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FG 7142- and Restraint-Induced Alterations in the Ataxic Effects of Alcohol and Midazolam in Rats Are Time Dependent

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AUSTIN, M., V. MYLES, P. L. BROWN, B. MAMMOLA AND R. C. DRUGAN. *FG 7142- and restraint-induced alterations in the ataxic effects of alcohol and midazolam are time dependent.* PHARMACOL BIOCHEM BEHAV **62**(1) 45–51, 1999.—The purpose of this study was to examine whether acute stress exposure would alter the ataxic properties of midazolam or ethanol in rats. Rats were administered either vehicle or FG 7142 (10 mg/kg) and placed back in their home cages, or placed in restraining tubes for 90 min. Three and one-half or 24 h following injection all subjects were then administered an ataxic dose of either ethanol or midazolam and after 10 min, motoric impairment was assessed by rotarod performance. Neither FG 7142 administration nor restraint had an impact on rotarod performance 3-1/2 h later for ethanol nor 24 h later in response to midazolam. However, midazolam-induced ataxia was significantly modified 3-1/2 h following both restraint and FG 7142 exposure. Similarly, at the 24-h time point, both manipulations had a significant effect on ethanol-induced motor incoordination. Importantly, prior exposure to FG 7142 and restraint was without effect on rotarod performance in saline-treated subjects. Functional alterations in behavioral reactivity to low doses of two classes of CNS depressants by the acute stress of restraint and/or FG 7142 administration suggest the anxiogenic nature of these stressors may be the critical factor. © 1998 Elsevier Science Inc.

Anxiety Restraint stress Rotarod Ataxia Depressants Alcohol Benzodiazepine Motor incoordination

THE GABA–Benzodiazepine receptor complex (GABA–BDZ) is known to be the site of minor tranquilizer action (48,49,54,56) and plays a critical role in the pathophysiology of anxiety (11,15,32,35). This site is also exquisitely sensitive to environmental stress, including cohort removal (58), pup isolation from mothers in rats (5,31), handling stress (4), ambient or cold-water swim stress (28,58,60), defeat stress (45), and shock stress (4,19,20).

Just as the type, severity, and chronicity of stress cause alterations in the GABA–BDZ site (14), controllability of stress modulates the impact of stress on this site. Controllability of stress can be defined as the opportunity (or lack thereof) of an animal to perform an instrumental response to terminate an aversive event, such as shock (41). Uncontrollable, but not controllable, stress is associated with a reduction in muscimol-stimulated chloride ion flux and a reduction of [^{3}H]Ro 15-1788 binding to the BDZ receptor in vivo (19). Several classes of minor tranquilizers including benzodiazepines and barbiturates are known to bind at the GABA/BDZ site (48,49,56). Alcohol is known to change the transducing mechanism at the GABA/BDZ site (e.g., chloride flux) (55,57). Although ethanol has activity at many different sites in brain including adenosine, serotonin, and NMDA receptors (12,27), direct or indirect modification of the GABA receptor by agonists or antagonists can potentiate or attenuate ethanols actions on motor incoordination, respectively. However, the inability of ethanol to competitively inhibit [^{3}H]muscimol binding indicates the noncompetitive nature of alcohols effect at the GABA receptor (26).

Uncontrollable, but not controllable, stress can markedly change the binding kinetics of the GABA–BDZ receptor (19,20,22) as well as subsequent behavioral reactivity to alco-

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hol and valium, suggesting a functionally significant alteration in this drug recognition site by psychological aspects of the stress experience (17,24). Several investigators report marked differences in fear levels in controllable vs. uncontrollable stress conditions including: conditoned emotional response (13), contextually conditioned fear (46), and social interaction (53). An important role for the neurochemical sequella of fear or anxiety has been proposed in explanations of the effects of inescapable shock exposure including instrumental learning deficits (21,23,40,52), stress-induced analgesia (23,39), and activity deficits (53). Therefore, high levels of fear in the inescapable shock subjects may be responsible for their subsequent heightened reactivity to drugs that act at the GABA–BDZ site (17,18,24). The current study evaluates the role of experimentally and pharmacologically induced fear in lieu of a noxious physical stimulus in altering the behavioral reactivity to alcohol and midazolam.

GENERAL METHOD

Subjects

The subjects were male, Sprague–Dawley rats (250–300 g), obtained from Charles River Laboratories (Kingston, NY). Rats were housed four per cage in polyethylene tub cages, and were maintained on a 12 L:12 D cycle with ad lib access to food and water.

Apparatus

Restraining tubes were made of Plexiglas ($6.5 \times 3 \times 2\ 1/8''$, L × W × H). There were holes and slots on the tops of the tubes for circulation of air. Motor impairment was assessed by a rotarod treadmill 6 cm in diameter and 35 cm long (UGO Basile Biological Research Apparatus—Model #7700, 21025 Comerio, Varese, Italy). The rotarod had four equal areas that were partitioned off from one another and the rod rotated at a speed of 10 rpm.

Drugs

FG 7142 (*N*-methyl β-carboline-3-carboxamide) was obtained from Research Biochemicals Incorporated (RBI, Natick, MA). FG 7142 was injected intraperitoneally (IP) at a dose of 10 mg/kg based on previous studies that determined this to be an effective anxiogenic dose (21,43,53). The drug was injected in a suspension consisting of a 45% weight-to-volume solution of tissue solubilizer (2-hydroxypropyl-beta-cyclodextrin-HBC) and one drop of Tween 20 (Sigma Chemical Co, St. Louis, MO) per ml of vehicle. Vehicle was mixed up in an identical suspension and volume as was the drug. Ethanol (95% solution) was injected 10 min prior to rotarod testing as a 20% vol–vol (ethanol–distilled water) solution. The effective dose (0.6 g/kg, IP) employed was also determined from a previous dose–response analysis using the same rat strain and supplier (17). Midazolam hydrochloride (Versed–Roche) obtained in injectable form (Henry Schien, Inc., Port Washington, NY) was used. Rats were administered an effective dose (0.5 mg/kg, IP) 10 min prior to the rotarod test based on previous dose–response analysis using the same rat strain and supplier (17).

Procedure

Rats arrived at the University of New Hampshire Department of Psychology and were allowed to acclimate for 1 week prior to experimentation. Rats were weighed at the start of the experiment and randomly assigned to one of four groups: FG 7142–restraint, vehicle–restraint, FG 7142–home cage, or vehicle–home cage. Criterion training preceded testing. This involved training rats to run continuously on the rotarod for 2 min. If the animal fell off, it was immediately placed back on the rotarod until 2 min of continuous running was accomplished. After completion of this task, rats were placed in individual tub cages. FG 7142 or vehicle was injected intraperitoneally (IP) and rats were placed either in the restraining tubes or back in their home cages. After 90 min, the rats were taken out of the restraining tubes and individually placed in tub cages for an additional 2 h or placed back in their home cages for 24 h. This was to approximately the 2- or 24-h poststress interval used in previous experiments using the shock stress paradigm (17–19,22). The rats were evaluated again to ensure that they could pass the criterion test on the rotarod. Shortly thereafter, all animals were injected with an effective dose of either ethanol (0.6 g/kg, IP) or midazolam (0.5 mg/kg), and 10 min were allowed for adequate drug absorption (17). After 10 min had elapsed, the rat was tested on the rotarod to determine the level of motor incoordination. The subject was placed on the rotarod and the latency to fall off was recorded. A maximum of three successive trials were conducted. If a subject reached a maximum time of 300 s on the second trial after running for greater than 180 s on the first trial, no further trials were conducted. The mean of the two to three trials was taken as the rotarod score for each subject. This procedure is similar to those reported in the literature to measure drug-induced motor ataxia (12,17,44,47). This 300-s cutoff was established to allow for proper testing of subjects in all four groups at or near the 2- or 24-hour post-restraint time frame. All groups were run in a counterbalanced fashion, and the experimenter testing the subjects on the rotarod was blind to treatment condition. A schematic diagram of the general procedure for the first four experiments is shown in Fig. 1.

Statistical Analysis

The data were analyzed by a two-way analysis of variance (ANOVA). Mean differences were compared using Newman–Keuls post hoc comparisons after ANOVA. Two sample comparisons in the last two experiments were analyzed using a Students *t*-test.

EXPERIMENT 1: FG 7142 OR VEHICLE ADMINISTRATION COUPLED WITH RESTRAINT OR HOME-CAGE PLACEMENT AND THE MOTOR-INCOORDINATING EFFECTS OF ETHANOL 3-1/2 HOURS LATER

Results

The impact of FG 7142 or vehicle administration, coupled with either restraint or home-cage placement on ethanol motor ataxia 2 h following stress, is shown in Fig. 2. As can be seen in the figure, there is no effect of either drug treatment or restraint vs. home-cage condition on rotarod performance across groups. This observation was verified statistically by a two-way ANOVA. There was a nonsignificant effect of drug, ($F(1, 28) = 0.02$, $p > 0.9$; a nonsignificant effect of location [restraint vs. home cage, $F(1, 28) = 1.32$, $p > 0.26$; and a nonsignificant interaction, $F(1, 28) = 0.06$, $p > 0.81$].

EXPERIMENT 2: FG 7142 OR VEHICLE ADMINISTRATION COUPLED WITH RESTRAINT OR HOME-CAGE PLACEMENT AND THE MOTOR-INCOORDINATING EFFECTS OF ETHANOL 24 HOURS LATER

Time-dependent effects of FG 7142 administration are observed 24 h post administration when the drug is no longer in the organism (21). Although previous reports indicate that

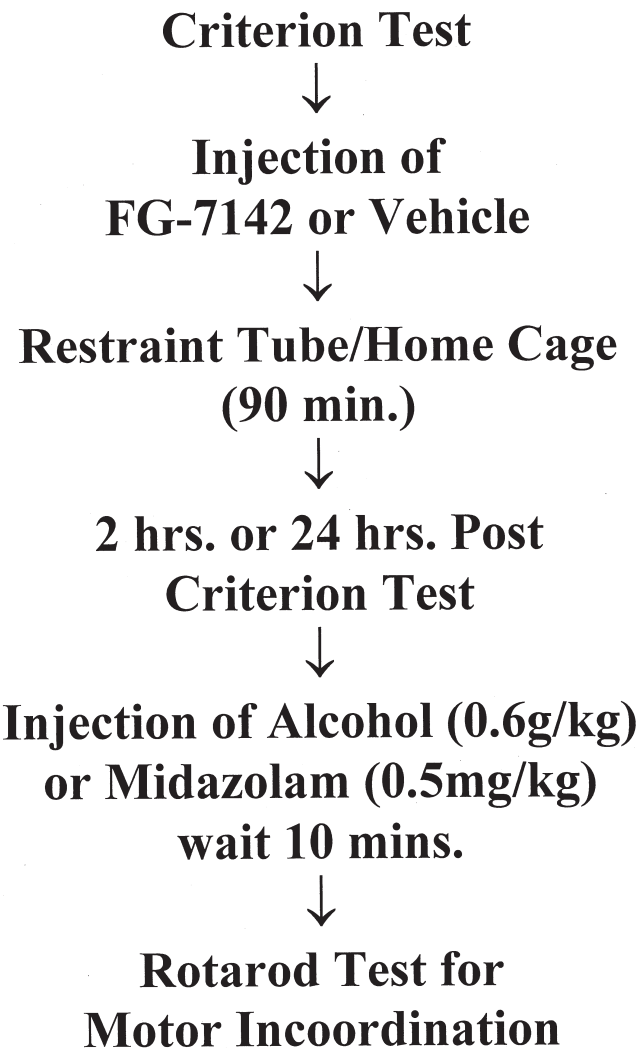

FIG. 1. Behavioral protocol for examining the impact of FG-7142 or vehicle coupled with either restraint or home cage placement on drug-induced motor ataxia.

the acute effects of FG 7142 last less than 2 h (37), this study examined the long delay effects of FG 7142 or vehicle injection coupled with restraint stress or home-cage placement on the motor-incoordinating effects of ethanol 24 h later.

Results

The effect of FG 7142 vs. vehicle administration and restraint or home-cage placement is shown in Fig. 3. As can be seen, the FG 7142 plus restraint group appears lower than all other groups. These observations were confirmed with a two-way ANOVA. The ANOVA indicated a significant drug effect, $F(1, 31) = 5.8$, $p < 0.03$, a nonsignificant location effect, $F(1, 31) = 3.24$, $p < 0.083$, and a nonsignificant drug × location interaction, $F(1, 31) = 0.22$, $p > 0.65$, Newman–Keuls mean comparisons after ANOVA indicated that the FG 7142/restraint group differed significantly from the vehicle/home-cage group ($p < 0.05$). All other groups were not different from one another.

To test the possibility that the 300-s cutoff resulted in an alteration of the normal distibution of the groups, a test of normality was conducted (Wilkes' test-BMDP 2D statistical package), and only the FG 7142 restraint group violated normality. However, a nonparametric test that does not assume normality (Kruska–Wallis ANOVA) was conducted, and a significant group effect was also obtained KW test statistic (8.63, $p < 0.04$). Multiple comparisons (Z-stat) also indicated that the FG 7142-restraint group was significantly different from the vehicle home-cage group ($p < 0.05$). Thus, the results were identical using either parametric or nonparametric analyses.

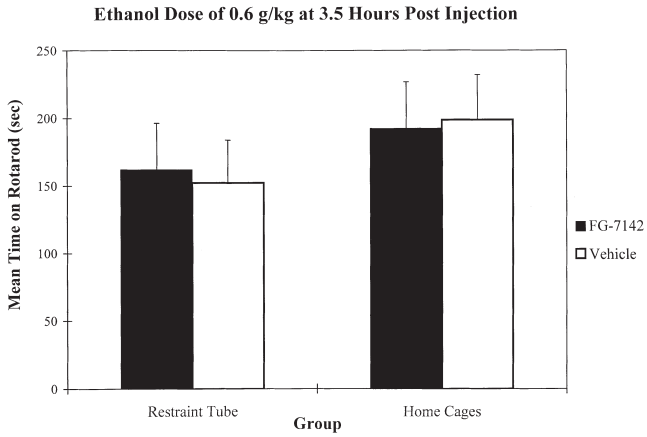

FIG. 2. Mean time spent on the rotatod in seconds for subjects 3-1/2 h following an injection of either FG 7142 (10 mg/kg, IP) or equivolume vehicle solution, and restrained or placed in an individual cage. Ten minutes prior to the rotarod test all subjects received an IP injection of 0.6 g/kg of ethanol. The histograms represent means (n = 8/group), and the vertical bars indicate SEMs.

EXPERIMENT 3: FG 7142 OR VEHICLE ADMINISTRATION COUPLED WITH RESTRAINT OR HOME-CAGE PLACEMENT AND THE MOTOR-INCOORDINATING EFFECTS OF MIDAZOLAM 3-1/2 HOURS LATER

Results

The effects of FG 7142 or vehicle injection, coupled with either restraint or home-cage placement on subsequent reactivity to midazolam-induced motor ataxia, are shown in Fig. 4. As can be seen, there appears to be a difference between groups in the mean time spent on the rotarod. This effect was confirmed by a two-way ANOVA. The ANOVA indicated a nonsignificant main effect of drug, $F(1, 84) = 0.06$, $p > 0.80$, a significant effect of location, $F(1, 84) = 7.02$, $p < 0.01$, and a nonsignificant interaction of drug × location, $F(1, 84) = 0.92$, $p > 0.33$. Subsequent Newman–Keuls mean comparisons after ANOVA indicated that the FG 7142/restraint group was significantly different from the FG 7142/home cage group ($p < 0.05$). No other group differences were significant.

As with Experiment 2, we tested the possibility that the 300-s cutoff may have violated normality of the group distributions. Test of normality (W-statistic) indicated that all four groups deviated from normality. Thus, similar to Experiment 2, a nonparametric test that does not assume normality, the Kruskal–Wallis Test, was conducted. The KW statistic indicated a similar significant group effect KW test statistic = 8.43, $p < 0.04$. Mean comparison tests (Z-stat) indicated a significant difference between the FG 7142 restraint and the FG 7142 home-cage group ($p < 0.05$). Again, the findings were similar whether analyzed by either parametric or nonparametric tests.

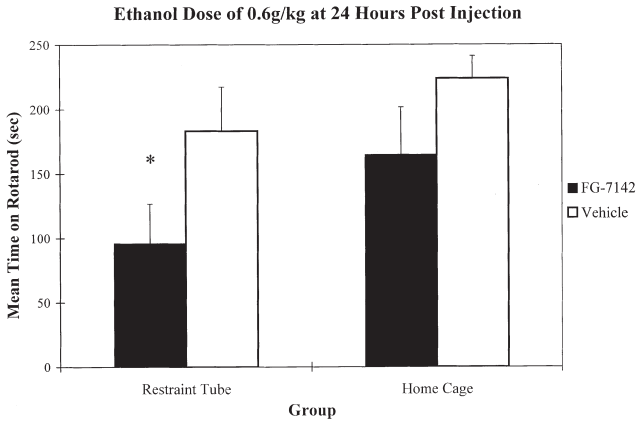

FIG. 3. Mean time spent on the rotarod in seconds for subjects 24 h following an injection of either FG 7142 or equivolume vehicle solution, and subsequent placement in either a restraining tube or a Plexiglas home cage. Ten minutes prior to the rotarod test all subjects received an IP injection of ethanol (0.6 g/kg). The histograms indicate means (n = 8–10/group), and vertical bars indicate SEMs. *Indicates significantly different from vehicle home-cage control as determined by Newman–Keuls mean comparisons ($p < 0.05$) after ANOVA.

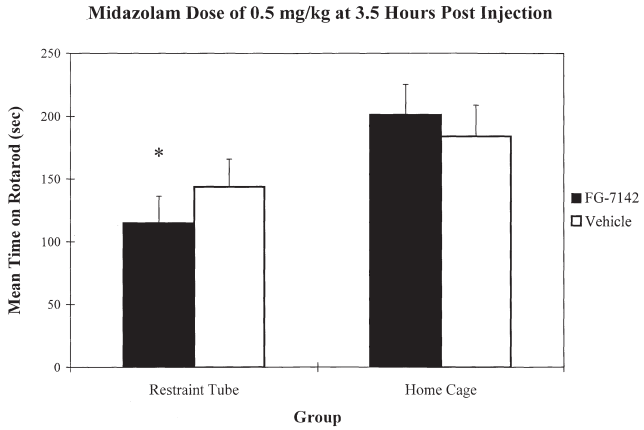

FIG. 4. Mean time spent on the rotarod in seconds for subjects 3-1/2 h following an IP injection of either FG 7142 or vehicle solution, and subsequent placement in either restraint tubes or a Plexiglas home cage. Ten minutes prior to the rotarod test all subjects were injected with midazolam (IP, 0.5 mg/kg). The histograms represent means (n = 20–25/group), and vertical bars indicate SEMs. *Indicates significantly different from the β-carboline home cage group, as determined by Newman–Keuls mean comparisons ($p < 0.05$) after ANOVA.

EXPERIMENT 4: FG 7142 OR VEHICLE ADMINISTRATION COUPLED WITH RESTRAINT OR HOME-CAGE PLACEMENT AND THE MOTOR-INCOORDINATING EFFECTS OF MIDAZOLAM 24 HOURS LATER

Because the effects of prior stress modified the reactivity to ethanol 24 h later in Experiment 2, we wanted to test a similar time course with midazolam reactivity. In our previous study evaluating the effects of stress controllability (17) both ethanol and midazolam reactivity were altered in a similar fashion. Both of these pieces of evidence suggest that testing at 24 h post-FG 7142 administration and/or restraint may be important.

Results

The results of Experiment 4 are shown in Fig. 5. As can be seen in the figure, it appears as though the vehicle home-cage group remains on the rotarod for a longer time than the other three groups (vehicle/restrained, FG 7142/home cage, and FG 7142/restrained), which do not appear to be different from one another. These observations were not confirmed by a two-way ANOVA. Although graphically similar to the ethanol 24-h data, no statistical significance was observed. There was a nonsignificant drug main effect, $F(1, 56) = 0.78$, $p = 0.38$, a nonsignificant location main effect, $F(1, 56) = 2.28$, $p = 0.139$, and a nonsignificant drug × location interaction, $F(1, 56) = 1.8$, $p = 0.187$.

EXPERIMENT 5: INTRINSIC ACTIONS OF FG 7142 WITH OR WITHOUT RESTRAINT ON THE ABILITY OF RATS TO PERFORM THE ROTAROD TASK FOLLOWING A SALINE INJECTION

The results reported herein suggest that following two different poststress time points, the behavioral reactivity to ethanol or midazolam is altered in certain stressed groups. However, it is possible that either FG 7142 and/or restraint exposure may influence ability of the subjects to perform the three trial rotarod test in the absence of ethanol. This impairment in longer duration running may not be apparent with the 2 min of continuous running required to pass the criterion test prior to minor tranquilizer administration. Experiments 5A and 5B tested the performance of the groups from Experiments 2 and 3, which were significantly different from one another in response to ethanol or midazolam, respectively, but tested the subjects in a nondrug condition (saline injection) prior to the rotarod test.

Experiment 5A

Post hoc Newman–Keuls comparisons from Experiment 2 indicated that the FG 7142/restrained group was significantly different from the vehicle/home-cage control. So, these were the two groups evaluated in Experiment 5A.

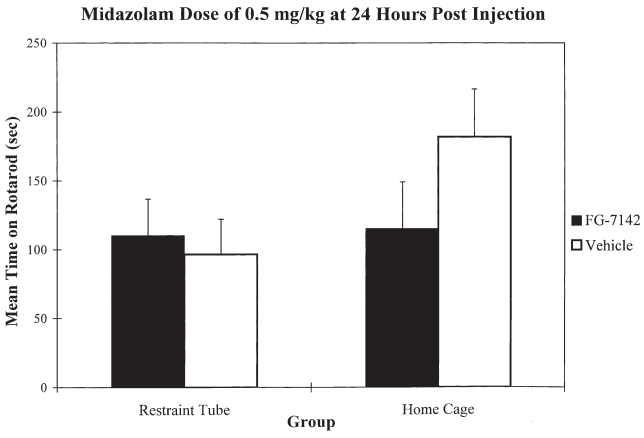

FIG. 5. Mean time spent on the rotarod in seconds for subjects 24 h following an injection of either FG 7142 or vehicle, and subsequent placement in either restraint tubes or a Plexiglas home cage. Ten minutes prior to rotarod test all subjects were injected (IP) with 0.5 mg/kg of midazolam. Histograms represent means (n = 12–14/group), and vertical bars indicate SEMs.

Experiment 5B

Post hoc Newman–Keuls comparisons from Experiment 3 indicated that the FG 7142/restraint group was significantly different from the FG 7142/home-cage control. Thus, these were the two groups evaluated in Experiment 5B.

METHOD

Subjects

Male Sprague–Dawley rats of the same strain and supplier as described in the previous experiments were used.

Apparatus

The restraint tubes and rotarod were identical to those previously described.

Procedure

All subjects were weighed and trained to criterion on the rotarod. Rats were then randomly assigned to one of two groups: FG 7142/restraint or vehicle/home-cage control (5A) or to FG 7142/restraint or FG 7142/home cage (5B). The FG 7142 groups were injected with 10 mg/kg of the drug and immediately placed in the restraint tubes or back in the home cage, while the vehicle group was injected with vehicle and immediately placed in a Plexiglass tub cage. After 90 min had elapsed, all groups were returned to their home cages. Twenty-four hours (5A) or 2 h (5B) later, all groups were again trained to criterion prior to injection of a saline solution and the three-trial rotarod test 10 min later.

Results

The results of Experiment 5A indates that there is no difference in the rotarod performance between the two groups (data not shown). These observations were confirmed using a Student's T-test, $t(14) = 1.013$, $p > 0.10$. Therefore, exposure to FG 7142, coupled with restraint, does not interfere with rotarod performance in comparison to the vehicle–home cage group 24 h later, 10 min following a saline injection.

The results of Experiment 5B also indicates no difference between the groups in their performance on the rotarod test (data not shown). These observations were statistically confirmed with a Student's *t*-test, $t(9) = 0.84$, $p > 0.4$. Thus, exposure to FG 7142, coupled with restraint compared to administration of FG 7142 and subsequent home cage placement, does not impair the ability of rats to perform the rotarod task 3-1/2 h later, 10 min following a saline injection.

GENERAL DISCUSSION

The results of the current study indicate that exposure to a nonpainful, yet anxiety-provoking experience can alter the behavioral reactivity to both ethanol and midazolam in a time-dependent fashion. The combination of FG 7142, coupled with restraint stress, appears to be sufficient to cause this change in ethanol and midazolam reactivity, resembling the effects of shock stress previously reported 2 h poststress (17). Experimental induction of fear or anxiety markedly influences the ataxic potency of two CNS depressants (midazolam and ethanol) known to interact with the GABA/BDZ site. These results confirm and extend previous findings showing a stress and alcohol interaction (17,24,50).

Ethanol is known to have actions at additional neurotransmitter systems in the brain. These interactions include 1) blockade of the NMDA receptor of the excitatory neurotransmitter, glutamate (38); 2) an antagonism at the 5-HT_3 serotonergic receptor (27); an interaction with the adenosine system (12); as well as changing intracellular cAMP by a direct action on G proteins (29). In addition to the direct action of ethanol at these other sites, the influence at the GABA/BDZ site may, in turn, influence these other sites indirectly because GABA is known to have an inhibitory influence on these sites in brain (27,33,38). Therefore, by using an inverse agonist at the GABA/BDZ site dies not exclude the possibility that the current observations may result from interactions of the GABAergic system with these other neurotransmitter or second-messenger systems in modifying motor coordination.

The time-dependent nature of FG 7142/restraint effects are different from previous work using shock stress (17), but marked procedural differences and/or the presence vs. absence of painful stimuli may be responsible for these differences. In addition, in the present study a dose response analysis for FG 7142, ETOH, or midazolam was not conducted. A dose–response curve may have yielded significant effects not presently observed. Effective doses were derived from previous stress controllability and motor ataxia studies using the same strain and rat supplier (17). The results of the current study should be viewed in this limited perspective of "threshold doses" used to produce anxiety and motor incoordination. Nonetheless, these results point to fear/anxiety as an important contributing factor in modifying an organisms reactivity to certain minor tranquilizers.

The ability of GABA/BDZ negative modulators (e.g., FG 7142, picrotoxin, Ro15-4513, or SR 95531) to exacerbate the depressant profile of ETOH has been reported in both stressful and nonstressful conditions (16). In addition, the high affinity BDZ antagonist Ro15-1788 (flumazenil), and the longer acting inverse agonist Ro19-4603 also block tolerance to the ataxic and sedative effects of ETOH 24–36 h after their administration (7,34). This interaction may be due to the intrinsic action of FG 7142 present in the animals 3-1/2 h but not 24 h later. However, this is rather unlikely, because the acute effects of FG 7142 at even higher doses (e.g., 20 mg/kg) are reported to last only 2 h (37), whereas in the current study the dose of 10 mg/kg is employed and testing occurs 3-1/2 h later. Finally, some studies indicate that the intrinsic actions of BDZ inverse agonists antagonize, rather than potentiate, the motor-incoordinating effects of ethanol (2,36), while other reports find no effect (3,55). Thus, the effects of the current study could be framed in such a way as restraint stress increases the ataxic effects resulting from prior FG 7142 activation and subsequent ETOH interaction at the GABA–BDZ site.

At the 3-1/2–h time point, it could also be argued that FG 7142 administration at a higher dose might directly compete with the binding of midazolam to the benzodiazepine receptor. Competitive inhibition is a concern for several reasons: 1) these sites (benzodiazepine/β-carboline) are known to be overlaping domains due to their competitive binding at the benzodiazepine receptor (6,8,25), and they have opposite actions on binding kinetics at the GABAa receptor (4,9,10,49,59), while both of their actions are reversed by the high-affinity BDZ receptor antagonists, flumazenil and CGS 8216 (11,30, 42,51). However, the present findings indicate that this potential confound does not occur. If the FG 7142 were directly competing with the midazolam at the BDZ/GABA receptor complex, then one would expect a diminution in the actions of midazolam at the 3-1/2–h time point. This is simply not the case. Careful inspection of Fig. 4 reveals that FG 7142 coupled with restraint enhances, rather than reduces, the ataxis prop-

erties of midazolam. The anxiety-potentiating effects of FG 7142 administration coupled with restraint appear to be the result of alterations in emotion and concomitant alterations in the GABA/BDZ site rather than direct antagonistic intrinsic actions of the FG 7142 on the actions of the minor tranquilizers tested.

These results show that ETOH, in a rather unique stress paradigm, is acting in a fashion similar to midazolam, suggesting a similar site of action, namely the GABA/BDZ receptor. In future studies it would be interesting to evaluate the self-administration profiles of the FG 7142/restraint vs. vehicle/home-cage subjects and observe their reactivity to the ataxic properties of ETOH and midazolam under a different schedule (e.g., response–contingent) of drug delivery. Nonetheless, the current results illustrate a significant influence of experimentally induced "stress" on an organism's response to several minor tranquilizers. This finding underscores the importance of nonpharmacological influences (e.g., predrug behavioral status) on the behavioral reactivity to drugs (1).

ACKNOWLEDGEMENTS

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