12,34).

GENERAL DISCUSSION

From these studies, it is clear that benzodiazepine inverse agonists can result in suppressed responding maintained by either ethanol or sucrose reinforcement. From our earlier work with Ro15-4513 using ethanol reinforcement (26), an approximate linear dose-effect relation was described, with significant reductions in responding occurring at doses of 0.3 mg/kg and greater. From the present work, it appears that sucrose-reinforced responding is less sensitive to the inverse agonists, as higher doses of Ro15-4513 are needed to decrease responding. In the concurrent situation, the failure of the 3 mg/kg dose of Ro15-4513 to suppress sucrose-reinforced responding while suppressing ethanol-reinforced responding supports this conclusion.

This effect upon ethanol-reinforced responding was not specific to Ro15-4513, as indicated by the effects of another inverse benzodiazepine agonist, FG 7142 (Experiment Four). In a pilot study using sucrose reinforcement, we found that doses of 7 mg/kg and 10 mg/kg of FG 7142 do not alter responding. Both of these doses were found to decrease ethanol-reinforced responding in

Experiment Four. Therefore, it appears that ethanol-reinforced responding is more sensitive to the effects of the benzodiazepine inverse agonists than is sucrose-reinforced responding.

There are several possible explanations which might account for this observed sensitivity difference. The first and most plausible explanation is that the two reinforcers tested were not equated in their efficacy, with sucrose having greater efficacy and thus requiring a larger dose. In comparing the data from studies in which ethanol or sucrose are presented individually as reinforcers, it appears that this is a valid explanation. In these studies, sucrose-reinforced responding (Experiment One), even when every attempt was made to adjust the sucrose concentration to equate response patterns, resulted in higher total session responding than that observed for ethanol-reinforced responding (26). This pattern of sucrose responding could indicate a potentially greater reinforcing efficacy for sucrose and support the efficacy hypothesis. However, the data from the concurrent study (Experiment Two) indicate that when rates of responding for both reinforcers are very close to equal, ethanol-reinforced responding is disrupted at doses which do not affect sucrose-reinforced responding. While this does not provide absolute refutation of the efficacy hypothesis, it suggests that qualitatively different interactions between the benzodiazepine system and each reinforcer could account for this differential sensitivity of ethanol reinforcement, and not just the behavioral efficacy for each reinforcer.

Another possible explanation of the differential effect of the Ro compound could be related to the effects of the benzodiazepine inverse agonists upon palatable fluid consumption (3). In these studies, intake of a preferred saccharin solution was decreased at doses of FG 7142 which had no effect upon quinine drinking. While there are many methodological differences between these studies and the present experiments, they suggest sucrose intakes should have been more sensitive to the palatability factors related to action of the inverse agonists. If one can assume that the palatability of the ethanol and sucrose solutions were approximately equal (based on the behavioral patterns seen in the concurrent conditions of Experiment Two), then it would be assumed from the palatability explanation that at the very least, equal suppression of both ethanol and sucrose should have resulted at a given dose of the Ro compound. Since this was clearly not the case, it seems unlikely that palatability factors alone can account for the present observation.

It is possible that the increased sensitivity of ethanol-reinforced responding to the inverse agonists is related to different activation processes involved with CNS mechanisms of reinforcement. It is most likely that the complex neural substrates which underlie reinforcement have many different activation and modulation components (28, 32, 35). Even when the measured behavioral activity for two different reinforcers appears similar, the CNS mechanisms involved for each reinforcer may be different. As shown by Rees and Balster (21), the inverse agonists can attenuate the discriminative properties of ethanol and oxazepam but not pentobarbital. It could also be that they have less effect upon certain classes of reinforcing stimuli, like sucrose. Thus, the effect of Ro15-4513 and FG 7142 on ethanol-reinforced responding suggests an important link to the benzodiazepine system as part of the reinforcement stimulus mechanism for ethanol. It appears that this link is less critical for sucrose reinforcement. This hypothesis is partially supported by examination of the nature of the response suppression found for each reinforcer. With ethanol, a dose-related decrease was observed, with increased suppression occurring as dose increased. With sucrose, an all or none dose function was found, with doses that had significant effects upon ethanol-reinforced responding not altering sucrose-reinforced behavior. As well with sucrose, the nature of the response pattern alteration suggested that at doses needed to result in any effect, a more general behavioral disruption occurred. With ethanol, the pattern of disruption at the low and moderate doses that did not affect sucrose-reinforced responding appeared more like a reduction in reinforcer efficacy rather than a general disruption of performance. It is possible that the effects of the inverse agonists on ethanol reinforcement altered the sensitivity of CNS reinforcement system to the relevant CNS cues related to ethanol reinforcement. No such effect at these low to moderate doses occurred for sucrose. This suggests that different parts of the brain reinforcement system may be influenced by different reinforcing stimuli, with ethanol more closely linked to the GABA-benzodiazepine receptor, a system on which it has a known action (1,34). This is not to say that sucrose reinforcement does not also utilize the GABA-benzodiazepine substrate for reinforcement, but this system may not be as important in the overall reinforcing efficacy of sucrose as it is for ethanol.

Since a variety of research has shown that ethanol does have an action at the GABA receptor complex, and that several of its actions can be affected by the inverse agonists, it is not surprising that ethanol-reinforced responding was altered by the inverse agonists. Given the role of the GABA receptor complex in the VTA (19), and the potential actions of the VTA in brain reinforcement mechanisms (27,35), it is possible that the effects observed for both sucrose and ethanol are related to this part of the reinforcement pathway. The difference in strength and type of effect upon ethanol and sucrose reinforcement by the inverse agonists suggests that different functions of this pathway may be involved in the actions of different reinforcers. Agents which can act upon this pathway from a variety of subsystems could result in behavior being influenced by the presentation of a given reinforcer. Thus, agents affecting opiate, catecholaminergic and GABAergic systems can all alter behavior. By using a more subtle analysis of behavior with a variety of reinforcers, perhaps a better understanding of how this complex neural system functions can be gained.

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