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ORIGINAL ARTICLE

Interaction between central effects of ethanol and melatonin in mice

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Abstract Effects of melatonin on acute toxicity of ethanol and ethanol-induced hypothermia, ethanol and thiopental sleeping time and ethanol-induced hypermotility were investigated in mice. The melatonin (1 and 5 mg/kg) was injected i.p. in single or repeated doses (7 days). The experiments were performed during the light phase of the light/dark cycle. It has been shown that a single dose of melatonin shortened duration of ethanol sleep and decreased ethanol-induced hyperactivity in mice. Melatonin given to mice for 7 days did not influence ethanol-induced effects. Melatonin prolonged thiopental hypnotic effect. The results of central interaction of ethanol and melatonin may be of antagonistic or synergistic nature.

Introduction

Melatonin, a pineal hormone, is an element of circadian rhythm and informs the body of time of day and type of season. Melatonin levels are high during the night and low during daytime. Few papers have been published of the research that aims at establishing the influence of melatonin on ethanol intake [1, 2] and the influence of ethanol on melatonin synthesis and its release [3, 4, 5, 6).

In this study we investigated the effect of ethanol combined with melatonin on the central nervous system of laboratory animals.

Materials and methods

Animals and treatment.

The experiments were carried out on Swiss mice, 18–24 g (males or both sexes). The animals were fed with granulated commercial laboratory chow ad libidum and had free access to water.

The animals were housed in group cages and were kept at 20°C room temperature, 12 hours light/dark cycle (light cycle from 02.00 untill 14.00) throughout the experiments and for 7 days prior to the study. The experiments were performed between 11.00 and 13.00. Melatonin (Sigma) was given intraperitoneally at the doses of 1 or 5 mg/kg (as isotonic saline solution) 45 min. before ethanol injection and in prolonged administration experiments at a dose of 1 mg/kg, twice daily (at 08.00 and 14.00). The last dose of melatonin was administered 22 hours before ethanol. Ethanol was given intraperitoneally as 20% v/w solution.

Acute toxicity.

Acute toxicity of ethanol was assessed with the method of Litchfield and Wilcoxon [7] using 17 groups of 6 mice in each. Mice were administered various volumes of ethanol and the number of dead animals was counted 24 hours later. After establishing LD_{50} value for ethanol alone, the LD_{50} value was measured in mice that were treated with melatonin acutely or chronically.

Ethanol hypothermia.

Body temperature was measured in male mice. Before experiments the mice were kept 10 or 5 in a cage at 20°C for 3 days and the experiments were carried out in the same room. Body temperature was measured in the rectum 3 times at 30 min. intervals and mean from these measurements was regarded as the basal initial temperature.

Ethanol was administered at a dose of 2.5 g/kg i.p. without melatonin or after acute or chronic treatment with melatonin. The body temperature was measured at 30 min. intervals for 2 hours after ethanol administration. Each group consisted of 10 mice.

Ethanol sleeping time.

Mice of both sexes received ethanol 4 g/kg ip with or without acute or chronic pretreatment with melatonin and time of absence of righting reflex was measured. Each group consisted of 10 or 20 mice.

Thiopental sleeping time.

Mice of both sexes received thiopental 75 mg/kg ip alone or thiopental with pretreatment with melatonin and time of absence of righting reflex was measured. Each group consisted of 10 or 20 mice.

Spontaneous locomotor activity.

Motility was recorded by placing mice in photoelectric actometers—Universal Motility Meter (UMM1-061 COMT Bialystok Poland) 27 cm in diameter and 16 cm high. Experiments were performed in special soundproof room.

The animals were not previously adapted to the apparatus. Locomotor activity was measured during 30 min period after administration of ethanol alone at a dose of 2 g/kg i.p. or after ethanol and melatonin pretreatment. Each group consisted of 10 mice.

Statistics.

The normality of distribution was checked by means of the Kolmogorow-Smirnow test, with Lillieforse correction and the variance equality was tested by the Fisher test. The statistical evaluation was performed by means of Mann-Whitney U-test by use of Statistica for Windows 4.0 program.

Results

It has been shown that melatonin in a dose of 5 mg/kg decreased acute toxicity of ethanol, whereas administered in a dose of 1 mg/kg or chronically for 7 days did not influence acute toxicity of ethanol (Tab. 1). A single dose of melatonin increased hypothermic effects of ethanol. This effect is especially clear when used in a dose of 1 mg/kg (Fig.1A). Melatonin administered for 7 days did not change ethanol hypothermia (Fig.1B). Melatonin administered in a dose of 1 mg/kg or 5 mg/kg, respectively,

30	60	90	120
-1.8	-1.5	-1.1	-0.6
-2.3	-1.9	-1.7	-1.1
-2.9	-1.8	-1.2	-0.8
30	60	90	120
-1.7	-1.3	-0.9	-0.6
-1.7	-0.9	-0.8	-0.4
30	60	90	120
-1.5	-0.8	-0.1	-0.1
0.8	0.9	0.9	1

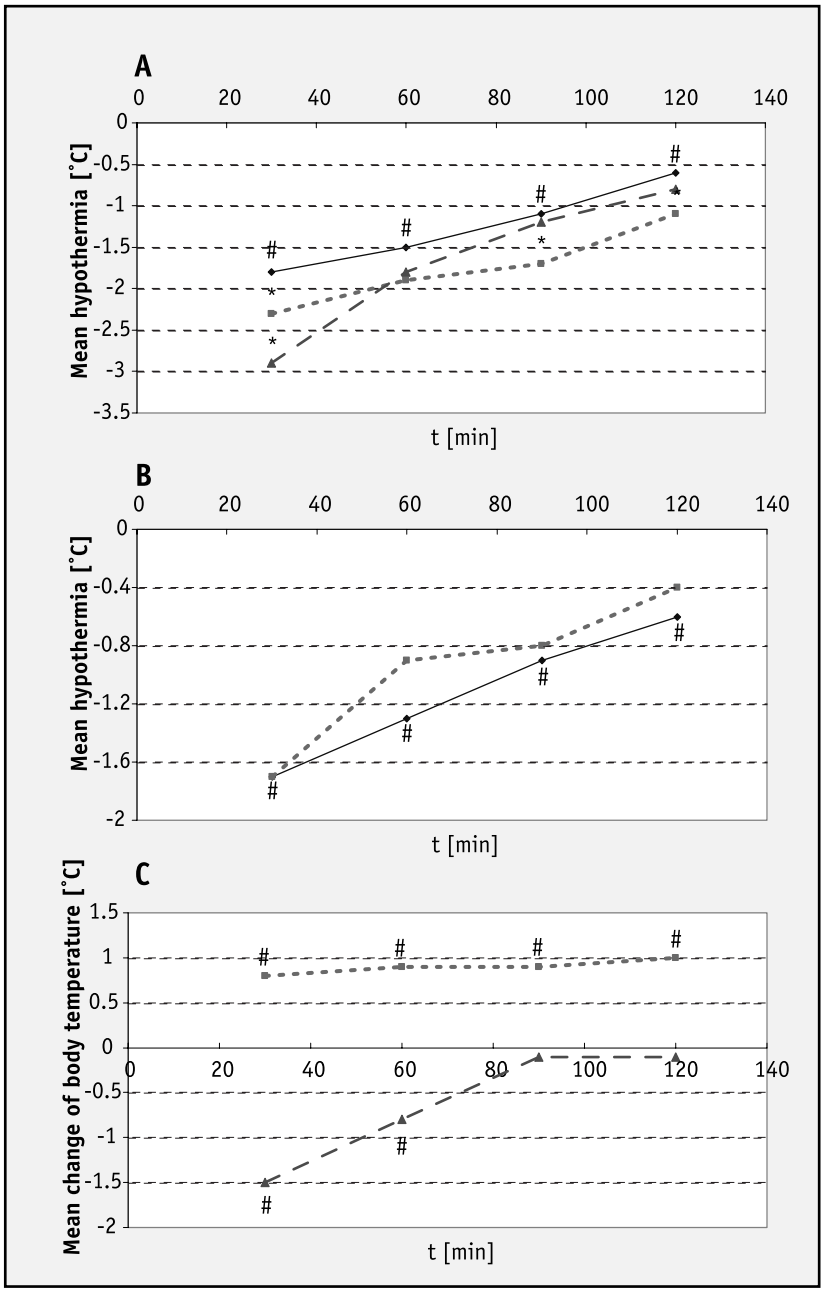

Fig. 1. The effect of treatment with melatonin on ethanol-induced hypothermia in mice (A, B)

A. acute experiments
B. chronic experiments (melatonin 1 mg/kg b.i.d. for 7 days)
C. mean changes of body temperature after acute treatment with melatonin
◆-◆ saline (control) ■-■ melatonin 1 mg/kg
▲-▲ melatonin 5 mg/kg

The results are presented as means ±SD
significantly different versus initial temperature $p < 0.05$ (Wilcox on matched pair test)
***** significantly different versus control $p < 0.05$ (Mann-Whitney U-test)

increased or decreased mice body temperature (Fig. 1C).

Melatonin (1 and 5 mg/kg) decreased ethanol-induced hypermotility in mice (Fig. 2A) and its repeated administration did not show such effect (Fig. 2A). Melatonin given alone in a dose of 1 mg/kg decreased spontaneous locomotor activity of mice (Fig. 2B). Ethanol given at a dose of 4 g/kg induced sleep lasting 17 min. on average (Fig. 3A). Melatonin (5 mg/kg) statistically significantly shortened duration of that sleep (Fig.3A). Melatonin administered for 7 days did not change duration of ethanol sleeping time (Fig.3B). Thiopental (75 mg/kg) induced sleep lasting 7.6 min. on average (Fig. 3C). Melatonin (5 mg/kg) prolonged duration of that sleep (Fig.3C).

Discussion

Conducted research showed that a single dose of melatonin administered during the light phase of the day/night cycle shortened duration of ethanol sleep, decreased acute toxicity of ethanol, increased ethanol hypothermia and

Table 1.
The influence of melatonin on acute toxicity of ethanol (LD_{50})

Treatment and dose	Ethanol LD50 (g/kg)	Confidence limit for p= 0.05
Saline (control)	6.5	(6.0–7.0)
Melatonin 1 mg/kg	6.5	(6.4–7.4)
Melatonin 5 mg/kg	7.5	(6.9–8.1)*
Melatonin 1 mg/kg b.i.d. for 7 days	6.7	(6.4–7.0)

All drugs were injected intraperitoneally
* Significantly different in comparison with the control (p< 0.05)

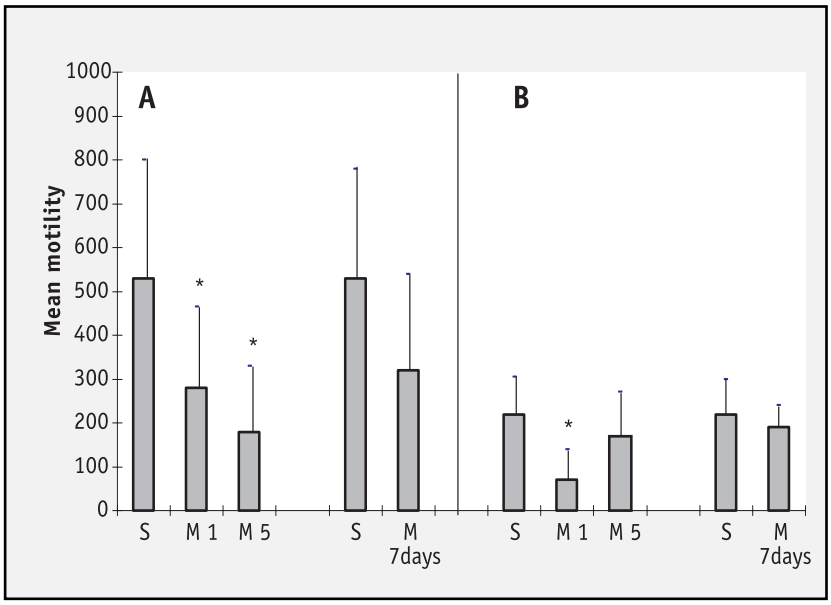

Fig. 2. The influence of melatonin and ethanol on motility in mice

A. mean changes of motility in mice after treatment with melatonin and ethanol
B. mean changes of motility in mice after treatment with melatonin
S–saline (control),
M1–melatonin 1mg/kg,
M5–melatonin 5 mg/kg
M7 days–melatonin 1 mg/kg
b.i.d. for 7 days
The data are shown as means ±SD * p < 0.05 (Mann-Whitney U-test)

	X	X+SD
S	17	25
M_1	18.7	29
M_5	10.5	20.3
S	17.8	21
M_{7days}	13	24.5
S	7.5	9.5
M_5	9.6	12
S	530	800
M_1	280	465
M_5	180	330
S	530	780
M_{7days}	320	540
S	220	305
M_1	70	140
M_5	170	270
S	220	300
M_{7days}	190	240

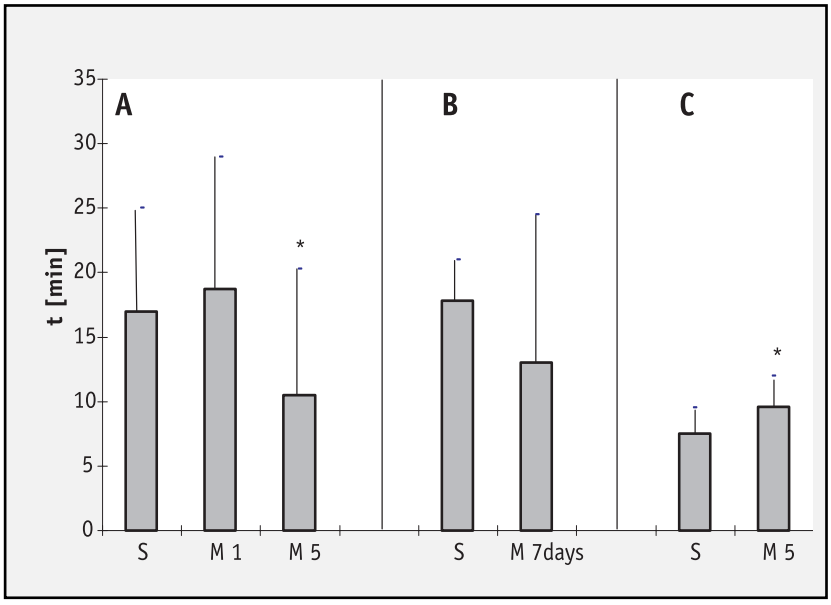

Fig. 3. A., B. Ethanol sleeping time
A–acute,
B–chronic experiments
C–thiopental sleeping time (acute experiments)
S–saline (control),
M1–melatonin 1 mg/kg,
M5–melatonin 5 mg/kg,
M7 days–melatonin 1 mg/kg
b.i.d. for 7 days
The data are shown as means ±SD * p < 0.05 (Mann-Whitney U-test)

reduced ethanol-induced hyperactivity in mice. Melatonin given to mice for 7 days b.i.d. (light phase) did not influence ethanol-induced effects.

Another part of the research conducted with the use of thiopental (also in light phase of the day/night cycle) showed that melatonin prolonged its hypnotic effect. Thus, there is a difference in melatonin influence on the duration of ethanol-induced or thiopental-induced sleep. There are no published data in the literature concerning interaction of melatonin and ethanol, but there are some papers on melatonin's influence on the action of barbiturates.

Sugden [8] showed that melatonin in a dose of 20 mg/kg i.p. prolonged duration of sleep induced by either pentobarbitone or barbitone in mice and in rats. The author believes that observed potentialization of action of these two compounds does not depend on pharmacokinetic effects, because barbitone is a barbiturate which is not metabolized by the liver but is excreted unchanged. Studies of melatonin and its analogues showed that these compounds prolonged duration of barbitone sleep in a dose-dependent manner [9]. Flumazenil blocked further prolongation of barbitone sleep produced by diazepam, but not by melatonin. Similarly an increase in pentobarbitone sleeping time was produced by the

cannabinoid receptor agonist (WIN 55212-2), but the cannabinoid receptor antagonist (WIN 56098) did not reduce that produced by melatonin. Thus melatonin action does not seem to be mediated through benzodiazepine or opioid receptors [10]. Arushanyan and Orhii [11] demonstrated that melatonin (0.01–10 mg/kg) potentiated the hypnotic effect of hexobarbital in mice both in the daytime and night.

Chronic administration of melatonin diminished barbiturates effects in experiments carried out during the day, but not in the experiments performed at night. These authors believe that pharmacological and pharmacokinetic effects may play an important role in this interaction (circadian changes in metabolism rate of the barbiturate). These researchers also demonstrate that melatonin given to nocturnal animals may not act as a hypnotic agent, but as a reverse one. In our experiments (the end of light phase of day/night cycle) we demonstrated that melatonin has different effects on ethanol sleep or thiopental sleep. Melatonin shortened ethanol sleep and reduced acute toxicity of ethanol. We believe that this is a pharmacokinetic interaction, but other mechanisms cannot be ruled out.

Depressant action of ethanol on the central nervous system may be explained on the basis of its action on GABA and NMDA receptors [10, 12, 13, 14].

Ethanol-induced increase of spontaneous locomotor activity is a result of ethanol-stimulated release of dopamine in CNS [15, 16]. In our experiments melatonin decreased ethanol hyperactivity in mice. This result seems to be in accordance with the results published by Zisapel et al. [17] who demonstrated that melatonin reduced dopamine release in rat hypothalamus slices.

Melatonin increased ethanol hypothermic effect and its action was most potent after a dose of 1 mg/kg. The influence of ethanol on the temperature set point in the brain depends on its multidimensional action (increase of GABAergic and serotonergic transmission, noradrenaline turnover and its influence on lipid membranes).

Central interaction of melatonin and ethanol is complex due to melatonin's role in circadian rhythm. Also the type of animals used for experiments may markedly influence the results.

Conclusions

1. Melatonin influences central action of ethanol.

2. The results of central interaction of ethanol and melatonin may be of antagonistic nature (decrease of inhibitory or stimulating effects) and synergistic nature (hypothermic influence).

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