Department of Pharmacodynamics, Medical University Łódź, Poland

Interaction between ethanol and diazepam in mice: chronobiological aspects

B. Pietrzak, E. Czarnecka

Received July 19, 2004, accepted November 25, 2004

Dr. Boguslawa Pietrzak, Department of Pharmacodynamics, Medical University, Muszyńskiego 1, 90-151 Łódź, Poland
bpietrzak@pharm.am.lodz.pl

Pharmazie 60: 761–764 (2005)

The mechanism of action of benzodiazepines and ethanol demonstrates that these agents can synergistically affect the central nervous system (CNS). The effects of both ethanol and diazepam are likely to depend on the time of the day when they were administered. Diazepam influence on ethanol-induced sleeping and hypothermic activity in mice as well as the influence of combined administration of these agents on spontaneous locomotor activity and coordination in mice (rota-rod) were investigated. Experiments were carried out in the light phase (10:00–12:00 h) and the dark phase (22:00–24:00 h). It was shown that ethanol-induced sleeping time was longer in the dark phase than the light phase, and that ethanol increased spontaneous locomotor activity both in the light and the dark. Ethanol-induced hypothermia was lower in the dark than in the light. Diazepam decreased locomotor activity more strongly in the dark phase than by day. It impaired the hypothermic action of ethanol in the light phase, but did not have such an effect in the dark phase. Diazepam prolonged ethanol-induced sleep in the light phase, enhanced its action on locomotor coordination and decreased the stimulating effect of ethanol on spontaneous locomotor activity in mice. The chronobiological effect of the interaction between diazepam and ethanol seems to be of practical importance (sleep and motor coordination).

1. Introduction

One of the new pharmacology specialities is chronopharmacology. It deals with examination of the relationship between timing of drug administration and drug efficacy. Basic concepts of chronopharmacology involve chronesthesy (circadian changeability of receptor sensitivity to drugs) and chronokinetics (circadian rhythms of drug availability, biotransformation and excretion processes). Circadian variations in drug efficacy (chronergy) are the consequence of chronesthesy and chronokinetics.

Ethanol has a broad range of actions on many neurotransmitter systems. The depressant actions of ethanol in the brain are related in part to facilitation of gamma-aminobutiric acid (GABA) neurotransmission via its interaction with the benzodiazepine/GABA receptor complex.

The benzodiazepines are a family of anxiolytic and hypnotic drugs. When taken concurrently with ethanol, a pharmacodynamic interaction may occur, potentiating the central nervous system depression produced by either drug.

Diazepam is a popular and frequently used benzodiazepine, also indicated in the alcohol abstinence syndrome. The GABA-ergic mechanism associated with the action of both benzodiazepines and ethanol can lead to their synergistic action. The synergistic effect of benzodiazepines and ethanol has been shown in experimental (Van-Steveninck et al. 1996; Vanover et al. 1999; Vanover 1999; Storustovu and Ebert 2003; Voss et al. 2003) and clinical research (Eves and Lader 1989; Hrbek et al. 1989; Van-Steveninck et al. 1996).

Chronobiological aspects of ethanol and diazepam action may have essential practical importance. The aim of the study was to observe interactions between ethanol and diazepam during the day/night cycle (light and dark phase).

2. Investigations and results

2.1. Ethanol sleeping time

Ethanol administered to mice in a dose of 4 g kg^{-1} produced sleep lasting about 12 min in a light phase. In a dark phase sleep was longer and lasted on average 21 min. Diazepam prolonged ethanol-induced sleep in the light phase only (Fig. 1).

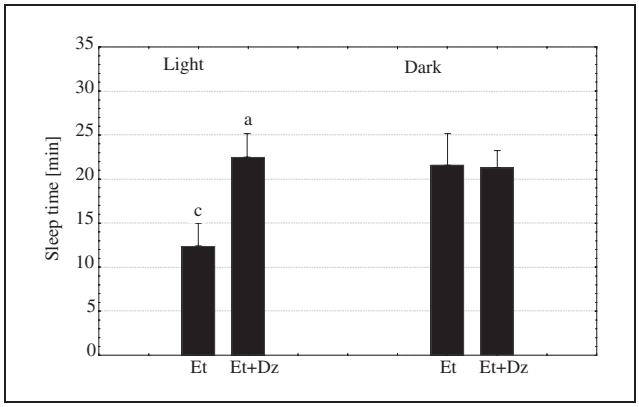

Fig. 1: The effect of treatment with diazepam on ethanol sleeping time in mice. Et – ethanol, Dz – diazepam 0.5 mg kg^{-1}. Ethanol was given at a dose of 4 g kg^{-1} i.p. ten minutes after diazepam. The data are shown as means ± SEM; a $p < 0.05$ versus ethanol group, c $p < 0.05$ light versus dark

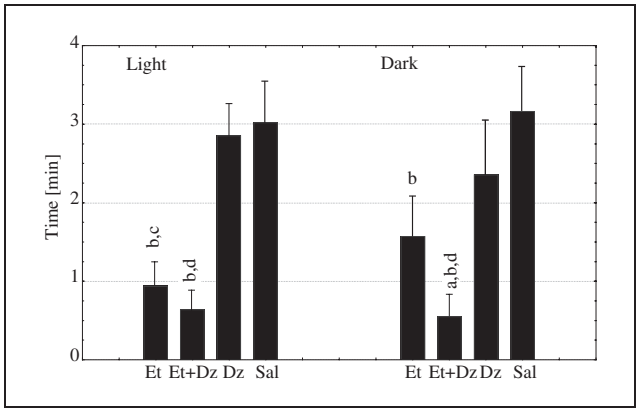

Fig. 2: The effect of treatment with diazepam on ethanol impairs motor coordination (rota-rod) in mice. Mean time of mice performance on the rod after treatment with: Et – ethanol (1.5 g · kg^{-1}), Dz – diazepam 0.5 mg kg^{-1}, Sal – saline (0.9% NaCl). The data are shown as means ± SEM, a $p < 0.05$ versus ethanol group, b $p < 0.05$ versus saline group, c $p < 0.05$ light versus dark, d $p < 0.05$ versus diazepam group

2.2. Rota-rod performance

Ethanol impairs motor coordination in mice both in the light and the dark phase, however, it acted more obviously in the light phase. Diazepam administered in the dark phase before ethanol resulted in stronger impairment of motor coordination than was observed after ethanol given alone (Fig. 2).

2.3. Spontaneous locomotor activity

Ethanol injected at a dose of 2 g · kg^{-1} increased spontaneous locomotor activity in mice both in the light and the dark phase. Diazepam alone clearly decreased mouse spontaneous locomotor activity in the dark. Diazepam administered before ethanol significantly decreased mouse spontaneous locomotor activity. This activity was apparent in both the light and dark phases (Fig. 3).

2.4. Ethanol induced hypothermia

Ethanol administered at a dose of 2.5 g · kg^{-1} produced a marked decrease in mouse body temperature. Mean hypothermia at 30 min following administration was 4.3 °C and at 120 min 3 °C below the baseline value in the light phase, and in the dark phase 3.2 and 1.2 °C, respectively

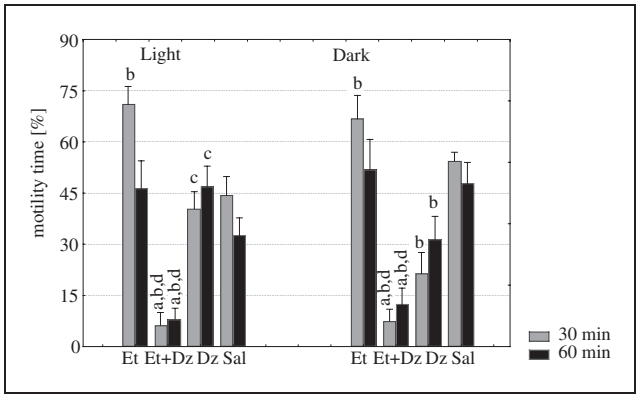

Fig. 3: The influence of diazepam and ethanol on motility in mice. Et – ethanol (2.0 g kg^{-1}), Dz – diazepam 0.5 mg kg^{-1}, Sal – saline (0.9% NaCl). Results are presented as means ± SEM, a $p < 0.05$ versus ethanol group, b $p < 0.05$ versus saline group, c $p < 0.05$ light versus dark, d $p < 0.05$ versus diazepam group

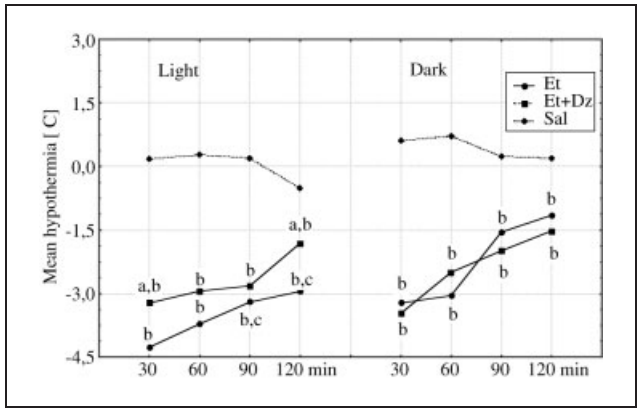

Fig. 4: The effect of treatment with diazepam on ethanol-induced hypothermia in mice. Et – ethanol (2.5 g · kg^{-1}), Dz – diazepam 0.5 mg kg^{-1}, Sal – saline (0.9% NaCl). Results are presented as means, a $p < 0.05$ versus ethanol group, b $p < 0.05$ versus saline group, c $p < 0.05$ light versus dark

(Fig. 4). In the dark, hypothermia 90 and 120 min after ethanol administration was statistically significantly lower than in the light phase. Diazepam given before ethanol in the light phase decreased the hypothermic action of ethanol, whereas it did not exert such an influence in the dark phase (Fig. 4).

Diazepam alone decreased mouse body temperature in the range from 1.4 °C to 0.4 °C in a similar way in both light and dark phases.

3. Discussion

The present study has shown that some actions of ethanol are associated with circadian rhythms. The sleeping action has been found to be stronger in the dark than in the light phase, however, during the light phase ethanol impairs locomotor coordination to a greater extent and its hypothermic action is more evident.

Ethanol affects different neurotransmitters, specific ion channel and receptors. It enhances GABA-ergic inhibition increasing the action of γ-aminobutyric acid (GABA) on GABA-A receptors (similarly to benzodiazepines) (Korpi 1994). This mechanism of action leads to intensification of the CNS depression produced by ethanol and by benzodiazepines. Benzodiazepines give a marked enhancement of ethanol induced motor impairment and increased ethanol sleep time in animals (Vanover et al. 1999; Voss et al. 2003).

The chronobiological aspect of ethanol action has a double signifance. The first is the influence of single-dose ethanol taken at different times of day and night. The second problem concerns the effect of chronically administered ethanol on biological rhythms.

Investigations on the chronobiological effect of ethanol action have been mostly performed in animals and more rarely in humans. Reinberg (1992) defined circadian changes in the psychological results of ethanol action. They were the greatest at 23:00 h and the least at 11:00 h, however, blood pressure values were not associated with day and night rhythms. Yap et al. (1993) determined ethanol concentration in blood and air breathed out by 10 healthy volunteers. Ethanol reached its highest blood concentration at 9:00 h.

Experiments on rats revealed that the pharmacokinetics of low but not high ethanol doses are associated with the circadian rhythm. Maximum elimination was observed in

the dark, i.e. the rats' active phase (Piekoszewski 1997). It was also stated that the highest activity of alcohol dehydrogenase and hepatic microsomal enzymes (MEOS) occurs just at the time of highest activity of animals (Sturtevant and Garber 1980). Williams et al. (1993) evaluated the development of tolerance to the hypothermic activity of ethanol administered in dark and light phases in the mouse and the rat. Tolerance developed only in animals that were given ethanol in the light phase. Baird et al. (1998) studied the effect of ethanol on body temperature and locomotor activity in rats in relation to alcohol dose and time of its administration. After a single dose of ethanol these parameters were measured for 48 h. Ethanol was administered 4 times a day (every 6 h starting at 1:00 h). A dose of 1 g/kg did not cause significant hyperthermia, significant hyperthermia occurring only after doses of 2 g · kg^{-1} at 13:00 h and 19:00 h. The greatest temperature decrease following ethanol administration was recorded at 19:00 h after both dosess, i.e. 1 g · kg^{-1} and 2 g · kg^{-1}. The greatest decrease in locomotor activity was also observed at 19:00 h, however, this change was statistically significant only at the dose of 2 g · kg^{-1}. Administration of ethanol dose of 2 g · kg^{-1} in the afternoon resulted in a shortening of the locomotor activity phase in animals.

In rats with free choice of ethanol the highest uptake was observed during the initial dark phase and correlated with an increase in activity (Sturtevant and Garber 1980; Gauvin et al. 1997). In humans consumption of the highest ethanol doses has been observed in the final part of the light phase and correlates with the end of locomotor activity (Sturtevant et al. 1976; Minors and Waterhouse 1980). The authors suggest there is not only an indirect but also a direct mechanism for the effect of alcohol on the biological clock.

Our study on diazepam reveals its association with circadian rhythm and its effect on spontaneous locomotor activity in mice, which has been found to be lower in the dark than the light phase after diazepam treatment. Yannielli et al. (1996) demonstrated that the antianxiety action of diazepam is related to circadian rhythm, being more pronounced between 16:00 h and 20:00 h. Golombek et al. (1993) in their study on rats in a plus maze test observed the highest anxiolytic action of diazepam by night and the lowest by day. Diazepam influenced induction of somatosensory potentials in rats to a greater extent in the light than the dark phase (Todorova 1992). Diazepam decreases nocturnal secretion of melatonin in humans (Monteleone et al. 1989). A high diazepam dose (10 mg kg^{-1}) inhibits expression of the *mPer1* gene (the gene responsible for circadian rhythms of life processes) in the mouse cerebellum, which is associated with a decrease in their locomotor activity (Akiyama et al. 1999).

In the present study evaluating the interaction of ethanol and diazepam in the dark and light phases we have demonstrated that diazepam decreased the hypothermic activity of ethanol in the light but not the dark phase. Diazepam decreased the stimulating effect of ethanol (2.5 g kg^{-1}) on mouse locomotor action to a similar extent in both phases. Ethanol in the dosage used in our study increased spontaneous locomotor activity in mice, which is associated with a higher level of dopamine secretion in the CNS (Gesse 1985, Brodie 1990). Diazepam prolonged ethanol-induced sleep in the light phase and enhanced its effect on motor co-ordination in mice in the dark phase.

The Chronobiological aspect of the diazepam and ethanol interaction seems to have practical importance (sleep and motor coordination).

4. Experimental

4.1. Animals and treatment

Treatment experiments were carried out on BALBc male mice (28–35 g). The mice were housed in group cages under normal laboratory conditions: temperature of 20–21 °C, light/dark cycle (light phase 6.00–18.00, dark phase 18.00–6.00). The experiments in the dark phase were carried out in red light. The animals had free access to commercial chow food and water. All experiments were performed between 10:00–12:00 h (light phase) and 22:00–24:00 h (dark phase). Ethanol was given intraperitoneally (ip) as a 20% w/v solution. Diazepam (0.5 mg kg^{-1}) (Relanium "Polfa") was administered intraperitoneally (i.p.) 10 min before ethanol. Control mice received isotonic solution in corresponding volume. Each group studied consisted of 10 mice.

4.2. Ethanol sleeping time

Mice received ethanol (4 g kg^{-1} i.p.) with or without pretreatment with diazepam and the duration of absence of righting reflex was measured (Czarnecka and Pietrzak 2002; Pietrzak and Bogucka-Kubik 2002).

4.3. Rota-rod performance

The mice were placed on a rod 17 mm in diameter rotating at 11 rpm. Before the session mice were pretested on the apparatus. For this experiment mice which remained on the rod for at least one minute were selected. Ethanol was given at a dose of 1.5 g kg^{-1} i.p. with or without pretreatment with diazepam (10 min before ethanol). The times the mice remained on the rod were measured.

4.4. Spontaneous locomotor activity

Motility was recorded by placing mice in photoelectric actometers (Universal Motility Metter, Protex, Poland) and locomotor activity was measured by a computer-aided device. Motility was expressed as the percentage of locomotor activity within the tested time. Experiments were performed in a special sound-proof room. The animals were not previously adapted to the apparatus. Locomotor activity was measured in two 30-min periods starting after administration of ethanol (2 g · kg^{-1} i.p.).

4.5. Ethanol induced hypothermia

Body temperature was measured in the rectum twice at 30-min intervals and the mean of these measurements was regarded as the basal initial temperature. Following ethanol administration at a dose of 2.5 g · kg^{-1} i.p., body temperature was measured at 30-min intervals for 2 h and the difference from the initial value was recorded.

4.6. Statistics

The statistical analysis of parameters was performed using the Statistica version 5.0 Stat-Soft program. The homogeneity of variance was tested by Bartlett's test. Statistical evaluation was performed using two-way Anova (for ethanol sleep time, rota-rod performance, locomotor activity), or three-way Anova (temperature) and post-hoc comparisons were performed with the NIR test. All parameters were considered statistically significantly different if $p < 0.05$.

References

Akiyama M, Kirihara T, Takahashi S, Minami Y, Yoshinobu Y, Moriya T, Shibata S (1999) Modulation of *mPerl1* gene expression by anxiolytic drugs in mouse cerebellum. Br J Pharmacol 128: 1616–1622.

Baird TJ, Briscoe RJ, Vallety M, Vanecek SA, Holloway FA, Gauvin DV (1998) Phase-response curve for ethanol alteration in circadian rhythms of temperature and activity in rats. Pharmacol Biochem Behav 61: 303–315.

Brodie MS (1990) Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. Brain Res 508: 65–69.

Czarnecka E, Pietrzak B (2002) Influence of captopril and enalapril on some central effects of ethanol. Acta Pol Pharm 59: 71–75.

Eves FF, Lader MH (1989) The effects of alcohol on psychological functions in normal volunteers after 8 days' treatment with pipequaline (PK 8165), diazepam and placebo. Eur J Clin Pharmacol 36: 47–52.

Gauvin DK, Baird TJ, Vanecek SA, Briscoe RJ, Vallet M, Holloway FA (1997) Effects of time-of day and photoperiod phase shifts on voluntary ethanol consumption in rats. Alcohol Clin Exp Res 21: 817–825.

Gesse GL (1985) Low doses of ethanol activate dopaminergic neurons in ventral tegmental area. Brian Res 348: 201–203.

Golombek DA, Martini M, Cardinali DP (1993) Melatonin as an anxiolytic in rats: time dependence and interaction with the central GABAergic system. Eur J Pharmacol 237: 231–236.

Hrbek J, Macakova J, Komenda S, Siroka A, Rypka M (1989) On acute effects of some drugs on the higher nervous activity in man. Ethanol (0.5 g/kg) and its interaction with diazepam (5 mg) and meclophenoxate (100 mg). Part LVIII. Acta Univ Palacki Olomuc Fac Med 121: 189–233.

Korpi ER (1994) Role of GABA-A receptors in the action of alcohol and in alcoholism: recent advances. Alcohol Alcohol 29: 115–129.
Minors DS, Waterhouse JM (1980) Aspects of chronopharmacokinetics and chronergy of ethanol in healthy man. Chronobiol 7: 465–480.
Monteleone P, Forziati D, Orazzo C, Maj M (1989) Preliminary observations on the suppression of nocturnal plasma melatonin levels by short-term administration of diazepam in humans. J Pineal Res 6: 253–258.
Piekoszewski W, Rutkowska A, Brandys J (1997) Effect of ethanol dose on its chronopharmacokinetics in the rats. Acta Pol Pharm – Drug Research 54: 401–404.
Pietrzak B, Kubik-Bogucak E (2002) Influence of mianserin on some central effects of ethanol. Pharm Res 46: 47–54.
Reinberg A (1992) Circadian changes in psychological effects of ethanol. Neuropsychopharmacology 7: 149–156.
Storustovu S, Ebert B (2003) Gaboxadol: *in vitro* interaction studies with benzodiazepines and ethanol suggest functional selectivity. Eur J Pharmacol 467: 49–56.
Sturtevant FM, Sturtevant RP, Scheving LE, Pauly JE (1976) Chronopharmacokinetics of ethanol II. Circadian rhythm in rate of blood level decline in single subject. Naunyn Schmiedebergs Arch Pharmacol 293: 203–208.
Sturtevant RP, Garber SL (1980) Light-dark and feeding regimens affect circadian phasing of blood-ethanol decay rates. Pharmacol Biochem Behav 13: 637–642.
Todorova A, Boyagieva M (1992) Effects of diazepam on somatosensory evoked potentials in rats at two different periods of day. Acta Physiol Pharmacol Bulg 18: 49–54.
Vanover KE, Suruki M, Robledo S, Huber M, Wieland S, Lan NC, Gee KW, Wood PL, Carter RB (1999) Positive allosteric modulators of the GABA(A) receptor: differential interaction of benzodiazepines and neuroactive steroids with ethanol. Psychopharmacology 141: 77–82.
Vanover KE (1999) Interaction of ethanol with excitatory amino acid receptor antagonists in mice. Eur J Pharmacol 368: 137–142.
Van-Steveninck AL, Geischke R, Schoemaker RC, Roncari G, Tuk B, Pieters MS, Breimer DD, Cohen AF (1996) Pharmacokinetic and pharmaco-dynamic interactions of bretazenil and diazepam with alcohol. Br J Clin Pharmacol 41: 565–573.
Voss J, Sanchez C, Michelsen S, Ebert B (2003) Rotarod studies in the rat of the GABAA receptor agonist gaboxadol: lack of ethanol potentiation and benzodiazepine cross-tolerance. Eur J Pharmacol 482: 215–222.
Williams RL, Soliman KFA, Mizinga KM (1993) Circadian variation in tolerance to the hypothermic action of CNS drug. Pharmacol Biochem Behav 46: 283–288.
Yannielli PC, Kanterewicz BI, Cardinali DP (1996) Daily rhythms in spontaneous and diazepam-induced anxiolysis in Syrian hamsters. Pharmacol Biochem Behav 54: 651–656.
Yap M, Mascord DJ, Starmer GA, Whitfield J (1993) Studies on the chronopharmacology of ethanol. Alcohol Alcohol 28: 17–24.