Pharmacology Biochemistry & Behavior, Vol. 30, pp. 501–510. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

REVIEW

Molecular Interactions of Ethanol With GABAergic System and Potential of RO15-4513 as an Ethanol Antagonist

M. K. TICKU AND S. K. KULKARNI

Department of Pharmacology, University of Texas Health Science Center 7703 Floyd Curl Drive, San Antonio, TX 78284-7764

Received 3 March 1988

TICKU, M. K. AND S. K. KULKARNI. *Molecular interactions of ethanol with GABAergic system and potential of RO15-4513 as an ethanol antagonist.* PHARMACOL BIOCHEM BEHAV 30(2) 501–510, 1988.—The behavioral and biochemical effects of ethanol in man and animals have been investigated for a long time. A role of catecholamines in the central stimulatory action and during withdrawal has been envisaged, but more recent observations have revealed the involvement of inhibitory synaptic transmitter, GABA, in the actions of ethanol. Ethanol-induced motor incoordination, hypnosedation, antianxiety, and anticonvulsant actions are reported to be GABA-mediated. Involvement of the GABA system has been implicated in ethanol withdrawal-induced seizures in animals. More direct evidences using Cl^- influx studies in synaptoneurosomes and spinal neuronal culture studies confirm such a mode of action of ethanol, probably influencing the chloride channel modulation at the GABA-benzodiazepine receptor ionophore complex. RO15-4513 (ethyl-8-azido-5,6-dihydro-5-methyl-6-Oxo-4H-imidazo [1,5-α], [1,4] benzodiazepine-3-carboxylate), a novel imidazobenzodiazepine, an analogue of the classical benzodiazepine antagonist is reported to possess alcohol antagonistic properties. RO15-4513 reverses both the behavioral and biochemical effects of ethanol, including the action of GABA-induced Cl^- fluxes. But its potential clinical application may be restricted due to its inverse agonistic property. The present review focuses on the GABA-linked behavioral and biochemical actions of ethanol and discusses the potential of RO15-4513 as an alcohol antagonist.

Ethanol RO15-4513 GABA-benzodiazepine receptor complex Chloride channels

ETHANOL is perhaps the oldest and most widely consumed and abused psychotropic drug known to mankind. The pharmalogical profile of ethanol is very similar to that of barbiturates and benzodiazepines. All the three classes of drugs, ethanol, barbiturates, and benzodiazepines (BZ) share some pharmacological properties in common such as antianxiety, anticonvulsant and muscle relaxant actions. Higher doses of ethanol cause behavioral depression and even narcosis or anaesthesia, an action again shared by barbiturates and benzodiazepines [35]. It has also been demonstrated that tolerance develops to ethanol, barbiturates, and benzodiazepines in humans and animals [6,8]. A cross tolerance between these drugs, namely between ethanol and barbiturates, and ethanol and benzodiazepines is also documented in the literature [8,35]. Furthermore, anxiety and withdrawal seizures are involved in the etiology of alcoholism [88], and benzodiazepines and barbiturates are indicated in the management of alcohol withdrawal syndrome [37]. These observations suggest that a common modulatory pathway may be involved in the central action of these three categories of drugs. Since benzodiazepines are now known to act through a γ-aminobutyric acid (GABAergic) mechanism [91] and barbiturates have a binding site in the GABA-BZ receptor complex [79,121], most of the recent investigations are focused in studying the effect of ethanol on GABAergic pathways. In this article an attempt has been made to review some of the behavioral and biochemical effects of ethanol that are supposed to be linked to GABAergic transmission. An attempt is also being made to review the literature on RO15-4513 (ethyl-8-azido-5,6-dihyrdo-5-methyl-6-Oxo-4H-imidazo [1,5α], [1,4] benzodiazepine-3-carboxylate), a novel imidazobenzodiazepine known to be a potential alcohol antagonist. This review may facilitate further research on these areas.

ETHANOL AND CENTRAL CATECHOLAMINES

Although the ability of ethanol to alter central nervous system (CNS) function has been speculated long back, the exact cellular mechanism of action, tolerance, and withdrawal due to ethanol are far from clear. Until recently, ethanol was considered to be a general CNS depressant without affecting any specific neurotransmitter mechanisms. In the early 1970's, however, attempts were made to explain some of the behavioral and biochemical changes due to ethanol on the basis of changes in catecholamine metabolism in the CNS, a view which still receives support from certain quarters, as adrenergic drugs are employed in the management of alcohol withdrawal syndrome [11]. Catecholamine involvement, particularly in the stimulatory action of ethanol and some withdrawal behavioral effects, was reported initially by Engel [27]. Pretreatment of animals with α-methyl-p-tyrosine (AMPT), an inhibitor of catecholamine biosynthesis, blocked the locomotor stimulatory action of low doses of ethanol [15], and this effect of AMPT was reversed by administration of catecholamine precursor, l-dopa [26]. Other dopamine agonists also exhibited similar action on ethanol-induced locomotor effect [2,17]. Ethanol also decreased dopamine function [64] and chronic alcohol consumption has been shown to increase dopamine receptor binding [101]. Several recent studies, both behavioral and biochemical, have shown that acute administration of ethanol enhances the metabolism of central catecholamines [14, 16, 28, 110, 112]. More interestingly, while catecholamine receptor antagonists blocked the stimulatory effects of ethanol [67], a lipid soluble alpha$_1$ receptor agonist antagonized the hypnotic effect of ethanol in mice [83]. The fact that norepinephrine turnover is increased in psychiatric symptoms during alcohol abstinence in man [11] and that clonidine, a central alpha$_2$ adrenoceptor agonist which down regulates catecholamine release, is effective in counteracting alcohol abstinence syndrome in man and animals substantiates further the role of central catecholamines in the action of ethanol [7, 62, 63, 92, 107, 128]. However, a catecholaminergic mechanism does not explain the CNS depressant effects of ethanol which may involve the inhibitory neurotransmitter, GABA.

ETHANOL AND GABA NEUROTRANSMISSION

Recent neurophysiological and several behavioral observations have demonstrated the involvement of inhibitory synaptic transmission in the mode of action of ethanol and an ethanol-evoked adaptive reduction in CNS inhibition as a result of diminished GABA neurotransmission in the development of physical dependence and withdrawal syndrome [52, 89, 119]. The presynaptic inhibitory effects of ethanol on GABAergic transmission were demonstrated long ago in cat spinal cord [5], frog spinal cord [22] and more recently, in feline cerebral cortex [89] and in the substantia nigra [84]. Ethanol potentiated the inhibition of cortical neurons by GABA without affecting the inhibition caused by glycine, serotonin, or dopamine. It also potentiated the inhibition of single cortical neurons by electrical stimulation of the surface of the cerebral cortex, an action believed to be mediated by endogenous GABA [24,61]. These studies speculated that ethanol most likely acted on the postsynaptic membrane, reminiscent of the mechanism of GABA potentiation by benzodiazpeine, probably modulating a site on or near GABA binding site.

TABLE 1

ANTICONVULSANT EFFECT OF ETHANOL WHEN STUDIED AGAINST DIFFERENT CHEMOCONVULSANTS IN RATS*

Treatment (mg/kg, IP)	Onset (min ±SD)	Total Seizure Duration (min ±SD)	Mortality (%)
Bicuculline (4)	1.8 ±0.8	6.4 ±1.4	100
Picrotoxin (10)	5.0 ±1.8	15.8 ±3	100
Strychnine (4)	2.5 ±0.5	5.2 ±0.8	100
Ethanol (2 g/kg) + Bicuculline	2.3 ±0.6	—	0
Ethanol (2 g/kg) + Picrotoxin	16 ±1.0‡	17.0 ±3	70
Ethanol (2 g/kg) + Strychnine	2.3 ±0.5	48 ±27.0†	75

*As abstracted from Rastogi and Ticku [98].

†$p < 0.01$ and ‡$p < 0.001$ as compared to corresponding per se effect of convulsant, respectively.

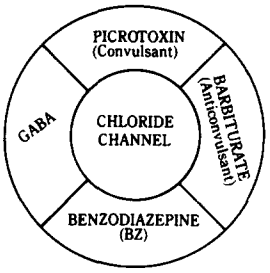
Ethanol-Induced Motor Impairment and GABAergic Transmission

Impairment of motor coordination (ataxia) is a well-known effect of ethanol on CNS [35,126]. GABA is known to be involved in the central control of motor coordination [125] and a functional relationship exists between GABA neurotransmission and motor incoordination during ethanol intoxication. Bicuculline, a GABA receptor antagonist, diminished ethanol-induced impairment of performance on a tilting plane task in rats [45]. Similarly, inhibition of GABA transaminase with aminooxyacetic acid which increased whole-brain GABA concentrations, enhanced ethanol-induced impairment [45,66]. However, Frye and Breese [32] failed to observe any simple relationship between the degree of motor impairment caused by ethanol and changes in GABA concentrations in different brain areas, although direct manipulation of GABA receptor activity modulated motor incoordination caused by ethanol.

Withdrawal-Induced Convulsions and Anticonvulsant Action of Ethanol and GABAergic Transmission

Another area of intensive research interest is the involvement of GABAergic system in ethanol withdrawal-induced audiogenic seizures in laboratory animals [1, 20, 33, 34, 38, 44]. Clinical observations supported this view as seizure-susceptible alcoholics showed reduced GABA concentrations in their CSF during acute ethanol withdrawal [36, 42, 60, 114]. Interestingly enough, all the ethanol withdrawal syndromes are not suppressed by activation of GABA receptors, indicating involvement of additional receptor system(s). Convulsions on handling and tremors in rats and monkeys were not susceptible to GABA receptor activation [33, 38, 113]. On the other hand, audiogenically-induced seizures were suppressed by GABAergic drugs [34]. Susceptibility to seizures induced in rodents by electroshock, kindling, or CNS excitants were markedly reduced by discrete microinjections of GABAmimetic drugs into substantia nigra [51, 52, 76, 77]. Intranigral administration of muscimol, a GABA$_A$ agonist, produced a dose-related suppression of audiogenic motor convulsions which accompanied with-

BZ/GABA RECEPTOR IONOPHORE COMPLEX



SITES OF DRUG ACTION

GABA RECEPTORS	BZ RECEPTORS	PICROTOXIN SITE	BARBITURATE SITE	COUPLING MODULATOR(S)
GABA	BZ Agonists	Picrotoxin	Barbiturates	Ethanol
Muscimol	BZ Antagonists	TBPS	(+)Etomidate	
Bicuculline	R015-4513 (?)	PTZ	Pyrazolopyridines	
R-5135 (?)	β-Carbolines (?)	R05-3663	Cyclopyrazolones	

FIG. 1. Different sites of drug action at the GABA-benzodiazepine receptor ionophore complex (modified from [121]). A possible mode of action of ethanol involving chloride coupling (modulatory site) has been envisaged.

drawal from chronic ethanol administration indicating the importance of GABA receptive neurons in the vicinity of the substantia nigra in the mediation of withdrawal seizures [39]. Electrophysiological and ablation studies have also implicated substantia nigra in the propagation of seizure activity [40,48]. Similarly, microinjections of GABAmimetics or lesions of the inferior colliculus and medial septal nucleus reduced susceptibility to audiogenic seizures in rats with a genetic predisposition for epileptic responses to loud noises [25,57]. More recent studies indicated that the inferior colliculus is important in GABAmimetic suppression of audiogenic seizures and that reduced GABAergic activity in this nucleus may be responsible for the increased susceptibility to audiogenic seizures in rats during ethanol withdrawal [34].

In an attempt to compare the anticonvulsant profile of drugs which facilitated GABAergic transmission, Rastogi and Ticku [98] studied the anticonvulsant profile of ethanol against various chemoconvulsants (Table 1). Furthermore, ethanol in combination with pentobarbital or diazepam was able to suppress chemoconvulsions due to bicuculline, picrotoxin, or strychnine [98]. Similarly, subeffective doses of pentobarbital and diazepam, together or when combined with a single ineffective dose of ethanol, offered protection against maximal electroshock seizures in rats [97]. In another study, Liljequist and Engel [68] reported that ethanol antagonized the convulsions produced by pentylenetetrazole, strychnine, bicuculline, and picrotoxin in a dose-dependent manner, ethanol being more potent in suppressing the convulsions produced by pentylenetetrazole and the glycine receptor antagonist strychnine. The ability of ethanol to antagonize strychnine-induced seizures may not be suprising in view of strychnine's reported GABA antagonistic activity [103], and close homology between GABA and glycine gated chloride channels [43]. These studies taken together suggested that individual withdrawal convulsive signs due to ethanol are unique and result from distinct types of CNS adaptive response to ethanol, possibly within the specialized regions of the brain. The anticonvulsant property of smaller doses of ethanol in combination with ineffective doses of other facilitators of GABAergic transmission provided protection against seizures and mortality induced by chemoconvulsants which act via GABA system [98]. These studies support the notion that drugs acting at different sites on the oligomeric GABA receptor complex can potentiate each other's effect in vivo (Fig. 1, [98]).

Ethanol-Induced Narcosis and GABAergic Transmission

Behavioral studies involving ethanol, GABAmimetics, and GABA antagonists indicated that GABAmimetics lengthened ethanol narcosis while picrotoxin shortened ethanol narcosis in mice [80]. GABA antagonists, bicuculline and picrotoxin, shortened motor incoordinating effects of ethanol and its potentiation by baclofen and aminooxyacetic acid [80]. The cage convulsant, isopropylbicyclophosphate, which binds to the picrotoxin site of the GABA receptor complex, significantly reduced the duration of loss of righting reflex induced by ethanol [82]. Bicuculline, the GABA antagonist, reduced the duration of ethanol-induced sleep in a dose-dependent manner, the highest dose (8 mg/kg) of bicuculline completely reversing ethanol narcosis [68]. Both bicuculline and picrotoxin further enhanced the hypothermic response due to ethanol [68]. Martz and co-workers [80] further observed genetic differences in ethanol response and related it to the differences in GABA sensitivity. In another study, pharmacologically-relevant concentrations of ethanol (15–50 mM) potentiated muscimol stimulation of ^{36}Cl uptake in cerebellum of long sleep line mice, but had no effect in cerebellum of short sleep lines [3]. However, it failed to alter stimulated chloride flux of hippocampal microsacs from either line. The genetic differences in ethanol hypnosis were related to differences in the sensitivity of GABA operated chloride channels to ethanol, since alcohol elimination rates

TABLE 2

EFFECT OF ACUTE AND CHRONIC TREATMENT OF ETHANOL ON GABA BENZODIAZEPINE RECEPTOR-IONOPHORE COMPLEX IN THE BRAIN TISSUES OF RATS AND MICE

Ethanol Treatment	Brain Areas	GABA-Binding: Low Affinity Site	GABA-Binding: High Affinity Site	Reference
I. Acute				
4 g/kg, IP; rats and mice	whole brain	↑	—	[116,117]
4 g/kg, IP; rat	cerebellum	↑	↑	[101]
	striatum, cerebral cortex substantia nigra	—	—	[101]
4 g/kg, PO; rat		—	—	[124]
Chronic				
10% in drinking fluid for 14 days; mouse	whole brain	↓		[117]
7% in drinking water for 21 days; rats	whole brain	—	—	[116]
During withdrawal	cerebral	↓	—	[116]
7% for 7 days, PO; rats	cortex	↓	↓	[123,124]
6% in drinking water for 21 days; rats	striatum	↑	↑	[101]
II. [^{3}H]-Benzodiazepine		Benzodiazepine-Binding		
in vitro: membranes		no effect		[41]
soluble		increases the affinity		[23]
Chronic		no effect		[55,99]
Withdrawal		no effect		[99]
III. [^{35}S]TBPS		TBPS Binding		
in vitro		inhibition (↓ B_{max})		[115]
chronic		no effect		[99,115]
withdrawal		no effect		[99,115]

were virtually identical [19,75]. The ED_{50} values of the GABA agonists in the bar holding test were approximately twice as large for the long sleep lines as for the short sleep lines. However, whole-brain GABA levels and GABA uptake kinetics did not differ in the lines [18,51].

Ethanol and the GABA-Benzodiazepine Receptor-Ionophore Complex

While the above cited behavioral and electrophysiological studies suggest that ethanol potentiated GABAergic neurotransmission, there are conflicting evidences supporting a direct action of ethanol at the GABA receptor. The GABA/benzodiazepine/barbiturate receptor complex is an oligomeric protein consisting of several subunits with multiple allosteric binding sites that are associated with a Cl^- channel [91,104] (Fig. 1). Benzodiazepines and barbiturates bind to this complex and indirectly regulate GABA receptor-mediated Cl^- conductance resulting in membrane hyperpolarization [105]. Since ethanol shared several of its behavioral effects with that of barbiturates and benzodiazepines, it was speculated that the GABA/benzodiazepine receptor-coupled chloride channel could be the site of action of ethanol in brain [21, 52, 69, 118]. Greenberg and co-workers [41] reported that the addition of ethanol to brain membranes in vitro had no effect either on [^{3}H]diazepam binding to the BZ receptors or [^{3}H]muscimol binding to the GABA receptor. However, ethanol has been reported to increase [^{3}H]diazepam binding to detergent solubilized benzodiazepine receptors [23] and to decrease the binding of t-butyl-bicyclophosphoro[^{35}S]thionate (TBPS) to a site closely associated with the Cl^- channel in both rat and mouse brain membranes [70, 95, 106]. Since ethanol inhibition of TBPS binding in vitro occurs at lethal concentrations, it is unlikely that this interaction is responsible for the pharmacological effects of ethanol, however, it is likely that this effect may be

involved in ethanol intoxication. Acute ethanol administration produced an increase in the binding capacity of the low affinity GABA receptor sites in both C57 and DBA mice [117]. The acute effect was also observed in rat brain membranes [116]. In contrast, other workers reported a decrease in specific [^{3}H]GABA binding in cerebellum with no change in other brain areas after acute ethanol treatment [101].

The effect of chronic administration of ethanol on GABA receptor sensitivity varied depending upon the method of ethanol administration and animal species. Chronic ethanol administration (10% v/v) for 14 days has been reported to produce a decrease in B_{max} of the low affinity GABA receptor sites in C57 mice [117]. Similarly, decreases in the affinity of low affinity GABA receptor site was reported following chronic treatment of mice with a higher concentration (30% v/v) of ethanol [122]. However, rats chronically treated with ethanol (7%) in a lipid diet for 21 days did not exhibit any changes in the binding constants of the high or low affinity GABA receptor sites as compared to pair-fed controls, suggesting a possible tolerance to the continuous presence of ethanol at the GABA synapse [116]. During withdrawal from ethanol, the low affinity GABA binding site in the whole rat brain membranes was significantly lower as compared to pair-fed controls and there was a good correlation with onset of audiogenic seizures in these ethanol withdrawn groups [116]. Volicer and co-workers [123,124] have observed similar changes in cerebral cortex of rats not only for the low affinity site, but for the high affinity GABA site as well. Intranigral administration of muscimol suppressed the audiogenic motor convulsions in a dose-dependent manner in ethanol-withdrawn animals, further substantiating the modulatory role of GABA in ethanol abstinence seizures [39]. Further, the affinity (K_D) and the number of receptor sites for (B_{max}) [^{3}H]diazepam binding (Table 2) was not altered in chronic ethanol-treated animals [55,99]. However, a decrease in the K_D and B_{max} of diazepam binding following chronic ethanol has been reported [31,124]. Similarly, several earlier workers have reported variable levels, increases, or decreases of brain GABA levels following acute or chronic ethanol administration [30, 66, 111]. More recent studies have, however, indicated that the affinity or the binding capacity of [^{3}H]flunitrazepam or [^{35}S]TBPS was not altered by chronic treatment with ethanol or during withdrawal from ethanol [99]. Neither the enhancing effect of GABA on the binding of [^{3}H]flunitrazepam nor its inhibiting effect on the binding of [^{35}S]TBPS were affected by chronic treatment with ethanol or its withdrawal at 24 hr [99]. As ethanol protected convulsions induced by 1,4-benzodiazepine [96], a compound known to produce its convulsant effect at the level of picrotoxin site [78, 93, 127], it was further speculated that ethanol may affect the coupling mechanism at the chloride channel by one or the other way in inducing changes in receptor sensitivity (Fig. 1).

Although several alcohols increased [^{3}H]diazepam binding in the Lubrol-solubilized fraction, the rank order of this enhancing effect did not correlate either with their lipid:water partition coefficients or with pharmacological potency in producing physical dependence [23], and this interaction was postulated to be responsible for some of the pharmacological effects of ethanol such as the antianxiety, sedative, and muscle relaxant effects. However, since ethanol's interaction with [^{3}H]BZ receptors to membranes cannot be demonstrated in a reproducible manner, the solubilization studies have not been pursued further.

Recently, several workers have demonstrated the use of synaptoneurosomes or microsacs, a subcellular brain preparation with pre- and postsynaptic elements for the study of GABA regulated chloride flux in vitro [4, 46, 50, 109]. These preparations responded in a pharmacologically appropriate manner for a chloride channel coupled to a GABA receptor [3,4]. Physiologically relevant concentrations of ethanol (15–50 mM) potentiated muscimol stimulation of $^{36}Cl^-$ uptake in cerebellum of certain strains of mice without having any effect on hippocampal microsacs [3,4]. Ethanol, like pentobarbital, stimulated $^{36}Cl^-$ uptake into the synaptoneurosomes in concentrations that are within the range observed during acute intoxication and these effects were sensitive to blockade by both bicuculline and picrotoxin [110], but not to other neurotransmitter receptor antagonists. It also showed a biphasic effect in that at higher concentrations there was decrease in ethanol-stimulated $^{36}Cl^-$ uptake. Such biphasic effects of ethanol on chloride-flux are in line with its effect on brain concentrations and turnover of GABA.

Studies conducted in the past to explore the possible mechanism of action of ethanol on $^{36}Cl^-$ flux tend to suggest that ethanol could cause perturbation of membrane lipids, leading to an increased membrane fluidity [85,108]. Therefore, an effect of ethanol on membrane lipids may alter the microenvironment of GABA/barbiturates-receptor-coupled Cl^- channel, resulting in an increase in Cl^- conductance [110]. Another in vitro model system to study GABA synaptic pharmacology utilizes intact cultured spinal cord neurons. These neurons have GABA-gated Cl^- channels [65,119] and central BZ receptors that are coupled to GABA, picrotoxin and barbiturate receptor sites [81]. In mouse spinal cord cultured neurons ethanol potentiated GABA-mediated $^{36}Cl^-$ influx and at higher concentrations it directly activated Cl^- channels in these neurons [120]. Both the potentiating and direct effects of ethanol were blocked by bicuculline and picrotoxin, suggesting the involvement of the same GABA-gated Cl^- channels in the action of ethanol ([120], Mehta and Ticku in preparation). Further, the effect of ethanol was specific on GABA-gated Cl^- channels, since ethanol did not effect glycine-induced $^{36}Cl^-$ influx in the same cells (Mehta and Ticku, in preparation). Regardless of the exact molecular mechanism of how ethanol stimulated $^{36}Cl^-$ transport, GABA receptor-mediated (low concentrations) or directly (high concentrations), large numbers of evidences, behavioral, electrophysiological, biochemical, and Cl^- flux studies suggested that the pharmacological profile of ethanol action, i.e., antianxiety, hypnotic, anticonvulsant, and withdrawal response centers around GABA/barbiturate-mediated Cl^- transport mechanism, a possible chloride channel modulator (Fig. 1).

RO15-4513, AN IMIDAZOBENZODIAZEPINE AND ETHANOL ANTAGONISM

Antagonism of Behavioral Responses

Ro15-4513 is an azido analogue of the classical benzodiazepine receptor antagonist RO15-1788 (Fig. 2) with an ability to bind to benzodiazepine receptors [87,102]. Although it did not have any per se behavioral effects (up to 10 mg/kg) in animals, prior administration of it completely reversed behavioral effects on ethanol and protected the animals against intoxication and mortality due to massive doses (5–15 g/kg, PO) of ethanol [10, 29, 94, 109]. Under identical conditions the benzodiazepine receptor antagonist RO15-4513 was ineffective in doses up to 30 mg/kg to reverse ethanol effects. The protective effect of RO15-4513 was not related to its effects on blood ethanol concentrations [10,29].

TABLE 3

PROCONVULSANT EFFECTS OF RO15-4513 AS STUDIED AGAINST BICUCULLINE

Treatment (mg/kg, IP)	Onset (sec ±SEM)	Mortality Time (min ±SEM)	Mortality (/n)	Severity of Convulsions
Bicuculline (1)	—	—	—	No convulsions
(4)	59±5.77	14±1.62	4/7	4/7 ++
(8)	36±2.32	8±0.63	10/10	+++
RO15-4513 (1) + Bicuculline (4)	28±2.25*	15±1.75	4/4	++
RO15-4513 (1) + Bicuculline (8)	13±3.00*	6±0.84	6/6	+++
RO15-4513 (4) + Bicuculline (8)	16±1.35*	2.8±0.20*	5/5	+++

*$p < 0.001$ as compared to corresponding bicuculline per se effect.

The quick onset of myoclonic jerks, mortality, and severity of convulsions are taken as index of proconvulsant effect.

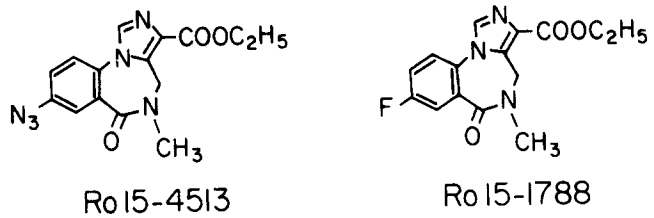


FIG. 2 Chemical structures of RO15-4513 and RO15-1788.

However, a recent study has failed to observe such a reversal of ethanol-induced narcosis in CD-1 mice, whether given before or after ethanol administration [47]. In another behavioral paradigm, RO15-4513 reversed the incoordinating effect of ethanol in mice as studied in a rotarod test [49]. The effect was sensitive to reversal by benzodiazepine antagonists. In the same study, however, it failed to reverse ethanol-induced hypothermia in mice. But it was able to reverse both muscle incoordination as well as hypothermic effects of pentobarbital in mice, though not at the same dose level [49]. RO15-4513 and the benzodiazepine receptor inverse agonist FG-7142 reversed the reductions in the number of head dips caused by ethanol in mice in the holeboard apparatus [71]. Higher doses partially reversed the locomotor stimulant actions of ethanol. However, the doses of RO15-4513 required were relatively less as compared to FG-7142 [71]. The receptor binding data supported these behavioral responses as FG-7142 had a much lower affinity for benzodiazepine receptors than RO15-4513 [12]. Pretreatment of rats with Ro15-4513 blocked the anticonflict activity of low doses of ethanol (but not pentobarbital) as well as the behavioral intoxication observed with higher doses of ethanol [109]. These effects were completely blocked by benzodiazepine receptor antagonists [109]. In another study RO15-4513 (1–6 mg/ kg) blocked the ethanol effect on operant conflict behavior. However, the reversal effect was not totally selective towards ethanol as the anticonflict actions of pentobarbital and chlordiazepoxide were equally sensitive to reversal by RO15-4513 [13]. We have recently observed that RO15-4513 (4 mg/kg) was able to reverse the anticonvulsant actions of ethanol (2 g/kg) against both bicuculline (8 mg/kg) and picrotoxin-induced (10 mg/kg) chemoconvulsions in rats (unpublished observations). The tonic extensor phase which was blocked by ethanol in bicuculline-convulsions was reversed by RO15-4513, but the onset of myoclonic jerks and mortality were not affected. In the case of picrotoxin convulsions RO15-4513 also reversed the protective effect of ethanol on the onset of myoclonic jerks, and the mortality. There was no difference between onset of convulsions in rats treated with RO15-4513, ethanol, and picrotoxin as compared to controls receiving picrotoxin alone. At the same dose level it failed to reverse the protective effect of pentobarbital (20 mg/kg) against picrotoxin (10 mg/kg).

Proconvulsant Actions of RO15-4513

Unlike the classical benzodiazepine receptor antagonist, RO15-4513 has been reported to possess inverse agonistic property [9,90]. When administered alone RO15-4513 lowered seizure threshold to bicuculline, pentylenetetrazole and RO5-3663 [74]. It had no such effect on the seizure threshold of other chemoconvulsants like picrotoxin, strychnine, caffeine, and quipazine. In another convulsive model, we also observed a proconvulsant effect of RO15-4513 even at small doses of 1 mg/kg in rats against bicuculline chemoconvulsions. It not only potentiated the subconvulsive dose (1–4 mg/kg) of bicuculline, but at the convulsive dose (8 mg/kg) of bicuculline it quickened the onset, serverity, and mortality due to the convulsant (Table 3). In these studies, the latency period of 5 to 15 min did not have any significant effect on the proconvulsant action of RO15-4513 in rats.

Reversal of Ethanol Effect on Chloride Flux

It is well documented that ethanol augmented GABA-stimulated chloride flux in whole-brain microsac preparations (see above section). RO15-4513 selectively antagonized ethanol-stimulated chloride uptake in isolated rat brain vesicles in a benzodiazepine receptor antagonist sensitive manner, an action not shared by other inverse agonists [109]. In C57BL/6J mouse spinal cord cultured neurons, RO15-4513 and FG-7142 blocked both the GABA enhancing and direct

effect of ethanol on $^{36}Cl^-$ influx [120]. Both RO15-4513 and FG-7142 reversed ethanol effect at concentrations lower than those that exhibit inverse agonistic activity in the $^{36}Cl^-$ influx assay in cultured neurons (unpublished data, Mehta and Ticku). These observations supported the view of $GABA_A$ receptor-mediated action of ethanol and its reversal by RO15-4513.

However, binding studies have indicated that [^{3}H]Ro15-4513, besides binding to central BZ receptors, also bound to an additional protein (P57) in cerebellum [102], suggesting a unique site for this ligand. However, in vitro ethanol does not inhibit the binding of [^{3}H]RO15-4513 in cortex, cerebellum, or hippocampus (Mahtre and Ticku, unpublished observations). Further, since RO15-4513 reverses ethanol potentiation of GABA-induced $^{36}Cl^-$ influx in regions other than cerebellum, these studies rule out the involvement of the unique binding site (P57 protein) in the actions of RO15-4513.

RO15-4513 and Future Developments

Development of an alcohol antagonist would have a far reaching clinical application. However, some of the recent observations of RO15-4513 have seriously questioned the clinical utility of RO15-4513 itself. Most of the reversal of ethanol effects, including the anticonvulsant actions, could be due to functional antagonism as RO15-4513 possessed inverse agonistic property even in as small as 0.75 mg/kg dose in mice [74] and 1 mg/kg dose in rats (unpublished data from our lab). Moreover, in most of the animal species its action is short-lived. Once the drug effect (due to RO15-4513) is washed out, the animals go back to sedation (ptosis) as with high doses of ethanol or pentobarbitone. One recent observation in squirrel monkeys has shown that chronic administration of (1 mg/kg, PO) RO15-4513 produced severe tremors in seven out of eight monkeys, two even exhibiting tonic-clonic convulsions [86]. This species could be exceptionally sensitive to the action of RO15-4513, however, no such intrinsic effects are reported in rodents, although a very brief period of attack and aggressive behavior is seen when animals are in groups after the intraperitoneal administration of RO15-4513. Furthermore, Lister and Karanian [72] observed RO15-4513-induced seizures in DBA/2 mice undergoing alcohol withdrawal, cautioning the potential application of this compound as alcohol antagonist, as alcohol withdrawal is known to produce withdrawal seizures in certain populations. Although the metabolic disposition of RO15-4513 is not known, it appears to have a short half-life and azido compounds are known to be carcinogenic in nature which may further limit its use once its exact metabolic pathway is known. In all the behavioral studies so far reported, the pharmacokinetic parameters of ethanol were not altered by RO15-4513 which may have serious implications on the untowards effects of ethanol on liver and brain, and also the possible legal and moral implications. In spite of these limitations, the pharmacology, particularly the alcohol-reversal effect of RO15-4513, has caught the imagination of medicinal chemists and enthused pharmacologists and alcohol researchers equally; to look for the development of compounds like RO15-4513 with alcohol antagonistic property in man to sober the intoxication effects without producing undesired inverse agonistic actions [58,73].

ACKNOWLEDGEMENTS

We thank Ms. Diana Reese for excellent secretarial support. This work was supported by NIAAA Grant No. AA04090.

REFERENCES

1. Aaronson, L. M.; Hinman, D. J.; Okamoto, M. Effects of diazepam on ethanol withdrawal. J. Pharmacol. Exp. Ther. 221:319–325; 1982.
2. Abbott, M. E.; Dudek, B. C. Behavioral effects of apomorphine and ethanol in lines of mice which differ in neurosensitivity to alcohol. Soc. Neurosci. Abstr. 7:104.9; 1981.
3. Allan, A. M.; Harris, R. A. Gamma-aminobutyric acid and alcohol actions: Neurochemical studies in long sleep and short sleep mice. Life Sci. 39:2005–2015; 1986.
4. Allan, A. M.; Harris, R. A. γ-Aminobutyric acid agonists and antagonists after chloride flux across brain membranes. Mol. Pharmacol. 29:497–505; 1986.
5. Banna, N. R. Potentiation of cutaneous inhibition by alcohol. Experientia 25:619–620; 1969.
6. Belleville, R. E.; Frazer, H. F. Tolerance to some effects of barbiturates. J. Pharmacol. Exp. Ther. 120:469–474; 1957.
7. Bjorkquist, S. E. Clonidine in alcohol withdrawal. Acta Psychiatr. Scand. 52:256–263; 1975.
8. Boisse, N. R.; Okamoto, M. In: Righer, H.; Crabbe, J. C., eds. Alcohol tolerance and dependence. Amsterdam: Elsevier/North Holland Biomedical Press; 1980:265–292.
9. Bonetti, E. P.; Polc, P.; Pieri, L. An azido analogue of the benzodiazepine antagonist RO15-1788 (RO15-4513) behaves as a partial inverse benzodiazepine agonist. Neurosci. Lett. [Suppl] 18:530; 1984.
10. Bonetti, E. P.; Burkard, W. P.; Gabl, M.; Mohler, H. The partial inverse benzodiazepine antagonist RO15-4513 antagonizes acute ethanol effects in mice and rats. Br. J. Pharmacol. 86:463; 1985.
11. Borg, S.; Kvande, H.; Mossberg, D.; Sedvall, G. Central nervous noradrenaline metabolism in alcoholics during long-term abstinence. Alcohol Alcohol. 18:321–323; 1983.
12. Braestrup, C.; Honore, T.; Nielsen, M.; Petersen, E. N.; Jensen, L. H. Ligands for benzodiazepine receptors with positive and negative efficacy. Biochem. Pharmacol. 33:859–862; 1984.
13. Britton, K. T.; Koob, G. F. Ethanol antagonists RO15-4513 is not selective for ethanol. Alcohol.: Clin. Exp. Res. 11:206; 1987 (abstract).
14. Bustos, G.; Roth, R. H. Effect of acute ethanol treatment on transmitter synthesis and metabolism in central dopaminergic neurons. J. Pharm. Pharmacol. 28:580–582; 1976.
15. Carlsson, A.; Engel, J.; Sversson, T. H. Inhibition of ethanol-induced excitation in mice and rats by α-methyl-p-tyrosine. Psychopharmacologia 26:307–312; 1972.
16. Carlsson, A.; Lindquist, M. Effect of ethanol on the hydroxylation of tyrosine and tyrptophan in rat brain in vivo. J. Pharm. Pharmacol. 25:437–440; 1973.
17. Carlsson, A.; Engel, J.; Strömbom, U.; Svensson, T. H.; Waldeck, B. Suppression by dopamine-agonists of the ethanol-induced stimulation of locomotor activity and brain dopamine synthesis. Naunyn Schmiedebergs Arch. Pharmacol. 283:117–128; 1974.
18. Chan, A. W. K. Gamma-aminobutyric acid in different strains of mice: Effect of ethanol. Life Sci. 19:597–604; 1976.
19. Collins, A. C. In: The development of animal models as pharmacogenetic tools. NIAAA Monograph, Washington, DC; 1981: p. 161.

20. Cooper, B. R.; Virk, K.; Ferris, R. M.; White, H. L. Antagonism of the ethanol susceptibility to audiogenic seizures during alcohol withdrawal in the rat by γ-aminobutyric acid (GABA) and GABAmimetic agents. J. Pharmacol. Exp. Ther. 209:396–403; 1979.
21. Cott, J.; Carlsson, A.; Engel, J.; Lindquist, M. Suppression of ethanol-induced locomotor stimulation by GABA-like drugs. Naunyn Schmiedebergs Arch. Pharmacol. 295:203–209; 1976.
22. Davidoff, R. S. Alcohol and presynaptic inhibition in an isolated spinal cord preparation. Arch. Neurol. 28:60–63; 1973.
23. Davis, W. C.; Ticku, M. K. Ethanol enhances [^{3}H]diazepam binding at the benzodiazepine-γ-aminobutyric acid receptor ionophore complex. Mol. Pharmacol. 20:287–294; 1981.
24. Dreifuss, J. J.; Kelly, J. S.; Krnjevic, K. Cortical inhibition and γ-aminobutyric acid. Exp. Brain Res. 9:137–154; 1969.
25. Duplisse, B. R.; Picchoini, A. L.; Chin, L.; Consroe, P. F. Relationship of the inferior collicules and gamma-aminobutyric acid (GABA) to audiogenic seizures in the rat. Fed. Proc. 33:468; 1974.
26. Engel, J. Neurochemical aspects of the euphoria-induced by dependence-producing drugs. In: Idestöm, C. M., ed. Recent advances in the study of alcoholism. Amsterdam: Excerpta Medica; 1977:16–22.
27. Engel, J.; Strömbom, U.; Svensson, T. H.; Waldeck, B. Suppression by α-methyltyrosine of ethanol-induced locomotor stimulation: Partial reversal by l-dopa. Psychopharmacologia 37:275–279; 1974.
28. Fadda, F.; Argiolas, A.; Melis, M. R.; Serra, G.; Gessa, G. L. Differential effect of acute and chronic ethanol on dopamine metabolism in frontal cortex, caudate nucleus and substantia nigra. Life Sci. 27:979–986; 1980.
29. Fadda, F.; Mosca, E.; Colombo, G.; Gessa, G. L. Protection against ethanol mortality in rats by imidazobenzodiazepine RO15-4513. Eur. J. Pharmacol. 136:265–266; 1987.
30. Ferrari, R. A.; Arnold, A. The effect of central nervous system agents on rat GABA levels. Biochem. Biophys. Acta 52:361–367; 1961.
31. Freund, G. Benzodiazepine receptor loss in brains of mice after chronic ethanol consumption. Life Sci. 27:987–992; 1980.
32. Frey, G. D.; Breese, G. R. GABAergic modulation of ethanol-induced motor impairment. J. Pharmacol. Exp. Ther. 223:750–756; 1982.
33. Frey, G. D.; McCown, T. J.; Breese, G. R. Differential sensitivity of ethanol withdrawal signs in the rat to GABAmimetics: Blockade of audiogenic seizures but not forelimb tremors. J. Pharmacol. Exp. Ther. 226:720–725; 1983.
34. Frey, G. D.; McCown, T. J.; Breese, G. R. Characterization of susceptibility to audiogenic seizures in ethanol-dependent rats after microinjection of γ-aminobutyric acid (GABA) agonists into the inferior colliculus, substantia nigra or medial septum. J. Pharmacol. Exp. Ther. 227:663–670; 1983.
35. Gilman, A. G.; Goodman, L. S.; Rall, T. W.; Murad, F. The pharmacological basis of therapeutics. New York: Macmillan Pub. Co.; 1985:338–386.
36. Goldman, G. D.; Volicer, L.; Gold, B. I.; Roth, R. H. Cerebrospinal fluid GABA and cyclic nucleotides in alcoholics with and without seizures. Alcohol: Clin. Exp. Res. 5:431–434; 1981.
37. Goldstein, D. B. Alcohol withdrawal reactions in mice: Effects of drugs that modify neurotransmission. J. Pharmacol. Exp. Ther. 186:1–9; 1978.
38. Goldstein, D. B. Sodium bromide and sodium valproate: Effective suppressants of ethanol withdrawal reactions in mice. J. Pharmacol. Exp. Ther. 208:223–227; 1979.
39. Gonzalez, L. P.; Hettinger, M. K. Intranigral muscimol suppresses ethanol withdrawal seizures. Brain Res. 298:163–166; 1984.
40. Gorant, D. S.; Gale, K. Lesions of substantia nigra protect against experimentally induced seizures. Brain Res. 273:156–161; 1983.
41. Greenberg, D. A.; Cooper, E. C.; Gordon, A.; Diamond, I. Ethanol and the γ-aminobutyric acid-benzodiazepine receptor complex. J. Neurochem. 42:1062–1068; 1984.
42. Greenblatt, D. J.; Greenblatt, M. Which drug for alcohol withdrawal? J. Clin. Pharmacol. 12:429–431; 1972.
43. Grenningloh, G.; Rienitz, A.; Schmitt, B.; Methfersel, C.; Zensen, M.; Begreuther, K.; Gundelfinger, E.; Betz, H. The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. Nature 328:215–220; 1987.
44. Guerrero-Figueroa, R.; Merrill, M.; Rye, D. Electrographic and behavioral effects of diazepam during alcohol withdrawal stage in cat. J. Pharmacol. 9:143–150; 1970.
45. Hakkinen, J-M.; Kulonen, E. Ethanol intoxication and γ-aminobutyric acid. J. Neurochem. 27:631–633; 1976.
46. Harris, R. A.; Allan, A. M. Functional coupling of GABA receptors to chloride channels in brain membranes. Science 228:1108–1110; 1985.
47. Hatch, R. C.; Jernigan, A. D. Effect of intravenously administered putative and potential antagonists of ethanol on sleep time in ethanol narcotized mice. Life Sci. 42:11–19; 1988.
48. Hayashi, T. A physiological study of epileptic seizures following cortical stimulation in animals and its application to human clinics. Jpn. J. Physiol. 3:46–64; 1953.
49. Hoffman, P. L.; Tabakoff, B.; Szabo, G.; Suzdak, P. D.; Paul, S. M. Effect of an imidazobenzodiazepine, RO15-4513, on the incoordination and hypothermia produced by ethanol and pentobarbital. Life Sci. 41:611–619; 1987.
50. Hollingsworth, E. B.; McNeil, E. T.; Burton, J.; Williams, R. W.; Daly, W.; Creveling, C. R. Biochemical characterization of a filtered synaptoneurosome preparation from guinea pig cerebral cortex: Cyclic adenosine 3′:5′ monophosphate generating systems, receptors, and enzymes. J. Neurosci. 5: 2240–2253; 1985.
51. Howerton, T. C.; Marks, M. J.; Collins, A. C. Norepinephrine, gamma-aminobutyric acid, and choline reuptake kinetics and the effects of ethanol in long-sleep and short-sleep mice. Subst. Alcohol Actions Misuse 3:89–99; 1982.
52. Hunt, W. A. The effect of ethanol on GABAergic transmission. Neurosci. Biobehav. Rev. 7:87–95; 1983.
53. Idarola, M. J.; Gale, K. Cellular compartments of GABA in brain and their relationship to anticonvulsant activity. Mol. Cell Biochem. 39:305–330; 1981.
54. Idarola, M. J.; Gale, K. Substantia nigra: Site of anticonvulsant activity mediated by gamma-aminobutyric acid. Science 218:1237–1240; 1982.
55. Karobath, M.; Rogers, J.; Bloom, F. Benzodiazepine receptor remain unchanged after chronic ethanol administration. Neuropharmacology 19:125–128; 1980.
56. Karoum, F.; Wyatt, R. J.; Majchrowicz, E. Brain concentrations of biogenic amine metabolites in acutely treated and ethanol-dependent rats. Br. J. Pharmacol. 56:403–411; 1976.
57. Kesner, R. P. Subcortical mechanisms of audiogenic seizures. Exp. Neurol. 15:192–205; 1966.
58. Kolata, G. New drug counters alcohol intoxication. Science 234:1198–1199; 1986.
59. Koob, G.; Braestrup, C.; Britton, K. T. The effects of FG-7142 and RO15-1788 on the release of punished responding produced by chlordiazepoxide and ethanol in the rat. Psychopharmacology (Berlin) 90:173–178; 1986.
60. Kramp, P.; Rafaelson, O. J. Delirium tremens: A double-blind comparison of diazepam and barbital treatment. Acta Psychiatr. Scand. 58:174–190; 1978.
61. Krnjevic, K.; Randic, M.; Straughan, D. W. An inhibitory process in the cerebral cortex. J. Physiol. (Lond.) 184:16–48; 1966.
62. Kulkarni, S. K. Clonidine and abstinence syndrome. Drugs Today (in press).
63. Kulkarni, S. K.; Parale, M. P.; Kulkarni, G. K. Clonidine in alcohol withdrawal: A clinical report. Methods Find. Exp. Clin. Pharmacol. 9:697–698; 1987.
64. Lai, K.; Makous, N. L.; Horita, A.; Leung, H. Effects of ethanol in turnover and function of striatal dopamine. Psychopharmacology (Berlin) 61:1–9; 1979.

65. Lehoullier, P. F.; Ticku, M. K. Benzodiazepine and β-carboline modulation of GABA-stimulated $^{36}Cl^-$ influx in cultured spinal cord neurons. Eur. J. Pharmacol. 135:235–238; 1987.
66. Leitch, C. J.; Buckes, D. J.; Siegman, F. S.; Guthrie, G. D. Possible role of GABA in the development of tolerance to alcohol. Experientia 33:496–497; 1977.
67. Liljequist, S.; Berggren, U.; Engel, J. The effect of catecholamine receptor antagonists on ethanol-induced locomotor stimulation. J. Neural. Transm. 50:57–67; 1981.
68. Liljequist, S.; Engel, J. Effects of GABAergic agonists and antagonists on various ethanol-induced behavioral changes. Psychopharmacology (Berlin) 78:71–75; 1982.
69. Liljequist, S.; Engel, J. The effects of GABA and benzodiazepine receptor antagonists on the anticonflict actions of diazepam or ethanol. Pharmacol. Biochem. Behav. 21:521–525; 1984.
70. Liljequist, S.; Culp, S.; Tabakoff, B. Effect of ethanol on the binding of [^{35}S]t-butylbicyclophosphorothionate to mouse brain membranes. Life Sci. 38:1931–1939; 1986.
71. Lister, R. G. The benzodiazepine receptor inverse agonists FG-7142 and RO15-4513 both reverse some of the behavioral effects of ethanol in a holeboard test. Life Sci. 41:1481–1489; 1987.
72. Lister, R. G.; Karanian, J. W. RO15-4513 induces seizures in DBA/2 mice undergoing alcohol withdrawal. Alcohol 4:409–411; 1987.
73. Lister, R. G.; Nutt, D. J. Is RO15-4513 a specific alcohol antagonist? Trends Neurosci. 10:223–225; 1987.
74. Lister, R. G.; Nutt, D. J. Interactions of the imidazodiazepine RO15-4513 with chemical convulsants. Br. J. Pharmacol. 93:210–214; 1988.
75. McIntyre, T. D.; Alpren, H. P. Reinterpretation of the literature indicates differential sensitivities of long-sleep and short-sleep mice are not specific to alcohol. Psychopharmacology (Berlin) 87:379–389; 1985.
76. McNamara, J.; Idarola, M. J.; Rigsbee, L.; Galloway, M. Kindled motor seizures are abolished by microinjections of muscimol into the ventral midbrain tegmentum. Soc. Neurosci. Abstr. 8:86; 1982.
77. McNamara, J. O.; Rigsbee, L. C.; Galloway, M. T. Evidence that substantia nigra is crucial to neural network of kindled seizures. Eur. J. Pharmacol. 86:485–486; 1983.
78. MacNeil, D. A.; D'Amico, J. A.; Horst, W. D.; O'Brien, R. A.; Spirit, N. Influence of two atypical benzodiazepines RO5-3663 and RO5-4864 on GABAergic mechanisms. Soc. Neurosci. Abstr. 9:412; 1983.
79. Maksay, G.; Ticku, M. K. Dissociation of [^{35}S]t-butylbicyclophosphorothionate binding differentiates convulsant and depressant drugs that modulate GABAergic transmission. J. Neurochem. 44:480–486; 1985.
80. Martz, A.; Deitrich, R. A.; Harris, R. A. Behavioral evidence for the involvement of γ-aminobutyric acid in the actions of ethanol. Eur. J. Pharmacol. 89:53–62; 1983.
81. Mehta, A. K.; Ticku, M. K. Characteristics of flunitrazepam binding to intact primary cultured spinal cord neurons and its modulation by GABAergic drugs. J. Neurochem. 49:1491–1497; 1987.
82. Mendelson, W. B.; Martin, J. V.; Wagner, R.; Roseberry, C.; Skolnick, P.; Weissman, B. A.; Squires, R. Are the toxicities of pentobarbital and ethanol mediated by the GABA-benzodiazepine receptor-chloride ionophore complex? Eur. J. Pharmacol. 108:63–70; 1985.
83. Menon, M. K.; Kodama, C. K. Antagonism of the hypnotic effect of ethanol in mice by an alpha-1 adrenoceptor agonist. Neuropharmacology 24:927–920; 1985.
84. Mereu, G.; Gessa, G. L. Low doses of ethanol inhibit the firing of neurons in the substantia nigra, pars reticulata: A GABAergic effect? Brain Res. 360: 325–330; 1985.
85. Michaelis, E. K.; Chang, H. H.; Roy, S.; McFaul, J. A.; Zimbrick, E. Ethanol effects on synaptic glutamate receptor function and on membrane lipid organization. Pharmacol. Biochem. Behav. 18:1–6; 1983.
86. Miczek, K. A.; Weerts, E. M. Seizures in drug treated animals. Science 235:1127; 1987.
87. Möhler, H.; Sieghart, W.; Richards, J. G.; Hunkeler, W. Photo-affinity labeling of benzodiazepine receptors with a partial inverse agonist. Eur. J. Pharmacol. 102:191–192; 1984.
88. Nerviano, N. F.; Gross, W. F. A multivariate delineation of two alcoholic profile types on the 16 pf. J. Clin. Psychol. 29:371–374; 1973.
89. Nestoros, J. N. Ethanol specificity potentiates GABA-mediated neurotransmission in feline cerebral cortex. Science 209:708–710; 1980.
90. Nutt, D. J.; Lister, R. G. The effect of the imidazodiazepine RO15-4513 on the anticonvulsant effects of diazepam, sodium pentobarbital and ethanol. Brain Res. 413:193–196; 1987.
91. Olsen, R. W. Drug interactions at the GABA receptor-ionophore complex. Annu. Rev. Pharmacol. Toxicol. 22:245–277; 1982.
92. Parale, M. P.; Kulkarni, S. K. Studies with α_2-adrenoceptor agonists and alcohol abstinence syndrome in rats. Psychopharmacology (Berlin) 88:237–239; 1986.
93. Polc, P.; Schaffner, R. Electrophysiological effects of the peripheral type benzodiazepine receptor ligand RO5-4864 in cat spinal cord and rat hippocampus. Neurosci. Lett. [Suppl] 14:3288; 1983.
94. Polc, P. Interactions of partial inverse benzodiazepine agonists RO15-4513 and FG-7142 with ethanol in rats and cats. Br. J. Pharmacol. 86:465; 1985.
95. Ramanjaneyulu, R.; Ticku, M. K. Binding characteristics and interaction of depressant drugs with [^{35}S]t-butylbicyclophosphorothionate, a ligand that binds to the picrotoxinin site. J. Neurochem. 42:221–229; 1984.
96. Rastogi, S. K.; Ticku, M. K. A possible role of a GABAergic mechanism in the convulsant action of RO5-4864. Pharmacol. Biochem. Behav. 23:285–288; 1985.
97. Rastogi, S. K.; Ticku, M. K. Involvement of a GABAergic mechanism in the anticonvulsant effect of pentobarbital agonist maximal electroshock-induced seizures in rats. Pharmacol. Biochem. Behav. 22:141–146; 1985.
98. Rastogi, S. K.; Ticku, M. K. Anticonvulsant profile of drugs which facilitate GABAergic transmission on convulsions mediated by a GABAergic mechanism. Neuropharmacology 25:175–185; 1986.
99. Rastogi, S. K.; Thyagarajan, R.; Clothier, J.; Ticku, M. K. Effect of chronic treatment of ethanol on benzodiazepine and picrotoxin sites on the GABA receptor complex in regions of the brain of the rat. Neuropharmacology 25:1179–1184; 1986.
100. Reggiani, A.; Barbaccia, M. L.; Spano, P. F.; Trabucchi, M. Dopamine metabolism and receptor function after acute and chronic ethanol. J. Neurochem. 35:34–37; 1980.
101. Reggiani, A.; Barbaccia, M. K.; Spano, P. F.; Trabucchi, M. Acute and chronic ethanol treatment on specific [^{3}H]-GABA binding in different rat brain areas. Psychopharmacology (Berlin) 67:261–264; 1980.
102. Sieghart, W.; Eichinger, A.; Richards, J. G.; Möhler, H. Photo-affinity labeling of benzodiazepine receptor proteins with the partial inverse agonist [^{3}H]RO15-4513: A biochemical and autoradiographic study. J. Neurochem. 48:46–52; 1987.
103. Simmonds, M. A. Classification of some GABA antagonists with regard to site of action and potency in slices of rat cuneate nucleus. Eur. J. Pharmacol. 80:347–358; 1982.
104. Skolnick, P.; Rice, K. C.; Barker, J. L.; Paul, S. M. Interactions of barbiturates with BZ receptors in the central nervous system. Brain Res. 233:143–156; 1982.
105. Squires, R. F. editor. GABA and benzodiazepine receptors. Vol. I and II. Boca Raton: CRC Press, Inc.; 1988.
106. Squires, R. F.; Casida, J. E.; Richardson, M.; Saederup, E. [^{35}S]t-butylbicyclophosphorothionate binds with high affinity to brain sites coupled to the γ-aminobutyric acid-A and ion recognition sites. Mol. Pharmacol. 23:326–336; 1983.
107. Strömbom, U.; Svensson, T.; Carlsson, A. Antagonism of ethanol's central stimulation in mice by small doses of catecholamine-receptor agonists. Psychopharmacology (Berlin) 51:293–299; 1977.

108. Strong, R.; Wood, G. W. Membrane properties and aging: In vivo and in vitro effects of ethanol on synaptosomal γ-aminobutyric acid (GABA) release. J. Pharmacol. Exp. Ther. 229:726–730; 1984.
109. Suzdak, P. D.; Glowa, J. R.; Crawley, J. N.; Schwartz, R. D.; Skolnick, P.; Paul, S. M. A selective imidazobenzodiazepine antagonist of ethanol in the rat. Science 234:1243–1247; 1986.
110. Suzdak, P. D.; Schwartz, R. D.; Skolnick, P.; Paul, S. M. Ethanol stimulates γ-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes. Proc. Natl. Acad. Sci. USA 83:4071–4075; 1986.
111. Systinsky, I. A.; Guzikov, B. M.; Gomanko, M. V.; Eremin, V. R.; Konovalova, N. N. The gamma-aminobutyric acid system in brain during acute and chronic ethanol intoxication. J. Neurochem. 25:43–48; 1975.
112. Tabakoff, B.; Hoffmann, P. L. Alterations in receptors controlling dopamine synthesis after chronic ethanol ingestion. J. Neurochem. 31:1223–1229; 1978.
113. Tarika, J. S.; Winger, G. The effects of ethanol, phenobarbital and baclofen on ethanol withdrawal in the Rhesus monkey. Psychopharmacology (Berlin) 70:201–208; 1980.
114. Thompson, W. L. Management of alcohol withdrawal syndromes. Arch. Intern. Med. 138:278–283; 1978.
115. Thyagarajan, R.; Ticku, M. K. The effect of *in vitro* and *in vivo* ethanol administration on [^{35}S]t-butylbicyclophosphorothionate binding in C57 mice. Brain Res. Bull. 15:343–345; 1985.
116. Ticku, M. K. The effects of acute and chronic ethanol administration and its withdrawal on γ-aminobutyric acid receptor binding in rat brain. Br. J. Pharmacol. 70:403–410; 1980.
117. Ticku, M. K.; Burch, T. Alterations in GABA receptor sensitivity following acute and chronic ethanol treatment. J. Neurochem. 34:417–423; 1980.
118. Ticku, M. K.; Burch, T. P.; Davis, W. C. The interaction of ethanol with the benzodiazepine-GABA receptor-ionophore complex. Pharmacol. Biochem. Behav. 18:15–18; 1983.
119. Ticku, M. K.; Lowrimore, P.; Lehoullier, P. Ethanol enhances GABA-induced $^{36}Cl^-$ influx in primary spinal cord cultured neurons. Brain Res. Bull. 17:123–126; 1986.
120. Ticku, M. K.; Mehta, A. H. Ethanol enhances GABA receptor gated $^{36}Cl^-$ influx in cultured spinal cord neurons. Alcohol: Clin. Exp. Res. 12:314; 1988.
121. Ticku, M. K.; Rastogi, S. K. Barbiturate-sensitive sites in the benzodiazepine-GABA receptor-ionophore complex. In: Roth, S. H.; Miller, W. K., eds. Molecular and cellular mechanisms of anesthetics. New York: Plenum Press; 1980:179–188.
122. Unwin, J. W.; Taberner, P. V. Sex and strain differences in GABA receptor binding after chronic ethanol drinking in mice. Neuropharmacology 19:1257–1259; 1980.
123. Volicer, L. GABA levels and receptor binding after acute and chronic ethanol administration. Brain Res. Bull. 5:809–813; 1980.
124. Volicer, L.; Biagioni, T. M. Effect of ethanol administration and withdrawal on GABA receptor binding in rat cerebral cortex. Subst. Alcohol Actions Misuse 3:31–39; 1982.
125. Wachtel, H.; Anden, N-E. Motor activity of rats following intracerebral injections of drugs influencing GABA mechanisms. Naunyn Schmiedebergs Arch. Pharmacol. 302:133–139; 1978.
126. Wallgren, H.; Barry, H., editors. Actions of alcohol, Vol I. New York: Elsevier; 1970.
127. Weissman, B. A.; Cott, J.; Hummer, D.; Paul, S.; Skolnick, P. Electrophysiological and pharmacological actions of the convulsant benzodiazepine RO5-4864. Eur. J. Pharmacol. 97:257–263; 1984.
128. Wilkins, A. J.; Jenkins, W. J.; Steiner, J. A. Efficacy of clonidine in treatment of alcohol withdrawal state. Psychopharmacology (Berlin) 81:78–80; 1983.