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Hypnotic activity of melatonin: involvement of semicarbazide hydrochloride, blocker of synthetic enzyme for GABA

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KEY WORDS melatonin; sleep; semicarbazide; glutamic acid; GABA

ABSTRACT

AIM: To assess the effect of semicarbazide hydrochloride (SCZ), the blocker of synthetic enzyme for GABA, on the hypnotic activity of melatonin. **METHODS:** Righting reflex method in mice and electroencephalography (EEG) in rats were used to determine effects of SCZ on sleep and hypnotic activity of melatonin. **RESULTS:** Melatonin displayed a marked hypnotic activity both in righting reflex experiment and EEG recording. SCZ had no influence on sleep parameters in mice and rats when it was used alone. However, it blocked the sleep-potentiation effect of melatonin in mice. SCZ also inhibited melatonin-induced increase in total sleep time, slow wave sleep time, and paradoxical sleep time, and prevented melatonin-induced decrease in awake time in rats. **CONCLUSION:** SCZ antagonized the hypnotic activity of melatonin. It is thought that the hypnotic activity of melatonin is mediated by GABAergic system.

INTRODUCTION

In recent years, a number of neuropharmacological effects of melatonin, including locomotor activity, hypnotic activity, analgesia, anti-convulsive, and anti-anxiety, *etc*, have been revealed, and melatonin exerted a depressive influence on the central nervous system (CNS)[1]. Among them, the well-described effect of melatonin on CNS was sedation and hypnotic activity. Data have shown hypnotic activity of melatonin both in animals[2,3] and human[4]. However, little is known about the mechanism(s) of hypnotic activity of melatonin.

Previous studies reported that the inhibitory activity of melatonin on brain might be produced by increasing the inhibitory neurotransmitter activity[1]. It has shown that the GABAergic system is involved in some central action of melatonin[5]. At present, most of studies on neuropharmacological effects of melatonin–GABAergic interaction in CNS were focused on melatonin and GABA-BZP receptor complex. It was reported that the locomotor activity[6], anxiolytic activity[7], anti-convulsant activity[8], and analgesia[9] of

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melatonin were blocked by pre-treatment with flumazenil, an antagonist of the benzodiazepine (BZP) ligand site on the $GABA_A$ receptor. However, no direct evidence showed whether GABAergic system or which site of the GABAergic system was involved in hypnotic activity of melatonin.

γ-Aminobutyric acid (GABA) is an important inhibitory neurotransmitter in central nervous system. It has been reported that GABA levels in the posterior hypothalamus were increased during sleep[10]. Microinjections of muscimol, a potent GABA agonist, in the middle and anterior parts of the posterior hypothalamus induced long-lasting behavioral and electroencephalographic signs of sleep with short latency[11]. Perfusion with melatonin prevented the daytime reduction in GABA in the neostriatum of rats[12]. Melatonin augmented $^{36}Cl^-$ influx by potentiating GABA-induced increase of chloride ion uptake[13]. These data demonstrate that there is a possible link between GABA levels and hypnotic activity of melatonin.

The aim of the present study was to determine whether semicarbazide hydrochloride, a blocker of synthetic enzyme for GABA, blunted the hypnotic activity of melatonin in mice and rats by behavioral and electrophysiological methods, and to elucidate the relationship of synthetic enzyme for GABA with hypnotic activity of melatonin.

MATERIALS AND METHODS

Animals Male Swiss mice (18 −24 g) and male Wistar rats (250 −300 g) (Grade II, Certificate № 042) were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University. Mice and rats were housed in a 12 h light/dark cycle (lights on from 08:00 to 20:00) at room temperature 22 ℃±2 ℃ with food and water *ad libitum*. Animals were fasted 12 h before drug administration.

Materials Melatonin, obtained from Changzhou Medical Technique Development Company, was suspended in 0.3 % sodium carboxymethylcellulose (CMC) and administered ig to mice. In electroencephalogram (EEG) recording experiment, melatonin was dissolved in corn oil and administered ip to rats. Semicarbazide hydrochloride (Shanghai Chemical Reagent Factory) was dissolved in 0.9 % saline.

Implantation of electrodes and polygraphic recording Electrodes for polygraphic recording of EEG and electromyogram (EMG) were implanted in rats as described by Timo-Iaria *et al*[14]. Male rats were anaesthetized with chloral hydrate (400 mg/kg, ip). Four stainless steel screw electrodes were threaded through the skull into the surface of the parietal cortices for subsequent bipolar EEG recordings. Two electrodes were placed over the left hemisphere and the other two at the same position over the right hemisphere (limb area, occipital cortex area). To record EMG, 2 silver wire electrodes were inserted bilaterally into the dorsal neck muscles. The leads from all electrodes were then soldered to the skull with dental cement.

After surgery, animals were housed in individual cages in a 12 h light/ dark cycle. Experiments were carried out at least 1 week after electrode implantation. At the start point of recording, the rat was injected ip with melatonin or corn oil. Recordings began at 08:00 and lasted for 4 h. Time to sleep onset (TSO), total sleep time (TS), slow wave sleep time (SWS), paradoxical sleep time (PS), and awake time (W) were recorded.

Recording was made by an 8-channel physiological recorder (RM6300, Nihon Kohden, Japan) at a chart speed of 25 mm/s. The half-amplitude frequency response was set at 1 to 35 Hz for the EEG and 30 to 75 Hz for the EMG.

Measurement of sleeping time in mice Mice were injected with SCZ (125 mg/kg, ip) or saline (ip), and 5 h after the injection, animals were administered with melatonin (15, 30 mg/kg, ig) or 0.3 % CMC. After a further 20 min, sodium pentobarbital (40 mg/kg, ip) was injected. The absence of the righting reflex was considered as the sleep onset and the duration of the loss of the righting reflex was recorded as the sleeping time[15]. All the experiments were performed at room temperature 25℃±1℃.

Statistical analysis The results were expressed as mean±SD. Data were analyzed by two-way analysis of variance (ANOVA) and Duncan's test for mul-

tiple comparisons between groups. $P<0.05$ were considered statistically significant.

RESULTS

Effect of SCZ on melatonin potentiation of sodium pentobarbital-induced sleeping time in mice Melatonin (15, 30 mg/kg) markedly potentiated sodium pentobarbital-induced sleeping time. SCZ by itself (125 mg/kg) was unable to modify sodium pentobarbital-induced sleeping time, but completely inhibited melatonin (30 mg/kg) potentiation of sodium pentobarbital-induced sleeping time (Tab 1). There was a significant interaction between melatonin (30 mg/kg) and SCZ, F (1, 29) =4.63, $P<0.05$.

Tab 1. Effect of SCZ on melatonin potentiation of sodium pentobarbital-induced sleeping time in mice. n = 10. Mean±SD. $^cP<0.01$ *vs* control+NS group. $^eP<0.05$ *vs* melatonin (30 mg/kg) +NS group.

Group	Dose/mg·kg^{-1}	Sleeping time/min	
		+ NS	+ SCZ
Control	-	38±33	41±11
Melatonin	15	97±36^c	110±54
	30	146±54^c	92±30^e

+ NS: control+NS or melatonin+NS. + SCZ: control+SCZ or melatonin+SCZ.

Effect of SCZ on hypnotic activity of melatonin by EEG analysis in rats Rats were given SCZ (87.5 mg/kg, ip) or saline 5 h before administration of melatonin (2.5, 10 mg/kg, ip). TS, SWS, PS, W, and TSO were recorded. The results showed that melatonin (10 mg/kg) markedly increased TS, SWS, and PS, but decreased W and TSO. Although SCZ (87.5 mg/kg) was unable to modify these sleep indices when it was used alone, it effectively inhibited melatonin-induced increase in TS, SWS, and PS. SCZ also prevented melatonin-induced decrease in W. However, SCZ had no influence on TSO when used either alone or with melatonin (10 mg/kg) (Tab 2). Two-way ANOVA showed a significant interaction between melatonin (2.5 mg/kg) and SCZ on SWS, F (1, 15)=5.37, $P<0.05$. Melatonin (10 mg/kg) and SCZ on PS, F (1, 14)=11.36, $P<0.01$.

DISCUSSION

Potentiation of sodium pentobarbital-induced sleeping time was a classical method and common practice to study the sleep-promoting effect in mice[2], and the rat was most used in studying hypnotic activity of melatoin for EEG analysis[3]. Melatonin potentiated sodium pentobarbital-induced sleep in Swiss mice. In EEG recording, melatonin also increased SWS, PS, and TS, and decreased TSO and W in Wistar rats. The present results demonstrated that melatonin displayed a significant hypnotic activity not only in mice but also in rats.

Previous studies were reported that one of the mechanisms of the inhibitory activity of melatonin on CNS was its stimulating effect on inhibitory neurotransmitter activity, especially on GABA. Melatonin exhib-

Tab 2. Effect of SCZ on hypnotic activity of melatonin in rats. n = 5-7. Mean±SD. $^cP<0.01$ *vs* control. $^fP< 0.01$ *vs* melatonin (2.5 mg/kg). $^iP<0.01$ *vs* melatonin (10 mg/kg). TSO: time to sleep onset ; TS: total sleep time; SWS: slow wave sleep time; PS: paradoxical sleep time; W: awake time.

Group	Dose/mg·kg^{-1}	TSO/min	TS/min	SWS/min	PS/min	W/min
Control	-	82±28	61±19	59±16	1.0±1.3	180±20
Melatonin	2.5	60±17	85±12	84±11	1.2±1.7	155±12
Melatonin	10.0	25±4^c	118±15^c	111±18^c	7±3^c	122±15^c
SCZ	87.5	73±40	56±32	53±28	0.25±0.5	184±32
Melatonin+SCZ	2.5+87.5	72±37	38±7^f	38±7^f	0.0±0.0	202±7^f
Melatonin+SCZ	10.0+87.5	57±34	70±16^i	70±16^i	0.10±0.17^i	170±16^i

ited GABA-like effect and potentiated the effect of GABA on the neuronal activity. Xu *et al* reported that melatonin administered ip significantly increased hypothalamic concentrations of GABA[16]. A single melatonin injection significantly augmented GABA turnover in several brain regions at least in part due to a stimulatory effect on the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD)[17]. GABA is produced from glutamic acid decarboxylation in the brain. Inhibition of GAD activity can markedly decrease the level of GABA. Yoneda *et al*[18] reported that SCZ-HCl (125 mg/kg), a blocker of GAD, decreased the cerebral GABA contents by 32 % in mice. SCZ was usually used as convulsant in previous study. In the present study, SCZ was for the first time studied in its effect on sleep and hypnotic activity of melatonin. Our results demonstrated that the administration of SCZ, at the dosage of 125 mg/kg, seemed to be devoid of pharmacological activity on sleep in mice, but it indeed blunted the sleep-potentiation effect of melatonin in mice. SCZ (87.5 mg/kg) also blocked melatonin-induced increase in TS, PS, and SWS, and decrease in W in rats. However, SCZ did not influence melatonin-induced decrease in TSO. The decrease in TSO induced by melatonin might be mediated by other mechanism.

Melatonin is synthesized and secreted by the pineal gland during the dark period of the light-dark cycle and able to act on specific receptors in or out central nervous system to elicit its different effects. Melatonin produces phase-shifting response and influences circadian rhythmicity through binding to MT_{1b} receptor in suprachiasmatic nucleus (SCN) in rodent[19]. However, acute inhibitory action of melatonin is related to MT_{1a} receptor in SCN[19]. Hypnotic activity of melatonin is attributed to increase inhibitory neurotransmitter or some humoral factors. It seems from above results that melatonin administration may activate GAD through MT_{1a} receptor, and then exhibit its hypnotic activity. Certainly, the minute mechanism of hypnotic activity of melatonin remains to be investigated.

In summary, the present study showed that the hypnotic activity of melatonin was blunted by SCZ, the inhibitor of GABA synthesizing enzyme, although SCZ by itself did not influence sleep by behavioral and electrophysiological experiments in mice and rats. It is thought that the hypnotic activity of melatonin is mediated by GABAergic system and GAD plays a significant role in this process.

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GABA合成酶抑制剂盐酸氨基脲对褪黑激素催眠作用的影响

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关键词　褪黑激素；睡眠；氨基脲；谷氨酸；GABA

目的：观察GABA合成酶抑制剂盐酸氨基脲对褪黑激素催眠作用的影响．方法：采用小鼠协同戊巴比妥钠睡眠法和大鼠脑电图描记法测定盐酸氨基脲对睡眠和褪黑激素催眠作用的影响．结果：褪黑激素对小鼠和大鼠均具有明显的催眠作用．盐酸氨基脲单独使用对小鼠和大鼠的睡眠无影响，但能明显阻断褪黑激素对戊巴比妥钠引起的小鼠睡眠时间的延长，并且明显抑制褪黑激素引起的大鼠总睡眠时间，慢波睡眠时间，快波睡眠时间的增加和觉醒时间的减少．结论：盐酸氨基脲能明显拮抗褪黑激素的催眠作用，提示褪黑素的催眠作用由GABA能系统介导．

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