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Influence of ethanol on the myorelaxant effect of diazepam in rats

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Interaction of ethanol with benzodiazepines can lead to enhanced therapeutic anxyolytic, sedative and hypnotic effects but can also augment unwanted effects such as drowsiness, confusion, amnesia and impaired coordination. In this study we investigated the interaction between ethanol and diazepam and its influence on muscle strength in rats using the grip-strength meter. Three doses of ethanol (0.4, 0.6 and 0.8 g kg^{-1} of body mass) and diazepam (0.75, 1.5 and 7.5 mg kg^{-1} b.m.) were used in experiments. Single substances and their combinations were tested. The myorelaxant effect of ethanol, measured as grip force (expressed in grams), was dose-dependent. The lowest dose (0.4 g kg^{-1} b.m.) failed to affect muscle strength while the strongest effect was observed with the highest dose of ethanol (0.8 g kg^{-1} b.m.) and it lasted for 75 min. Diazepam dose-dependently reduced muscle strength too. However, when ethanol was combined with diazepam (1.5 mg kg^{-1} b.m.), more enhanced muscle relaxation occurred than by either drug alone. Namely, two lower doses of ethanol (0.4 and 0.6 g kg^{-1} b.m) enhanced the myorelaxant effect of diazepam by additional 26 and 46%, respectively, when measured after 15 min. The most pronounced myorelaxation was recorded when the highest dose of ethanol (0.8 g kg^{-1} b.m.) was combined with diazepam: from complete muscle relaxation observed after 15 min, it gradually decreased to 91% at the 45th min and to 24% at the 105th min after the beginning of the treatment.

The results of this preclinical investigation showed that ethanol enhanced the muscle relaxant effect of diazepam in rats. This enhancement as well as duration of the effect was dependent on the applied dose of ethanol.

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Acute ingestion of ethanol combined with benzodiazepines is widespread and is responsible for several toxicological interactions, which can have significant clinical implications. Ethanol is used as a social »drug« and is legal and freely available. However, it could be very dangerous, especially because of its ability to influence the pharmacokinetics or pharmacodynamics of many prescribed and over-the-counter therapeutic agents, for example, benzodiazepines, barbiturates, some antidepressants, antipsychotics, analgetics, antibiotics. These interactions can lead to a changed therapeutic effect because ethanol can influence absorption and metabolism or interfere with the drug at its site of action. However, the results of interactions between ethanol and therapeutic agents depend on the amount and duration of alcohol consumption (acute or chronic alcohol intake) (1).

On the other hand, benzodiazepines are extensively used in the pharmacotherapy of anxiety disorders and sleep disturbances throughout the world. In addition to anxiolytic, sedative and hypnotic effects, benzodiazepines, especially diazepam, have a well documented and therapeutically valuable myorelaxant effect. Therefore, diazepam is used to treat chronic muscle spasms, such as example chronic back pain and spasticity resulting from birth injury, cerebral vascular disease, or spinal cord lesions. Benzodiazepines reduce the muscle tone by central action that is independent of their sedative effect. The drugs reduce the background tone of the muscle without seriously affecting its ability to contract transiently under voluntary control (2). Clinical applicability of this class of drugs is limited in distressed patients with a current or prior history of alcoholism. However, combined use of anxiolytics and ethanol is widespread and well documented (3). Acute ingestion of alcohol combined with benzodiazepines, especially diazepam as one of the commonly prescribed benzodiazepine, is responsible for several toxicological interactions that can have significant clinical implications. Fatal poisoning involving coadministration of these substances continues to be a serious problem (4). Unfortunately, benzodiazepines are often prescribed without adequate information concerning the patient's ethanol use. It should be stressed that ethanol is able to enhance myorelaxation and impair coordination, especially if combined with other central nervous system depressants, which considerably affects manual skills and also driving performance.

Therefore, in the present study we investigated the intensity of the myorelaxant effect of acutely administered different doses of diazepam and ethanol alone in rats using the grip-strength meter. Further, the dose- and time-dependence of ethanol's influence on myorelaxant action of diazepam, when used concomitantly, was investigated. The employed doses of these substances were chosen to be lower than the doses commonly used in other preclinical experiments. Namely, the aim of the present study was to find the lowest dose of ethanol that could significanty enhance the myorelaxant effect of diazepam.

EXPERIMENTAL

Animals

The experiments involved female Wistar rats, approximately three months old and weighing about 180 ± 10 g, under standard laboratory conditions (temperature 22 °C with changes of light and dark every 12 hours and constant access to food and water). The rats were divided into 9 groups, 5–7 animals in each group.

Tested substances

Diazepam solution (Normabel® ampoules, Belupo, Croatia) was diluted in 0.9% NaCl. Diazepam (0.75, 1.5 and 7.5 mg kg^{-1} b.m.) was injected intraperitoneally in a volume of 0.5 mL. Three doses of ethanol were used: 0.4, 0.6 and 0.8 g kg^{-1} b.m. These were administered intraperitoneally as 0.5, 0.75 or 1 mL of 15% (*V/V*) solution per animal, respectively.

Each rat within a treated group received a single dose of diazepam or ethanol 15 min before measurement. In combined treatments, diazepam was applied 5 min before ethanol. The same animals were used for control measurements and received 0.5 mL saline only.

Method

Muscle strength was measured using the grip-strength meter method (Ugo Basile, Italy). The apparatus measures the pull force (expressed in grams) necessary to overcome the animal's forelimbs grip-strength to the bar connected to a force transducer. This forelimb grip-strength procedure is accepted as a useful screening test for identification of the potential muscle relaxant properties of drugs (5). The animal is placed over a plate, in front of a grasping trapeze, which is the arm of a force transducer connected to the peak amplifier. When pulled by the tail, the animal grasps the trapeze. Namely, rodents instinctively grab anything they can to try to stop this involuntary backward movement. After the animal loses its grip on the grasping trapeze, the peak preamplifier automatically stores the peak pull force achieved by the forelimbs and shows it on the liquid crystal display. Control values for each group of animals were obtained measuring the grip force before treatments with the tested substances.

In the present study, measurements of muscle strength started 15 min after the last intraperitoneal application of tested substances and lasted until complete recovery of muscle strength.

Statistical analysis

Results are presented as mean ± SE. Significance of the difference between the results was determined using the Newman-Keuls test for independent groups. Difference between means was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The rodent grip-strength test, employed in this study, represents a useful screening test for identification of the potential muscle relaxant properties of different substances (6). Also, the effects of drugs, toxins, diseases, ageing or neural damage may be assessed using this test.

The present experiments in rats, involving the grip-strength test, showed that myorelaxant effect of ethanol alone was dose- and time-dependent. The strongest response was obtained with the dose of 0.8 g kg^{-1} b.m. and lasted for 75 min. The maximal reduc-

Table I. Time profile of the myorelaxant effect of ethanol

Ethanol dose (g kg^{-1} b.m.)	Grip strength (g) after the application of ethanol (min)[a]				
	0	15	45	75	105
0.4	364.2 ± 8.6	346.5 ± 20.5	385 ± 20.8	370.3 ± 15.3	NM
0.6	360.3 ± 16.2	277.1 ± 32.1[b]	348 ± 23.7	363.7 ± 23.9	NM
0.8	354.6 ± 18.4	160.7 ± 8.3[c]	217.3 ± 17.2[c]	298.3 ± 7.2[b]	328.8 ± 12.2

NM – not measured.
[a] Mean ± SE, n = 5–7.
Statistically significant differences compared to the control (t = 0): [b] $p < 0.05$, [c] $p < 0.001$.

tion of the pull force was 56%. While the medium dose of ethanol (0.6 g kg^{-1} b.m.) significantly changed muscle strength after the first 15 min, the lowest dose (0.4 g kg^{-1} b.m.) failed to affect the grip force (Table I).

Some previous studies have shown the importance of both dose and time dependency on ethanol action. Thus, Durcan and Lister (7) found that the effects of ethanol (1.2 g kg^{-1} b.m.) on the behaviour of mice on the holeboard and the plus-maze, as models to investigate exploratory and locomotor behaviours, vary with the dose and time of administration of ethanol and these changes do not always follow the same pattern.

The muscle relaxant effect of diazepam was also dose- and time-dependent. The strongest effect was obtained with the dose of 7.5 mg kg^{-1} b.m. while the lowest used dose (0.75 mg kg^{-1} b.m.) did not significantly change the animal's muscle strength (Table II). The mean pull force with the highest dose was reduced by about 80% when measured 15 min following the drug administration while in the 75th min a 58% reduction was obtained.

The percent change of grip-force measured before and after the treatment with tested substances is presented in Fig. 1. In combined treatments, the influence of different doses of ethanol on intensity of the muscle relaxant effect of diazepam was investigated. Since the highest dose of diazepam (7.5 mg kg^{-1} b.m.) produced robust myorelaxation when applied alone, the dose of diazepam whose effect was evident but less pronounced was chosen; therefore, for combined treatments, diazepam in the dose of 1.5 mg kg^{-1} b.m. was used.

Table II. Time profile of the myorelaxant effect of diazepam

Diazepam dose (mg kg^{-1} b.m.)	Grip strength (g) after the application of diazepam (min)[a]				
	0	15	45	75	105
0.75	353.2 ± 8.6	356.5 ± 18.5	365.7 ± 21.8	NM	NM
1.5	334.9 ± 14.2	170.1 ± 22.1[c]	222.5 ± 19.7[b]	271.7 ± 18.9	314.8 ± 15.6
7.5	364.9 ± 6.2	75.4 ± 8.3[c]	106.5 ± 11.2[c]	152.3 ± 17.2[c]	305.4 ± 12.2

[a] NM – not measured.
Mean ± SE, n = 5–7.
Statistically significant difference compared to the control (t = 0): [b] $p < 0.05$, [c] $p < 0.001$.

When diazepam in the dose of 1.5 mg kg^{-1} b.m. was applied concomitantly with each of the three doses of ethanol, a significantly augmented myorelaxant effect was obtained in rats. This enhancement of muscle relaxation depended on the ethanol employed dose (Table III). Thus, a significantly increased muscle relaxant effect was observed for combinations of diazepam (1.5 mg kg^{-1} b.m.) with ethanol at the doses of 0.4 and 0.6 g kg^{-1} b.m., especially in the first 15 min ($p < 0.01$, compared to diazepam and ethanol alone, respectively). For example, these doses of ethanol enhanced the muscle relaxant effect of diazepam by additional 26% and 45%, respectively, after the first 15 min. Combination of diazepam and ethanol of 0.8 g kg^{-1} b.m. produced a more robust myorelaxant effect, which lasted longer than the effect of either substance alone. Namely, complete muscle relaxation was observed after 15 min of experiment and it decreased to 91% in the 45th min and to 24% in the 105th min after the beginning of combined treatment (Fig. 1).

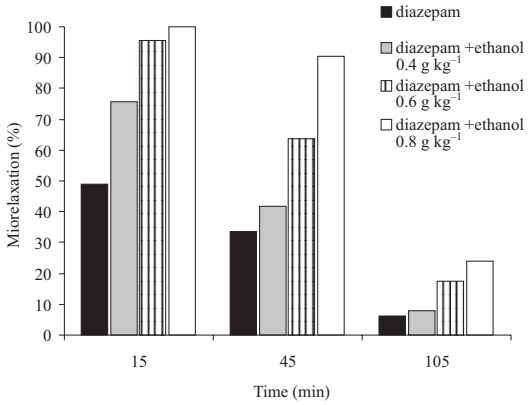

Fig. 1. Time profile of the combined myorelaxant effect of diazepam (1.5 mg kg^{-1} b.m.) and ethanol.

Since combined use of ethanol and benzodiazepines represents a major problem in practice, there are many preclinical as well as clinical investigations dealing with all aspects of this potentially fatal interaction. Some recent studies investigated benzodiazepines-induced impairment of motor performance using the rat rotarod model (8), mice horizontal wire task (9) or radiotelemetric method to test electromyografic activity in animals (10). When ethanol was combined with different benzodiazepines and with non-benzodiazepine, zolpidem, a marked augmentation of the effects of these drugs was obtained. However, doses of ethanol used in these studies were higher (above 1 g kg^{-1} b.m.) than the doses employed in the present study.

Although ethanol is supposed to act non-specifically as a general central nervous system (CNS) depressant, several cellular mechanisms of its action are postulated, such as inhibition of calcium-channel opening, inhibition of N-methyl-D-aspartate (NMDA) type glutamate receptors and enhancement of gamma-aminobutyric acid (GABA) action (2). The last mechanism is common to ethanol and benzodiazepines; namely, both substances act as agonists of GABA type A (GABA-A) receptors. These receptors are ligand--gated chloride channels that mediate inhibitory synaptic transmission in CNS. Diazepam acts by binding to a specific regulatory site on the GABA-A receptor, thus enhanc-

Table III. Time profile of the combined myorelaxant effect of diazepam and ethanol

Combined treatment	Grip strength (g) after the application of substances (min)[a]				
	0	15	45	75	105
Diazepam (1.5 mg kg^{-1} b.m.) + ethanol (0.4 g kg^{-1} b.m.)	354 ± 18	85.9 ± 23.3[c]	206.6 ± 21.3[b]	318.8 ± 14.1	325 ± 20.8
Diazepam (1.5 mg kg^{-1} b.m.) + ethanol (0.6 g kg^{-1} b.m.)	364.2 ± 16.7	16.6 ± 10.3[c]	132.5 ± 19.7[c]	253.8 ± 14.7[b]	301 ± 31.3
Diazepam (1.5 mg kg^{-1} b.m.) + ethanol (0.8 g kg^{-1} b.m.)	352.6 ± 19	0[c]	33.4 ± 8.4[c]	220.7 ± 19[c]	268.5 ± 16.9[b]

[a] Mean ± SE, n = 5–7.
Statistically significant difference compared to the control (t = 0): [b] $p < 0.05$, [c] $p < 0.001$.

ing the effect of GABA in CNS. However, subtypes of GABA-A receptor exist in different brain regions and differ in their sensitivity to various benzodiazepines (11). Ethanol enhances the action of GABA on GABA-A receptors in a similar way as do benzodiazepines. Its action is, however, weaker and less consistent and no obvious effect on inhibitory synaptic transmission in the CNS has been demonstrated for ethanol.

Recent studies have clearly demonstrated that GABA-A receptors play an important role in ethanol dependence, and functional properties of these receptors are altered following chronic ethanol administration (12). A recent study in humans tested the acute behavioural effects of two benzodiazepines, triazolam and temazepam, alone and in combination with a moderate dose of ethanol (0.5 g kg^{-1} b.m.), which corresponds to 2–3 standard drinks (a single drink usually contains about 8–12 g of ethanol). These results showed that ethanol alone caused little performance impairment, while its combination with benzodiazepines produced robust performance impairment that might diminish an individual's ability to respond adequately to unexpected demands (13).

The results of the present study are in agreement with the preclinical as well as clinical investigations, which showed more pronounced effects when benzodiazepine was combined with ethanol. Since some of the goals of our experiments were to find the lowest dose of ethanol that could affect significantly the muscle relaxant effect of diazepam as well as to investigate the dose and time-dependence of this interaction, we used much lower doses of both substances, compared to those usually used in preclinical investigations.

Therefore, the present preclinical study investigating myorelaxant effects of ethanol and diazepam separately or concomitantly showed that: (i) the effects of both ethanol and diazepam were dose- and time-dependent; (ii) when applied concomitantly, ethanol enhanced significantly the muscle relaxant effect of diazepam in the dose-dependent and time-dependent manner. This effect lasted longer in combined treatment than with either substance alone; (iii) ethanol's ability to enhance the myorelaxant effect of diazepam is evident even at doses as low as 0.4 g kg^{-1} b.m. in rats.

CONCLUSIONS

The marked enhancement of diazepam-induced muscle relaxation by ethanol, observed in the present investigation, confirmed the great danger in practice of the interaction between these two substances, not only because of an enhanced CNS depressant effect, but also because of impaired muscle activity which could affect work and motor performance.

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S A Ž E T A K

Utjecaj etanola na miorelaksirajuće djelovanje diazepama u štakora

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Konzumacija etanola može dovesti do pojačanog anksiolitičkog, sedativnog i hipnotskog djelovanja benzodiazepina, a može i pojačati njihove neželjene reakcije, kao što su vrtoglavica, konfuzija, amnezija i poremećena koordinacija. Cilj ovog rada bio je ispitati miorelaksirajući učinak diazepama i etanola kod štakora koristeći »metodu jačine stiska«. Također se željelo utvrditi u kojoj dozi te u kolikoj mjeri primjena etanola može pojačati djelovanja diazepama. Stoga su u ispitivanjima korištene po tri doze etanola (0,4, 0,6 and 0,8 g kg^{-1} tjelesne mase, t.m.) i diazepama (0,75, 1,5 and 7,5 mg kg^{-1} t.m.). Miorelaksirajuće je djelovanje ispitivano nakon jednokratne primjene obje supstance pojedinačno kao i u njihovoj kombinaciji. Miorelaksirajući učinak etanola kao i diazepama pojedinačno bio je ovisan o dozi. Što je doza bila veća, to je učinak bio jači, a vrijeme njegova trajanja bilo je dulje. Kod istodobne primjene diazepama (1,5 mg kg^{-1} t.m.) s različitim dozama etanola, miorelaksirajuće se djelovanje značajno pojačalo i produljilo u odnosu na ispitivane supstance pojedinačno. Pritom je učinak bio najjače izražen u prvih 15 min, a potom je postupno slabio. Naime, u tom je vremenu najmanja doza etanola korištena u ovom radu pojačala miorelaksirajuće djelovanje diazepama za dodatnih 26%, srednja za čak 46% dok je u kombinaciji s najvećom dozom etanola nastupila potpuna mišićna relaksacija koja je postupno, tijekom vremena, slabila da bi 105 min nakon primjene supstancija iznosila oko 24%.

Stoga rezultati ovog pretkliničkog ispitivanja pokazuju da etanol, ovisno o dozi, pojačava miorelaksirajuće djelovanje diazepama kod eksperimentalnih životinja. Ono je trajalo dulje što je doza etanola u kombinaciji s diazepamom bila veća.

Ključne riječi: diazepam, etanol, interakcija, mišićna snaga

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