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Selective Antagonism of Acute Ethanol-Induced Motor Disturbances by Centrally Administered Ro 15-4513 in Mice

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DAR, M. S. *Selective antagonism of acute ethanol-induced motor disturbances by centrally administered Ro 15-4513 in mice.* PHARMACOL BIOCHEM BEHAV 42(3) 473–479, 1992.—Results of the present investigation demonstrated that Ro 15-4513 when given ICV selectively antagonized ethanol-induced motor disturbances at doses that did not produce motor incoordination and lacked proconvulsant activity. Ro 15-4513 in 10-, 15-, and 22-ng doses antagonized, roughly in a dose-dependent manner, ethanol-induced motor incoordination. The 10-ng dose produced an optimal effect with nearly complete antagonism within 30 min postethanol. The higher, 15 and 22 ng, doses of Ro 15-4513 antagonized, as well as probably reversed, ethanol-induced motor incoordination. The stimulation and inhibition of spontaneous motor activity by 1 and 2 g/kg IP ethanol, respectively, were also selectively antagonized by Ro 15-4513. Neither an alteration in the latency and/or duration of pentylenetetrazol-induced convulsions nor an antagonism to sodium pentobarbital-induced motor incoordination and inhibition of spontaneous motor activity by Ro 15-4513 at dose levels that showed antiethanol effects were observed. Only the 150-ng dose of Ro 15-4513, which exhibited intrinsic activity as proconvulsant, attenuated sodium pentobarbital-induced motor incoordination. When given alone at doses higher than those used in motor coordination experiments, Ro 15-4513 markedly increased spontaneous motor activity dose dependently.

Ethanol Motor incoordination Spontaneous motor activity Intracerebroventricular
Selective Antagonism Ro 15-4513

THE early (2,22) and subsequent (27) reports that the partial inverse benzodiazepine agonist Ro 15-4513 antagonizes behavioral effects of acute ethanol was followed by tremendous interest to investigate the extent and mechanism of ethanol antagonism by this drug. So far, the available data suggest that Ro 15-4513 antagonizes many but not all the behavioral effects of ethanol. However, whether this antagonism is selective to ethanol or observed when Ro 15-4513 pretreatment is given with other CNS depressants is still debatable (2,3,17,27).

Ro 15-4513 has been shown to reverse a number of behavioral effects of ethanol including motor incoordination and loss of righting reflex (2,12,28), as well as locomotor stimulation (19). An important issue concerns whether the ability of Ro 15-4513 to antagonize ethanol effect is exclusively due to its intrinsic behavioral effects that oppose those of ethanol rather than specific antagonism. Reports have been published supporting the specificity of antagonism of ethanol's effects by Ro 15-4513 at doses without any observable intrinsic action (1,27). On the other hand, several studies have shown that Ro 15-4513 acts as an ethanol antagonist only when the ethanol effects were opposite in direction to the intrinsic actions of Ro 15-4513 (4,16,19).

Throughout the present investigation, Ro 15-4513 pretreatment was given ICV into either of the lateral ventricles. The ICV route of administration was selected because Ro 15-4513 has never been tested by this route as an antagonist of motor-disturbing effects of ethanol. Since very low doses of Ro 15-4513 were expected to be administered by ICV route, we thought perhaps it may be possible that the specific and intrinsic effects of Ro 15-4513 at these low ICV dose levels may be amenable to clear separation. Such an approach, in our opinion, may help clarify the confusion regarding the selectivity and specificity of Ro 15-4513 as an antagonist of ethanol. In addition, support for the above-stated objective of the present study was sought by experiments in which Ro 15-4513 was tested as a possible antagonist of sodium pentobarbital-induced motor incoordination and inhibition of spontaneous motor activity (SMA). Also, the presence or absence of pro-

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convulsant effect of Ro 15-4513 at doses used in the motor coordination and SMA studies was investigated by evaluating the effect of its pretreatment (ICV) on the latency to onset and lethality of pentylenetetrazol-induced convulsions to further clarify its specificity as an antagonist to ethanol-induced motor disturbances. The primary objective of the present investigation, therefore, was to clarify if Ro 15-4513 given directly into either of the brain lateral ventricles would antagonize acute ethanol (IP)-induced motor incoordination and inhibition of SMA at dose levels that would not exhibit any intrinsic behavioral and/or proconvulsant actions or antagonize pentobarbital-induced motor impairment. We would also carry out dose-response studies to observe if the antagonism by Ro 15-4513 of ethanol-induced motor disturbances occurred in a dose-related manner. In addition, the results of the present investigation would establish whether or not the antagonism by Ro 15-4513 of ethanol-induced motor disturbances is mediated centrally.

METHOD

Male CD-1 mice, 5 weeks old and weighing approximately 25 g, were purchased from Charles River, Inc. (Raleigh, NC). Mice were housed individually after surgical implantation of permanent indwelling stainless steel guide cannulae into one of the cerebral lateral ventricles. They were maintained on a 12 L : 12 D cycle and allowed ad lib access to food and water.

Surgery

The permanent indwelling guide cannulae were implanted stereotaxically under chloral hydrate (450 mg/kg; IP) into either of the lateral cerebral ventricles with the skull surface in the horizontal plane. The implantation of the guide tubes was according to the coordinates from *A Stereotaxic Atlas of the Albino Mouse Forebrain* (26) as follows: AP, 0.2 mm (bregma); ML, ± 1.4 mm; and DV, -2.4 mm from the surface of the skull. The guide cannulae were made from 23-ga stainless steel tubing (Small Parts Inc., Miami, FL), cut to 10 mm length, and blunted. These cannulae were lowered to desired depth through the appropriately located craniotomy holes. The guide cannulae were anchored to the cleaned and dried cranial surface with the help of fast-drying dental cement (Durelon, Premier Dental Products Company, Norristown, PA). At least 5 days were allowed for animals to recover from surgery and the effects of the anesthetic before their use in a behavioral study. Each animal postoperatively received 300 U Durapen (benzathine and procaine penicillin G suspension) subcutaneously to prevent possible infection. The cannula implantation was conducted strictly under aseptic conditions, using providone-iodine swab sticks (Operand, Redi-Products, Prichard, WV) to clean the skull surface and autoclaving the surgical tools, burrs of drill, and guide cannulae.

Drugs

Ro 15-4513 was dissolved in ACSF with the aid of dimethylsulfoxide (DMSO). The final concentration of DMSO in the solutions of various doses of Ro 15-4513 used in the present study ranged from 0.125% (10-ng dose) to 1.88% (150-ng dose). The Ro 15-4513 solutions were stored in glass tubes completely covered with black vinyl tape to protect the drug from light and were kept in the freezer. Sodium pentobarbital and pentylenetetrazol (Sigma Chemical Co., St. Louis, MO) were dissolved in normal saline. Ro 15-4513 was a gift from Hoffman-La Roche and Company (Basel, Switzerland) and provided by Drs. Imhof and Eigenmann. The administration of Ro 15-4513 was always by ICV route in a fixed injection volume of 5 μl over a 60-s period, during which animals were able to move about freely in their cages. At the time of ICV administration, an injector cannula (30-ga tubing) was connected to a 25-μl Hamilton microsyringe by PE-10 (Clay Adams) polyethylene tubing and the Ro 15-4513 injected by Harvard Pump Model 22 (Harvard Apparatus, MA) into a group of five mice in a single motor coordination or SMA experiment. Sodium pentobarbital and pentylenetetrazol were administered IP in a volume of 10 ml/kg. Ethanol was prepared as 12% w/v solution in normal saline and was always injected IP 20 ml/kg. The ACSF contained (mM): NaCl 127; KCl 2.55; $CaCl_2$ 0.95; $MgCl_2$ 0.94; and $Na_2S_2O_5$ 0.05.

Histology

Correct placement of the ICV cannula was verified after every experiment by injecting 5 μl cresyl violet stain through the guide cannula. Animals were killed immediately after injection and brains were removed and sectioned to assess the spread of the stain within the ventricular system. Animals in which even diffusion of the stain was observed throughout the entire ventricular system (both lateral ventricles, third ventricle, and aqueduct of sylvius) were included in the analysis of the behavior experimental data. More than 95% of cannula implantations were successful.

Motor Coordination

A standard mouse rotorod (UGO Basil, Varese, Italy) calibrated for a fixed 20 rpm speed was used for the evaluation of degree of motor incoordination. Five mice were simultaneously evaluated for motor coordination. Before the actual experiment, mice were acclimatized to the treadmill and prescreened such that each animal stayed on the rotorod for the preset criterion of 180 s. It was essential to test motor coordination of each mouse before its use in rotorod study in case of an inborn defect (e.g., cerebellar). Normal motor coordination was defined as the ability of each animal to stay on the rotorod consecutively for the arbitrarily selected time of 180 s. This constituted the basis to evaluate the effect of a drug pretreatment on ethanol-induced motor incoordination. Successfully screened animals were injected (ICV) with Ro 15-4513 or ACSF and 2 min later with a test dose of ethanol or sodium pentobarbital. This was followed by an evaluation of motor coordination, each mouse serving as its own control. Starting from the moment of ethanol or pentobarbital injection, the index of motor coordination was evaluated every 15 min in a 60-min experimental period. The degree of motor incoordination was expressed as an activity ratio defined as the ratio of time the mouse stayed on the rotorod after Ro 15-4513/ACSF + ethanol/sodium pentobarbital treatment to the time (180 s) before such treatment. There was, thus, a fixed common denominator of 180 s in all motor coordination experiments that permitted intergroup statistical comparison of the activity ratio data. An activity ratio of 1 (maximum value) or near 1 indicated normal motor coordination and a decreasing activity ratio indicated increasing motor incoordination. For each drug dose, at least two separate motor coordination experiments (total of 10 mice) were conducted. The other details of the procedure have been reported previously (5–8).

SMA

SMA of individual animals was measured (one mouse per activity monitor). In a single SMA experiment, five mice were

used simultaneously using five Automex 2S animal activity monitors (Columbus Instruments, Columbus, OH). Prior to actual SMA experiments, each mouse while still in its original housing cage was injected with Ro 15-4513 or ACSF ICV followed 2 min later by IP injection of the test dose of ethanol/sodium pentobarbital or saline. Immediately after ethanol/pentobarbital/saline administration, animals while still in their original individual housing cages were placed on the activity monitors and their SMA recorded every 15 min for a 60-min experimental period (four recordings). The activity counts from animals that received ACSF + saline (IP) treatment constituted the control SMA value. The SMA experiments were conducted in a dedicated room where the only light source was a 25-W red lamp. The background noise was offset by the use of a white noise generator. Again, as in motor coordination experiments, for each dose of a drug at least two separate SMA experiments (total of 10 mice) were conducted.

Convulsion Study

The clonic phase of the convulsions was characterized by the appearance of tremors, increased motor activity, facial twitching, piloerection, and clonic forelimb seizures. The presence of tonic extension of hindlimbs associated with generalized clonic and tonic convulsions of both limbs constituted the tonic phase (18). The effect of three different doses of Ro 15-4513 given ICV on the convulsive effect of pentylenetetrazol (IP) was investigated. To the control group (n = 18), ACSF was given ICV and 2 min later pentylenetetrazol, 120 mg/kg IP, was injected. Similarly, three additional convulsive experiments were conducted in which ICV pretreatment of each dose (10, 75, and 150 ng/5 μl) of Ro 15-4513 to six animals was followed by the same IP dose of pentylenetetrazol. Each animal was observed individually for the time of onset and duration of convulsions, as well as for mortality. Any decrease in the time of onset of convulsions and/or the duration of convulsions, as well as mortality rate, would be an indication for the proconvulsant effect of Ro 15-4513.

Data Analysis

The data from the motor coordination and SMA experiments were subjected to analysis of variance (ANOVA) with repeated measures to test for the significance of interaction between treatment groups and time periods. This statistical analysis was performed using the actual time periods rather than the activity ratios and using the Crunch Statistical Package version 3 (Crunch Software Corp., Oakland, CA). This was followed by one-way ANOVA and Newman-Keuls post-hoc analysis, at each evaluation time in the case of motor coordination data and only at 60 min in the case of SMA data, to determine the significance of differences between the treatment groups. Data obtained from the convulsion study were statistically analyzed by Students' t-test. A $p \leq 0.05$ was taken as a measure of significance.

RESULTS

The test dose, 2 g/kg IP, of ethanol used throughout the motor coordination and SMA experiments in the present investigation was selected based upon a separate dose-response study (data not shown). The basis of the selection of this test dose was its ability to produce significant motor incoordination and inhibition of SMA with little or no sedation. Administration of the test dose of ethanol resulted in a maximal motor incoordination within 15 min, followed by a gradual recovery. Recovery was 83% of normal motor coordination at 60 min postethanol [Fig. 1; ACSF (vehicle) + ethanol curve] and usually complete by 90 min postethanol (data not shown).

As stated in the Method Section, Ro 15-4513 or vehicle (0.125-1.88% DMSO in ACSF) in the entire study was administered ICV, followed 2 min later by IP administration of the

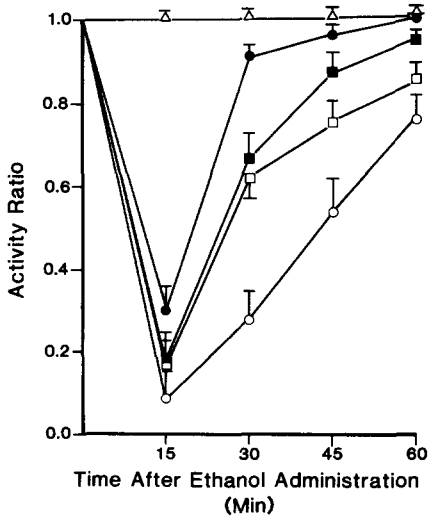

FIG. 1. Effect of pretreatment of various doses of Ro 15-4513 administered ICV 2 min before ethanol (2 g/kg, IP) on ethanol-induced motor incoordination in mice. Each point represents the mean ± SEM of at least 10 mice (○), ACSF 5 μl + EtOH; (●), Ro 15-4513 10 ng/5 μl + EtOH; (□), Ro 15-4513 15 ng/5 μl + EtOH; (■), Ro 15-4513 22 ng/5 μl + EtOH; (△), Ro 15-4513 22 ng/5 μl + saline 10 ml/kg.

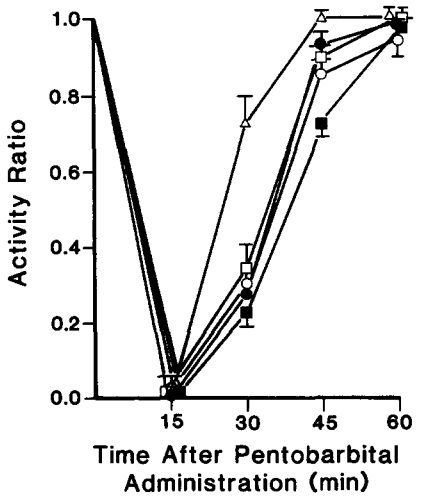

FIG. 2. Effect of various doses of Ro 15-4513 administered ICV 2 min before sodium pentobarbital (6 mg/kg, IP) on sodium pentobarbital-induced motor incoordination. Each point represents the mean ± SEM of at least 10 mice. (○), ACSF 5 μl + pentobarbital; (●), Ro 15-4513 10 ng/5 μl + pentobarbital; (□), Ro 15-4513 22 ng/5 μl + pentobarbital; (■), Ro 15-4513 75 ng/5 μl + pentobarbital; (△), Ro 15-4513 150 ng/5 μl + pentobarbital.

test dose of ethanol or 6 mg/kg sodium pentobarbital or 120 mg/kg pentylenetetrazol or saline. Figure 1 shows that Ro 15-4513 (10, 15, and 22 ng) dose dependently attenuated ethanol-induced motor incoordination. There was a significant, $F(12, 228) = 3.868$, $p < 0.00001$, interaction between drug treatment and time periods. All three doses of Ro 15-4513 markedly attenuated ethanol-induced motor incoordination at 30-, 45-, and 60-min postethanol evaluation periods. After 10-ng Ro 15-4513 pretreatment, there was a significant time and drug treatment interaction, $F(3, 132) = 15.404$, $p < 0.00001$. A significant [ANOVA followed by planned comparisons of the means yielded: $F(1, 44) = 45.162$, $p < 0.00001$, to $F(1, 44) = 4.593$, $p < 0.03$] attenuation of ethanol-induced motor incoordination at 30, 45, and 60 min postethanol evaluation time periods was observed. The 15- and 22-ng doses of Ro 15-4513 also exhibited significant [$F(3, 129) = 4.534$, $p < 0.004$, and $F(3, 159) = 4.521$, $p < 0.004$, respectively] interactions between time and drug treatments. Marked attenuation of ethanol-induced motor incoordination at 30-, 45-, and 60-min postethanol time periods with 15- and 22-ng doses [ANOVA followed by planned comparisons of the means yielded: $F(1, 43) = 11.90$, $p < 0.001$, to $F(1, 43) = 4.171$, $p < 0.04$, and $F(1, 53) = 8.642$, $p < 0.004$, to $F(1, 53) = 4.234$, $p < 0.05$, respectively] was observed. Animals in the 10-ng Ro 15-4513 pretreatment group regained 92% of their normal motor coordination within 30 min postethanol compared to 28% in control animals and nearly complete recovery of their normal motor coordination by 45 min postethanol. In 15- and 22-ng Ro 15-4513 pretreatment groups, animals regained 63 and 68%, respectively, of their normal motor coordination by 30 min, 87 and 76%, respectively, within 45 min, and 96 and 86%, respectively, by 60 min postethanol compared to 28, 57, and 75% at the three time periods, respectively, in vehicle + ethanol control group. No effect on normal motor coordination was noted when the 22-ng dose of Ro 15-4513 was followed by saline instead of ethanol (Fig. 1).

Figure 2 shows that pretreatment with 10-, 22-, and 75-ng doses of Ro 15-4513 did not alter sodium pentobarbital-induced motor incoordination. However, a significant, $F(3, 66) = 8.492$, $p < 0.0001$, time and drug treatment interaction was observed in the 150-ng pretreatment dose group. The attenuation by 150-ng dose of Ro 15-4513 of pentobarbital-induced motor incoordination at 30- and 45-min postpentobarbital evaluation time periods was marked and statistically significant [ANOVA followed by planned comparisons of the means yielded: $F(1, 22) = 13.956$, $p < 0.001$, $F(1, 22) = 13.429$, $p < 0.001$, and $F(1, 22) = 5.491$, $p < 0.02$, respectively]. The 150-ng pretreatment dose of Ro 15-4513 when administered alone followed by saline instead of sodium pentobarbital produced overt behavioral changes such as inability to walk on the rotorod, clonic jerking, and seizures. There was, however, a lack of an antagonism of pentobarbital-induced motor incoordination by 10-, 22-, or 75-ng pretreatment doses of Ro 15-4513 with no significant interaction between time and drug treatment, $F(9, 117) = 0.907$, $p > 0.5$.

The results of Ro 15-4513 pretreatment on ethanol-induced stimulation and inhibition of SMA are presented in Fig. 3. The biphasic effect of ethanol on SMA are well known. Therefore, two different doses of ethanol were used to study the effect of Ro 15-4513 on ethanol-induced stimulation and inhibition of SMA. Ethanol, 1 g/kg, exhibited significant (ANOVA followed by planned comparisons of the means yielded: $p < 0.0001$) increases in SMA compared to vehicle + saline control group (S vs. S + E_1 in Fig. 3). The increase in SMA was markedly attenuated (ANOVA followed by planned comparisons of the means yielded: $p < 0.002$) by the

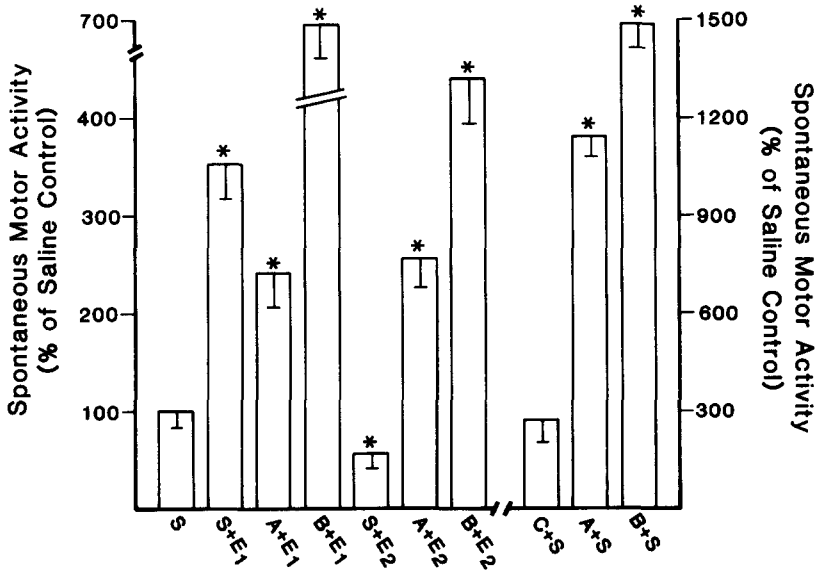

FIG. 3. Effect of ICV administration of Ro 15-4513 on ethanol (IP)-induced stimulation and inhibition of spontaneous motor activity. Each bar represents mean ± SEM of at least 10 mice. S, saline; E_1, EtOH 1 g/kg; E_2, EtOH 2 g/kg; A, Ro 15-4513 75 ng/5 μl; B, Ro 15-4513 150 ng/5 μl; C, Ro 15-4513 10 ng/5 μl. *$p < 0.00001$–$p < 0.002$ (compared to saline control group).

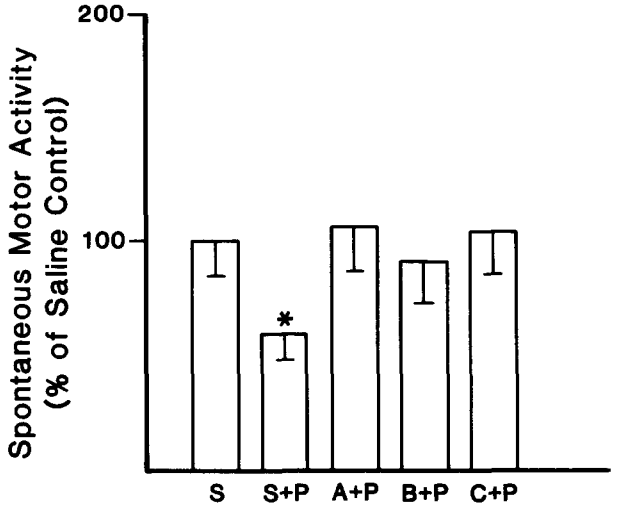

FIG. 4. Effect of Ro 15-4513 ICV pretreatment on sodium pentobarbital (IP)-induced inhibition of spontaneous motor activity. Each bar represents mean ± SEM of at least 10 mice. S, saline; P, sodium pentobarbital 6 mg/kg; A, Ro 15-4513 10 ng/5 μl; B, Ro 15-4513 22 ng/5 μl; C, Ro 15-4513 75 ng/5 μl. *$p<0.05$ (compared to saline control group).

75-ng dose of Ro 15-4513 (S + E_1 vs. A + E_1 in Fig. 3) but not by 10- and 22-ng doses of Ro 15-4513 (pilot experiments; data not shown). The 150-ng dose of Ro 15-4513, on the other hand, markedly (ANOVA followed by planned comparisons of the means yielded: $p < 0.0001$) enhanced the ethanol-induced increase in SMA (S + E_1 vs. B + E_1 in Fig. 3). However, the ethanol dose of 2 g/kg produced nearly 45% inhibition of SMA compared to vehicle + saline control group (ANOVA followed by planned comparisons of the means yielded: $p < 0.001$; S vs. S + E_2 in Fig. 3). The ethanol-induced inhibition of SMA was not only blocked but reversed by the 75-ng dose of Ro 15-4513 as animals exhibited a 155% increase in SMA compared to saline-injected animals (ANOVA followed by planned comparisons of the means yielded: $p < 0.00001$; S + E_2 vs. A + E_2 in Fig. 3). Ethanol-induced inhibition of SMA was not altered by 10 and 22 ng Ro 15-4513 (pilot experiments; data not shown). The increase in SMA was 340% in animals given 150 ng Ro 15-4513 + ethanol 2 g/kg compared to vehicle + saline controls (ANOVA followed by planned comparisons of the means yielded: $p < 0.0003$; S + E_2 vs. B + E_2 in Fig. 3). When 10-, 75-, and 150-ng pretreatment doses were administered followed by saline injection instead of ethanol, a marked (ANOVA followed by planned comparisons of the means in each case yielded: $p < 0.001$ and $p < 0.00001$, respectively) increase in SMA was observed in a dose-dependent manner in 75- and 150-ng dose groups (S vs. A + S and B + S in Fig. 3) but not in the 10-ng group (S vs. C + S; Fig. 3).

To test if Ro 15-4513 selectively antagonizes ethanol-induced inhibition of SMA, experiments in which animals received 10, 22, and 75 ng Ro 15-4513 followed by 6 mg/kg IP test dose of pentobarbital were conducted. As evident from Fig. 4, there was no significant attenuation of pentobarbital-induced inhibition of SMA by any Ro 15-4513 dose used.

To test if the doses of Ro 15-4513 that antagonized ethanol-induced motor disturbances in the present study possessed any proconvulsant activity, the effect of ICV pretreatment of lowest, 10 ng, and highest, 75 ng, antiethanol doses was investigated on the onset and duration of seizures and/or lethality by pentylenetetrazol. In addition, the proconvulsant effect of 150 ng Ro 15-4513, a dose much higher than the doses that antagonized ethanol's motor-impairing effects in the present study, was also evaluated. Table 1 shows that only the 150-ng dose of Ro 15-4513 significantly ($p < 0.05$) decreased the seizure onset latency compared to the ACSF + pentylenetetrazol group.

DISCUSSION

In the present investigation, the pharmacology of the partially negative ligand for benzodiazepine binding site Ro 15-4513 was investigated in terms of its site of action (peripheral vs. central) and its selectivity in attenuating and reversing ethanol-induced motor disturbances. Considerable interest has been focused on the ability of Ro 15-4513 to antagonize the effects of ethanol. In addition, several reports have demonstrated the intrinsic behavioral activity of this compound, such as reduction of exploratory head-dipping (14) and an increase in locomotor activity (24).

In motor coordination experiments, very low doses (10, 15, and 22 ng) of Ro 15-4513 markedly attenuated ethanol-induced motor incoordination roughly in a dose-dependent fashion (Fig. 1). More interestingly, the highest dose (22 ng) of Ro 15-4513 did not show any effect on normal motor coor-

TABLE 1

EFFECT OF Ro 15-4513 PRETREATMENT (ICV) ON PENTYLENETETRAZOL (IP)-INDUCED CONVULSIONS IN MICE

Treatment Groups	Convulsion Onset Latency (seconds)	Lethality Time (seconds)
ACSF[a] + pentylenetetrazol (n = 18)	51.25 ± 2.54	212.73 ± 58.65
Ro 15-4513 10 ng + pentylenetetrazol (n = 6)	52.46 ± 9.78	367.66 ± 71.29
Ro 15-4513 75 ng + pentylenetetrazol (n = 6)	44.50 ± 8.10	196.50 ± 40.35
Ro 15-4513 150 ng + pentylenetetrazol (n = 6)	42.00 ± 1.80[b]	217.33 ± 65.40

Values are expressed as mean ± SEM. Administration (ICV) of ACSF or Ro 15-4513 pretreatment was followed 2 min later by pentylenetetrazol (120 mg/kg, IP).

[a]Artificial cerebrospinal fluid.

[b]$p < 0.05$ compared to ACSF + pentylenetetrazol group.

dination when administered alone (Fig. 1). The graphic presentation of the rotorod data in Fig. 1 do not precisely show the dose dependency of the attenuation of ethanol-induced motor incoordination by Ro 15-4513. As shown in Fig. 1, the 10-ng dose of Ro 15-4513 exhibited the maximum attenuation of ethanol-induced motor incoordination, followed by that observed after 15- and 22-ng pretreatment doses. The rotorod procedure for evaluation of motor coordination did not permit graphic expression of the true dose–response relationship. The anticipated greater antagonism by 15- and 22-ng doses of Ro 15-4513 compared to the 10-ng dose consequently was not evident from the dose–response data in Fig. 1, possibly due to the reversal of ethanol-induced motor incoordination. Animals pretreated with 15 and 22 ng Ro 15-4513 experienced antagonism, as well as most likely some reversal of ethanol-induced motor incoordination. The possible reversal of ethanol-induced motor incoordination in these animals compared to animals that received pretreatment of 10 ng Ro 15-4513 caused difficulty in their walking on the rotorod because of their frequent jumping off the rotorod.

The marked attenuation by ICV-administered Ro 15-4513 of ethanol-induced motor incoordination demonstrates, perhaps for the first time, a central mechanism by which this partially negative ligand for benzodiazepine binding site antagonizes and reverses this effect of ethanol. The ICV doses of Ro 15-4513 were very low and even if any drug escaped from the CNS into systemic circulation no behavioral effect would be expected as relatively much higher doses of Ro 15-4513 were used systemically in studies published by various investigators (14,18,24,27). In addition, the ICV doses (10, 15, 22, and 75 ng) of Ro 15-4513 selected in this study did not produce any overt behavioral effect when injected systemically (data not shown).

In the present investigation, the selectivity of Ro 15-4513 in attenuating and reversing the motor-incoordinating effect of ethanol was suggested when it failed to antagonize sodium pentobarbital-induced motor incoordination (Fig. 2). Whereas the maximum attenuation of ethanol-induced motor incoordination was seen after the 10-ng pretreatment dose of Ro 15-4513, there was no attenuation of pentobarbital-induced motor incoordination even after the 75-ng dose of Ro 15-4513 (Fig. 2). Only a much higher, 150 ng, dose of Ro 15-4513 produced a statistically significant attenuation of motor incoordination by pentobarbital (Fig. 2). The test dose of pentobarbital produced motor incoordination comparable to that produced by the test dose of ethanol and was selected based upon a separate dose–response relationship between various doses of pentobarbital and degree of motor incoordination (data now shown). Nevertheless, the 150-ng dose of Ro 15-4513, which did antagonize pentobarbital-induced motor incoordination, was also found to be proconvulsant and exhibited intrinsic behavioral activity including clonic jerking and seizures (Table 1). Based on pentylenetetrazol study (Table 1), only the 150-ng dose of Ro 15-4513 resulted in a statistically significant decrease in the onset time of pentylenetetrazol-induced seizures. The absence of intrinsic proconvulsant activity after 10- and 75-ng doses of Ro 15-4513 (Table 1) together with lack of any motor incoordination by 10-, 15-, and 22-ng doses of Ro 15-4513 (Fig. 1) suggested a selectivity of Ro 15-4513, at least within this dose range, as an antagonist of ethanol-induced motor incoordination.

Ro 15-4513 also attenuated ethanol-induced stimulation and inhibition of SMA (Fig. 3). The well-known biphasic effects of ethanol on SMA were observed using smaller and higher doses of ethanol. The smaller ethanol dose produced a significant increase in SMA that was significantly attenuated by 75 ng Ro 15-4513 (Fig. 3), in agreement with others (15). However, the higher, 150 ng, dose further markedly increased ethanol-induced stimulation of SMA (Fig. 3). Again, the intrinsic proconvulsant activity of this high dose of Ro 15-4513 may be responsible for further stimulating ethanol-induced increase in SMA. The higher ethanol dose, on the other hand, significantly inhibited SMA, which was reversed by Ro 15-4513 (Fig. 3). The doses (75 and 150 ng) of Ro 15-4513 that reversed the ethanol-induced inhibition of SMA also exhibited dose-dependent marked increases in SMA when injected alone (Fig. 3). The selectivity of Ro 15-4513 in attenuating ethanol-induced inhibition of SMA was, therefore, suggested by a lack of an attenuation of sodium pentobarbital-induced inhibition of SMA by nonproconvulsive doses (10 and 75 ng) of Ro 15-4513 (fig. 4).

According to several reports (21,23), negative ligands for benzodiazepine-binding sites can antagonize, partially or completely, the behavioral effects of barbiturates. These reports, in fact, support the observations that the negative ligands for benzodiazepine binding sites attenuate and barbiturates accentuate the effects of GABA (10,25). In addition, GABA antagonists such as pentylenetetrazol and picrotoxin oppose the effects of barbiturates (11). Many studies (10,13,20), including ours (9), have suggested the participation of GABA in the mediation of CNS effects of acute and chronic ethanol. Ethanol specifically has been shown to enhance the GABA-mediated neurotransmission (20), whereas GABA antagonists such as bicucculine antagonize ethanol-induced central behavioral effects (27). Therefore, it could be predicted that the negative ligands for benzodiazepine binding sites would also antagonize and reverse the ethanol-induced behavioral effects. Indeed, in the present investigation a partially negative ligand, Ro 15-4513, has been found to reverse the acute ethanol-induced motor incoordination and stimulation and inhibition of SMA at dose levels that did not cause any motor incoordination nor were proconvulsant. The ICV route of administration especially permitted evaluation of very small doses of Ro 15-4513 that systemically showed no effect but markedly attenuated ethanol-induced motor deficits yet lacked intrinsic activity, thereby permitting the demonstration as well as separation of its selective antiethanol effects from its intrinsic activity. In the present study, all doses of Ro 15-4513 used failed to antagonize the motor incoordination and inhibition of SMA by test doses of sodium pentobarbital except the highest (150 ng) dose, which exhibited intrinsic proconvulsant activity. Therefore, to observe the selectivity of Ro 15-4513 in antagonizing and reversing motor deficit by ethanol the selection of its dose appears to be of critical importance. By administering Ro 15-4513 by the ICV route, it was possible, based upon the present investigation, to fulfill the dual objectives of delineating Ro 15-4513's selectivity as an antiethanol drug as well as its central site of action. Ro 15-4513's selectivity to attenuate and reverse ethanol-induced behavioral effects may be related to its ability to selectively inhibit ethanol- but not pentobarbital-stimulated chloride uptake as had been demonstrated by Suzdak et al. (27). In the present study we similarly observed that Ro 15-4513, at least in the dose range used, interacted selectively with ethanol but not with sodium pentobarbital. It should be noted that other partially negative ligands for the central benzodiazepine binding sites such as FG-7142 and ÃCCE lacked the ability to block ethanol-induced intoxication, thereby strongly suggesting that the se-

lectivity of Ro 15-4513 as an antiethanol compound is not primarily a result of its inverse agonist properties (27).

In conclusion, the results of the present study point that the motor incoordination and inhibition of SMA produced by ethanol may be mediated through the GABA-gated chloride ion channels within the CNS, which is selectively antagonized by Ro 15-4513 at a nonproconvulsive dose range.

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