ELSEVIER

European Journal of Pharmacology 317 (1996) 15–20

ejp

Ro 15-4513 potentiates, instead of antagonizes, ethanol-induced sleep in mice exposed to small platform stress

Paavo Pokk [a], Sture Liljequist [b], Alexander Zharkovsky [a,*]

[a] *Department of Pharmacology, University of Tartu, EE 2400 Tartu, Estonia*

[b] *Department of Clinical Neuroscience, Division of Drug Dependence Research, Karolinska Institute, S-17176 Stockholm, Sweden*

Received 28 August 1996; accepted 3 September 1996

Abstract

The effects of ethanol and the benzodiazepine receptor ligand ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo-[1,5*a*] [1,4] benzodiazepine-3-carboxylate (Ro 15-4513), were examined in NMRI mice exposed to small platform stress. This model contains several factors of stress, like rapid eye movement (REM) sleep deprivation, isolation, immobilization, falling into water and soaking. In control mice, ethanol exerted an anxiolytic effect in the plus-maze, but did not further enhance the anxiolytic-like effects induced by small platform stress. Ro 15-4513 antagonized ethanol-induced sleep in control animals, but enhanced the hypnotic and lethal actions of ethanol in small platform stressed mice. Small platform stress did not alter the characteristics (K_D and B_{max}) of [^{3}H]Ro 15-4513 binding to cerebellar membranes. Muscimol-stimulated $^{36}Cl^-$ uptake into brain microsacs was significantly reduced in cortex from small platform stressed animals. Ethanol had no effect on $^{36}Cl^-$ uptake into brain microsacs from cortex or cerebellum. It is proposed that small platform stress alters the activity of the γ-aminobutyric acid $(GABA)_A$ receptor-chloride ionophore complex, causing changes in the interaction between ethanol and Ro 15-4513.

Keywords: Stress; Ethanol; Ro 15-4513; Plus-maze; Sleep; $^{36}Cl^-$ uptake; [^{3}H]Ro 15-4513

1. Introduction

It is largely accepted that ethanol and benzodiazepines share a number of neuropharmacological and behavioural characteristics. Thus both compounds are known to produce anxiolytic, sedative, muscle relaxant, anticonvulsant and hypnotic actions (Korpi, 1994). Furthermore, both ethanol and benzodiazepines have been shown to enhance γ-aminobutyric acid (GABA)-evoked chloride uptake in brain microsacs (Allan and Harris, 1986; Suzdak et al., 1986; Mehta and Ticku, 1994), although this effect of ethanol has not been confirmed by all investigators (Mihic et al., 1992b). While it is well established that classical benzodiazepine receptor agonists enhance the pharmacological effects of ethanol, there is considerable evidence implicating that the atypical benzodiazepine receptor ligand ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo-[1,5*a*] [1,4] benzodiazepine-3-carboxylate (Ro 15-4513) is able to antagonize certain effects of ethanol, in particular its sedative/hypnotic actions (Lister, 1987; Glowa et al., 1989; Naijm and Karim, 1993; Meng and Dar, 1994; Dar, 1995; Petry, 1995).

Although the nature of interaction between ethanol and Ro 15-4513 remains unclear, some authors have suggested that antagonistic actions of Ro 15-4513 might be due to its partial inverse benzodiazepine receptor agonistic properties (Lister and Nutt, 1987; Glowa et al., 1989). Results from recent binding studies have revealed that Ro 15-4513 binds to two distinct benzodiazepine recognition sites, one of which has been shown to be sensitive to diazepam, whereas the other is recognized as diazepam-insensitive (Malminiemi and Korpi, 1989; Uusi-Oukari and Korpi, 1990; Wong and Skolnick, 1992; Wong et al., 1995). The possibility that diazepam-insensitive recognition sites play a significant role for the pharmacological actions of ethanol is suggested by findings showing that a point mutation at the α6 subunit of diazepam-insensitive benzodiazepine receptor has significant effects on ethanol- and benzodiazepine-induced motor impairment in genetically determined non-tolerant rats (Malminen and Korpi, 1988).

Accumulating data suggest that environmental stressors may act as potent modulators of the functional properties

* Corresponding author. Tel.: (372-7) 465-296; Fax: (372-7) 465-423.

PII S0014-2999(96)00711-X

of $GABA_A$ receptor-chloride ionophore complex in the brain (Biggio et al., 1990; Mihic et al., 1992a; Barbaccia et al., 1996). For example, it has been shown that pharmacological agents, like benzodiazepines, barbiturates and ethanol, which act by enhancing the activity of the $GABA_A$ receptor-chloride ionophore complex, are able to reduce behavioural and biochemical changes seen after acute or chronic stress exposure (Carden and Hofer, 1990; Drugan et al., 1990; Pohorecky, 1990; Breschi et al., 1995).

In our previous experiments we have used the small platform technique as a stress model. Using this technique animals are selectively deprived of rapid eye movement (REM) sleep, but also exposed to other stress factors such as isolation, immobilization and falling into water. 24 h exposure to small platform stress causes an increase in locomotor activity and anxiolytic-like effect in the plus-maze test that last for about 60 min.

In a recent series of investigations we found a reduction in the anxiolytic properties of diazepam but an enhancement of diazepam-induced sedation in mice exposed to stress (Pokk and Zharkovsky, 1995). Using receptor binding assays, we demonstrated that exposure to stress induced an increase in the number of cortical benzodiazepine receptors in brain membranes as well as a reduction in GABA-stimulated $^{36}Cl^-$ uptake by forebrain microsacs. However, none of the described biochemical changes could be observed in animals exposed to isolation-, swimming- or large platform-induced stress, suggesting that a specific form of stress, perhaps related to a selective deprivation of REM sleep, is induced by the small platform stress situation (Pokk et al., 1996).

The main objective of this study was to investigate (1) the effect of ethanol and its interactions with Ro 15-4513 in animals exposed to small platform stress, (2) the effect of ethanol on the muscimol-evoked $^{36}Cl^-$ uptake in small platform stressed animals, (3) the effect of small platform stress on the binding of [^{3}H]Ro 15-4513 to diazepam-insensitive benzodiazepine receptors.

2. Materials and methods

2.1. Animals

Naive male albino mice (NMRI strain, Grindex Breeding Center, Riga, Latvia) weighting 25–30 g were used throughout the study. Mice were maintained at 20 ± 2°C with water and standard laboratory food ad libitum. Mice were housed 20 per cage and exposed to a 12/12 h light/dark cycle. Lights were on from 07.00 a.m. to 07.00 p.m.

2.2. Stress methods

2.2.1. Group I – naive control

Animals were kept grouped in their home cages for 24 h.

2.2.2. Group II – small platform-induced stress

Animals were exposed to the small platform according to the method described previously (Asakura et al., 1994) Mice were individually placed for 24 h on the small platform (3 cm high, 3.5 cm in diameter) which was fixed at the center of a plastic chamber (20 cm diameter, 40 cm high) and was surrounded by water (1 cm deep) at 22°C.

In order to eliminate the effect of stress due to handling each animal was handled twice a day for 7 days before the start of experiments.

2.3. Drugs

Ethanol was diluted in saline and injected i.p. 20 min before the plus-maze test. To control mice vehicle was administered 20 min before the plus-maze test. The benzodiazepine receptor inverse agonist Ro 15-4513 was kindly donated by Hoffmann-LaRoche (Basel, Switzerland). Ro 15-4513 was suspended in saline with a drop of Tween-80 and injected i.p. 5 min before the administration of ethanol. Injection volume was adjusted with saline to 0.1 ml per 10 g body weight.

2.4. The plus-maze test

The plus-maze test was carried out according to File et al. (1989). The plus-maze consisted of two open (8 × 17 cm) and two closed arms (8 × 17 × 30 cm), which were connected by a central platform (8 × 8 cm). Mice were placed on the central platform facing an open arm. During 5 min the number of entries made onto the open and into the closed arms and the time spent on the open and in the closed arms were measured. On the basis of these data the percentage of entries made onto the open arms and the percentage of time spent on the open arms were calculated.

2.5. Sleeping time

Sleeping time was measured as the time elapsed between the loss and the regaining of the righting reflex, the experimental criteria being that the animal had to regain its righting reflex 3 times within 1 min.

2.6. Muscimol- and ethanol-induced $^{36}Cl^-$ uptake

Each group consisted of 10–12 animals. The procedure for preparation of microsacs and $^{36}Cl^-$ uptake assay were performed as described previously (Allan and Harris, 1986). In different experiments whole brain minus cerebellum or cerebellum was homogenized by hand (10–12 strokes) in ice-cold assay buffer [145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 10 mM glucose and 10 mM (4-(2-hydroxyethyl)-1-pipirazinethanesulfonic acid (HEPES) adjusted to pH 7.5 with Tris base], using a glass-Teflon homogenizer. The homogenate was washed twice at 900 × *g* for 15 min using a Sorvall SA 600 rotor. The final pellet was resuspended in an assay buffer yielding a preparation

containing 12–14 mg protein per ml of suspension. Aliquots (100 μl) were incubated in a shaking water bath at 34°C for 5 min. Ten minutes prior to incubation 100 μl of assay buffer or various concentrations of ethanol were added to aliquots. Following this incubation, various concentrations of muscimol (0.3, 3.0 and 30.0 μM) and 50 nCi of $^{36}Cl^-$ in a total volume of 0.2 ml were added. Three seconds following the addition of $^{36}Cl^-$, the incubation was terminated by the addition of 4 ml of ice-cold assay buffer containing 100 μM picrotoxin and rapidly filtered through GF/B glass fiber filters. Filters were washed with an additional 12 ml of ice-cold assay buffer containing 100 μM picrotoxin and placed into vials for scintillation counting. The amount of $^{36}Cl^-$ taken up by the microsacs was expressed in nmol/mg protein/3 s.

Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as external standard.

2.7. [^{3}H]Ro 15-4513 binding

[^{3}H]Ro 15-4513 binding was carried out by the method of Wong and Skolnick (1992). Mice were killed by decapitation. Brains were taken out and the cerebella were dissected, weighted and disrupted (Polytron Pt 1200, setting 6, 10 s) in 60 volumes of phosphate buffer (pH 7.3). Homogenates were centrifuged at 20000 × g for 20 min (4°C), resuspended in 60 volumes of buffer and recentrifuged. This washing procedure was repeated 5 more times. [^{3}H]Ro 15-4513 binding was determined in a total volume of 1.0 ml consisting of: 0.1 ml of tissue (approx. 100 μg of protein), 0.1 ml [^{3}H]Ro 15-4513 (final concentration 0.4375–14.0 nM) and 0.6 or 0.7 ml of buffer. Diazepam-insensitive binding was determined as a difference between the binding in the presence of 10 μM diazepam and nonspecific binding in the presence of 10 μM Ro 15-1788 + 10 μM of diazepam.

Incubations was carried out at 0–4 °C in the dark. Incubations were initiated by the addition of tissue and terminated after 60 min by adding 4 ml of ice-cold buffer and filtration through Whatman GF/B filters using a vacuum pump. Filters were additionally washed with 2 × 4 ml of buffer.

2.8. Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA), where small platform stress was taken as one factor and effect of drugs as another. Post-hoc statistical analysis was done using the Bonferroni test.

3. Results

3.1. The effect of ethanol in the plus-maze test

As noted previously (Pokk and Zharkovsky, 1995) animals exposed to small platform stress displayed less anxiety in the plus-maze test (Fig. 1) evidenced as an increase in the percentage of time spent ($P < 0.05$) and the percentage of entries made ($P < 0.05$) onto the open arms.

In control mice, ethanol caused a dose-dependent (0.25, 0.5 and 1.0 g/kg) increase of both percentage of time spent on the open arms [$F(3,35) = 4.1$, $P < 0.05$] and the percentage of entries made onto the open arms [$F(3,35) =$

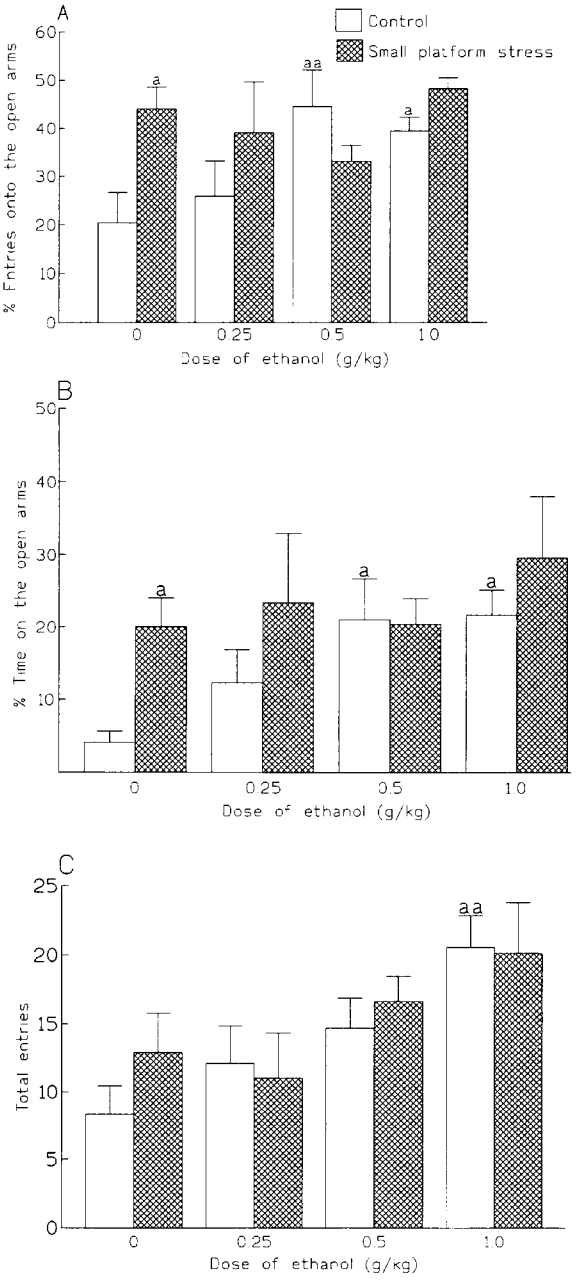

Fig. 1. The effect of ethanol on the behaviour of control and small platform stressed mice in the plus-maze test. A: The percentage of entries made onto the open arms. B: The percentage of time spent on the open arms. C: Total number of entries made in the plus-maze test. The values are mean ± S.E.M. The number of animals was 8–10 in each group. [a] $P < 0.05$, [aa] $P < 0.01$ as compared with control/saline (Bonferroni test).

Table 1
Sleeping time (min) after the administration of ethanol and Ro 15-4513 in control and small platform stressed mice

Drugs, dose	n	Control	n	Small platform stress
Ethanol 3.0 g/kg	12	14.9 ± 6.5	11	74.1 ± 17.9 [a]
Ethanol 4.0 g/kg	17	95.3 ± 16.5	16	219.5 ± 30.2 [aa]
Ethanol 4.0 g/kg + Ro 15-4513 0.5 mg/kg	8	53.5 ± 15.1	8	210.2 ± 36.1
Ethanol 4.0 g/kg + Ro 15-4513 1.0 mg/kg	8	26.5 ± 10.2 [b]	8	260.9 ± 28.6
Ethanol 4.0 g/kg + Ro 15-4513 3.0 mg/kg	8	10.5 ± 5.4 [bb]	8	332.6 ± 29.8 [b]

The values are mean ± S.E.M. n = number of animals. [a] $P < 0.05$; [aa] $P < 0.01$ vs. corresponding control; [b] $P < 0.05$, [bb] $P < 0.01$ vs. ethanol 4.0 g/kg (Bonferroni test).

2.9, $P < 0.05$]. Ethanol also increased the total number of entries [$F(3,35) = 4.8$, $P < 0.01$].

In contrast, ethanol had no significant effects on the percentage of entries made onto the open arms or on the percentage of time spent on the open arms in small platform stressed mice (Fig. 1). Furthermore, two-way ANOVA did not reveal any significant interaction between stress and ethanol on the measures of anxiolytic activity in the plus-maze.

3.2. The effect of ethanol on sleeping time

Small platform stress enhanced ethanol-induced sleep [$F(1,74) = 125.5$, $P < 0.01$] (Table 1). In control mice, Ro 15-4513 dose-dependently decreased the ethanol-induced sleeping time [$F(3,38) = 6.9$, $P < 0.01$] (Table 1). In small platform stressed mice, however, the lower doses of Ro 15-4513 (0.5 and 1.0 mg/kg) had no effect, whereas the high dose (3.0 mg/kg) caused a significant enhancement of the hypnotic actions of ethanol. Further analysis by two-way ANOVA revealed a significant interaction between small platform stress and the effects of Ro 15-4513 [$F(3,74) = 6.2$, $P < 0.01$]. It should be also mentioned that the combined administration of ethanol with a high dose of Ro 15-4513 (3.0 mg/kg) caused lethal effects (four of eight animals) in small platform stressed animals. No such effect was observed in control animals. Death of animals was due to failure to wake up.

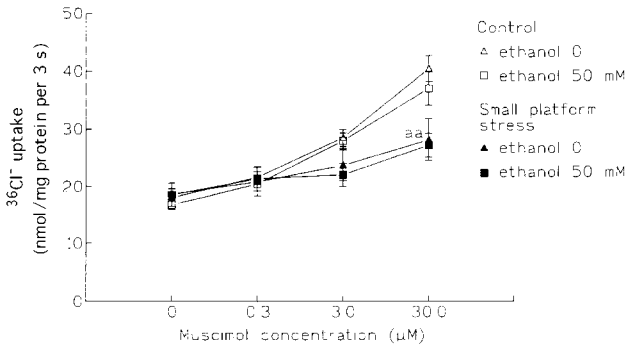

Fig. 2. The effect of ethanol on the muscimol-induced $^{36}Cl^-$ uptake in the whole brain minus cerebellum of control and small platform stressed mice. The values are mean ± S.E.M. of 6–7 experiments. The effect of muscimol (30 μM) in small platform stressed mice was significantly lower ([aa] $P < 0.01$, Bonferroni test) as compared with control mice.

3.3. Effect of ethanol on muscimol-stimulated $^{36}Cl^-$ uptake

Muscimol induced a concentration-dependent (0.3, 3.0 and 30.0 μM) increase of $^{36}Cl^-$ uptake in both cortical and cerebellar microsacs (Table 2; Fig. 2). Furthermore, the stimulatory effect of muscimol on $^{36}Cl^-$ uptake was significantly attenuated in cortical microsacs from small platform stressed animals [$F(3,68) = 5.9$, $P < 0.01$], whereas we were unable to demonstrate any corresponding differences in the $^{36}Cl^-$ uptake into cerebellar microsacs (Table 2). Furthermore, addition of ethanol (25, 50 and 100 mM) did not alter basal or muscimol-induced $^{36}Cl^-$ uptake in any of the brain structures studied regardless whether the examinations were carried out in control or small platform stressed mice (Table 2 and Fig. 2).

Table 2
The effect of ethanol on the muscimol-induced ^{36}Cl uptake in the cerebellum of control and small platform stressed mice

Concentration of drugs	Control	Small platform stress
0	16.62 ± 0.69	17.31 ± 0.56
Muscimol 3.0 μM	25.40 ± 1.75	22.97 ± 1.64
Muscimol 3.0 μM + ethanol 25 mM	24.91 ± 1.78	23.12 ± 1.12
Muscimol 3.0 μM + ethanol 100 mM	25.45 ± 1.65	23.67 ± 1.28

The values are mean ± S.E.M. of 4 experiments. The uptake is expressed in nmol/mg protein per 3 s.

Table 3
[^{3}H]Ro 15-4513 binding in the cerebellum of control and small platform stressed mice

	Control	Small platform stress
Total specific binding		
B_{max} (fmol/mg protein)	2009.4 ± 204.8	2203.7 ± 599.8
K_d (nM)	5.75 ± 1.30	8.00 ± 1.64
Diazepam insensitive binding		
B_{max} (fmol/mg protein)	669.1 ± 113.1	661.4 ± 118.8
K_d (nM)	6.12 ± 1.50	6.51 ± 1.92
Diazepam sensitive binding		
B_{max} (fmol/mg protein)	1438.6 ± 589.6	1569.8 ± 572.1
K_d (nM)	6.70 ± 2.72	8.73 ± 2.19

The values are mean ± S.E.M. of 4–6 experiments.

3.4. [^{3}H]Ro 15-4513 binding

In preliminary experiments we were unable to demonstrate any diazepam-insensitive binding in other brain areas except cerebellum.

Small platform stress did not affect the binding parameters (B_{max} and K_d) of [^{3}H]Ro 15-4513 to either diazepam-sensitive receptors or diazepam-insensitive receptors in the cerebellum (Table 3).

4. Discussion

The major new finding in this study is the observation that exposure to small platform stress altered the behavioural consequences following the administration of ethanol alone and together with the benzodiazepine receptor inverse agonist, Ro 15-4513.

We found, as demonstrated many times before (Glowa et al., 1989; Dar, 1995) that the hypnotic actions of ethanol are dose-dependently antagonized by Ro 15-4513 in control animals. However, we also report that the administration of Ro 15-4513 in doses which antagonized ethanol-induced sleep in control animals, caused a dose-dependent potentiation of the hypnotic effects of ethanol in animals exposed to small platform stress. These observations were further substantiated by the observations that the combined treatment with ethanol plus Ro 15-4513 caused lethal effects in animals exposed to small platform stress with no such effect observed in control animals.

Since small platform stress produced a marked potentiation of ethanol-induced sleep we wanted to compare the influence of stress on the effects of ethanol on anxiety in the plus-maze situation. In line with previous reports, ethanol produced a dose-dependent attenuation of anxiety in control animals (Glowa et al., 1989; Dudek et al., 1994; June and Lewis, 1994), an effect that was accompanied by a significant increase in the locomotor activity of the animals. Administration of ethanol, in doses which reduced anxiety in control animals did not alter any of the anxiety parameters in small platform stress animals. However, this lack of effects of ethanol can perhaps not be directly attributed to a reduction of the pharmacological effects of ethanol. Statistical analysis was unable to demonstrate any interaction between the effects of ethanol and small platform stress. Since small platform stress by itself caused a marked attenuation of anxiety in the plus-maze (this study, Pokk and Zharkovsky, 1995) as well as in the hole-board test (Pokk and Zharkovsky, unpublished), the most likely assumption for the lack of effect of ethanol is that a further decrease of anxiety should not be expected due to the 'floor' effect for reducing anxiety which in certain situations can be observed in this behavioural paradigm.

In accordance with other studies (Wong and Skolnick, 1992), in preliminary experiments we were unable to demonstrate any diazepam-insensitive binding in other brain areas except cerebellum. In experiments performed with brain membranes from cerebellum we found no evidence for the idea that small platform stress alters the binding characterstics of [^{3}H]Ro 15-4513 in this brain area. These findings suggest that the recognition sites for [^{3}H]Ro 15-4513 are not directly involved in the altered effects of Ro 15-4513 or for the enhanced sensitivity to ethanol in small platform stressed animals.

In our experiments we compared the effects of muscimol and/or ethanol on $^{36}Cl^-$ uptake into microsacs from cortex and cerebellum of control and small platform stressed animals. In confirmation with a previous study (Pokk et al., 1996) small platform stress reduced GABA receptor agonist-stimulated $^{36}Cl^-$ uptake into microsacs from cortex but did not affect this in cerebellum.

Ethanol in concentrations (25–100 mM) similar to those found in the brain after its systemic administration in doses of 2–4 g/kg (Allan and Harris, 1989; Melchior and Allen, 1992) did not affect basal or muscimol-stimulated $^{36}Cl^-$ uptake into microsacs from either cortex or cerebellum. These data were in agreement with those of Mihic et al. (1992b), but at variance with some other reports (Allan and Harris, 1986; Suzdak et al., 1986; Mehta and Ticku, 1994).

In conclusion, the exposure to small platform stress results in a reduction of GABA agonist stimulated $^{36}Cl^-$ uptake in cortical brain tissue and increases the number of cortical benzodiazepine receptors labelled by [^{3}H]flunitrazepam (Pokk et al., 1996). The binding of another benzodiazepine ligand, [^{3}H]Ro 15-4513, to cerebellar membranes remains unchanged.

An obvious explanation would be that the increased sensitivity to the hypnotic effect of ethanol could be related to the increased number of benzodiazepine receptors seen in cortex of small platform exposed animals. However, the changes in the benzodiazepine receptor density after the stress exposure cannot explain the observed paradoxical enhancement of the neurotoxic effects of ethanol by Ro 15-4513.

It has been proposed in the literature that benzodiazepine receptors can exist in different conformational states and the effect of drugs depends on the 'resting state' or 'setpoint' of the receptors (File, 1984; File and Pellow, 1986). According to this hypothesis changes in the effects of the partial inverse agonist Ro 15-4513 could be explained by the shift of benzodiazepine receptors toward agonist conformation. However, in our previous experiments (Pokk et al., unpublished) Ro 15-4513 in doses of 0.5–10.0 mg/kg exerted a similar anxiogenic effect in the plus-maze and hole-board tests in both control and small platform stressed mice. Another possible explanation is that the coupling between recognition sites of the $GABA_A$ receptor-chloride ionophore complex is an important determinant of the antagonistic actions of Ro 15-4513 on the behavioural effects of ethanol. However, to which extent

changes in the coupling mechanisms between specific GABA and/or benzodiazepine recognition sites and/or the receptor coupled channel activity could account for the observed effects of ethanol and Ro 15-4513 remains to be further investigated.

Acknowledgements

This study was supported by the Estonian Scientific Foundation (Grants 2410 and 2373) and by the Karolinska International Research and Training Program (KIRT).

References

Allan, A.M. and R.A. Harris, 1986, Gamma-aminobutyric acid and alcohol actions: neurochemical studies of long sleep and short sleep mice, Life Sci. 39, 2005.

Allan, A.M. and R.A. Harris, 1989, A new alcohol antagonist: phaclofen, Life Sci. 45, 1771.

Asakura, W., K. Matsumoto, H. Ohta and H. Watanabe, 1994, Monoamine depletion attenuates the REM sleep deprivation-induced increase in clonidine response in forced swimming test, Pharmacol. Biochem. Behav. 49, 79.

Barbaccia, M.L., G. Roscetti, M. Trabucchi, M.C. Mostallino, A. Concas, R.H. Purdy and G. Biggio, 1996, Time-dependent changes in rat brain neuroactive steroid concentrations and $GABA_A$ receptor function after acute stress, Neuroendocrinology 63, 166,

Biggio, G., A. Concas, M.G. Corda, O. Giorgi, E. Sanna and M. Serra, 1990, GABAergic and dopaminergic transmission in the rat cerebral cortex: effect of stress, anxiolytic and anxiogenic drugs, Pharmacol. Ther. 48, 121.

Breschi, M.C., C. Martini, R. Scatizzi, R. Cristofani, G. Giannaccini, E. Martinotti and A. Lucacchini, 1995, Benzodiazepine agonists reverse the effects of noise exposure on central benzodiazepine receptors and cardiac responsiveness, Life Sci. 57, 1131.

Carden, S.E. and M.A. Hofer, 1990, Independence of benzodiazepine and opiate action in the suppression of isolation distress in rat pups, Behav. Neurosci. 104, 160.

Dar, M.S., 1995, Antagonism by intracerebellar Ro 15-4513 of acute ethanol-induced motor incoordination in mice, Pharmacol. Biochem. Behav. 52, 217.

Drugan, R.C., P.V. Holmes and A.P. Stringer, 1990, Pentobarbital blocks the stress-induced decrease in [^{3}H]Ro 5-4864 binding in rat kidney, Brain Res. 535, 151.

Dudek, B.C., T. Tritto and K.A. Underwood, 1994, Genetic influences on locomotor activating effects of ethanol and sodium pentobarbital, Pharmacol. Biochem. Behav. 48, 593.

File, S.E., 1984, Behavioural effects of pentylenetetrazole reversed by chlordiazepoxide and enhanced by Ro 15-1788, Naunyn-Schmiedeberg's Arch. Pharmacol. 326, 129.

File, S.E. and S. Pellow, 1986, Intrinsic actions of the benzodiazepine receptor antagonist Ro 15-1788, Psychopharmacology 88, 1.

File, S.E., L.J. Wilks and P.S. Mabbutt, 1989, Withdrawal, tolerance and sensitization after a single dose of lorazepam, Pharmacol. Biochem. Behav. 31, 937.

Glowa, J.R., J. Crawley, P.D. Suzdak and S.M. Paul, 1989, Ethanol and the GABA receptor complex: studies with the partial inverse benzodiazepine receptor agonist Ro 15-4513, Pharmacol. Biochem. Behav. 31, 767.

June, H.L. and M.J. Lewis, 1994, Interactions of Ro 15-4513, Ro 15-1788 (flumazenil) and ethanol on measures of exploration and locomotion in rats, Psychopharmacology 116, 309.

Korpi, E.R., 1994, Role of $GABA_A$ receptors in the actions of alcohol and in alcoholism: recent advances, Alcohol Alcohol. 29, 115.

Lister, R.G., 1987, Interactions of Ro 15-4513 with diazepam, sodium pentobarbital and ethanol in a holeboard test, Pharmacol. Biochem. Behav. 28, 75.

Lister, R.G. and D.J. Nutt, 1987, Is Ro 15-4513 a specific alcohol antagonist?, Trends Neurosci. 10, 223.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193, 265.

Malminen, O. and E.R. Korpi, 1988, GABA/benzodiazepine receptor/chloride ionophore complex in brains of rat lines selectively bred for differences in ethanol-induced motor impairment, Alcohol 5, 239.

Malminiemi, O. and E.R. Korpi, 1989, Diazepam-insensitive [^{3}H]Ro 15-4513 binding in intact cultured cerebellar granule cells, Eur. J. Pharmacol. 169, 53.

Mehta, A.K. and M.K. Ticku, 1994, Ethanol enhancement of GABA-induced $^{36}Cl^-$ influx does not involve changes in Ca^{2+}, Pharmacol. Biochem. Behav. 47, 355.

Melchior, C.L. and P.M. Allen, 1992, Interaction of pregnanolone and sulfate with ethanol and pentobarbital, Pharmacol. Biochem. Behav. 42, 605.

Meng, Z.-H. and M.S. Dar, 1994, Intrastriatal Ro 15-4513 functionally antagonizes ethanol-induced motor incoordination and striatal adenosinergic modulation of ethanol-induced motor incoordination in rats, J. Pharmacol. Exp. Ther. 271, 524.

Mihic, S.J., B.N.M. Van Berckel, B.F. O'Dowd, T. Nguyen and P.H. Wu, 1992a, Effects of sedatives on GABA-mediated chloride flux into cerebral cortical microsacs prepared from emotional and non-emotional mice, Eur. J. Pharmacol. 218, 283.

Mihic, S.J., P.H. Wu and H. Kalant, 1992b, Potentiation of γ-aminobutyric acid-mediated chloride flux by pentobarbital and diazepam but not ethanol, J. Neurochem. 58, 745.

Naijm, R.A. and K.H. Karim, 1993, Prevention of ethanol-induced gastric damage by the imidazobenzodiazepines Ro 15-4513 and Ro 15-3505 in rats, Pharmacol. Biochem. Behav. 45, 597.

Petry, N.M., 1995, Ro 15-4513 selectively attenuates ethanol, but not sucrose and reinforced responding in a concurrent access procedure: comparison to other drugs, Psychopharmacology 121, 192.

Pohorecky, L.A., 1990, Interaction of ethanol and stress: research with experimental animals – an update, Alcohol Alcohol. 25, 263.

Pokk, P. and A. Zharkovsky, 1995, The effects of drugs acting at GABA-benzodiazepine-barbiturate receptor complex on the behaviour of sleep-deprived mice, Pharmacol. Toxicol. 76, 23.

Pokk, P., T. Kivastik, D. Sobol, S. Liljequist and A. Zharkovsky, 1996, Is upregulation of benzodiazepine receptors a compensatory reaction to reduced GABAergic tone in the brain of stressed mice?, Naunyn-Schmiedeberg's Arch. Pharmacol. (in press).

Suzdak, P.D., R.D. Schwartz, P. Skolnick and S.M. Paul, 1986, Ethanol stimulates γ-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes, Proc. Natl. Acad. Sci. USA 83, 4071.

Uusi-Oukari, M. and E.R. Korpi, 1990, Diazepam-sensitivity of the binding of an imidazobenzodiazepine, [^{3}H]Ro 15-4513, in cerebellar membranes from two rat lines developed for high and low alcohol sensitivity, J. Neurochem. 54, 1980.

Wong, G. and P. Skolnick, 1992, High affinity ligands for 'diazepam-insensitive' benzodiazepine receptors, Eur. J. Pharmacol. 225, 63.

Wong, G., M. Uusi-Oukari, H.C. Hansen, P.D. Suzdak and E.R. Korpi, 1995, Characterization of novel ligands for wild-type and natural mutant diazepam-insensitive benzodiazepine receptors, Eur. J. Pharmacol. 289, 5.