

INFLUENCE OF CLONAZEPAM AND CARBAMAZEPINE ON ALCOHOL WITHDRAWAL SYNDROME, PREFERENCE AND DEVELOPMENT OF TOLERANCE TO ETHANOL IN RATS

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Influence of clonazepam and carbamazepine on alcohol withdrawal syndrome, preference and development of tolerance to ethanol in rats.
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The effects of clonazepam (0.3 and 1.0 mg/kg or 0.1 mg/kg, *b.i.d.*, 5 days) and carbamazepine (50 and 100 mg/kg or 12.5 and 50 mg/kg *b.i.d.*, 5 days) on alcohol withdrawal syndrome in rats were investigated. Moreover, the influence of clonazepam (0.3 mg/kg, single dose, or repeated doses for 8 days) and carbamazepine (50 mg/kg, single dose, or repeated doses for 8 days) on the development of tolerance to ethanol was also examined. To study the influence of clonazepam and carbamazepine on preference to ethanol, both drugs were administered for 5 days during the last week of the experiment, (clonazepam at 0.1 mg/kg, *b.i.d.*, *ip* and carbamazepine at 12.5 mg/kg, *b.i.d.*, *ip*).

Clonazepam and carbamazepine administered at single doses as well as multiple doses diminished the symptoms of withdrawal syndrome. Clonazepam did not prevent the development of tolerance to sleep-inducing and hypothermal action of ethanol, while carbamazepine prevented the development of tolerance to hypnotic effect of ethanol. Carbamazepine clearly reduced preference to ethanol (significantly vs. the control group and vs. the baseline values). Clonazepam also diminished preference to alcohol, but only in comparison with baseline values.

Key words: *clonazepam, carbamazepine, ethanol, withdrawal, preference, tolerance, rats*

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INTRODUCTION

Alcohol abuse still remains an important problem and its consequences may be life threatening. There are various methods to treat abstinence syndrome, but in spite of tremendous efforts to find treatments which could lead to decreased preference for ethanol (ETOH) or that could safely and effectively treat the withdrawal syndrome, there is still a need for search for new compounds to treat ETOH dependence as well.

As the clinical picture of ethanol withdrawal often includes tremor or even seizures, the drugs, which have anxiolytic and anticonvulsant properties, are used to treat alcohol abstinence. Both clonazepam and carbamazepine have been used in clinical practice to alleviate the symptoms of alcohol dependence or withdrawal. However, various clinical guidelines are inconsistent as far as the first-line treatment of alcohol withdrawal is considered [1, 13, 16, 23, 24].

Some authors suggest benzodiazepines for first-line treatment of alcohol withdrawal, especially in the severe cases, while others show that carbamazepine may have some advantages as its administration can start before cessation of alcohol intake, and it may decrease the intensity of dysphoria and seizure frequency [4]. Others have indicated that carbamazepine may increase ETOH-induced hyperactivity [5].

The suggested mechanism of action of both drugs is thought to consist in the augmentation of GABAergic transmission, while carbamazepine seems to possess some other advantageous pharmacological properties, like enhancement of adenosine receptors function [8], antagonism to NMDA receptors [12], increased sensitivity of serotonin and opioid receptors, which may contribute to additional anxiolytic, anticonvulsant, antidepressant, antimanic and mood-stabilizing effects [15, 21].

Prolonged use of ETOH may produce ETOH tolerance, that in turn requires the subjects to consume higher doses of ETOH, which then may cause organic brain damage. Alcohol-induced epilepsy may further develop and anticonvulsant medication is needed in such cases. On the other hand, sporadic, excessive consumption of alcohol is not uncommon among epileptic patients, therefore, detailed knowledge of interactions of antiepileptic drugs with ETOH is crucial. Thus, patients' safety may depend on the adequate choice of an antiepileptic drug. ETOH clearly interacts with GABA [2,

3] and excitatory amino acid (EAA) neurotransmitter systems [10, 11, 14].

Prolonged use of ETOH induces hypoactivity of GABAergic system and, due to organic disturbances, epileptiform pattern in EEG recordings is observed. Thus, antiepileptic drugs may diminish or stop kindling. Clonazepam enhances function of serotonergic receptors, which may facilitate anxiolytic effect of endogenous serotonin or exogenous "serotonergic" drugs [28]. Therefore, we decided to evaluate the influence of two commonly used drugs: clonazepam and carbamazepine on the course of ETOH dependence, withdrawal and tolerance to ETOH in rats, in order to elucidate their pharmacological differences and compare their theoretical usefulness.

MATERIALS and METHODS

Animals and treatment

The experiments were carried out on male Wistar rats (180–220 g). They were housed in group cages under normal laboratory conditions (temperature of 20–21°C, natural day/night cycle) and they had free access to commercial chow and water (except for the specified periods of some tests). All experiments were performed between 11.00 a.m. and 02.00 p.m. ETOH was given intraperitoneally (*ip*) or intragastrically as a 20% w/v solution or as a 5% w/v solution and 8% w/v solution in a free choice period during the preference test.

In alcohol withdrawal tests clonazepam (Clonazepam; Polfa, Tarchomin, Poland) was given *ip* at doses of 0.3 mg/kg and 1.0 mg/kg 60 min before the test or *b.i.d.*, *ip*, 0.1 mg/kg for five days while carbamazepine (Amizepin; Polfa, Starogard Gdański, Poland) was administered *ip* at doses of 50 mg/kg and 100 mg/kg, 60 min before the test or *b.i.d.*, *ip*, 12.5 mg/kg for five consecutive days. In chronic experiments the last dose of the studied drug was given 24 h before the test.

In the examination of tolerance, clonazepam was given *ip* for 8 days at a dose of 0.3 mg/kg and carbamazepine was given also *ip* for 8 days at a dose of 50 mg/kg.

In ETOH preference tests, clonazepam was given *ip*, *b.i.d.* for 5 days, a dose of 0.1 mg/kg during the fifth week of the experiment while carbamazepine was given in the same manner at a dose of 12.5 mg/kg.

The studied drugs were given *ip* in 1% methylcellulose suspension.

The experiments were carried out in strict accordance with Polish governmental regulation concerning experiments on animals.

Alcohol abstinence syndrome

Rats were given intragastrically a priming dose of 5.0 g/kg of ETOH, while the subsequent doses (three per day) were adjusted depending on the behavioral signs of intoxication according to Majchrowicz [26]. ETOH was given for next four days (three times daily at 09.00–10.00, 14.00–15.00 and 21.00–22.00, total daily doses ranging from 9 g/kg to 15 g/kg. The study drugs were given *ip* 60 min before assessing abstinence syndrome (acute experiments). In chronic experiments the animals received the drugs for 5 days. The abstinence syndrome was evaluated (24 h after the last dose of the drug and 18 h after the last dose of ETOH) by assessing body and tail tremor and rigidity; moreover, audiogenic seizure response (ASR) was also recorded. ASR was elicited with an electric buzzer (100 dB) and the intensity of the response was scored in 10-point scale, according to Jobe et al. [22]. While assessing the intensity of seizures, a particular attention was drawn to the type of seizures, whether they occurred with adduction or extension of anterior or posterior extremities and if they appeared following one encircling or two in the cage. Body and tail tremor and rigidity were assessed according to the method of Majchrowicz [26]. The 3-point scale was used: 0 – indicated no tremor or rigidity, 1 – mild tremor or rigidity and 2 – represented the highest intensity of tremor or rigidity. Motility was recorded by placing rats in photoelectric actometers (Universal Motility Meter, UMM-1-061 COTM Białystok, Poland). Experiments were performed in a special sound-proof room. The animals were not previously adapted to the apparatus. Locomotor activity was measured during 30 min, starting 30 min after ETOH administration with or without acute or repeated administration of the study drugs.

Each group consisted of 8 to 10 rats.

Ethanol preference

In order to obtain animals preferring ETOH, a procedure developed by Dyr and Kostowski [9] was used. Seventy rats were used for the experiment. Each group consisted of 6 to 8 rats. During

the first week of experiments rats received intragastrically 20% (w/v) ETOH solution twice daily (at 09.00–10.00 and 16.00–17.00), 5 g/kg. They had free access to commercial chow and tap water. During the next two weeks the animals had free access to the tap water only for one hour per day; during the remaining 23 h the only source of fluid was 5% (w/v) ETOH solution (second week of experiment) and then 8% ETOH solution (third week of experiment). The consecutive two weeks were the baseline consumption measurement period (fourth week) and the treatment period (fifth week). During that time the animals were presented with a free choice (two bottles) between water and 8% ETOH solution. Measurement of fluid and refilling were performed every day (at 09.00–11.00) when the positions of water and ETOH bottles were exchanged. A high and a low preference group was selected on the basis of the fluid intake during the fourth week of the experiment (i.e. during the baseline consumption measurement period, five consecutive everyday measurements from Monday to Friday). The total fluid intake in g/kg/24 h and absolute ETOH consumption in g/kg/24 h were calculated. The ETOH preference was expressed as the percentage of ETOH solution (8%) consumed against total fluid consumption. The rats with a mean intake of ETOH solution exceeding 50% of their total fluid intake were considered as the high-preferring group (HP), while the low-preferring group (LP) was made up of rats with a mean consumption of ETOH solution less than 20% of their total daily fluid intake. The rats not fulfilling any of these criteria were discarded. After gathering of the baseline consumption data, the animals in each group were treated (two times daily, at 09.00 and 20.00 for 5 consecutive days, Monday to Friday) with clonazepam (0.1 mg/kg, *ip*, *b.i.d.*) or with carbamazepine (12.5 mg/kg *ip*, *b.i.d.*) for 4 consecutive days, while one separate group received methylcellulose. The measurement of the fluid intake and ETOH intake in this period was performed in the same way as in the preceding baseline consumption measurement period. The results from the treatment period were compared with those obtained during the baseline measurement period.

Induction of ethanol tolerance

The tolerance to hypnotic and hypothermic effects of ETOH was induced according to Majchrowicz [26] with some modifications. Each group

consisted of 8 to 10 rats. In the rats pretreated with clonazepam or carbamazepine for 8 days, ETOH was administered on the 5th day of the experiment. On this day the rats received 5 g/kg *ip* of ETOH, and their sleeping time was determined (the sleeping time was measured as time to regain a righting reflex) and body temperature was measured in the rectum with an Ellab electric thermometer. The initial temperature was measured 3 times and the mean from these measurements was regarded as a basal initial temperature. During the following 3 days rats received ETOH orally at a dose of 5 g/kg and on the fifth day ETOH was given *ip*. After the last treatment, 24 h after the last dose of the study drugs for pretreated rats, the sleeping time and body temperature were measured. The comparison was made between the results obtained on the 1st and 5th day of ETOH administration.

Statistics

The normality of distribution was checked by means of Kolmogorow-Smirnow test, with Lilliefors correction and then variance equality was tested by Fisher's test. The statistical evaluation was performed by means of Mann-Whitney U-test or Wilcoxon matched pair test, by use of Statistica for Windows 4.0 program.

RESULTS

Alcohol abstinence syndrome

Rats which had been treated with ETOH for 5 days, developed alcohol dependence. They showed the symptoms of alcohol abstinence syndrome (marked hypermotility, body and tail tremor and rigidity, aggression, piloerection) 18 h after cessation of ETOH administration. In this period, the animals had decreased seizure threshold to audiogenic stimuli. The symptoms were best to observe and measure 18 h after discontinuation of alcohol administration. The intensity of body and tail tremor and rigidity scored according to 3-point scale was: body rigidity 1.78 pt., tail rigidity 1.56 pt., body tremor 1.22 pt., tail tremor 1.44 pt. ASR was scored at 1.44 pt.

Clonazepam given at higher dose decreased all examined symptoms of abstinence syndrome while at a dose of 0.3 mg/kg it decreased only the intensity of tail tremor (Fig. 1). Carbamazepine administered at both doses diminished intensity of body

and tail tremor, while at a lower dose it increased body and tail rigidity. Acute administration of clonazepam and carbamazepine at all examined doses decreased the intensity of ASR (Fig. 1).

Chronic administration of clonazepam or carbamazepine did not influence ASR, but decreased the intensity of body and tail rigidity and body and tail (clonazepam only) tremor (Fig. 1).

Ethanol preference

Out of 62 animals, 24 rats fulfilled the criterion for inclusion into the group of high preference, while 18 animals composed the low preference group. There was no significant difference in body weight between the preferring and non-preferring group. Treatment with clonazepam did not lower the ETOH preference (in high-preferring group of rats) in comparison to the control group, but only in comparison with baseline values. Carbamazepine clearly decreased ETOH preference not only in comparison with baseline values, but also in relation to the control group. Neither clonazepam nor carbamazepine changed ETOH intake or preference in low-preferring group (Fig. 2). Clonazepam and carbamazepine failed to influence total fluid intake in HP and LP rats.

Induction of ethanol tolerance

In control rats tolerance to hypnotic effect of ETOH developed in the course of five-day treatment: the sleeping time was shorter on the last day than on the first day of treatment. On the first day of treatment with ETOH, it evoked sleep lasting 58 min, while after 5 days of treatment it caused sleep lasting 30 min. Clonazepam given at a dose of 0.3 mg/kg did not prevent the development of tolerance either to hypothermic or to hypnotic effects of ETOH. Carbamazepine prevented the development of tolerance to sleep-inducing and hypothermic effect of ETOH (Fig. 3).

DISCUSSION

Prolonged treatment with alcohol exerts multiple adaptive changes in neurotransmission. The characteristic outcome of these changes is withdrawal syndrome, which is a manifestation of disturbed balance and some adaptive and compensative mechanisms in neurotransmission that occur during alcohol intake. Some mechanisms have

been claimed to be responsible for this situation, including disturbed GABA [28], NMDA [18, 19], adenosine [7, 8], opioid [20], dopaminergic [5] and noradrenergic neurotransmission [10, 22]. Mechanisms of ETOH preference are even more unclear and seem to be due to compensative and adaptive changes in the brain produced by alcohol intake.

Some clinical reports suggest the efficacy of clonazepam and carbamazepine in treating ETOH withdrawal [15, 21]. Benzodiazepines were proved to be effective without any doubt, and numerous studies have suggested usefulness of carbamazepine too [27]. However, even now, years after first time these drugs have been used in clinical set-

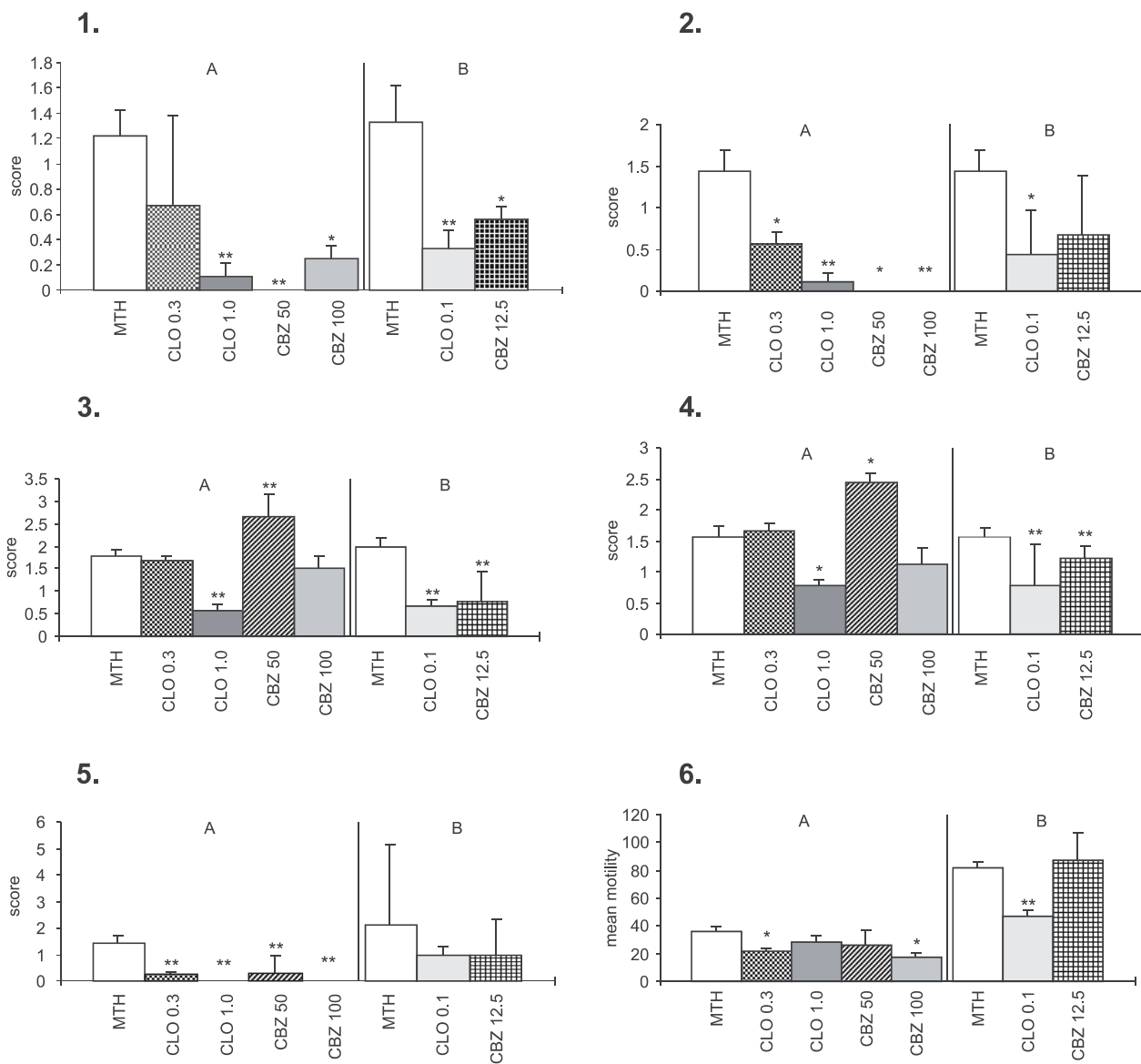


Fig. 1. The influence of acute (A) and chronic (B) treatment with clonazepam and carbamazepine on alcohol abstinence syndrome in rats. 1 – body tremor, 2 – tail tremor, 3 – body rigidity, 4 – tail rigidity, 5 – audiogenic seizure response, 6 – locomotor activity; MTH – control (methylcellulose), CLO – clonazepam at 0.3 mg/kg, 1.0 mg/kg, or 0.1 mg/kg *b.i.d. ip*, CBZ – carbamazepine at 50 mg/kg, 100 mg/kg or 12.5 mg/kg *b.i.d. ip*. Clonazepam and carbamazepine were given 60 min before the test (acute experiments) or in chronic experiments (5 days, *b.i.d.*) last dose of study drugs was injected 18 h before the test. Results are presented as means ± SEM (Mann-Whitney U-test); * p < 0.05, ** p < 0.01 vs. control (methylcellulose) group

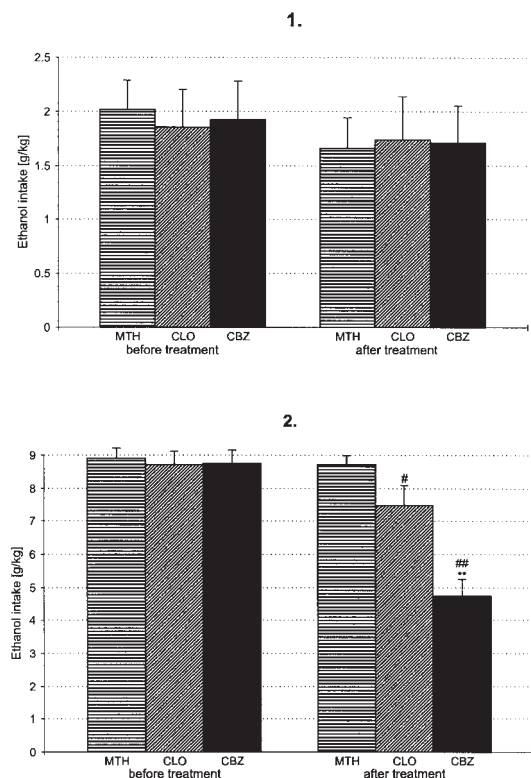


Fig. 2. The influence of clonazepam and carbamazepine on ethanol preference. 1 – low preferring group, 2 – high preferring group, MTH – methylcellulose (control), CLO – clonazepam (0.1 mg/kg, *b.i.d.* for 5 days, *ip*), CBZ – carbamazepine (12.5 mg/kg, *b.i.d.* for 5 days, *ip*). Results are presented as means \pm SEM; * $p < 0.05$, ** $p < 0.01$ vs. control (methylcellulose) group (Mann-Whitney U-test). Significant difference in comparison with the initial value, # $p < 0.05$, ## $p < 0.01$ (Wilcoxon matched pair test)

tings, it remains uncertain if some of the benzodiazepines show clear advantages over the others in the class or if their use may be stopped in favor of some other compounds like carbamazepine or valproate. The potent action of clonazepam is thought to be due to its strong agonistic action at GABA_A receptors. Other studies have shown that chronic ETOH intake may decrease number and sensitivity of GABA_A receptors [3, 4]. Thus, clonazepam beneficial effect is understandable and clear. Carbamazepine, however, may increase GABAergic transmission in more indirect way [29, 30]. It has also been suggested that carbamazepine efficacy in preventing occurrence of seizures in ETOH withdrawal, may be due to its “anti-kindling” effect [4]. Prolonged treatment with carbamazepine may potentiate adenosine binding to adenosine receptors. It has been shown that adenosine receptor antagonists may induce seizures whereas adenosine receptor agonists exert antiepileptic effects [7, 8].

There have been some reports that ETOH anticonvulsant effect may also be due to its agonistic action at non-mu opioid receptors [20]. Carbamazepine has been suggested to increase sensitivity of opioid receptors too [20].

Thus, complex mechanism of action of carbamazepine may account for its wider spectrum of actions. In our experiments, we have shown that acute administration of clonazepam diminishes symptoms of ETOH withdrawal. This may be understood on the basis of GABAergic theory which

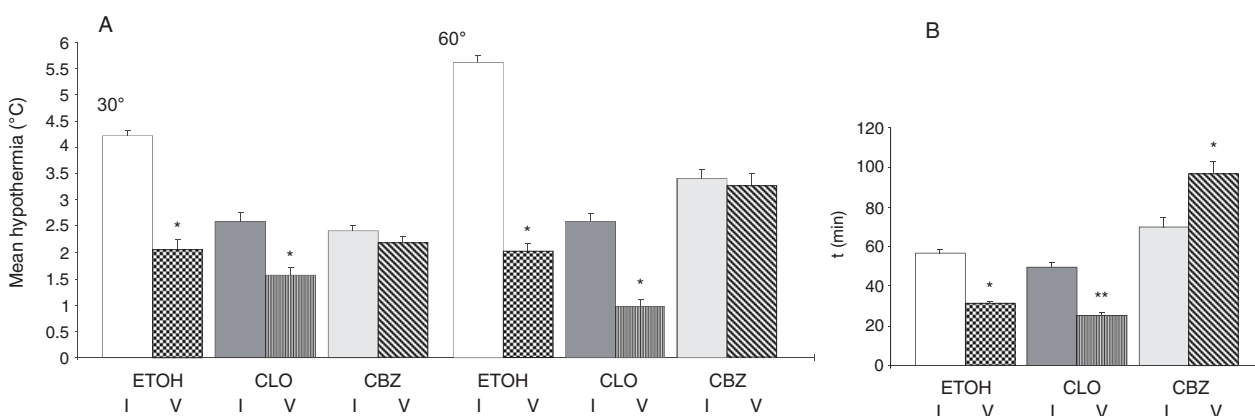


Fig. 3. The effect of treatment with clonazepam and carbamazepine on the development of tolerance to hypothermic (A) and hypnotic (B) effects of ethanol (ETOH) in rats. Clonazepam and carbamazepine were administered for 8 days. ETOH was given from the 5th to the 9th day of the experiment. The comparison was made between the results obtained on the first and the fifth day of ETOH administration (that are 5th and 9th day of the experiment). The temperature was measured 30 and 60 min after injection of ETOH. I, V – first and fifth day of ETOH treatment; CLO – clonazepam at 0.3 mg/kg, *b.i.d.*; CBZ – carbamazepine at 50 mg/kg, *b.i.d.* Results are presented as means \pm SEM; * $p < 0.05$ (Mann-Whitney U-test) significant difference in comparison with the results of the first day of treatment with ETOH

claims that acute ETOH administration increases activity of GABA_A receptors. Also prolonged administration of clonazepam was shown to decrease the intensity of ETOH withdrawal syndrome. Prolonged administration of carbamazepine decreased body and tail rigidity and body tremor, but did not influence total locomotor activity. Some researchers demonstrated that prolonged carbamazepine treatment increased motility during ETOH withdrawal, therefore, they did not recommend its use in alcohol-dependent individuals [17]. Our study did not show this effect, but duration of carbamazepine administration was relatively short (5 days).

During this time symptoms of withdrawal are most evident. It has been shown that ETOH prevents occurrence of withdrawal and benzodiazepines and carbamazepine which partly has pharmacological properties similar to ETOH, may substitute for some symptoms that occur during the ETOH withdrawal. Ballenger and Post [4] have proposed that anti-kindling effect of carbamazepine may contribute to its superior way of action over benzodiazepines. They have also proposed that this mechanism of action may be responsible for decreased preference to ethanol.

Chronic administration of clonazepam increases the number and activity of 5-HT₁ receptors [28]. Increased serotonergic transmission is believed to reduce ETOH preference, although clinical studies confirmed this hypothesis only in depressed patients who abused ETOH. In our study clonazepam itself was shown to be ineffective in decreasing ETOH preference, but this result may be due to its short administration in this model, since its augmenting effects appear usually after more than two weeks of treatment.

In our experiments, carbamazepine decreased ETOH intake in comparison to control group and to initial values, whereas clonazepam administration decreased only ETOH intake in comparison to initial values. This may be the result of wider pharmacological properties of carbamazepine.

Treatments with carbamazepine, but not with clonazepam, prevented occurrence of tolerance to hypothermic effect of ETOH and prolonged the ETOH-induced sleeping time. It has been previously reported by others that carbamazepine treatment may be associated with increased time to regain a righting reflex and increased motor incoordination [6, 31]. It seems, however, to be in dis-

crepancy with carbamazepine ability to increase motility in animals and possibly in humans too.

CONCLUSIONS

1. Both clonazepam and carbamazepine reduced intensity of ETOH withdrawal syndrome.
2. Carbamazepine, but not clonazepam, prevented the development of tolerance to ETOH and diminished ETOH preference.
3. Clonazepam should be used in short-time treatment schedules and its withdrawal tapered cautiously. Carbamazepine may be a useful tool in the treatment of craving and preference to ETOH since possessing anticonvulsant activity it may be used even in the treatment of early stages of ETOH withdrawal.

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