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Effects of melatonin injection on running-wheel activity and body temperature differ by the time of day

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Abstract

Melatonin, which is released from the pineal gland, influences many physiological events concerned with circadian rhythm. Body temperature levels and rhythmicity are tightly coupled with locomotor activity. To understand the functions of melatonin, we determined the effects of melatonin injection on locomotor activity measured by running-wheel activity and body temperature in rats. The rats were kept under a 12-h light and 12-h dark lighting condition, with the light on at zeitgeber time 0 (ZT 0, correspond to 7:00 JST). Melatonin injection, between ZT 3 and ZT 5 (light period) and between ZT 15 and ZT 17 (dark period), attenuated the wheel-running activity in a dose-dependent manner (10 μg to 1 mg/100 g body weight [bw]). A significant attenuation of activity by melatonin was recognized when injected at ZT 8, ZT 14 and ZT 20. After the injection of melatonin, the animal's body temperature was elevated at ZT 2 and ZT 8 (during light), while it fell at ZT 14 and ZT 20 (during darkness). We propose a plausible explanation underlying the observed changes in body temperature during the light and dark periods accompanying the suppression of activity induced by melatonin.

Keywords: Melatonin; Wheel-running activity; Body temperature; Circadian rhythm

1. Introduction

The synthesis and release of the pineal hormone melatonin shows a circadian rhythm that is regulated by light and the circadian pacemaker located in the suprachiasmatic nucleus (SCN) (Moore, 1996). The neuronal signals of light that inhibits the melatonin synthesis are relayed from the SCN to the pineal gland via a pathway through the hypothalamus and the superior cervical ganglia, which is the origin of the sympathetic projections to the pineal gland (reviewed by Moore, 1996; Weaver, 1999).

A pineal feedback control of SCN activity is suggested by several factors: (1) the presence of high-affinity melatonin binding sites in the SCN (Dubocovich, 1995), (2) phase- and dose-dependent inhibition of the SCN activity (Gillette and McArthur, 1996; McArthur et al., 1991) and (3) a time-dependent phase shift in the electrical activity rhythm of the SCN after exposure to melatonin in vitro (McArthur et al., 1991). Furthermore, melatonin inhibits Arg-vasopressin (AVP), which is contained in one class of output neurons in the SCN (Buijs and Kalsbeek, 1993; Isobe et al., 2001a; Morin, 1993). In contrast, AVP suppresses the melatonin release from the pineal gland (Isobe et al., 2001b).

Melatonin has been reported to affect behavior, such as inducing sleep or a hypnotic property (Holmes and Sugden, 1982; Mirmiran and Pevet, 1986), and decreasing activity in rodents (Golombek et al., 1991; Sugden, 1983). Enhanced performance in motor coordination, i.e., rotorod by melatonin injection, was noted during the dark phase (Poulos and Borlongan, 2000). Melatonin is synthesized in the pineal gland during the dark phase and the daytime administration of melatonin induces arousal (elevates motor activity) in nocturnal rodents (Hastings et al., 1992). Accordingly, the timing of administration is important for neuroendocrine effects of melatonin (Golombek et al., 1991; Hastings et al., 1992).

Locomotor activity is tightly linked to the regulation of core body temperature. Melatonin is considered to affect both the regulations of locomotor activity and body temperature and their rhythmicity. Melatonin intervenes in heat-stress tolerance and in setting the central body temperature in mice and humans (Cagnacci et al., 1997; Haim and Zisapel, 1997). In chickens, melatonin caused a dose-

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Table 1
Wheel running activity during 20 min after the saline and melatonin injection

	ZT 2	ZT 8	ZT 14	ZT 20
Saline	86 ± 30.6	91.2 ± 46.5	120.8 ± 31.8[#]	107.9 ± 46.7
Melatonin	72.23 ± 54.1	72.0 ± 39.2 *	89.0 ± 48.3 *	81.3 ± 45.8 *
df	10	10	10	10
F	3.125	1.407	2.337	1.035
P	.069	.046	.021	.049

* $P < .05$ compared with the saline value.
[#] $P < .05$ compared with the value at ZT 2 in saline injection.

dependent reduction in body temperature accompanied by an increase in the peripheral skin temperature (Rozenboim et al., 1998). Melatonin induced hyperthermia only during the photophase (light period), while the responses were poor during the scotophase (dark period) in rats (Padmavathamma and Joshi, 1994). A consensus has not yet been reached on the effect of melatonin on body temperature in relation to the changes in locomotor activity, especially with regard to differences in the time of day.

In the present study, we determined the effects of melatonin on locomotor activity and body temperature at different times of day. Simple behavioral thermoregulation and sensitivities did not explain the day–night changes in body temperature in response to melatonin.

2. Materials and methods

2.1. Animals

Male Wistar rats (SLC) that were born and kept under the lighting cycle of 12-h light and 12-h dark (LD cycle) were used. The body weight of rats ranged 150–180 g (6–8 weeks age). The rats were kept two animals per one transparent plastic cage (home cage) in an animal room. Food and water were available ad libitum. The light on at ZT 0 (07:00 JST). Time is expressed in zeitgeber time (ZT), where ZT 12 corresponds to the offset of light at 19:00 JST. The animals were habituated to the handling for three times before each experiment. All experimental procedures were carried out in accordance with the guidelines of Nagoya City University Medical School for use of animals and research.

2.2. Locomotor activity

Locomotor activity was measured (Locomotor Activity Meter, Natsume, Japan) for wheel-running activity under a constant ambient temperature (25 ± 1 °C) and humidity (50–70%) set in the climatic room. The circumference of the wheel was 100 cm. A cumulative counter recorded the amount of activity over a 20-min period. During the recording of wheel-running activity, food and water were deprived (Isobe, 1997). The animal was allowed to access to the wheel only at the time of measurement.

2.3. Body temperature

Body temperature was measured using a rectal thermometer with an accuracy of 0.1 °C, inserted 5 cm beyond the anal sphincter. Before each measurement, the thermometer tip was first dipped into an antiseptic solution at 40–41 °C and a stable rectal temperature could be recorded within 10 s of insertion. Measurements were obtained within 1 min of picking the animal up before it was released to the home cage (Isobe, 1997; Isobe and Ohara, 1987).

2.4. Dose-dependent wheel-running activity

Two groups of six test rats were used. Melatonin was injected intraperitoneally at doses of 10 μg, 100 μg or 1 mg/100 g body weight (bw) between ZT 3 and ZT 5 (light

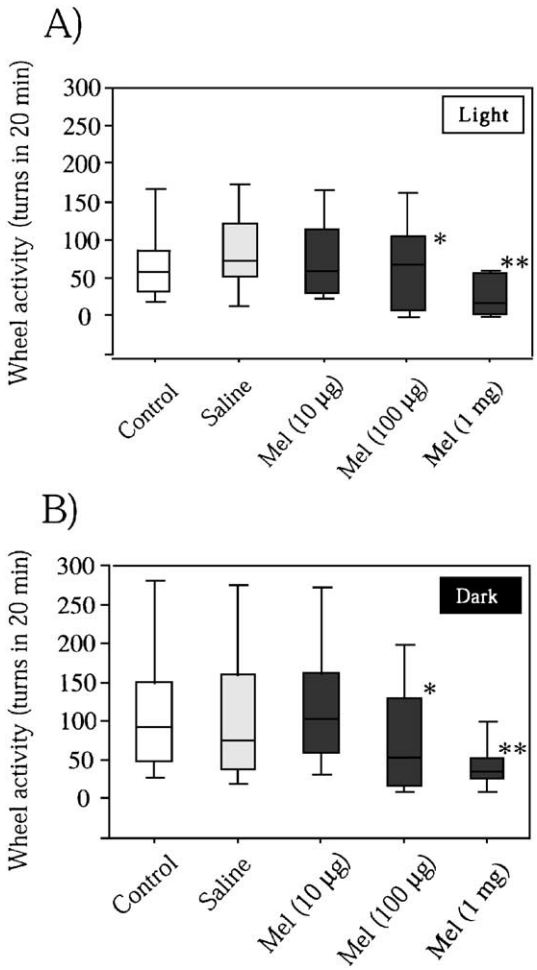

Fig. 1. The different responses during the light (A) and dark (B) periods to the melatonin dose in attenuating the effect on wheel running activity for 20 min. A rectangle indicates the 25–75% reliable range with the median value is shown as a horizontal line. The vertical line attached to the box indicates the 10–90% reliable range of activity. The same six animals were used in each dose of melatonin. Control: no injection. Saline: containing 0.1% ethanol. Melatonin was injected (intraperitoneally) at doses of 10 μg, 100 μg and 1 mg/100 g bw. * $P < .05$, ** $P < .01$ compared with the saline values.

period, one group) or between ZT 15 and ZT 17 (dark period, another group). Melatonin (Sigma, Japan) was dissolved in ethanol (final 1:1000) and diluted with saline. The melatonin concentration was 20 μg, 200 μg and 2 mg/ml. For the control, no injection was performed. Saline (containing 0.1% ethanol) and melatonin were injected at 0.5 ml/100 g bw. Using a random-number table randomized the sequence of treatment and activity measurements for the saline, melatonin and control. The injection was administered once a day to the same animal.

2.5. Diurnal variations in wheel-running activity and body temperature changes

2.5.1. Differences in wheel activity by the time of day

Two groups of six rats were used. Based on the dose–response results, we chose 100 μg/100 g bw for the subsequent experiment. The effects of saline and melatonin on wheel-running activity were compared at ZT 2 and ZT 14 (six rats, first group), or at ZT 8 and ZT 20 (six rats, second group). Immediately after the saline or melatonin injection, each animal was set on the wheel and the wheel turns were counted for a 20-min period. The injection sequence of saline and melatonin at ZT 2 and ZT 14 or at ZT 8 and ZT 20 on the same rat was randomized using a random-number table. The injection of saline or melatonin was administered once per day to the same animal. The averages of the wheel-running activity at ZT 2 and ZT 14 or at ZT 8 and ZT 20 in each rat receiving a saline injection were set at 100%. The differences in the effects of melatonin on wheel activity by the time of day were compared against the value (percentage) of the saline in the same rat.

2.5.2. Changes in body temperature by the time of day

Four groups, two groups for melatonin and two groups for saline, of rats were used in total for analyzing the body temperature changes (Table 1). When measuring the diurnal variation of wheel-running activity independently, the effects of melatonin and saline on body temperature were measured at ZT 2 and ZT 14 or ZT 8 and ZT 20 in the two groups (six rats in each). Based on the dose change in the suppression of the wheel-running activity, we chose 100 μg/100 g bw for the analysis of body temperature changes. Immediately after the injection of melatonin or saline, each rat spent 20 min in the wheel. The time of injection was randomized for each rat. The rectal temperature was measured twice: 1.5 h before the rat was set on the wheel and immediately after it was removed from the wheel. The effect on body temperature due to wheel turning was not considered, since the rectal temperature of each rat was measured before the animal was placed in the wheel. The rectal temperature 1.5 h before the rat entered the wheel showed the "before" values of temperature at each expected time (Isobe, 1997). The body temperature measurements obtained at ZT 2 and ZT 14 or at ZT 8 and ZT 20 in the two groups were compared in both the melatonin and saline groups.

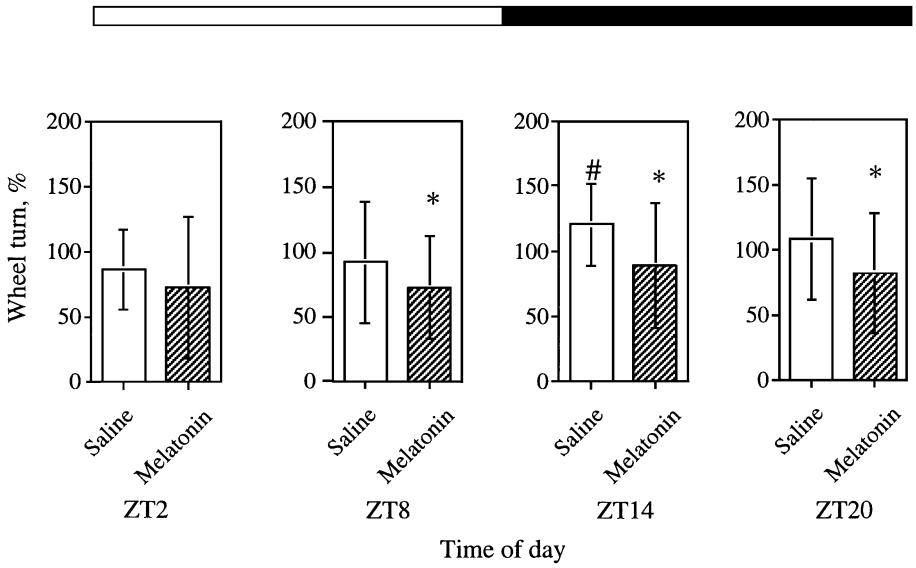

Fig. 2. Diurnal variations in melatonin's inhibitory effect on wheel running activity during a 20-min period. Each rat was placed on the wheel immediately after the injection of saline or melatonin (100 μg/ 100 g bw). The average of the wheel running activity in each rat at ZT 2 and ZT 14 (first group) or at ZT 8 and ZT 20 (second group) was set as 100%. Time is expressed in zeitgeber time (ZT), where ZT 12 corresponds to the offset of lights (19:00 JST). The wheel activity was expressed as a percentage of the average value. The open and oblique-lined columns represent the wheel activity after the injection of saline and melatonin, respectively. Values are expressed as means ± S.E.M. ($n = 6$). Findings from the two groups of six rats were combined in the figure. The horizontal white and black bars represent the light and dark periods, respectively. Statistical analysis was performed using Student–Newman–Keul's test after the two-way ANOVA. * $P < .05$ compared with the saline value at each time. $^{\#}P < .05$ compared with the value at ZT 2 in saline injection. Original data are the same as in Table 1.

2.6. Data analysis

Significance in the differences in the wheel-running activity and body temperature in the same group of rats was analyzed by ANOVA. Significance in the differences between groups was analyzed by Student–Newman–Keul's test. In analyzing the significance of dose, Mann–Whitney's U test was used. Differences with $P < .05$ were considered statistically significant.

3. Results

3.1. Suppression of running-wheel activity by melatonin

Melatonin attenuated the wheel-running activity within 20 min dose-dependently at a range of 10 μg to 1 mg/100 g bw (Fig. 1). The dose-responses to melatonin were similar during the light and dark period. The attenuation of wheel-running activity appeared to be larger during the dark period than during the light period, however, no significant differences were detected by Student's t test (different group of animals). Animals showed increased activity after the saline injection, but it was not significant. The numbers of wheel turns when no drug was applied (control) was higher at night (112 ± 44) than during day (57 ± 23, $P < .05$). Five minutes after entering the wheel, the rats turned the wheel vigorously, then subsequently reduced their wheel-running activity for 20 min. These behavioral responses to the wheel were observed not only after the injection of saline, but also after melatonin injections and no injection (control). The effect of each dose of melatonin on the activity level showed similar tendencies, without exception, in each rat.

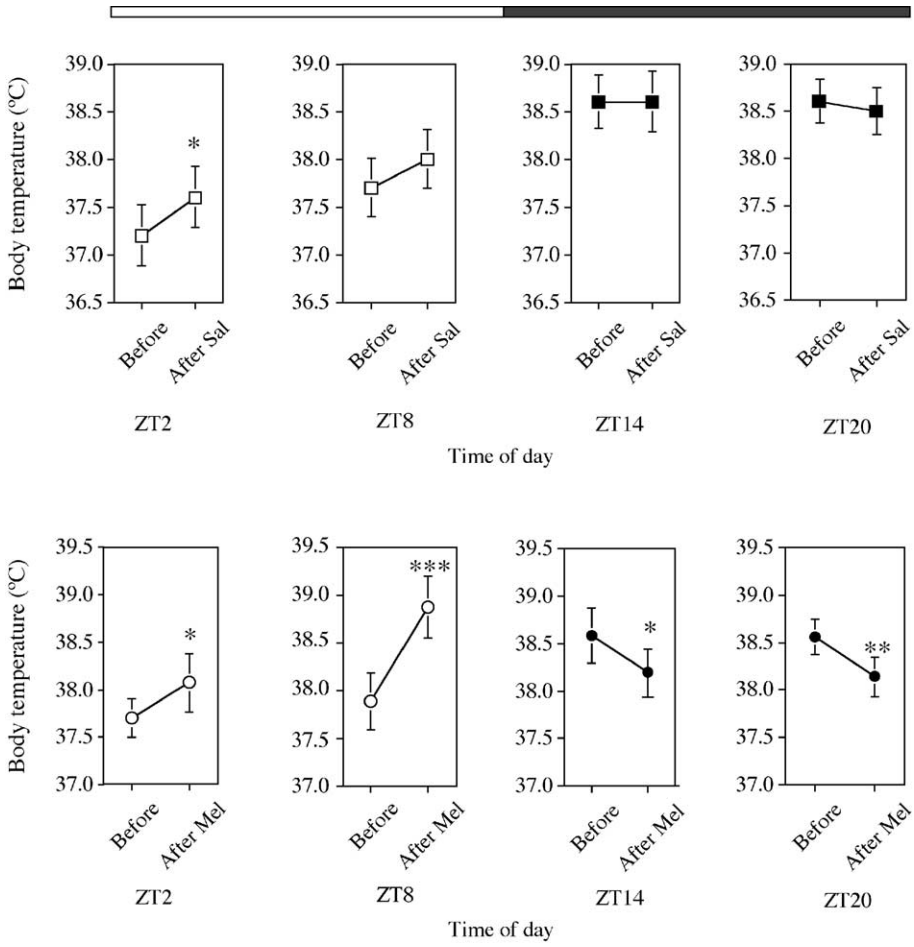

Fig. 3. Diurnal variations in the effect of saline (top) or melatonin (bottom) on body temperature. The findings from the two groups, in each drug application series, were combined. Temperatures in the melatonin (100 μg/100 g bw) or saline groups at ZT 2 and ZT 14 differed from those at ZT 8 and ZT 20 (Table 1). Immediately after the injection of melatonin or saline, each rat spent 20 min in the wheel. The body temperature was measured twice, 1.5 h before (Before) and 20 min after the injection of saline or melatonin (after Sal or after Mel). Open and closed circles or squares indicate the temperature measured during the light and dark periods, respectively. Values are expressed as means ± S.E.M. ($n = 6$). * $P < .05$, ** $P < .01$, *** $P < .005$ compared with the before value, analyzed by Student–Newman–Keul's test after two-way ANOVA.

3.2. Suppression of wheel-running activity is different by the time of day

The diurnal variations in wheel activity within 20 min in response to melatonin were compared with those of the saline group. Melatonin (100 μg/100 g bw) significantly suppressed wheel activity at ZT 8, ZT 14 and ZT 20, when compared with saline by Student–Neuman–Keul's test (Table 1 and Fig. 2). At ZT 2, the tendency of suppression was clear, but the significance level was small ($F=3.125$, $df=10$, $P=.0591$, $P<.1$). In rats injected with saline, the nocturnal response to the wheel was higher compared with the activity at ZT 2 and ZT 14 ($P<.05$). These findings suggest that melatonin suppressed activity more during the active period during darkness.

3.3. The differences in the body temperature changes after the administration of melatonin by the time of day

The effect of melatonin (100 μg /100 g bw) on body temperature differed by the time of day (Fig. 3). Twenty minutes after the melatonin injection, the body temperature increased at ZT 2 (before Mel: 37.70 ± 0.20, after Mel: 38.07 ± 0.31; $P<.05$) and ZT 8 (before Mel: 37.88 ± 0.30, after Mel: 38.87 ± 0.33; $P<.001$), during the light period, when the body temperature usually shows lower circadian levels (Fig. 3). However, during the dark period, it decreased at ZT 14 (before Mel: 38.59 ± 0.29, after Mel: 38.06 ± 0.31; $P<.05$) and ZT 20 (before Mel: 38.56 ± 0.19, after Mel: 38.14 ± 0.21; $P<.01$), when the body temperature and melatonin levels are higher in nocturnal animals. After the saline injection, animals spent 20 min in the wheel and body temperature increased significantly only at ZT (before Sal; 37.2 ± 0.32, after Sal: 36.7 ± 0.32; $P<.05$). Saline did not increase the body temperature during the dark period (Fig. 3).

Any fluctuation in body temperature caused by handling and inserting of the thermo-probe had recovered within 1.5 h. The expected body temperature was reached after the cessation of wheel-running activity and after handling and melatonin injection as reported previously (Isobe, 1997). The initial differences in body temperature by the time of day suggest the intrinsic circadian drift of the temperature.

4. Discussion

4.1. Attenuation of running wheel activity throughout 1 day by melatonin

The response to saline on the wheel-running activity was different by the time of day (Fig. 2). Wheel-running activity was enhanced during both the dark and light periods. The light period corresponds to the time at which spontaneous locomotor activity is low, reflecting the circadian rhythm. A significantly higher response to the wheel was observed at ZT 14, during the dark period. The injection of saline transiently facilitated the running activity, which was attenuated by melatonin in a dose-dependent manner (Figs. 1 and 2). Melatonin decreased the wheel activity during the light and dark periods in all rats. The tendency toward higher activity during the dark period, reflecting the intrinsic rhythmicity, was attenuated by the melatonin. Diurnal changes during the early phase (0–120 min) of locomotor activity was previously reported (Golombek et al., 1991). The intraperitoneal injection of melatonin at a lower dose (0.1 mg/kg bw) inhibited the early phase of activity at 12:00 and 20:00 h, but not at midnight (Golombek et al., 1991). The effect of melatonin at noon and early night is commonly explained by the sedative effects of the melatonin, as shown in previously established psychological tests for sedative, hypnotic and analgesic activities (Holmes and Sugden, 1982; Sugden, 1983).

The rotorod performance, maintaining a balance on the rotating rod, is higher during the dark period than under the light (Poulos and Borlongan, 2000). The rotorod performance increased after the melatonin administration (Poulos and Borlongan, 2000). It is interesting that the effects of melatonin on rotorod performance and wheel-running activity (this study) are opposite. To clarify the mechanism of the different effect of melatonin is a further problem.

The present findings showing diurnal variations after melatonin administration suggest phase shifting of the activity rhythm. The daily administration of pharmacological doses of melatonin, but not lower doses, entrains the free-running circadian locomotor activity rhythm of rats in constant darkness (Cassone et al., 1986; Redman et al., 1983). However, the present animals were limited to the LD conditions. Moreover, melatonin was administered only twice during the experiment in a random administration time-sequence. The possibility that the decrease in activity was induced by melatonin is unlikely to be due to phase shifts.

4.2. Diurnal variations in body temperature changes against melatonin

Melatonin induced hypothermia during the dark period and hyperthermia during the light (Fig. 3). The effect was not so simple as in the wheel-running activity. Melatonin inhibits thirst and lipopolysaccharide-induced fever by reducing free radicals (nitric oxide) and the excessive formation of prostaglandin and cytokines (Nava et al., 1997). This decrease in prostaglandin in turn decreased body temperature (Nava et al., 1997). In chickens, melatonin caused a dose-dependent reduction in body temperature accompanied by a peripheral skin-temperature increase (Rozenboim et al., 1998). Melatonin-induced hyperthermia during daytime was also reported at a lower dose of 25 μg in rats (Padmavathamma and Joshi, 1994). In humans, the administration of melatonin during the light period decreased the body temperature by about 0.3–0.4 °C and

the suppression of melatonin at night enhanced body temperature by about the same magnitude (Cagnacci et al., 1997). In rats, the same tendency that a decrease in body temperature during the dark period and increase during the light period was observed (Fig. 3).

It is likely that melatonin's ability to reduce body temperature is exerted mainly in the hypothalamus, where the thermoregulatory center is located. The nocturnally elevated level of melatonin affects the activity levels and body temperature differently in humans and rodents. In rodents, if melatonin is administered at night, as in the present study, the melatonin concentration overrides the higher basal level. Consequently, during darkness, the activity level is attenuated by the melatonin and causes the decrease in body temperature in parallel. However, melatonin administered during daytime, when melatonin levels are usually low, decreases the activity accompanied with an increase in body temperature. The acute thermoregulatory effects and the acute locomotor-activity-attenuating effect induced by melatonin might be independent. The entrainment of melatonin's circadian activity rhythm depends upon the hypothalamic SCN (Cassone et al., 1986). It is reasonable to assume that the thermoregulatory effects of melatonin injected intraperitoneally are mediated via the SCN in the hypothalamus. The effects of melatonin on the SCN, output for the control of locomotor activity and body temperature changes during the daytime and nighttime, might be explained in relation to AVP (Buijs and Kalsbeek, 1993).

Intraventricularly administered AVP caused hypothermia accompanying suppression of novelty-induced behavior (Drago et al., 1997; Pittman and Wilkinson, 1992). The decrease in AVP release from the SCN by melatonin was dependent on the time of day. The AVP decrease was greater during the light period, when the spontaneous AVP in the SCN is high (Isobe et al., 2001a). The decrease in hypothermic neuropeptide (AVP), induced by the melatonin, might increase the body temperature during the light period, but not during the darkness.

The running-wheel cage is widely used to analyze the circadian activity rhythm. The wheel activity in relation to the food intake (Boer et al., 1990), injections of cocaine (Ito et al., 1997) and amphetamine (Bradbury et al., 1987), were reported, however, no consensus has been reached. The trigger and motivating mechanism of the wheel activity is requires clarification. The wheel cage facilitates the phase-shifting effect of a light pulse (Turek, 1989). Phase advance by manual injections of melatonin is effective, but there is no effect with remote injection (Hastings et al., 1992). An environmental or pharmacological stimulus induces phase shifts and period change (Morin, 1993). A short-term increase in locomotor activity occurs when a dark pulse in constant light shifts the circadian activity rhythm (Van Reeth and Turek, 1989). Resynchronization to a shifted light–dark cycle can be greatly accelerated if animals are made more active by triazolam (facilitate the wheel-activity in rodents) injections or by confinement to a new wheel (Mrosovsky and Salmon, 1987; Van Reeth and Turek, 1989). Although an animal's motivation to start and continue wheel-running activity is not yet clear, several neurotransmitters, neuropeptides and related circuits should be considered (Moore, 1996; Morin, 1993). If the nature of the burst of activity is clarified, we can uncover, at least in a part, the mechanisms of phase shifting and the circadian controlling systems.

In conclusion, intraperitoneally administered melatonin deceased the running wheel activity at ZT 2, ZT 8, ZT 14 and ZT 20 in the light–dark condition. Melatonin increased and decreased the body temperature during light and dark conditions, respectively. Additional studies are warranted to determine the mechanisms underlying these variations in running wheel activity and body temperature produced by melatonin.

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