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## ETHANOL AND BENZODIAZEPINES. THE INFLUENCE OF CGS 8216 ON THE ETHANOL-INDUCED HYPOTHERMIA AND MOTOR INCOORDINATION IN MICE AND RATS

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Ethanol has pharmacological profile very similar to benzodiazepines which facilitate GABA-ergic neurotransmission. In addition, a lot of ethanol-induced effects are partially antagonized by Ro 15-4513, a benzodiazepine inverse agonist. In our study, the influence of CGS 8216, another benzodiazepine inverse agonist, on the hypothermic (3.5 g/kg in mice, 3.0 g/kg in rats) and disturbing the motor coordination (3.2 g/kg in mice, 2.5 g/kg in rats, aerial righting reflex) effects of ethanol was investigated. The hypothermic effects of ethanol were antagonized in mice, and significantly attenuated in rats by CGS 8216 (10 and 20 mg/kg). Ethanol-induced motor incoordination was significantly diminished by 10 and 20 mg/kg of CGS 8216 in mice but not in rats. These data suggest that some effects of ethanol may result from the intensification of benzodiazepine/GABA-ergic activity. In addition, they let us presume that the activity of CGS 8216 is connected with a benzodiazepine receptor named BZ-1 or omega 1. The results indicate the need of further work on the benzodiazepine inverse agonists for use in treatment of ethanol poisoning.

**Key words:** *Ethanol, hypothermia, motor incoordination, CGS 8216, mice, rats.*

### INTRODUCTION

Several electrophysiological, behavioral, and biochemical studies indicate that some of the central effects of ethanol may be mediated *via* facilitation of gamma-aminobutyric acid (GABA) transmission, similar to a group of drugs such, as benzodiazepines (BZs) and barbiturates (1). However, the oneway action of ethanol and BZs has not been confirmed by all clinical investigations. Ethanol-induced depression is not potentiated by chlordiazepoxide (2) but the ethanol-induced motor incoordination is intensified by it (3). Most of the acute effects of ethanol can be potentiated by diazepam (4) and other BZs with predominant hypnotic properties.

Some of the ethanol-induced changes in behavior, e.g. an anxiolytic-like action (5) or motor incoordination (6, 7) are antagonized by Ro 15-4513 — the

benzodiazepine receptor partial inverse agonist. Ro 15-4513 has also been found to reverse the hypnotic (8) and hypoactive (9) activity of ethanol in animals and protect them against ethanol-induced intoxication (10).

Czernik et al (11) described CGS 8216 — pyrazoloquinoline derivative as a potent brain BZ receptor antagonist, and Langer and Arbilla (12) classified it as a selective antagonist of omega 1 subtype of this receptor. CGS 8216 markedly inhibits the binding of BZs to their receptors and antagonizes the characteristic effects of these drugs (11, 13). The compound given alone has shown some behavioral properties in being proconvulsive (14), anxiogenic (15) or mnemonic-enhancing (16) and therefore it has been defined as a weak inverse agonist of BZ receptors (17).

The mechanism of action of ethanol is not fully understood. It is presumed that the effects of ethanol on the central nervous system may be due to an alteration of the action of some neurotransmitters. The results of studies obtained by Criswell et al (18) show that ethanol may enhance GABA effects *via* influence on GABA<sub>A</sub>/BZ izoreceptor (also named BZ-1 or omega 1) in the brain. This GABA<sub>A</sub> izoreceptor (composed of the alfa-1, beta-2 and gamma-2 subunit) which binds zolpidem (an agonist of BZ-1 or omega 1 receptor), is sensitive to ethanol and ethanol enhancement of GABA. Two forms of the gamma-2 subunit, short and long, have been shown to exist (19). Wafford et al (20) have proved that only gamma-2 long (which differs from gamma-2 short by the addition of 8 amino acids) confers ethanol sensitivity to recombinant GABA<sub>A</sub> receptor by enhancing the efficacy of GABA in opening the Cl<sup>-</sup> channel. Recently Miralles et al (21) have shown a high expression of long form of gamma-2 subunit of GABA<sub>A</sub> receptor in neurons of those regions of the rat brain where ethanol potentiated the inhibitory effects of GABA.

Taking the above data into account, it seems interesting to find out whether CGS 8216 — an antagonist of BZ-1 or omega 1 BZ receptor subtype is capable of changing some of the ethanol effects.

The ethanol-induced motor discoordination and hypothermia in man and animals is generally well known. Although the mechanism by which ethanol produces the above effects is hardly known, it is possible that GABA-ergic and/or benzodiazepine systems are involved. The therapy of acute ethanol poisoning is very difficult. Clinical trials are taken of the administration, among others, the benzodiazepine antagonist — flumazenil as an antidote for ethanol-induced coma, but with poor efficacy (22). Also benzodiazepine inverse agonist Ro 15-4513 is able to antagonize some ethanol effects but the antagonism is not specific to ethanol. It also reverses the effects of barbiturates and benzodiazepines (23). Therefore, estimation of the antagonistic activity of other inverse agonist to ethanol effects seems to be needed for clinical therapy.

## MATERIAL AND METHODS

Experiment were carried out on male Wistar rats (160—220 g) and male Albino Swiss mice (17—25 g). The animals were kept 8—10 to a cage, at room temperature of  $20 \pm 1^\circ\text{C}$ , on natural day-night cycle (spring). Standard food (Bacutil, Motycz) and water were available ad libitum.

Ethanol (95% v/v — Polmos) was given intraperitoneally (ip) as 20% v/v of water solution in the doses of 3.2—3.5 g/kg in mice and 2.5—3.0 g/kg in rats. CGS 8216 (2-phenylpyrazolo [4.3-c]-quinolin-3(5H)-one) (Ciba Geigy) was administered subcutaneously (sc), as suspension in 0.5% methylcellulose (tylose) solution, in the doses of 10 and 20 mg/kg, 15 min before ethanol application. Control animals received only solvent. Experiments were carried out between 9<sup>00</sup> h and 15<sup>00</sup>.

*Choice tests*

Body temperature was measured in the rectum by means of a thermistor thermometer. The arithmetical mean of two measurements taken before drug injections was accepted as the initial value of temperature. Later on the measurements were taken at 30, 60, 90, 120 and 150 min after ethanol administration. A constant ambient room temperature of  $22 \pm 0.5^\circ\text{C}$  was maintained throughout the study.

Motor incoordination was investigated in the aerial righting reflex test of Frye et al (24). The animals were placed on the rod in back down position and allowed to fall free twice. The rod elevation was 3, 6, 9 up to 42 cm for mice and 5, 10, 15 up to 50 cm for rats. If the animals did not land correctly (on four paws) in both trials, the rod was elevated by 3 cm for mice or 5 cm for rats, and the test was repeated till positive results were scored.

*Statistical analysis*

The obtained data were elaborated statistically with Mann-Whitney U-test (motor incoordination) and the two-way analysis of variance followed by Duncan's test (body temperature).

## RESULTS

Ethanol, 3.5 g/kg in mice and 3.0 g/kg in rats, significantly decreased body temperature of animals ( $F = 47.553$ ;  $df\ 5/45$  and  $F = 37.717$ ;  $df\ 5/35$  respectively) (*Fig. 1 and 2*).

In mice, the ethanol hypothermic effects were abolished by 20 mg/kg of CGS 8216 ( $F = 14.485$ ;  $df\ 5/45$ ) and significantly diminished by 10 mg/kg ( $F = 44.313$ ;  $df\ 5/45$ ). Body temperature of mice was significantly ( $F = 2.723$ ;  $df\ 5/45$ ) elevated by 20 mg/kg of CGS 8216 given alone (*Fig. 1*).

Ethanol-induced hypothermia was significantly decrease by 10 and 20 mg/kg of CGS 8216 ( $F = 10.718$ ;  $df\ 5/35$  and  $F = 13.126$ ;  $df\ 5/35$  respectively) in rats. CGS 8216 (10 mg/kg) given alone did not affect body temperature of rats (*Fig. 2*).

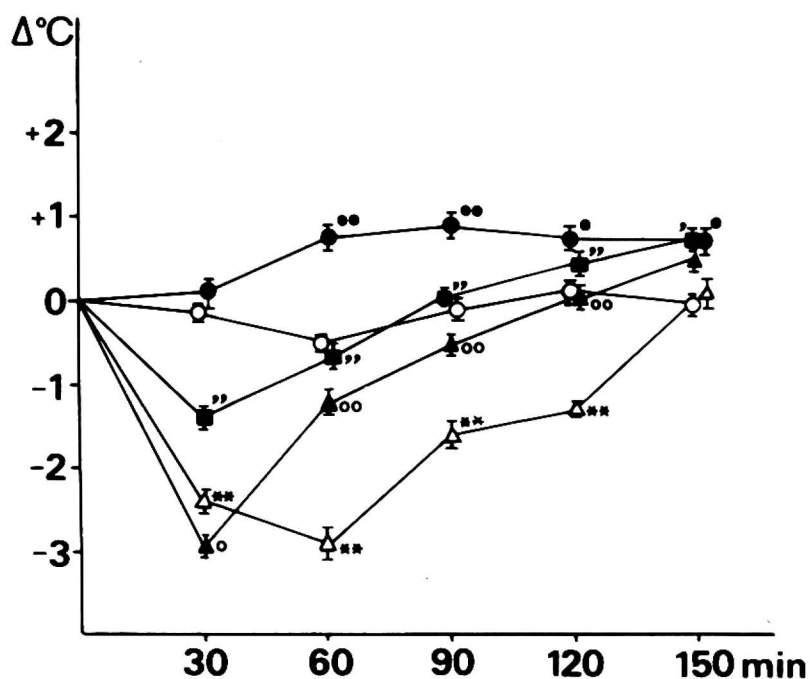


Fig. 1. The influence of CGS 8216 (CGS) on hypothermic effects of ethanol (EtOH) in mice.  $\circ$ — $\circ$  — control,  $\Delta$ — $\Delta$  — 0.5% tylose + EtOH 3.5 g/kg,  $\blacktriangle$ — $\blacktriangle$  — CGS 10 mg/kg + EtOH,  $\blacksquare$ — $\blacksquare$  — CGS 20 mg/kg + EtOH,  $\bullet$ — $\bullet$  — CGS 20 mg/kg + 0.9% NaCl \*\*— $p < 0.01$  vs control,  $o$ — $p < 0.05$ ,  $oo$ — $p < 0.01$  vs tylose + EtOH, '— $p < 0.05$ , ''— $p < 0.01$  vs tylose + EtOH,  $\bullet$ — $p < 0.05$ ,  $\bullet\bullet$ — $p < 0.01$  vs control (Duncan test). Each point represents the mean  $\pm$  SEM for a group of 10 mice.

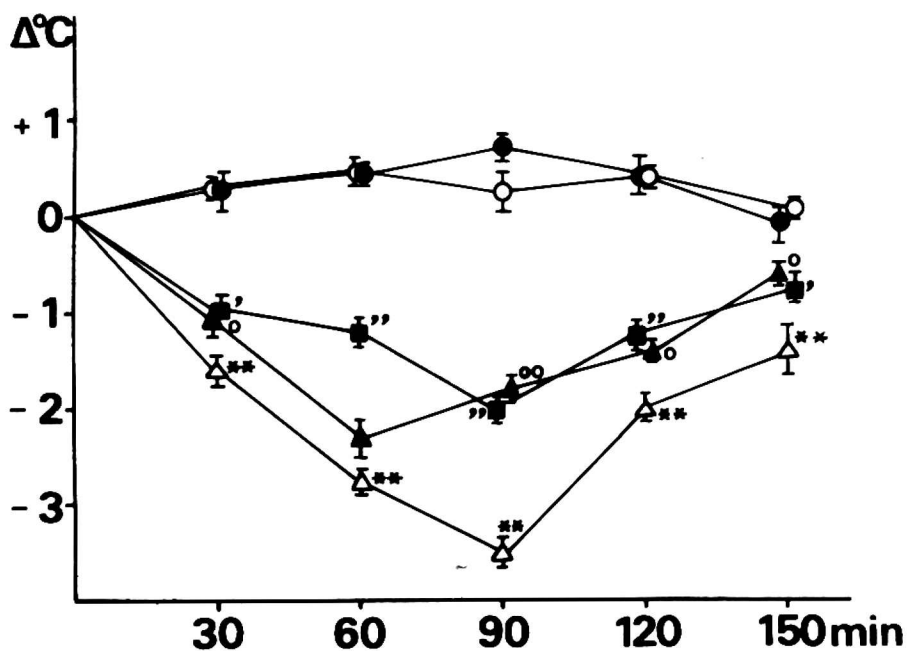


Fig. 2. The influence of CGS 8216 (CGS) on hypothermic effects of ethanol (EtOH) in rats.  $\circ$ — $\circ$  — control,  $\Delta$ — $\Delta$  — 0.5% tylose + EtOH 3.0 g/kg,  $\blacktriangle$ — $\blacktriangle$  — CGS 10 mg/kg + EtOH,  $\blacksquare$ — $\blacksquare$  — CGS 20 mg/kg + EtOH,  $\bullet$ — $\bullet$  — CGS 10 mg/kg + 0.9% NaCl \*\*— $p < 0.01$  vs control,  $o$ — $p < 0.05$ ,  $oo$ — $p < 0.01$  vs tylose + EtOH, '— $p < 0.05$ , ''— $p < 0.01$  vs tylose + EtOH (Duncan test). Each point represents the mean  $\pm$  SEM for a group of 8 rats.

Ethanol (3.2 g/kg in mice and 2.5 g/kg in rats) significantly disturbed motor coordination, and this was reflected in an increase in the elevation of the rod securing the proper landing posture of animals (Fig. 3 and 4). CGS 8216, given in the doses of 10 and 20 mg/kg, significantly diminished the above ethanol effect in mice (Fig. 3). In rats, ethanol-induced impairment of motor

coordination was not altered by 10 mg/kg, and was markedly attenuated by 20 mg/kg of CGS 8216 (Fig. 4). CGS 8216 given alone in the doses of 20 mg/kg in mice and 10 mg/kg in rats did not affect the motor coordination of the animals (Fig. 3 and 4).

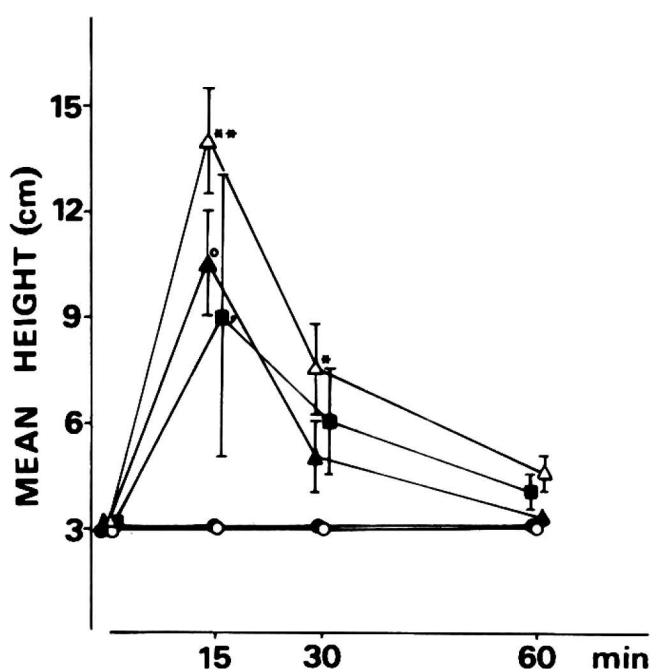


Fig. 3. The influence of CGS 8216 (CGS) on disturbance of motor coordination evoked by ethanol (EtOH) in mice measured as mean high necessary to „successful landing”.  $\circ-\circ$  — control,  $\Delta-\Delta$  — 0.5% tylose + EtOH 3.2 g/kg,  $\blacktriangle-\blacktriangle$  — CGS 10 mg/kg + EtOH,  $\blacksquare-\blacksquare$  — CGS 20 mg/kg + EtOH,  $\bullet-\bullet$  — CGS 20 mg/kg + 0.9% NaCl \* —  $p < 0.05$ , \*\* —  $p < 0.001$  vs control,  $\circ$  —  $p = 0.1$ , ' —  $p = 0.1$  vs tylose + EtOH (Mann-Whitney U-test). Each point represents the mean  $\pm$  SEM for a group of 8 mice.

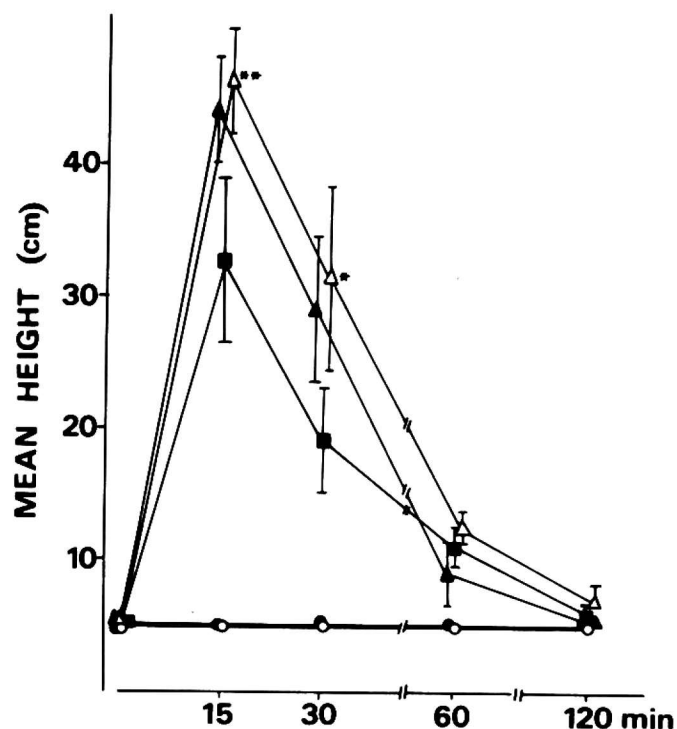


Fig. 4. The influence of CGS 8216 (CGS) on disturbance of motor coordination induced by ethanol (EtOH) in rats measured as mean high necessary to „successful landing”.  $\circ-\circ$  — control,  $\Delta-\Delta$  — 0.5% tylose + EtOH 2.5 g/kg,  $\blacktriangle-\blacktriangle$  — CGS 10 mg/kg + EtOH,  $\blacksquare-\blacksquare$  — CGS 20 mg/kg + EtOH,  $\bullet-\bullet$  — CGS 10 mg/kg + 0.9% NaCl \* —  $p < 0.05$ , \*\* —  $p < 0.01$  vs control, (Mann-Whitney U-test). Each point represents the mean  $\pm$  SEM for a group of 8 rats.

## DISCUSSION

Heat balance is centrally (heat production) and peripherally regulated (heat loss). One of the ethanol effects is hypothermia.

Neurotransmission of GABA is believed to be involved in the regulation of body temperature, although both a GABA-induced increase and decrease in body temperature have been reported. Dar and Wooles (25) have assumed, on the basis of results obtained from studies using aminooxyacetic acid (AOAA), bicuculline and ethanol, that the pathways and/or mechanism(s) for GABA-ergic and ethanol-produced hypothermia are different. Chan et al (26)

have shown that Ro 15-1788, a specific BZ antagonist, did not antagonize the hypothermic and sedative effects of ethanol in mice. These results are in agreement with other reports on the inability of Ro 15-1788 to antagonize some ethanol central actions (27, 28). The hypothermic effect of ethanol was also not blocked by Ro 15-4513 (7), and inverse BZ agonist with great potency to antagonize the central effects of ethanol (29).

Contrary to the above literature data, the results of our studies strongly demonstrate that CGS 8216, also termed a partial inverse agonist, decreased or inhibited hypothermia produced by ethanol in mice. In rats, the ethanol-induced hypothermia was diminished by CGS 8216, but the effect was markedly weaker than that observed in mice. The data enable us to say, that CGS 8216 is capable of antagonizing the ethanol-induced hypothermia, and to believe that the action may be, at least partially, a result of the interaction with omega 1 subtype of the BZ/GABA receptor complex. CGS 8216 appears to block selectively omega 1, while Ro 15-1788 blocks both omega 1 and omega 2 BZ receptor subtypes (12). The above differences in the selectivity of the antagonists may be the cause of their dissimilar influence on ethanol-induced hypothermia. The differences between the effects of CGS 8216 and Ro15-4513 on ethanol hypothermia is difficult to explain.

The ethanol-induced reduction of body temperature, after the large doses of alcohol beverages, may be dangerous for life (freezing). So, antagonizing of this hypothermia by CGS 8216 may be important for clinical practise.

Aerial righting reflex test is generally accepted and widely used. Its idea is based on the fact that an animal dropped from some height falls „on four paws”. Normal motor coordination is necessary for it. When motor coordination is disturbed the animal needs a greater height to land correctly. The test lets find out the degree of motor disturbance by estimating the height which is required for the animal to land correctly (on four paws).

The effects of ethanol resulting in impairment of motor function in animals may be related to the changes of GABA-ergic neurotransmission. It was shown that a decrease in GABA function caused by GABA antagonist — picrotoxin (30) and bicuculline (30, 31) induced inhibition, and an increase in GABA transmission, by AOAA (31), led to intensification of motor impairment produced by ethanol in rats. Similarly, Dar and Wooles (25) have shown that pretreatment with AOAA produced a significant potentiation of ethanol-induced motor incoordination in mice whereas pretreatment with bicuculline did not. Ethanol intoxication measured as impaired performance on an accelerating rotarod was also completely blocked by Ro 15-4513 (7).

In our study, ethanol-induced motor discoordination, tested in aerial righting reflex, was slightly, but in a dose dependent manner, attenuated by 10 and 20 mg/kg of CGS 8216 in mice ( $p = 0.01$ ) and in rats (statistically non significant results).

Bernard et al (32) have indicated the lack of influence of 20 mg/kg of CGS 8216 on the ethanol-induced impairment of rotarod performance in rats. Perhaps the slight divergences can be explained by the use of different methods to estimate the disturbances of motor function and the administration of a lower (1600 mg/kg) dose of ethanol. In our study ethanol was given in the dose of 2.5 g/kg in rats and 3.2 g/kg in mice. The ability of CGS 8216 to suppress motor disturbances was also found in the studies of Boast et al (13) and Shannon and Katzman (33). They have demonstrated the blocking influence of CGS 8216 on ataxic effects of diazepam as measured on the rotarod in rats. It is necessary to underline that the CGS 8216 given alone did not affect the motor coordination of animals both given in low doses of 0.3—3.0 mg/kg (in rotarod test) (33) and in high doses of 10 and 20 mg/kg (in aerial righting reflex test, our results).

In summary, the present data indicate that benzodiazepine inverse agonist, CGS 8216 can antagonize hypothermia and markedly attenuate the motor impairment produced by ethanol. The results seem to show that the studied effects of ethanol are, at least partially, a result of the intensification of BZ/GABA-ergic activity. Our behavioral experiments and literature data suggest that CGS 8216 may act via omega 1 or BZ-1 subtype of GABA/BZ receptor complex.

The obtained results suggest the need of further work on the benzodiazepine inverse agonists for in treatment of ethanol poisoning in human.

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