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Influences of a light–dark profile and the pineal gland on the hypnotic activity of melatonin in mice and rats

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Abstract

We have investigated the influences of the light–dark cycle and the pineal gland on the hypnotic activity of melatonin in rats and mice. The results showed that melatonin significantly shortened time to sleep onset and wakefulness time, increased slow wave sleep, paradoxical sleep, and total sleep time in rats during the light phase of a 12-h light:12-h dark cycle, by electroencephalogram recording. However, during the dark phase it had almost no significant sleep-promoting effect except shortened time to sleep onset. Melatonin exhibited more potent sleep-promoting effect in rats exposed to constant light compared with rats exposed to 12:12-h light:dark at 2000 h. Melatonin markedly prolonged sleeping time in the mice exposed to constant illumination. It was found that pinealectomy was an important factor that influenced the hypnotic activity of melatonin. When melatonin was administered to pinealectomized mice, the hypnotic activity of melatonin was more intense compared with sham-operated mice. These results demonstrated that the hypnotic activity of melatonin displayed a light-dependence manner. These results suggested that light exposure and the functional state of the pineal gland could substantially impact the hypnotic activity of melatonin at pharmacological dosage.

Introduction

Although the pineal gland was described some two thousand years ago, many of its functions remained obscure. The role of the pineal gland in the body has been revealed rapidly since melatonin was isolated and purified in 1958 (Lerner et al 1958). It is well known that melatonin as a darkness hormone is mainly synthesized and secreted by the pineal gland with high nocturnal and low diurnal levels and is sensitive to light exposure. One of the most important functions of endogenous melatonin is to transmit information concerning the light–dark cycle and it encodes a neuroendocrine time signal useful for learning circannual and circadian rhythmicity, including sleep-wake cycle (Reiter 1991). A number of behavioural effects of melatonin at pharmacological dosage have been described in mammals, including decrease in locomotor, sleep-promoting, anxiolytic, anti-convulsive and analgesic effects. Many papers reported that melatonin administration produced hypnotic-like action both in animals (Holmes & Sugden 1982) and man (Arendt et al 1984). However, some inconsistent results emerged. Melatonin ($60\,mg\,kg^{-1}$, i.p.) failed to produce significant sleep-promoting effect at any sleep stage in the cat (Chamblin 1973) and even reduced sleep after a low dose ($0.833\,mg\,kg^{-1}$, i.p.) in rats (Mendelson et al 1980). Stone et al (2000) reported that melatonin given at 2300 h had no significant clinical effect on nocturnal sleep in healthy individuals, but the hypnotic activity of melatonin in the early evening was similar to that of 20 mg temazepam. This showed that previous studies had not drawn a completely coincident conclusion. The hypnotic activity of melatonin at pharmacological dosage may be affected by different factors.

It is well known that light is a very important environmental factor that can influence the rhythmic phase or suppress endogenous melatonin production according to the time of light exposure. Bunnell et al (1992) reported that bright light before sleep on two consecutive nights significantly suppressed salivary melatonin concentration.

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When young sows were exposed to continuous illumination, no significant circadian changes in plasma melatonin were noted (Lewczuk & Przybylska-Gornowicz 2000). It was shown that diurnal rhythm of melatonin secretion was influenced by environmental light and the physiological function of melatonin was influenced by a light regime. However, it is still unclear whether at pharmacological dosage the hypnotic activity of melatonin is linked with the change of light signal. To clarify the relationship between them, we checked the effect of light phase, dark phase and continuous light on the intensity of the hypnotic activity of melatonin in mice and rats.

Moreover, melatonin concentration and effectiveness are affected by the status of the pineal gland. Pineal dysfunction significantly decreases melatonin concentration. Bubenik & Brown (1997) reported that serum melatonin concentrations of pinealectomized rats exhibited significantly lower values compared with sham-pinealectomized rats and control rats. Therefore, we have used the pinealectomized model to clarify the influence of pineal dysfunction on the hypnotic activity of melatonin at pharmacological dosage.

Materials and Methods

Animals

Male Swiss mice (18–22 g) and male Wistar rats (250–300 g) were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University. All animals were used in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China on November 14th, 1988. Mice and rats were housed in a 12-h light:dark cycle (light phase 0800–2000 h, 150 lx) or under constant light conditions (24-h light/day, 150 lx). An actogram was used as an additional index for phase. The room temperature was maintained at $22 \pm 2\,^{\circ}C$ with food and water freely available.

Materials

Melatonin (Changzhou Medical Technique Co, Jiangsu, China) was suspended in 0.3% carmellose sodium and administered orally to mice. In the electroencephalogram recording experiment, melatonin was dissolved in corn oil and injected intraperitoneally to rats.

Implantation of electrodes and polygraphic recording

Electrodes for the electroencephalogram (EEG) and electromyogram (EMG) were implanted in rats as described by Timo-Iaria et al (1970). Male rats were anaesthetized with chloral hydrate ($400\,mg\,kg^{-1}$, i.p.). 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, two 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. Before suturing the rat, antibiotic ointment was applied to the incision to prevent infection.

Following surgery, each rat was housed in individual cages in a 12-h light:dark cycle (light phase 0800–2000 h) or under constant light. Experiments were carried out at least one week after electrode implantation. To minimize the stress involved in experimental procedures, all rats were habituated to the separate recording chamber and the recording conditions (i.e. drug injection and cable attachment) for several days before the actual experiment.

The recording took place in the rat's home cage, where it could move freely to some extent on the day of the experiment. At the start point of recording, the rat was injected with either melatonin or corn oil. Recordings began at either the beginning of the light phase (0800 h) or the dark phase (2000 h) and lasted for 4 h. Waves of EEG were classified into three types: slow wave sleep, paradoxical sleep and wakefulness. Definitions for these three types of wave are described by Mirmiran & Pevet (1986). The total time of slow wave sleep and paradoxical sleep was defined as total sleep time. Time to sleep onset was defined as the time from injection to the occurrence of the first slow wave sleep episode.

The recording was made using an eight-channel physiological recorder (Nihon Kohden, Japan) at a chart speed of $25\,mm\,s^{-1}$. The half-amplitude frequency response was set at 1–35 Hz for the EEG and at 30–75 Hz for the EMG.

Pinealectomy

Procedure of pinealectomy in mouse was referenced to the method described by Hsieh & Ota (1969). The mouse was anaesthetized with pentobarbital sodium ($45\,mg\,kg^{-1}$, i.p.). The dorsum of the head was shaved after sterilization with alcohol. A $0.2\,cm^2$ incision was made over the occipital region. The distance between left side and sagittal suture was approximately 2 mm and the same distance between right side and sagittal suture. The bottom side was left from the lambdoid suture approximately 4 mm, and the upper side was left from the coronal suture approximately 3 mm. The right, left and upper sides were cut with a scalpel, but the bottom side was not. Caution was taken not to damage the cerebral cortex. The cranii was raised with forceps from the upper side. Another set of forceps approached to the site of the pineal gland and removed it. Slight bleeding was easily controlled with the aid of saline-moistened cotton pledgets pressed over the site for approximately 1 min. The small piece of bone removed was replaced and the incised skin was sutured and puffed with powder of penicillin. Following the surgery the mouse was allowed to recover for one week before use. After experimentation, the mouse was killed with sodium pentobarbital (i.p.), and examined to ascertain whether the pinealectomy had been performed correctly.

Measuring sleeping time in mice

Melatonin or 0.3% carmellose was administered orally to mice, 20 min later pentobarbital sodium (40 mg kg^{-1}) was injected intraperitoneally. The absence of the righting reflex was considered as the sleep onset and the duration of the loss of righting reflex was recorded as the sleeping time (Yang et al 1999). The experiment was performed at 25 ± 1 °C.

Statistical analysis

The results were expressed as the mean ± s.e. Data were analysed using a one-way analysis of variance followed by the Student–Newman-Keuls' test for multiple comparisons between groups. Differences with $P < 0.05$ were considered statistically significant.

Results

The effect of melatonin on EEG during the light phase compared with the dark phase in rats exposed to a 12-h light:dark cycle

When melatonin was injected during the light phase it significantly shortened time to sleep onset and wakefulness, and increased slow wave sleep, paradoxical sleep and total sleep time. However, when melatonin was injected during the dark phase all these sleep parameters were not significantly changed except for a decrease in time to sleep onset (Table 1).

The effect of melatonin on the EEG of rats exposed to constant light compared with those exposed to the 12-h light:dark cycle

In rats exposed to a 12-h light:dark cycle, beginning at 2000 h, it was found that melatonin revealed only very slight hypnotic activity. However, in the rats exposed to constant light, melatonin significantly shortened time to sleep onset and wakefulness, and increased slow wave sleep and total sleep time (Table 2).

Effect of melatonin on potentiation of pentobarbital sodium-induced sleep in mice exposed to constant light

Mice were exposed to constant light for 14 days before the study. The experiment began at 0800 h. Melatonin was administered 20 min before pentobarbital sodium injection (40 mg kg^{-1}). Melatonin (15 mg kg^{-1}) prolonged the sleeping time more significantly in mice exposed to constant light compared with mice housed in the 12-h light:dark cycle (Figure 1).

Table 1 The effect of melatonin on EEG during light phase in comparison with the dark phase in rats exposed to 12:12-h light:dark.

	Group	Dose (mg kg^{-1})	Time to sleep onset	Total sleep time (min)	Slow wave sleep (min)	Paradoxical sleep (min)	Wakefulness (min)
Light phase	Control	—	93.00 ± 11.28	57.88 ± 8.56	55.42 ± 7.06	1.05 ± 0.52	183.90 ± 9.10
	Melatonin	10	25.41 ± 1.73**	117.73 ± 6.83**	110.89 ± 7.90**	6.84 ± 1.35**	122.27 ± 6.83**
Dark phase	Control	—	83.84 ± 5.20	56.08 ± 5.92	53.26 ± 5.00	2.82 ± 1.18	184.00 ± 5.82
	Melatonin	10	57.10 ± 4.54$^{\#}$	72.31 ± 4.82	63.70 ± 4.06	8.61 ± 2.70	167.69 ± 4.82

Data were expressed as mean ± s.e.m. **$P < 0.01$ compared with control group (light phase), $^{\#}P < 0.05$ compared with control group (dark phase).

Table 2 The effect of melatonin on the EEG of rats exposed to constant light in comparison with a 12:12-h light:dark cycle.

	Group	Dose (mg kg^{-1})	Time to sleep onset (min)	Total sleep time (min)	Slow wave sleep (min)	Paradoxical sleep (min)	Wakefulness (min)
Light:dark 12:12-h	Control	—	83.84 ± 5.20	56.08 ± 5.92	53.26 ± 5.00	2.82 ± 1.18	184.00 ± 5.82
	Melatonin	10	57.10 ± 4.54*	72.31 ± 4.82	63.70 ± 4.06	8.61 ± 2.70	167.69 ± 4.82
Constant light	Control	—	44.12 ± 7.06	72.35 ± 5.85	70.85 ± 5.55	1.50 ± 0.80	167.53 ± 5.79
	Melatonin	10	28.32 ± 7.97†	107.63 ± 8.08**‡	100.92 ± 7.68**‡	6.71 ± 1.92	132.37 ± 8.08**‡

Data were expressed as mean ± s.e.m. *$P < 0.05$ compared with control group (12:12-h light:dark), **$P < 0.01$ compared with control group (constant light), †$P < 0.05$, ‡$P < 0.01$ compared with melatonin (12:12-h light:dark).

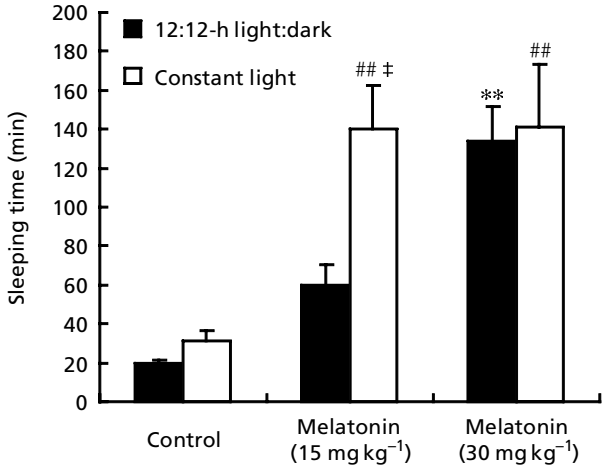

Figure 1 Effect of melatonin on potentiation of sodium pentobarbital-induced sleep in mice exposed to constant light. Data were expressed as mean ± s.e.m. $^{**}P < 0.01$ compared with control group (12:12-h light:dark), $\#\#P < 0.01$ compared with control group (constant light), $\ddagger P < 0.01$ compared with melatonin ($15\,mg\,kg^{-1}$) (12:12-h light:dark).

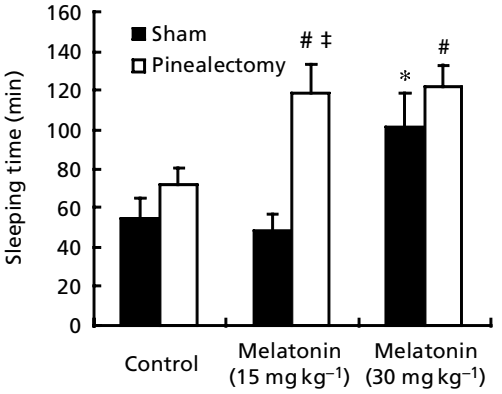

Figure 2 Effect of melatonin on potentiation of sodium pentobarbital-induced sleep in pinealectomized mice. Data were expressed as mean ± s.e.m. $^{*}P < 0.05$ compared with control group (sham), $\#P < 0.05$ compared with control group (pinealectomy), $\ddagger P < 0.01$ compared with melatonin ($15\,mg\,kg^{-1}$) (sham).

Effect of melatonin on potentiation of pentobarbital sodium-induced sleep in pinealectomized mice

Mice were pinealectomized one week before the study. The experiment began at 0800 h. Melatonin was administered 20 min before pentobarbital sodium injection ($40\,mg\,kg^{-1}$). Melatonin ($15\,mg\,kg^{-1}$) prolonged the sleeping time more effectively in pinealectomized mice compared with sham-operated mice (Figure 2).

Discussion

A lot of papers reported that melatonin administration had sedative and sleep-promoting effects in experimental animals and man. However, the hypnotic activity of melatonin may become weak or even disappear under some unsuitable conditions. We found that melatonin significantly prolonged slow wave sleep, paradoxical sleep and total sleep time, but shortened time to sleep onset and wakefulness during the light phase. When using EEG analysis in rats, we found that during the dark phase the hypnotic activity almost disappeared (except shortening time to sleep onset). This indicated that the hypnotic activity of melatonin was affected by the light–dark cycle and that constant light may play an important action in this process.

As to the mechanism of the influence of the light–dark profile on the hypnotic activity of melatonin, it was reported that a GABAergic system might be involved in sleep regulation. Gamma-aminobutyric acid (GABA) as an inhibitory transmitter can be a target for melatonin action (Coloma & Niles 1988; Xu et al 1995; Wu et al 1999). Melatonin administration enhanced binding affinity of [^{3}H]GABA- and [^{3}H]diazepam-binding in rat brain (Niles et al 1987). Many psychopharmacological effects of melatonin exhibited time-dependency and were blunted by co-injection with flumazenil, an antagonist of the benzodiazepine recognition site on the $GABA_A$ receptor (Golombek et al 1992, 1993). Recently, we proved that the hypnotic activity of melatonin was mediated through a synthetic enzyme for GABA (Wang et al 2002) and the benzodiazepine, picrotoxin, and GABA-binding site on the $GABA_A$ receptor (Wang et al 2003). Furthermore, it was reported that benzodiazepine binding was high during periods of sleep and low activity with a significant decrease occurring just before waking (Brennan et al 1985). These findings indicated that the difference of hypnotic activity of melatonin during the light and dark phase might be due to its ability to enhance central GABAergic transmission by modulating GABA-receptor activity.

The other possibility is that circadian rhythm of melatonin binding might play an important role. High-affinity binding of 2-[^{125}I]iodomelatonin in rat brain sections was high in the light phase and low during darkness, in inverse correlation to the serum melatonin rhythm (Tenn & Niles 1993). The density of [^{125}I]melatonin binding sites in the hypothalamus was maximal between 1000 and 1800 h and dropped sharply after the lights went off (Zisapel 1988). Masana et al (2000) reported that 2-[^{125}I]iodomelatonin binding in the suprachiasmatic nucleus in mice revealed circadian rhythms with highest levels of binding 2 h after lights on or at the beginning of the subjective day, respectively. When melatonin is administered during the light phase, it may bind to more melatonin binding sites to reveal strong hypnotic activity compared with that during the dark phase.

It is well known that melatonin is a darkness hormone and its secretion and function are sensitive to light exposure. Constant light abolished the nocturnal rise of 6-sulfatoxymelatonin, a metabolite of melatonin, and lowered it to daytime levels, so it was considered as a functional pinealectomy model (John et al 1992, 1994). Even though

melatonin level was lowered after constant light exposure, [^{125}I]melatonin binding site density was inversely increased in rats (Gauer et al 1993). In this study, we exposed the mice to constant light for 14 days before the experiment. Melatonin administration prolonged the sleeping time induced by pentobarbital sodium more significantly in mice exposed to constant light compared with mice housed in a normal light–dark cycle during the light phase. Similarly, using EEG recording, melatonin markedly shortened time to sleep onset and wakefulness, and increased slow wave sleep and total sleep time in rats exposed to constant light during the dark phase, even though it almost did not affect sleep parameters in normal rats during the same time. It can be seen that the hypnotic activity of melatonin at pharmacological dosage was enhanced not only during the rest period, but during the active period in the case of constant light-induced pineal dysfunction.

To investigate the hypnotic activity of melatonin related to the functional status of the pineal gland, a pinealectomy model was chosen. The administration of melatonin significantly prolonged pentobarbital sodium-induced sleeping time in pinealectomized mice compared with sham-operated mice. It was noted that pinealectomy caused a significant decrease in serum melatonin concentration compared with normal controls (Wu et al 1990), but the melatonin receptor density was significantly increased (Schuster et al 2001). Melatonin may have more chance to bind to the increased melatonin receptors in pinealectomized mice than sham mice. Our result indicated that the functional state of the pineal gland was an important element that could affect the hypnotic activity of melatonin.

From the above results, it was interesting to note that when endogenous melatonin was diminished due to constant light exposure or pinealectomy, the hypnotic activity of melatonin at pharmacological dosage was stronger than when the endogenous melatonin level was normal. The increased melatonin receptor is likely to be involved in the hypnotic action of melatonin in pineal dysfunction.

Conclusions

The hypnotic activity of melatonin in mice or rats was impacted by light signal and pineal gland. Melatonin prolonged the sleeping time significantly in pinealectomized animals or animals exposed to constant light compared with normal animals. It was apparent that the extent of hypnotic action of melatonin was related to the change of light signal and the functional status of the pineal gland.

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