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Antagonism by Intracerebellar Ro15-4513 of Acute Ethanol-Induced Motor Incoordination in Mice

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DAR, M. S. *Antagonism by intracerebellar Ro15-4513 of acute ethanol-induced motor incoordination in mice.* PHARMACOL BIOCHEM BEHAV 52(1) 217-223, 1995.—The possible antiethanol effect of intracerebellarly microinjected Ro15-4513 was investigated using motor incoordination as the test response. The results of this study further confirmed reports from this and other laboratories that this partially negative ligand of benzodiazepine selectively attenuated and nearly reversed the motor impairment of acute ethanol. The attenuation observed after microinjections of doses of 0.05, 0.1, and 0.5 ng was significant and dose related. There was no effect on normal coordination when the highest dose, 0.5 ng, was administered followed by saline instead of a test dose of ethanol. When 0.5 ng of Ro15-4513 alone was microinjected into the cerebellum, no significant change in the locomotor activity was observed. Even a 10-fold higher intracerebellar dose (5 ng) of Ro15-4513 administered alone produced no significant changes in locomotor activity. This suggests that attenuation of ethanol-induced motor incoordination was most likely due to the selective antiethanol effect of Ro15-4513 at the dose range used in the present investigation. The antiethanol effect of intracerebellar Ro15-4513 also reaffirmed the well-known belief that the cerebellum is an important brain region for ethanol's motor-impairing effect. The results also indirectly suggest the inhibition of $GABA_A$-gated chloride ion channel activity as the most likely basis of Ro15-4513's antiethanol effect.

Ethanol Motor incoordination Intracerebellar Ro15-4513 Selective antagonism

RECENTLY, Ro15-4513, a partial inverse agonist of benzodiazepine receptors, has been reported selectively to antagonize the motor-incoordinating effects of ethanol (6). This ability was only demonstrated when administered in a low dose range by the intracerebroventricular (ICV) route (6). However, at higher doses, Ro15-4513 exhibited an intrinsic activity [i.e., an increased spontaneous motor activity (6,27)] as well as a proconvulsant property (6). The ability of Ro15-4513 to produce increased locomotor activity is the basis of its classification as an anxiogenic compound (27). The antiethanol effect of Ro15-4513 includes many, but not all effects of ethanol (31). The ability to antagonize the ethanol-induced ataxia was among the earliest demonstrated antiethanol effects of Ro15-4513 (2,24). Subsequent studies (6,13) in which ICV-administered (1) as well as systemically injected (7) Ro15-4513 effectively antagonized acute ethanol-induced motor incoordination at relatively small doses confirmed the original observation. The compound, however, does possess significant intrinsic proconvulsant activity at higher doses (17,33). This has provided the basis for controversy over whether the antiethanol effects of Ro15-4513 represent its specific interaction with ethanol or its intrinsic activity (16,31).

Motor incoordination is one of the earliest and best-recognized effects of ethanol consumption in humans and laboratory animals. The cerebellum is a key motor area of the brain and is known to be important in motor coordination and motor control. Using the antiataxia effect of Ro15-4513 (2,6,13,24) as the basis, the effect of intracerebellar pretreatment with Ro15-4513 on acute ethanol-induced motor incoordination was evaluated. The data from these motor coordination experiments was intended to provide dual information: a) Ro15-4513 after direct microinjection into the cerebellum antagonized ethanol-induced motor incoordination; and, thereby b) suggested an involvement of cerebellum in the expression of ethanol's motor-incoordinating effect. Using ethanol-induced motor incoordination as the test response the present investigation was intended to confirm the antiataxia effect of Ro15-4513 after its direct microinfusion into the cerebellum and the possible involvement of the cerebellum in the mediation of this important effect of ethanol. To rule out any role of the intrinsic activity of Ro15-4513 in its antiataxia effect of ethanol, the effect of Ro15-4513-alone on spontaneous locomotor activity was also measured.

METHOD

Animals

Male CD-1 mice, weighing between 22 and 25 g, 5 weeks of age, were used in the present investigation. The animals were purchased from Charles River (Raleigh, NC). All mice in the present study were surgically implanted with permanent indwelling stainless-steel guide cannulae into the cerebellar cortex for direct microinjection of Ro15-4513 into the cerebellum. After surgery, the animals were individually housed in a controlled environment (ambient temperature 24 ± 1°C; 12 L : 12 D cycle, lights on at 0800 h) and fed commercial pellet food and tapwater ad lib.

Surgery

Under chloral hydrate (450 mg/kg, IP) anesthesia and strict aseptic conditions, mice were stereotaxically (David Kopf Instruments, Tujunga, CA) implanted with permanent indwelling guide cannulae (22 ga, 12 mm long) in the cerebellar cortical tissue with the skull surface in the horizontal plane (skull flat). The implantation of the guide cannulae was according to the coordinates of Slotnick and Leonard (30) as follows: AP −6.4 (bregma); ML, ±0.8 mm;, and DV −1.0 mm from the surface of the skull. The guide cannulae were lowered to the desired depth through the appropriately located craniotomy holes. After their placement, the guide cannulae were anchored using fast drying dental cement (Durelon; Premier Dental Products Co., Norristown, PA) to the cranial surface, which had been scraped clean of periosteum. Each animal was administered SC 3000 U of Crystiben (combination of benzathine and procaine penicillin G suspension; Solvay Veterinary, Inc., Princeton, NJ) immediately after surgery to prevent possible infection. The aseptic surgical procedure included routine scrubbing of the cranial surface with swab sticks impregnated with providine-iodine solution (Operand; Redi-Products, Prichard, WV) as well as autoclaving of the surgical instruments, guide cannulae, and drill burrs. A recovery period of 5 days was allowed for the cannulated animals before their use in the behavioral experiment. Appropriate stainless-steel wire plugs were used to occlude the guide cannulas during the 5-day recovery period.

Drugs

Ro15-4513 was received as a gift from Hoffman-La Roche and Company (Basel, Switzerland) and provided by Drs. Imhof and Eigenmann. It was solubilized with the aid of dimethylsulfoxide (DMSO) in artificial cerebrospinal fluid (ACSF) containing (mM): NaCl, 127.65; KCl, 2.55; $CaCl_2$, 0.05; $MgCl_2$, 0.94; $Na_2S_2O_5$, 0.05, at pH 7.4. The final concentration of DMSO in the solutions of various doses of Ro15-4513 used in the present work ranged between 0.032% (0.05 ng) and 0.32% (0.5 ng). The Ro15-4513 solution was prepared a few days before the experiments and stored at −70°C in glass tubes completely covered with black vinyl tape. The chloral hydrate was prepared in distilled water and injected as 5 ml/kg body wt. The ethanol solution (10% w/v) was prepared in normal saline (0.9%) and always injected as 20 ml/kg IP. Using five Hamilton microsyringes (25 μl) mounted on an infusion pump (Harvard Pump Model 22; Harvard Apparatus, South Natick, MA), Ro15-4513 solutions were infused at a constant rate of 100 nl (0.1 μl)/min. The injection cannulae were kept in the same position after complete infusion for an additional period of 60 s to ensure complete drug diffusion. The volume of intracerebellar drug-vehicle microinjection was kept constant at 100 nl (0.1 μl) throughout the entire investigation. Thus, the administration of Ro15-4513 by microinjection was done over a total period of 2 min during which the animals were able to move about freely in their home cages. Just before intracerebellar microinjection, the stainless-steel injection cannulas (30 ga, 0.31 mm diam.), cut to protrude 1.0 mm beyond the tips of the guide cannulae, were connected to the microsyringes by PE-10 (Clay Adams, Parsippany, NJ) polyethylene tubing. In a single motor coordination experiment the desired Ro15-4513 solution or vehicle was delivered by a microinfusion pump, as explained earlier, to a group of five mice. The microsyringe and part of PE-10 tubing connected to it were separately filled with water; the latter was separated from the Ro15-4513 solution or vehicle by a small air bubble in the PE-10 tubing. Proper drug delivery was adjudged by monitoring the movement of air bubble during the microinjection.

Intracerebellar Drug Microinjection

The infusion of Ro15-4513 solution into the cerebellum was targeted to bathe the superficial layers of the cerebellar cortex, primarily the molecular, Purkinje cell, and portions of the granular cell layers. Included in these layers were the neuronal cell types, their axons, and axonal terminals on which were localized the binding sites for adenosine, $GABA_A$, and various neurotransmitters alleged to mediate some of the CNS effects of ethanol. Consequently, these binding sites (directly and/or indirectly) may be the intended target(s) of Ro15-4513—in particular, the $GABA_A$-benzodiazepine-chloride ionophore complex. The infusion of 100 nl of various concentrations of Ro15-4513 appeared to be adequate to stimulate a sufficient concentration of its active sites. This was deemed so because significant antagonism to ethanol-induced motor incoordination was noted based on the motor coordination evaluation by the rotorod test in the present study.

Histology

Correct placement of the guide cannula, and consequently the delivery of the Ro15-4513 solutions, was verified by histology. At the conclusion of a motor coordination experiment, each animal was injected with 100 nl of india black ink through the guide cannula. Immediately afterward, we killed the animals and removed their brains. The isolated brains were fixed in 4% formalin solution for 24 h. The fixed brains were then frozen and cut into 40-μm coronal sections using Tissue-Tek II microtome cryostat (Miles, Naperville, IL). After the sections were mounted onto glass slides and stained with Cresyl Violet, they were viewed microscopically for the correct placement of the guide cannulae. Only data from animals in which histologic confirmation was made based on distribution of india ink within the cerebellum were included in the calculation of the results. The extent of tissue damage sustained due to an individual guide cannula implantation is shown by a representative histologic photomicrograph of cerebellar tissue in Fig. 1. There was little or no tissue damage observed resulting from cannula placement (Fig. 1). A schematic drawing (Fig. 2) shows the degree of dispersion from the microinjections as estimated by india ink diffusion. The circular shaded areas represent the dispersion of india ink. There was little variation within and between groups and treatments in the location of the microinjections, tissue damage due to cannula implantation, and in the extent of overall dispersion of Ro15-4513 microinjections, as evident in Figs. 1 and 2. There was a 98% success rate in the animals undergoing microsurgery.

FIG. 1. A representative histologic photomicrograph of the cerebellar cortex showing the position of guide cannula and the slight tissue damage due to its placement. The central dark area represents some of the india ink present.

Motor Coordination

The degree of motor coordination was evaluated by a mouse rotorod treadmill (UGO Basil, Varese, Italy) calibrated for a fixed speed of 24 rpm. Because only five mice could be evaluated at one time by the rotorod treadmill, a single motor coordination experiment was based on five animals. According to the rotorod experimental protocol, normal motor coordination was defined by the ability of the animals to remain on the rotorod for an arbitrarily selected time of 180 s. Fifteen to 30 min before the actual rotorod experiment, we acclimated the animals to the treadmill by placing them on it two to three times. Prescreening of animals was found to be essential to rule out possible inborn defects (such as cerebellar) or damage due to microsurgery. Nearly all animals met the prescreening criterion for motor evaluation. Only animals successful in the prescreening evaluation were used in the actual rotorod experiments and received an intracerebellar microinjection with vehicle or Ro15-4513 as pretreatment, followed by the test dose of ethanol after 2 min. Based on a separate dose-response study (data not included) 2 g/kg, IP, was the selected test dose of ethanol in the present investigation. The basis for the selection of the test dose was that it should produce a significant motor incoordination with little or no sedation in the animals.

In a typical rotorod experiment, the successfully prescreened animals were injected with the test dose of ethanol 2 min after the intracerebellar microinjection of either one of the doses of Ro15-4513 or vehicle. Following ethanol administration, the animals were evaluated for motor incoordination every 15 min for 60 min. The animals therefore served as their own control and were used only once in a rotorod experiment. Within a rotorod experiment, each animal received only one intracerebellar pretreatment with either one of the doses of Ro15-4513 or vehicle, followed 2 min later by an ethanol or saline injection. The other experimental details were similar to what was reported previously (5,7,8).

The degree of motor incoordination was expressed as an activity ratio, defined as the ratio between the time the animals were able to walk on the rotorod after the intracerebellar drug treatment and/or ethanol injection and the time the animals were able to walk, before drug and/or ethanol treatment, which was arbitrarily selected to be 180 s. Consequently, an activity ratio score of 1 was considered to be normal coordination according to our criterion, whereas a decreasing ratio indicated increasing motor incoordination. Although the actual walking time periods were used in the statistical analyses, the dose-response graphs were based on the activity ratio numbers.

Statistical Analyses

The motor coordination data from rotorod experiments were analyzed by analysis of variance (ANOVA) with repeated

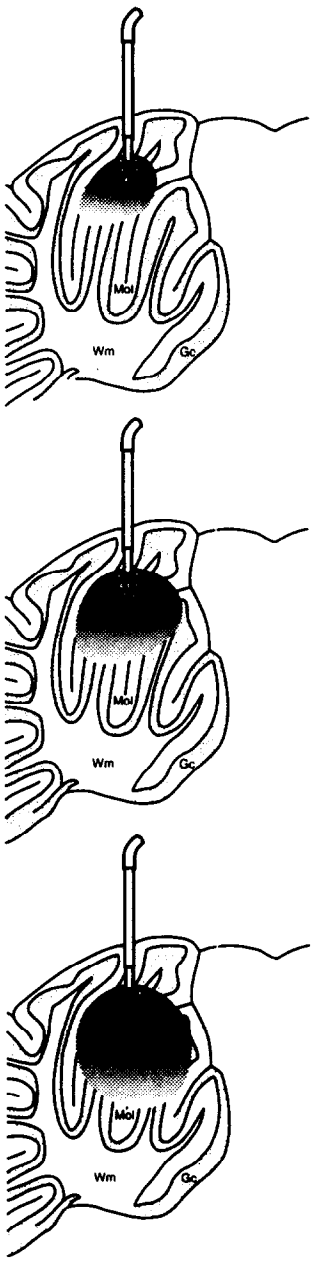

FIG. 2. The guide cannula placement and the degree of dispersion of india ink within the cerebellar cortex as an indicator of distribution of drug microinjections.

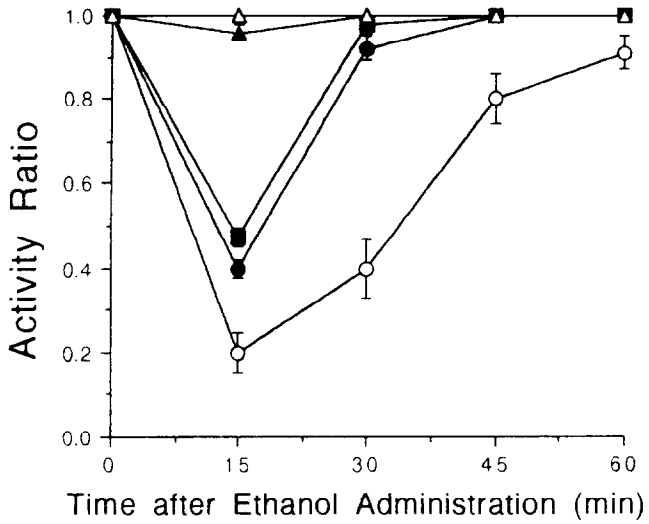

FIG. 3. The effect of various doses of Ro15-4513 administered intracerebellarly 2 min before ethanol (2g/kg, IP) on ethanol-induced motor incoordination in mice. Each point represents the mean ± SEM of at least 10 mice. ○, Vehicle, 100 nl + EtOH; ▲, Ro15-4513, 0.5 ng/100 nl + EtOH; ■, Ro15-4513, 0.1 ng/100 nl + EtOH; ●, Ro15-4513, 0.05 ng/100 nl + EtOH; △, Ro15-4513, 0.5 ng/100 nl + saline.

measures to check the significance of interaction between drug treatment and motor coordination evaluation periods. This was followed by one-way ANOVA and Newman-Keuls post-hoc analysis at each rotorod evaluation time to determine the significance of differences within the treatment groups. The statistical analysis was carried out using the Crunch Statistical Package Version 3.0 (Crunch Software Corporation, Oakland, CA). A $p < 0.05$ was taken to be significant.

Locomotor Activity Monitoring (LAM)

Using the Auto-Track Optoverimex Activity Monitoring System (Columbus Instruments, Columbus, OH), the effect on locomotor activity of the highest dose of Ro15-4513 used in the rotorod experiments was measured. Four activity monitors were available, so each LAM experiment was based on four animals. The animals were tested individually and acted as their own control. Prior to the actual LAM experiment, the animals were acclimated to the environment of the activity monitors for 5 min by transferring each animal from its home cage to an activity monitor. Baseline motor activity of each animal was recorded every 10 min for a 60 min test period (total of six recordings), which served as the control motor activity. Immediately following the measurement of baseline motor activity, the animals were given an intracerebellar injection of the highest dose (0.5 ng/100 nl) of Ro15-4513 or vehicle and placed in their respective activity monitors. After a 5-min acclimation period, their motor activity was recorded every 10 min for 60 min. The LAM was carried out in a sound- (white noise) and light- (red 25-W light from one side) controlled, dedicated room. The measurements by the Auto-Track System represented multivariate locomotor analysis with specific measures such as simultaneous measurements of ambulatory, stereotypical, and sudden burst movements. Two nonequivalent parameters (26) were analyzed: a) horizontal activity, which represented the total number of beam interruptions in the horizontal direction; and b) total distance traveled, which indicated the distance in centimeters traveled by the animal. The latter depended on the path taken. In the present investigation, only total distance traveled was used (Fig. 4), because changes in the horizontal activity were highly correlated with the total distance traveled as also reported by others (21).

RESULTS

The test dose of ethanol produced a significant degree of motor incoordination. Its onset was quick with a peak effect noted within 15 min of IP ethanol administration. There was no sedation observed at this ethanol dose. Ethanol-induced motor incoordination generally lasted 45–60 min postethanol. Animals always regained their normal motor coordination by 90 min (data not shown), with a return of nearly 90% of their normal motor coordination by 60 min postethanol [Fig. 3 ○, ACSF (vehicle) + ethanol curve].

Figure 3 shows the dose-dependent effect of intracerebellar pretreatment of Ro15-4513 on ethanol-induced motor incoordination. All three doses (0.05, 0.1, and 0.5 ng) of Ro15-4513 used in the rotorod experiments significantly attenuated the motor-incoordinating effects of ethanol. There was a significant [$F(12, 534) = 59.751$, $p < 0.0001$] interaction between pretreatments with various doses of Ro15-4513 and time periods. The attenuation of ethanol-induced motor incoordination by all three doses of Ro15-4513 was dose dependent and significant at three postethanol motor evaluation time periods, i.e., 15, 30, and 45 min (Fig. 3). For example, after the 0.05-ng dose of Ro15-4513, the interaction between treatment and time was significant [$F(3, 465) = 34.637$, $p < 0.0001$; Fig. 3, ●]. Attenuation of ethanol-induced motor incoordination by this dose was significant at 15, 30, and 45 min postethanol [ANOVA, followed by planned comparisons of the means yielded: $F(1, 155) = 48.882$, $p < 0.0001$ to $F(1, 155) = 7.266$, $p < 0.007$] but not at 60 min ($F(1, 155) = 2.961$, $p < 0.08$). Similarly, the higher doses, 0.1 and 0.5 ng, of Ro15-4513 produced significant interactions between time periods

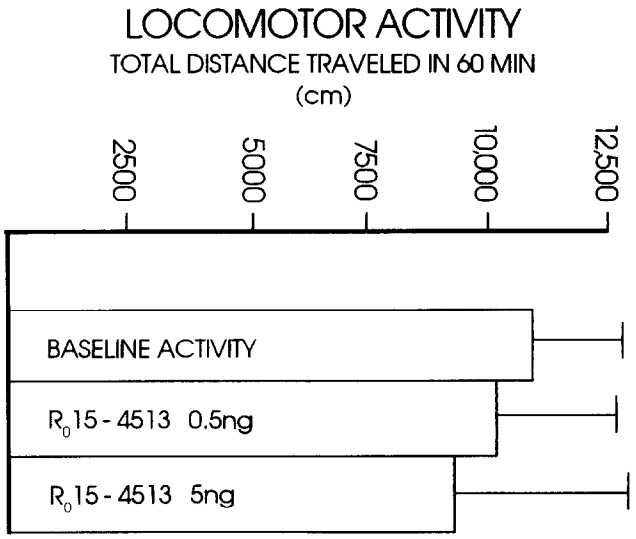

FIG. 4. The evaluation of the effect of highest doses of Ro15-4513-alone administered intracerebellarly on normal locomotor activity in mice. Each bar represents mean ± SEM of at least eight mice.

and drug pretreatments [$F(3, 468) = 48.119, p < 0.0001$ and $F(3, 465) = 101.690, p < 0.0001$, respectively; Fig. 3; ■ and ▲, respectively]. The attenuation of ethanol-induced motor incoordination by both of these doses was marked at 15-, 30-, and 45-min but not at 60-min postethanol evaluation periods (ANOVA, followed by planned comparisons of the means yielded: $F(1, 156) = 113.425, p < 0.0001$ to $F(1, 156) = 7.993, p < 0.005$ and $F(1, 155) = 710.926, p < 0.0001$ to $F(1, 155) = 7.266, p < 0.005$, respectively]. However, no change in the normal motor coordination was noted when the highest dose, 0.5 ng, of Ro15-4513 was administered intracerebellarly followed by IP saline instead of the test dose of ethanol (Fig. 3; △, curve).

We also investigated the effect on locomotor activity of the highest intracerebellar dose of Ro15-4513 (Fig. 4). The 0.5-ng dose, as well as a much higher 5-ng dose, produced no significant changes in locomotor activity relative to baseline control after intracerebellar microinjection. The total distance traveled by animals with and without intracerebellar Ro15-4513 was similar (Fig. 4).

DISCUSSION

The ability of Ro15-4513 to antagonize acute ethanol-induced motor incoordination was initially noted by Bonneti et al. (2) and subsequently confirmed in our laboratory (6) as well as by others (13). The results of the present investigation confirmed our previous report (6) that Ro15-4513 antagonized ethanol-induced motor incoordination as a result of its action within the CNS. The results also confirmed the relative specificity of the antiataxia effects of Ro15-4513 at the dose range employed in the rotorod experiments in the present investigation. The importance of the cerebellum in the control of normal motor functions and motor coordination is well established. The ability of Ro15-4513 to antagonize acute ethanol-induced ataxia significantly and dose dependently after its intracerebellar microinjection confirmed the role of the cerebellum not only in the control of normal motor functions and motor coordination but also in the expression of the motor-incoordinating effect of acute ethanol. There was no effect of Ro15-4513 alone on normal motor coordination at the dose levels used in the present study (Fig. 3, △, curve). This may suggest that the antiataxia effect of the drug was most likely selective for ethanol and not the result of its intrinsic activity, at least at these dose levels.

The selectivity of the antiataxia effect of Ro15-4513 was also suggested by the lack of any significant effect on locomotor activity of the animals of the highest intracerebellar dose (0.5 ng) of Ro15-4513 (Fig. 4). Furthermore, there was an absence of significant change in locomotor activity even after a much higher (5-ng) intracerebellar dose of Ro15-4513 alone. The rationale to test whether the highest antiataxic dose of Ro15-4513 (0.5 ng) used in the present study (Fig. 3) produced any increase in locomotor activity when given alone by the intracerebellar route was to clarify whether the observed antiataxic effect was independent of the intrinsic effect of Ro15-4513. Any contribution by the intrinsic effect of Ro15-4513 toward the observed antiataxic effect after ethanol, such as an increase in locomotor activity (27), should be reflected by an increase in locomotor activity due to Ro15-4513 alone. Because no increase in locomotor activity was observed after 0.5 ng, and after even a 10-fold increase in the intracerebellar (5 ng) dose of Ro15-4513 (Fig. 4), it was clear that antagonism by Ro15-4513 of ethanol-induced motor incoordination was selective at the doses used in the study (Fig. 3) and independent of the intrinsic activity of Ro15-4513. In the present investigation, therefore, intracerebellar Ro15-4513 exhibited its antiataxia effect independent of its intrinsic activity suggesting its selectivity for antiethanol effect at these dose ranges.

The possibility of any peripheral action of Ro15-4513 contributing to its antiataxia effect after its intracerebellar microinjection in ethanol-injected animals was almost negligible. The intracerebellar doses used in the motor coordination experiments were extremely small. Such low doses have never been used systemically and have never exhibited a response in any previously published reports. In fact, the doses used for systemic effects of Ro15-4513 were in the microgram to milligram per kilogram range as reported in the literature (13). In addition, histologic data (Figs. 1 and 2) confirmed that after intracerebellar administration of 100 nl of the Ro15-4513 solution, the drug remained well within the cerebellar cortex.

Ro15-4513's relative selectivity to antagonize and reverse ethanol-induced motor incoordination may be explained by its ability selectively to inhibit ethanol-induced increases in chloride uptake, as was demonstrated by Suzdak et al. (31). According to several reports (1,18,20,31), acute ethanol administration increased the activity of neuronal chloride channels linked to $GABA_A$ receptor. $GABA_A$ receptor generally has been accepted as the site of action of barbiturates and benzodiazepines. Motor impairment action of ethanol has been suggested to be due to its effects on $GABA_A$ receptor (11,32). However, it should be stated that results of several other studies (5,18,19) do not support the selectivity of Ro15-4513's antiethanol effect. These studies (5,18,19) concluded that Ro15-4513 acts as an ethanol antagonist only when the ethanol effects were opposite to the intrinsic actions of Ro15-4513. In addition, observations by Koob et al. (15) of the effects of Ro15-4513 on the anxiolytic and behavioral effects of ethanol are not consistent with those of Suzdak et al. (31). Nevertheless, the selectivity of Ro15-4513 in antagonizing the ataxic effect of ethanol observed in the present investigation was due to the extremely low dose range of intracerebellarly administered Ro15-4513. Ro15-4513 and the other negative ligands for benzodiazepine binding sites are known to attenuate effects of GABA, whereas barbiturates accentuate such effects (10,29). The GABA-antagonists picrotoxin and pentylenetetrazole oppose the effects of barbiturates (12). The attenuation by Ro15-4513 of the behavioral effects of GABA (10,29) and ethanol points toward the possible role of GABA in the motor-incoordinating effect of ethanol. In fact, many reports (10,14,20) including ours (9) have suggested the participation of GABA in mediating the CNS effects of acute and chronic ethanol. It is also known that ethanol enhances GABA-mediated neurotransmission (20), and GABA antagonists such as bicuculline, attenuate central behavioral effects of ethanol (31). In view of these literature reports, it is not surprising to observe that negative ligands for benzodiazepine binding sites would antagonize and reverse ethanol-induced motor incoordination. Indeed, in the present investigation, Ro15-4513, a partially negative ligand for benzodiazepine receptors, was found to significantly antagonize and even nearly reverse the ataxia produced by acute ethanol at dose levels that lacked any effect on normal motor coordination and locomotor activity. The use of very small doses of Ro15-4513 by the intracerebellar route permitted demonstration of its selective antiataxia effect as well as separation of this effect of Ro15-4513 from its intrinsic activity.

The subclass of the GABA receptor that most likely mediates ethanol's motor impairment action has been generally regarded to be $GABA_A$ (11,32). This subtype is not a G-

coupled receptor, but instead belongs to the ion channel-gated family of receptors. The activity of $GABA_A$-gated chloride channels in the neuronal tissues is increased during acute exposure to ethanol and is blocked or reversed by Ro15-4513 (18,20,31). Several types of $GABA_A$ receptor subunits have been identified (22); each exists in multiple forms. Various combinations of subunit forms may result in many subtypes of the $GABA_A$ receptor. One of the subunits (γ) has been suggested recently to be critical for the effects of ethanol, including motor impairment. Although partially negative ligands for benzodiazepine binding sites have been predicted to have antiethanol effects, the selectivity of Ro15-4513 as an antiethanol drug was further supported by the lack of ability of other partially negative ligands for benzodiazepine binding sites such as FG-7142 and ACCE to antagonize the effects of ethanol (31). Although Ro15-4513 binds to benzodiazepine receptor sites on the $GABA_A$ receptor complex, in the cerebellum a significant proportion of Ro15-4513 also binds to diazepam-insensitive sites on $GABA_A$ receptor complex (28,34), because it is not displaced by classical benzodiazepine agonist diazepam. The cerebellar diazepam-insensitive sites may mediate, at least in part, the antiethanol effect including ataxia of Ro15-4513 because of the selective binding of Ro15-4513 to these sites (34). The density of diazepam-insensitive sites is highest in the cerebellum. These unique cerebellar diazepam-insensitive sites on the $GABA_A$ receptor complex may be the first altered by ethanol, resulting in cerebellar ataxia. The subsequent effects of ethanol may then be expressed via the classical $GABA_A$-benzodiazepine-sensitive receptor sites (34).

Ro15-4513 reverses ethanol's effect on cerebellar Purkinje cell firing (23); the latter are especially sensitive to the actions of ethanol (4,25). Therefore, the antagonism by intracerebellar Ro15-4513 of ethanol-induced motor incoordination observed in the present investigation most likely resulted from its ability to reverse ethanol's effect on Purkinje cells, thereby enhancing the inhibitory effect of GABA and consequently closing the chloride ion channels gated by the $GABA_A$ receptor complex within the cerebellum. The selective inhibition of $GABA_A$-gated chloride ion channels occurred at the low dose range used in the present investigation and most likely independent of the intrinsic activity of Ro15-4513.

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