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RO15-4513 but not FG-7142 Reverses Anticonvulsant Effects of Ethanol Against Bicuculline- and Picrotoxin-Induced Convulsions in Rats

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KULKARNI, S. K. AND M. K. TICKU. *RO15-4513 but not FG-7142 reverses anticonvulsant effects of ethanol against bicuculline- and picrotoxin-induced convulsions in rats.* PHARMACOL BIOCHEM BEHAV **32**(1) 233–240, 1989.—The reversal of anticonvulsant effect of ethanol against chemoconvulsions by RO15-4513 was investigated in rats as this novel imidazobenzodiazepine (ethyl-8 azido-5, 6-dihydro-5-methyl-6-Oxo-4H-imidazo [1,5a] [1,4] benzodiazepine-3-carboxylate) is reported to antagonize the acute behavioral and biochemical effects of ethanol in animals. Reversal of ethanol effects on onset of myoclonic jerks, tonic extensor phase, mortality time and percent protection against mortality were compared with not only other anticonvulsant pentobarbital but also with another inverse agonist FG-7142. Pretreatment with RO15-4513 (4 mg/kg) reversed the protective effect of ethanol against bicuculline-induced tonic extensor phase and mortality (87%). This response was sensitive to reversal by RO15-1788 (10 mg/kg). However, onset of myoclonic jerks and duration of clonus were not significantly altered. It also reversed the effect against picrotoxin but the reversal against mortality was up to 50%. As compared to ethanol, RO15-4513 reversed partially the protective effect of pentobarbital against bicuculline- and picrotoxin-induced convulsions. FG-7142 failed to reverse the protective effect of ethanol and pentobarbital against bicuculline-induced tonic extensor phase although it reversed the effect against onset and mortality. It had no effect on the protective effect against picrotoxin-induced convulsions. Both RO15-4513 and FG-7142 possessed proconvulsant effects against bicuculline but not against picrotoxin. These observations suggest that RO15-4513 has a more preferential action against ethanol effects as compared to the other inverse agonist.

RO15-4513 FG-7142 Ethanol Chemoconvulsions RO15-1788 Rat

RECENT studies have indicated that the imidazobenzodiazepine RO15-4513 (ethyl-8 azido-5, 6-dihydro-5-methyl-6-Oxo-4H-imidazo [1,5a] [1,4] benzodiazepine-3-carboxylate) antagonizes the acute behavioral and biochemical effects of ethanol in animals (1, 11, 19, 28). RO15-4513 has also been reported to reverse the ability of ethanol to stimulate $GABA_A$-gated $^{36}Cl^-$ uptake in a benzodiazepine receptor antagonist sensitive manner in brain synaptoneurosomes and primary spinal neuronal cell cultures (26,28). RO15-4513 is also reported to possess proconvulsant properties in mice (12,18) and it reversed the increase in seizure threshold produced by diazepam, sodium pentobarbital or ethanol (14). The earlier reports from our laboratory have shown that ethanol possessed anticonvulsant property against different chemoconvulsants (22) and it was suggested that the anticonvulsant effect of ethanol may be related to its ability to infulence GABAergic transmission at the level of coupled chloride ion channels (20). In light of these observations, the present investigation was undertaken to study the selectivity of RO15-4513 to reverse the anticonvulsant property of ethanol, and compared to pentobarbital, a drug which also facilitates GABAergic transmission (21). Its effects were also compared with another β-carboline inverse agonist FG-7142 to know the relative specificity of RO15-4513 to antagonize the acute effects of ethanol in rats.

METHOD

Adult male Sprague-Dawley rats weighing 180–200 g were used. The animals were maintained at a constant room temperature (22±2°C) and under 12-hr light/dark cycle. They were allowed free access to food and water except during experimentation.

Chemoconvulsions

Bicuculline (1–8 mg/kg) and picrotoxin (1–10 mg/kg) were

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used for inducing chemoconvulsions in rats. Different phases of chemoconvulsions were observed and the duration of each phase was recorded with the help of a timer. Onset of myoclonic jerk, tonic flexion and extensor, clonus and mortality (both onset and % mortality) were recorded. Each animal was observed individually for the presence or absence of above phases of convulsions up to 2 hr and thereafter at 24 hr for mortality. The experiments were carried out between 10:00 and 16:00 hr. Bicuculline and picrotoxin produced severe tonic-clonic convulsions followed by 100% mortality at 8 and 10 mg/kg doses, respectively. At lower doses they were ineffective in producing 100% seizures. Therefore, these doses were used for interaction studies and the modification by RO15-4513 of the protective effects of ethanol and pentobarbital against onset of myoclonic jerks, tonic extensor phase and mortality (onset and % mortality) were compared.

Drug Treatment

Ethanol (2 g/kg as 20% w/v in saline) was administered 20 min before bicuculline or picrotoxin treatment. Pentobarbital was studied at 10, 15 and 20 mg/kg dose levels, and it was administered 30 min before the convulsant. RO15-4513 was studied at 1 and 4 mg/kg dose. It was given 5 or 15 min before ethanol or pentobarbital treatment. Since no significant difference in the action was observed when RO15-4513 was given 5 or 15 min prior to treatment, the latency period of 15 min was kept constant throughout the study. The benzodiazepine receptor antagonist RO15-1788 (10 mg/kg) was given 1 min before RO15-4513. The other inverse agonist FG-7142 was studied at 10 and 20 mg/kg dose and it was given 15 min before ethanol or pentobarbital.

Proconvulsant Studies

Although RO15-4513 did not exhibit any convulsions per se, it reduced the seizure threshold to chemoconvulsants. Therefore, experiments were designed to study the proconvulsant properties of both RO15-4513 and FG-7142. RO15-4513 was administered at 1 and 4 mg/kg doses and animals were challenged with different subconvulsive doses (1 and 4 mg/kg) of either bicuculline or picrotoxin. To another group even the convulsive dose of bicuculline was administered following RO15-4513 or FG-7142 treatment. Quick onset of myoclonic jerks and reduction in mortality time as compared to controls was considered as proconvulsant effect.

Statistics

The data are expressed as mean (sec or min) ±SEM. Each group consisted of a minimum of five observations. The results were analyzed by one-way analysis of variance with 95% confidence ($p<0.05$) as significance and also compared with Student's t-test.

Drugs

(+)Bicuculline, picrotoxin and pentobarbital sodium were purchased from Sigma Chemicals (St. Louis, MO). RO15-4513 was kindly provided to us by Professor W. Haefely (Hoffmann-La Roche, Basel) and RO15-1788 was a gift from Hoffmann-La Roche, Inc. (Nutley, NJ). Except pentobarbital, which was dissolved in distilled water, all other drugs were dissolved in dimethylsulphoxide and injected intraperitoneally (IP) in a constant volume of 1 ml/kg body weight. The doses selected were based on the earlier reports from this laboratory and refer to the salt or base as specified.

RESULTS

Anticonvulsant Effects of Ethanol Against Chemoconvulsions

Different doses of bicuculline (1–8 mg/kg) induced varying degrees of convulsions in rats, 8 mg/kg dose producing severe tonic-clonic convulsions with 100% mortality (Table 1). Bicuculline at 1 mg/kg dose did not induce any tonic-clonic convulsions whereas 4 mg/kg produced convulsions and mortality in 50% of animals. The onset (36±1.76 sec) and mortality (8±0.53 min) due to 8 mg/kg dose of bicuculline were quick (Table 1). Similarly, picrotoxin produced tonic-clonic convulsions at 10 mg/kg dose in 100% of animals (Table 2). However, the onset of convulsions (329±8 sec) and mortality (22±0.81 min) due to picrotoxin were delayed. At lower doses (1–4 mg/kg) picrotoxin did not produce tonic-clonic convulsions.

Pretreatment of animals with ethanol (2 g/kg) offered complete protection against tonic flexion and extensor phase of bicuculline convulsions. There was 33% protection against mortality and the onset of mortality was significantly delayed ($p<0.001$). However, there was no significant difference in the onset of myoclonic jerks due to bicuculline (Table 1). In case of picrotoxin convulsions, ethanol pretreatment not only delayed the onset of myoclonic jerks, but it completely abolished clonus, tonic extensor phase and offered 100% protection against mortality (Table 2). Only 3/7 animals showed mild myoclonic jerks and later recovered in 30 min.

Anticonvulsant Effect of Pentobarbital Against Chemoconvulsions

Doses of pentobarbital up to 10 mg/kg did not offer any protection against bicuculline-induced convulsions. The effect of 15 mg/kg dose of pentobarbital (40% protection) was comparable to ethanol (33% protection) against mortality, however, it did not significantly modify the tonic extensor phase of bicuculline-induced convulsions (Table 1). On the other hand, 20 mg/kg dose of pentobarbital offered full protection against bicuculline-induced convulsions. The onset of myoclonic jerks was significantly delayed and animals did not show clonic and tonic phases of the convulsions. Only one out of six rats died due to bicuculline convulsions (Table 1). Pentobarbital was more effective against picrotoxin-induced convulsions. It delayed the onset of myoclonic jerks and completely protected the animals against clonic, tonic extensor phase and mortality at 10 and 20 mg/kg dose (Table 2).

Effect of RO15-4513 Pretreatment on the Anticonvulsant Action of Ethanol

Pretreatment of animals with RO15-4513 15 min prior to ethanol significantly reversed the protective effect of ethanol against bicuculline-induced tonic extensor phase and mortality. There was 87% (7/8) mortality due to bicuculline in animals treated with RO15-4513 and ethanol as compared to ethanol treatment (66%). However, onset of myoclonic jerks and the duration of clonic convulsions were not significantly altered (Table 1). This reversal effect of RO15-4513 on tonic extensor phase was sensitive to blockade by RO15-1788 (10

TABLE 1

MODIFICATION BY RO15-4513 OF THE PROTECTIVE EFFECT OF ETHANOL AND PENTOBARBITAL AGAINST BICUCULLINE-INDUCED CONVULSIONS IN RATS

Treatment	Dose (mg/kg) IP	Duration of: Onset of Myclonic Jerks (sec ± SEM)	Clonus (sec ± SEM)	Tonic Flexion and Extensor (sec ± SEM)	Mortality Time (min ± SEM)	Nature and Severity Convulsions
Bicuculline	8	36 ± 1.76	435 ± 25	18 ± 0.92	8 ± 0.53	100% (17/17) mortality.
Ethanol + bicuculline	2 g 8	32 ± 3.81	988 ± 117†	0.00	23 ± 4.20†	No tonic extensor phase 33.33% protection from mortality (8/12).
Pentobarbital + bicuculline	15 8	51 ± 4.67	1135 ± 4.24	16 ± 2.72	26 ± 6.67	Mild myoclonic jerks and 40% protection from mortality (3/5).
Pentobarbital + bicuculline	20 8	59 ± 10.00	0.00	0.00	0.00	Mild myoclonic jerks and 83.33% protection (1/6) from mortality.
RO15-4513 + ethanol + bicuculline	1 2 g 8	34 ± 12.00	860 ± 172	6 ± 1.73†	15 ± 2.64	Reversal of ethanol-induced protection against tonic extensor phase. 57% protection from mortality (3/7).
*RO15-4513 + ethanol + bicuculline	4 2 g 8	29 ± 2.47	1019 ± 188	8 ± 0.77†	15 ± 2.88	Reversal of ethanol-induced protection against tonic extensors. 13% protection from mortality (7/8).
RO15-4513 + pentobarbital + bicuculline	4 15 8	42 ± 7.30	1150 ± 427	12 ± 10.60	20 ± 7.07	Myoclonic jerks, intermittent clonic convulsions and body shakes. 66.66% protection from mortality (2/6).
RO15-4513 + pentobarbital + bicuculline	4 20 8	32 ± 2.42	0.00	0.00	17 ± 7.88	Myoclonic jerks, intermittent popcorn-like convulsions and body shakes. 42.86% protection from mortality (3/7).

*Sensitive to reversal by RO15-1788 (10 mg/kg) given 1 min before RO15-4513. These animals showed no tonic extensors (0.00) and 40% mortality at 21 ± 1.66 min (n=5), an effect not significantly different from effect per se of ethanol.

†$p<0.001$ as compared to effect per se of convulsant or its combination with ethanol.

TABLE 2

MODIFICATION BY RO15-4513 OF THE PROTECTIVE EFFECTS OF ETHANOL AND PENTOBARBITAL AGAINST PICROTOXIN-INDUCED CONVULSIONS IN RATS

Treatment	Dose (mg/kg) IP	Onset of Myclonic Jerks (sec ± SEM)	Duration of Tonic Flexion and Extensor (sec ± SEM)	Mortality Time (min ± SEM)	Mortality /N	Nature and Severity of Convulsions
Picrotoxin	10	329 ± 8	16 ± 0.82	22.23 ± 0.81	17/17	Animals in hunch-like posture after the onset, show tonic flexion and extensor followed by brief clonus before death.
Ethanol + picrotoxin	2 g 10	525 ± 38*	0.00	0.00	0/7	3/7 showed abrupt myoclonic jerks and recovered in 30 min. 100% protection.
Pentobarbital + picrotoxin	10 10	395 ± 13*	0.00	0.00	0/8	Body tremors, mild myoclonic jerks in 5/8 animals. All recovered. 100% protection.
Pentobarbital + picrotoxin	20 10	518 ± 10*	0.00	0.00	0/6	Body tremors, mild myoclonic jerks, hunch-like posture. All recovered. 100% protection.
RO15-4513 + ethanol + picrotoxin	4 2 g 10	356 ± 37	4 ± 2.02*	67 ± 12.74*	3/6	Hunch-like posture, myoclonic jerks, tonic convulsions, 50% protection from mortality.
RO15-4513 + pentobarbital + picrotoxin	4 10 20	333 ± 25	15 ± 1.84*	60 ± 11.12*	4/6	Body tremors, hunch-like posture, tonic convulsions, 33% protection from mortality.
RO15-4513 + pentobarbital + picrotoxin	4 20 10	437 ± 33	0.00	0.00	0/7	Body tremors, mild jerks of the head, hunch-like posture. All recovered. 100% protection.

*$p < 0.001$ as compared to effect per se of convulsant or its combination with ethanol or pentobarbital.

TABLE 3

MODIFICATION BY FG-7142 OF THE PROTECTIVE EFFECT OF ETHANOL AGAINST BICUCULLINE- AND PICROTOXIN-INDUCED CONVULSIONS IN RATS

Treatment	Dose (mg/kg) IP	Duration of: Onset (sec ± SEM)	Clonus (sec ± SEM)	Tonic Flexion and Extensor (sec ± SEM)	Mortality Time (min ± SEM)	Nature and Severity of Convulsions
FG-7142 + ethanol + bicuculline	10 2 g 8	25 ± 4.45	1280 ± 304	0.00	22 ± 5.00	Tonic-clonic convulsions. No tonic flexion and extensor phase. 100% (6/6) mortality.
FG-7142 + ethanol + bicuculline	20 2 g 8	19 ± 2.46* (32 ± 3.81)	964 ± 181 (982 ± 117)	0.00 (0.00)	17 ± 3.00 (23 ± 4.20)	Severe tonic-clonic convulsions. No tonic flexion and extensor phase. 100% (7/7) mortality.
FG-7142 + ethanol + picrotoxin	10 2 g 10	624 ± 101	0.00	0.00	37 ± 8.13	Attain hunch-like posture, show intermittent popcorn-like convulsions. No clonus or tonic extensor phase. 57% mortality (4/7).
FG-7142 + ethanol + picrotoxin	20 2 g 10	488 ± 25 (525 ± 38)	0.00 (0.00)	0.00 (0.00)	20	Attain hunch-like catatonic posture and show intermittent tonic convulsions. 20% mortality (1/5).

Numbers in the parentheses indicate protective effect per se of ethanol against respective chemoconvulsant.
*$p<0.01$ as compared to the corresponding protective effect of ethanol.

mg/kg), a benzodiazepine receptor antagonist. In this group, like ethanol-treated rats, RO15-1788-pretreated animals did not exhibit any tonic extensor phase of bicuculline convulsions (n=5) and the mortality rate was 40%.

RO15-4513 also reversed the protective effect of ethanol against picrotoxin-induced convulsions. However, the reversal was partial, as there was only 50% reversal in the mortality rate compared to ethanol treatment (Table 2).

Effect of RO15-4513 Pretreatment on the Anticonvulsant Action of Pentobarbital

Pretreatment with RO15-4513 failed to modify the effect of 10 mg/kg dose of pentobarbital against bicuculline-induced convulsions. However, it partially reversed the protective effect of higher doses of pentobarbital. In animals treated with 15 mg/kg dose of pentobarbital the onset of mortality was quicker (20±7.07 vs. 26±6.67 sec), but the other parameters were not significantly modified by RO15-4513. The animals treated with 20 mg/kg dose of pentobarbital showed myoclonic jerks and popcorn-like convulsions and there was 57% mortality in this group (Table 1). Animals failed to exhibit clonus and tonic extensor phase. In case of picrotoxin convulsions, RO15-4513 significantly reversed the protective effect of pentobarbital (10 mg/kg) on onset of myoclonic jerks, tonic extensor phase and partially, mortality rate (66%). However, the protective effect of 20 mg/kg dose of pentobarbital was not reversed significantly (Table 2).

Effect of FG-7142 Pretreatment on the Anticonvulsant Action of Ethanol

Unlike RO15-4513, FG-7142 (10 and 20 mg/kg) pretreatment failed to reverse the protective effect of ethanol against tonic extensor phase although animals showed quick onset of myoclonic jerks (Table 3). In case of picrotoxin convulsions, on pretreatment with FG-7142, animals attained characteristic hunch-like catatonic posture and exhibited intermittent popcorn-like convulsions. Clonic and tonic extensor phase were absent. The protective effect of ethanol against picrotoxin-induced mortality was not significantly altered, at higher dose (20 mg/kg) of FG-7142 the mortality rate was only 20% (Table 3).

Similarly, FG-7142 failed to reverse the protective effect of pentobarbital against both bicuculline and picrotoxin. The protective effect of pentobarbital against mortality was reversed up to 33% in case of bicuculline convulsions and there was no reversal in case of picrotoxin convulsions (Table 4). Animals pretreated with FG-7142 attained hunch-like catatonic posture and stood on the hind limbs and occasionally showed clonic convulsions of the fore limbs. The tonic extensor phase was absent in these animals.

Proconvulsant Property of RO15-4513 and FG-7142

RO15-4513 did not exhibit any response per se at 1 and 4 mg/kg doses although immediately after the intraperitoneal

TABLE 4

MODIFICATION BY FG-7142 OF THE PROTECTIVE EFFECT OF PENTOBARBITAL AGAINST BICUCULLINE- AND PICROTOXIN-INDUCED CONVULSIONS IN RATS

Treatment	Dose (mg/kg) IP	Onset (sec ± SEM)	Mortality Time (min ± SEM)	Nature and Events of Convulsions
FG-7142 + pentobarbital + bicuculline	20 20 8	35 ± 2.75 NS (59 ± 10)	36 ± 8 (0.00)	Attain hunch-like posture, show intermittent popcorn-like convulsions, standing on hindlimbs and show forelimb clonus. NO tonic flexion and extensor phase. 33% mortality (2/6).
FG-7142 + pentobarbital + picrotoxin	20 10 10	342 ± 29 NS (395 ± 13)	0.00 (0.00)	Attain hunch-like catatonic posture and show mild intermittent tonic convulsions. 100% recovery (0/6) from the catatonic posture in 1 hr.

NS: Not significant as compared to the corresponding protective effect of pentobarbital against respective convulsant. The values are given in the parentheses.

administration of the drug, animals showed attack and aggressive behavior. This effect was short lived. RO15-4513 (1 mg/kg), however, potentiated the effects of bicuculline at subconvulsive doses (1 and 4 mg/kg). The onset of convulsions and mortality due to a convulsive dose (8 mg/kg) of bicuculline was further potentiated by pretreatment with 1 or 4 mg/kg dose of RO15-4513. Animals showed immediate onset of convulsions and mortality (Fig. 1). However, RO15-4513 failed to exhibit any proconvulsant effect against picrotoxin. It neither potentiated the response due to a subconvulsive dose (4 mg/kg) of picrotoxin nor did it quicken the onset of convulsions due to a convulsive dose of picrotoxin.

FG-7142 also exhibited proconvulsant effect against bicuculline. The effect was not, however, as pronounced as compared to RO15-4513. There was a delay in the onset of mortality. It also did not possess any proconvulsant property against picrotoxin (Table 5).

DISCUSSION

Recent experimental evidence suggests that neuropharmacological and behavioral actions of ethanol are mediated through the central inhibitory synaptic transmitter, GABA (3, 8, 9). Ethanol potentiated the inhibition of cortical neurons by GABA without affecting the inhibition caused by other putative neurotransmitter systems (17). Since ethanol shared several of its behavioral effects with that of barbiturates and benzodiazepines, it has been speculated that GABA/benzodiazepine receptor-coupled chloride channels could be the site of action of ethanol in the brain (4, 6, 10, 15, 23–25). More direct in vitro evidence using synaptoneurosomes indicated that at pharmacologically relevent concentrations ethanol potentiated muscimol stimulation of $^{36}Cl^-$ uptake (7,29) and these effects were sensitive to blockade by both

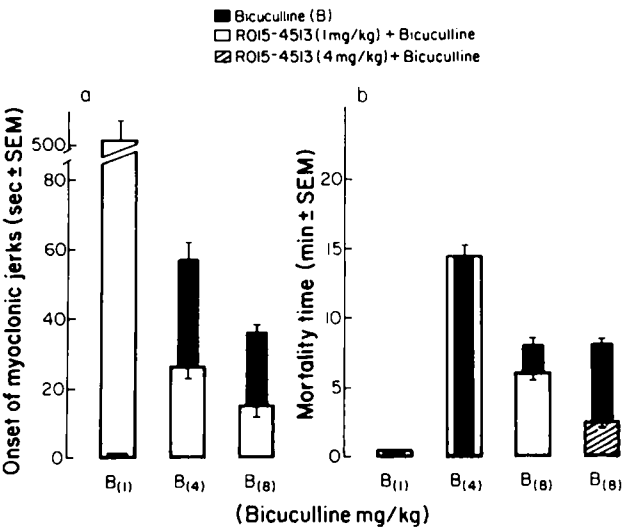

FIG. 1. Proconvulsant effect of RO15-4513 (1 and 4 mg/kg) as studied by the quick onset (a) of myoclonic jerks and mortality (b) time as compared to controls in rats. The drug was given 15 min prior to a challenge dose of bicuculline (B_1, B_4 or B_8 mg/kg).

bicuculline and picrotoxin (29). In the intact cultured spinal cord neurons, ethanol (5–100 mM) potentiated GABA-mediated $^{36}Cl^-$ influx and at higher concentrations (≥50 mM) it directly activated Cl^- channels in these neurons (26). Both the potentiating and direct effects were blocked by bicuculline and picrotoxin suggesting the involvement of the same

TABLE 5

ONSET, MORTALITY TIME AND % MORTALITY DUE TO BICUCULLINE OR PICROTOXIN AND AS MODIFIED BY PRETREATMENT WITH FG-7142 IN RATS

Treatment	Dose (mg/kg) IP	Onset of: Myoclonic Jerks (sec ± SEM)	Mortality (min ± SEM)	% Mortality	Response
FG-7142 + bicuculline	10 1	63 ± 18.77 (0.00)	0.00 (0.00)	0.00 (0.00)	Recovery
FG-7142 + bicuculline	10 4	27 ± 2.01* (58 ± 8.33)	15 ± 1.88 (14 ± 1.41)	100 (50)	Procon-vulsant
FG-7142 + bicuculline	10 8	28 ± 2.03* (37 ± 2.82)	13 ± 2.69 (8 ± 0.70)	100 (100)	Procon-vulsant
FG-7142 + picrotoxin	10 4	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	No pro-convul-sant
FG-7142 + picrotoxin	10 10	290 ± 32.01 (333 ± 9.24)	29 ± 2.76 (23 ± 0.97)	100 (100)	No pro-convul-sant

*$p<0.001$ as compared to value per se of the convulsant as indicated in the parentheses.

GABA-gated Cl channels in the action of ethanol. Thus, GABA/benzodiazepine receptor mediated Cl^- transport mechanism, possibly a chloride channel modulatory function, would explain some of the pharmacological actions of ethanol.

RO15-4513, an azido analogue of the classical benzodiazepine receptor antagonist RO15-1788 with an ability to bind to benzodiazepine receptors, is reported to reverse several of the acute behavioral and toxic effects of ethanol in mice and rats (5, 11, 29). It also antagonized ethanol-stimulated chloride uptake in isolated brain vesicles in a benzodiazepine receptor antagonist sensitive manner, an action not shared by other inverse agonists (28). However, in C57BL/6J mouse spinal cord cultured neurons, RO15-4513 and FG-7142 blocked the GABA enhancing and direct effect of ethanol on $^{36}Cl^-$ influx. Both exhibited inverse agonistic activity in the $^{36}Cl^-$ influx assay in cultured neurons (26). These observations, while supporting the view of $GABA_A$ receptor mediated action of ethanol and its reversal by RO15-4513, question the selectivity of RO15-4513 as a specific antagonist of ethanol action. Several recent behavioral observations supported such a view (2, 11, 16).

In the present study in doses (1–4 mg/kg) investigated, though devoid of any action per se, RO15-4513 significantly reversed the protective effect of ethanol against tonic extensor phase of bicuculline-induced convulsions. The protective effect against mortality was also significantly reversed. Unlike bicuculline convulsions, RO15-4513 reversed the protective effect of ethanol against picrotoxin-induced mortality up to 50% only. The onset of mortality was also significantly different in this group. The fact that RO15-4513 showed preferential reversal of ethanol action for bicuculline as compared to picrotoxin and that this effect was sensitive to blockade by RO15-1788, a benzodiazepine receptor antagonist, suggested that RO15-4513 acted at the benzodiazepine receptor site. Although the proximity of ethanol modulatory site on GABA/benzodiazepine complex (bicuculline vs. picrotoxin site) is debatable, ethanol perhaps more selectively modulates the coupling mechanism of Cl channels that are sensitive to GABA/benzodiazepine activation as compared to picrotoxin site. This would also be consistent with the observations that ethanol potentiates GABA-induced $^{36}Cl^-$ fluxes at much lower concentrations [5–100 mM; (26,28)] than those required to inhibit the binding to picrotoxin site [IC_{50}=500 mM; (10,23)]. Further support is also provided by the fact that ethanol decreases the K_m value of GABA in inducing ^{36}Cl influx in cultured neurons (Mehta and Ticku, unpublished observations). Moreover, the present study and other similar reports (14) have shown that RO15-4513 possessed proconvulsant property against bicuculline and not against picrotoxin. Similarly, it partially reversed the protective effect of smaller doses of pentobarbital but failed to reverse the protective effect of 20 mg/kg dose of the pentobarbital against picrotoxin-induced convulsions. The interaction of pentobarbital with the GABA receptors is more pronounced as compared to ethanol, and barbiturates are also known to inhibit excitatory transmission. Thus, the activation of the receptor ionophore complex by inverse agonist may not be sufficient enough to antagonize the effects of barbiturates.

It is rather surprising that RO15-4513 is reported to reduce the seizure threshold to pentylenetetrazole while having no proconvulsant action against picrotoxin both in rats (present study) and in mice (14). In addition to binding to central benzodiazepine binding site, [^{3}H]RO15-4513 has been reported to bind to an additional protein (P57) in cerebellum (27). However, the in vitro studies do not support the direct involvement of this unique site, since ethanol (up to 100 mM) did not inhibit the binding of [^{3}H]RO15-4513 in different rat brain areas (Mhatre and Ticku, unpublished observations). The present study also revealed that the reversal of anticonvulsant effect of ethanol by RO15-4513 may not solely be

related to the inverse agonistic property of the drug as speculated by other investigators (18), since the inverse agonist FG-7142 failed to reverse the protective effect of ethanol against tonic extensor phase. Further, FG-7142 failed to reverse the protective effect of ethanol and pentobarbital against picrotoxin-induced convulsions. In this respect, RO15-4513 differs from other inverse agonists. The present study, like the other earlier ones (1, 2, 13), cautions about the inverse agonistic property of the drug for the intended alcohol antagonistic property but suggest the site of action of RO15-4513 could be an ideal target for drugs possessing alcohol antagonistic property.

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