Thermoregulatory and soporific effects of very low dose melatonin injection

CAMERON J. VAN DEN HEUVEL,1 DAVID J. KENNAWAY,1 AND DREW DAWSON2

1Department of Obstetrics and Gynaecology, University of Adelaide, Medical School North, Adelaide, South Australia 5005; and 2The Centre for Sleep Research, University of South Australia, The Queen Elizabeth Hospital, Woodville, South Australia 5011, Australia

Van den Heuvel, Cameron J., David J. Kennaway, and Drew Dawson. Thermoregulatory and soporific effects of very low dose melatonin injection. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E249–E254, 1999.—The effect of a rapid increase in circulating melatonin on body temperatures and sleepiness was investigated in eight young adults at 1000. Melatonin administered intravenously at 10- and 30-µg doses, but not 3 µg, resulted in elevated plasma and saliva levels consistent with endogenous levels measured in adults at night. Melatonin at 10 and 30 µg significantly attenuated the daytime increase in rectal core temperature (P < 0.05 for both). The mean maximum rectal core temperature differences between saline and melatonin treatment were 0.11 ± 0.03°C, 0.16 ± 0.04°C, and 0.18 ± 0.04°C after the 3-, 10-, and 30-µg melatonin doses, respectively. All three doses significantly increased hand temperature compared with saline (P < 0.05) within 30 min. The mean maximum hand temperature differences were 0.72 ± 0.12°C (3 µg), 0.95 ± 0.15°C (10 µg), and 0.65 ± 0.11°C (30 µg). Foot temperature and subjective sleepiness measures did not change at any melatonin dose. The results suggest that daytime intravenous injection of melatonin to achieve normal nocturnal levels in young adults may produce significant thermoregulatory changes without soporific effects.

core temperature; hand temperature; sleepiness; intravenous administration; physiology

INTEREST in the potential physiological roles of the pineal neurohormone melatonin is rapidly increasing. In an attempt to understand what melatonin does in the body, many experiments since the early 1960s have investigated the physiological effects of melatonin administered to both animals (4, 5, 16, 23, 36) and humans (1, 10, 21, 27, 28). As the nocturnal production of melatonin typically coincides with the normal sleep period (3, 30), it has been suggested as an endogenous regulator of sleep propensity (reviewed in Ref. 12). It is currently presumed that the acute soporific effects observed after daytime melatonin administration are mediated by either or both thermoregulatory (13, 15, 17, 26, 32) and/or chronobiotic effects (25, 38) of the hormone.

With regard to a possible role in thermoregulation or sleep, the majority of recent studies administering melatonin to humans have utilized oral preparations of 0.1–100 mg, given during the day or evening (2, 6–8, 13, 15, 17–19, 24, 26, 39, 40). Only four of these studies, however, included low melatonin doses (0.1–0.3 mg) that elevated melatonin levels close to nocturnal physiological levels (13, 15, 39, 40). Daytime melatonin administration protocols have typically been used, because endogenous production is lowest during the daylight hours (3). Whereas oral daytime dosing has some obvious advantages in research and clinical settings, several authors (12, 29, 38) have suggested that mimicking the endogenous nocturnal melatonin plasma profile may better reveal the physiological effects of melatonin.

Recently, we found that a prolonged daytime infusion of melatonin, with a relatively slow onset to peak plasma levels, had significant effects on temperature and subjective sleepiness only at supraphysiological levels (32). In the present study, we examined the effects of a range of melatonin levels on body temperatures and subjective sleepiness when administered with a rapid systemic onset. As opposed to the steady infusion in our previous study, we aimed to achieve a short latency to peak melatonin levels with an intravenous bolus injection.

METHODS

Subjects. The study was approved by the Ethics of Human Research Committee at The Queen Elizabeth Hospital, on the basis of guidelines from the National Health and Medical Research Council of Australia and the Declaration of Helsinki. Eight subjects (4 male, 4 female) gave informed consent and attended the laboratory for four nonconsecutive bed rest sessions between 0800 and 1500. The subjects were aged 20–27 yr (means ± SE = 23.9 ± 0.7 yr), and body mass indexes (BMI) for females and males were 22.1 ± 0.5 and 27.7 ± 1.4 kg/m², respectively. Subjects were screened for medical, psychiatric, and sleep disorders with a battery of questionnaires and a 7-day sleep diary. Potential subjects were excluded if they exhibited any concurrent medical or psychiatric illness or occult sleep disorder or if they were taking any medication known to affect sleep, thermoregulation, or melatonin production. Female subjects participated only during the follicular menstrual phase.

Experimental protocol. Subjects were required to abstain from caffeine and alcohol for at least 24 h before and during each experimental session. On arrival before 0800 in each session, subjects had an intravenous cannula placed by medical staff in the antecubital vein of the nondominant forearm. Subjects were also fitted with a montage of thermistors for the measurement of body temperatures on the back of each hand (YSI-4499E, Yellow Springs Instruments, Yellow Springs, OH), the instep of both feet (YSI-4499E, Yellow Springs Instruments), and 10 cm into the rectum (Steri-Probe 4918, Cincinnati Sub-Zero Products, Cincinnati, OH). All thermistors were connected to a custom data collection and display and storage system comprising Workbench for Win-

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dows software and hardware from Strawberry Tree (Sunnyvale, CA).

At 1000 in each session, subjects received, in counterbalanced order, a 1-ml sterile intravenous injection of 0.9% saline or melatonin (Sigma Aldrich, Castle Hill, Australia) in the antecubital vein of the dominant (noncannulated) arm. Melatonin was dissolved in 0.9% saline, and each subject received three doses (3, 10, and 30 µg) in separate sessions. Sterile single-use injections were prepared by The Queen Elizabeth Hospital Pharmacy Production Unit between 24 and 72 h before the start of each session and were refrigerated until required. Each unmarked syringe was covered with black plastic until being used to protect the melatonin from possible degradation by light and to ensure that subjects and investigators were blinded to the treatment. Double blinding was ensured by data coding during data collection and analysis.

Plasma and saliva melatonin measurement. The cannula on the nondominant side of each subject was used to sample 10 ml of blood hourly from 0800 to 1500, with additional samples at 1015 and 1030. Blood samples were stored in heparinized tubes for up to 2 h at 4°C and then were centrifuged at 1,500 rpm for 10 min. Plasma was prepared and frozen at −20°C for later assay. Saliva samples were taken immediately after each plasma sample with plain salivettes (Sarstedt, Technology Park, Australia). After collection, the salivettes were centrifuged at 1,500 rpm for 10 min, and the saliva was stored at −20°C for later assay. The concentration of melatonin in 500-µl plasma and 200-µl saliva samples was determined with the Bühlmann radiommun assay kit (Bühlmann Laboratories AG, Allschwil, Switzerland), which measures melatonin by a double antibody assay on the basis of the Kennaway G280 anti-melatonin antibody (33, 35). Melatonin was extracted from plasma samples before assay with Bühlmann C18 reverse-phase columns, whereas saliva samples were assayed directly. The sensitivities of the assays were 4.3 and 8.6 pM for plasma and saliva, respectively. The intra- and interassay coefficients of variation (CV) of all assay runs were all <8.0 and <15.0% for plasma and saliva, respectively.

Subjective sleepiness. Coinciding with each saliva collection, subjects were presented with an unmarked linear sleepiness rating (LSR) sheet. The LSR was identical to the standard 100-mm visual analog scale developed by Carskadon and Dement (9) and used in our previous study (32). The LSR requires subjects to rate their level of alertness between the extremes of very wide awake (score 0) to very sleepy (score 100).

Statistical analysis. Melatonin levels across doses and time (between 1015 and 1500) were compared with a repeated-measures ANOVA (SuperANOVA for the Macintosh, version 4.5). The average hand and foot temperature measures (across both limbs) were collapsed into 30-min bins and expressed relative to the temperature at 1000 in each condition. Subjective sleepiness scores were also expressed relative to 1000. Temperature and sleepiness data between 1015 and 1500 were then analyzed with repeated-measures ANOVA, with two within-subject factors (dose and time). Planned means comparisons were performed where required, determining where statistically significant differences occurred.

RESULTS

Plasma melatonin. Plasma melatonin levels analyzed between 1015 and 1500 showed a nonsignificant trend to decrease over time, from 16.8 ± 4.0 pM (means ± SE) at 1015 to 8.7 ± 4.0 pM at 1500. After melatonin administration, highest observed plasma melatonin levels occurred at 15 min after injection at all melatonin doses (see Fig. 1). Highest observed melatonin levels reached 63.5 ± 20.1, 150.0 ± 25.7, and 569.1 ± 110.8 pM for the 3-, 10-, and 30-µg doses, respectively, compared with 16.8 ± 4.0 pM after saline. There was a significant effect of dose on plasma melatonin concentration (P < 0.05). Planned comparisons showed that plasma melatonin levels were significantly higher than in the saline condition after injection of 10- and 30-µg doses (P < 0.05) but not after 3 µg melatonin. The 10-µg dose produced mean plasma melatonin levels across the experimental session that were significantly higher than the 3-µg dose (P < 0.05) but significantly lower than the 30-µg melatonin dose (P < 0.05). Plasma melatonin remained elevated above levels in the saline condition up to and including 60 and 120 min after injection of 10 and 30 µg melatonin, respectively.

Highest observed melatonin levels in plasma did not correlate significantly with either body weight or BMI of subjects. Regression analyses were conducted on each melatonin dose separately, with analysis by weight yielding (nonsignificant) correlation coefficients of r = 0.38 (3 µg), r = 0.06 (10 µg), and r = 0.04 (30 µg). Regression analysis of highest observed plasma melatonin against BMI gave (nonsignificant) correlation coefficients of r = 0.43, r = 0.10, and r = 0.18 for the 3-, 10-, and 30-µg melatonin doses, respectively.

Saliva melatonin. Saliva melatonin levels after injection of the saline vehicle decreased nonsignificantly across the day, from 22.3 ± 4.3 pM at 0800 to 12.0 ± 4.1 pM at 1500. Repeated-measures ANOVA revealed a

![Fig. 1. Plot of plasma melatonin levels (means ± SE) for each dose group: ● saline; ○, 3 µg melatonin; ▲, 10 µg melatonin; □, 30 µg melatonin. Limit of detection in assay was 4.3 pM. Plasma melatonin levels were significantly elevated above saline control levels for 120 and 60 min after injection of 30 and 10 µg melatonin, respectively (P < 0.05).](http://ajpendo.physiology.org/DownloadedFrom/jpo269312177010957502179531758330178602873270217852702785247.png)
significant effect of melatonin dose on saliva melatonin levels analyzed between 1015 and 1500 (P < 0.05). Saliva melatonin levels increased significantly above those after saline injection at both the 10- and 30-µg melatonin doses (P < 0.05, see Fig. 2). Mean saliva melatonin levels in the 3-µg melatonin condition were not significantly different from those after saline injection. Highest observed saliva melatonin levels occurred at 15 min after melatonin injection in each condition and were 42.2 ± 6.1 pM (3 µg), 136.1 ± 41.8 pM (10 µg), and 321.4 ± 63.6 pM (30 µg) compared with saliva melatonin levels after vehicle injection at the same time (1015) of 13.1 ± 2.9 pM. The 10-µg dose produced mean saliva melatonin levels between 1015 and 1500 that were significantly higher than the 3-µg dose (P < 0.05) but significantly lower than the 30-µg melatonin dose (P < 0.05). Saliva melatonin remained significantly elevated above levels in the saline condition until 60 min after injection at both 10- and 30-µg doses.

Rectal core temperature. As can be seen in Fig. 3, injection with melatonin had a significant main effect on core temperature (P < 0.05). Melatonin had no significant effect on core temperature at the 3-µg dose but significantly attenuated the normal daytime increase in rectal temperature at both 10- and 30-µg doses (P < 0.05). Rectal temperature after 10- and 30-µg melatonin administration remained significantly lower than after saline vehicle administration for 300 min after injection (P < 0.05). The changes in rectal temperature relative to the saline condition between 15 and 300 min after injection (i.e., 1015–1500) were −0.16 ± 0.04°C (10 µg) and −0.18 ± 0.04°C (30 µg). The relative temperature change between 15 and 300 min after injection of 3 µg melatonin was −0.11 ± 0.03°C.

Regression analysis indicated that highest observed plasma melatonin levels across conditions were strongly and significantly correlated with the mean relative change in rectal temperature (r = 0.95, P < 0.0001). A log regression with equation y = 0.12 − 0.12 log(x) fit best to the data (Fig. 4).

Peripheral temperature.

HAND. Repeated-measures ANOVA revealed no difference in hand (or foot) temperature when “injected” and “noninjected” sides of the body were compared in the melatonin condition; therefore, hand (and foot) data were compared in all other analyses as a mean of both sides. Mean hand skin temperatures are plotted in Fig. 5, which shows there was a significant effect of melatonin dose (P < 0.05). ANOVA revealed that hand temperature increased significantly relative to injection of saline for 180 min after administration of 3-, 10-, and 30-µg melatonin doses (P < 0.05 at each dose). Planned comparisons revealed no significant differences between the mean hand temperature changes at different melatonin doses: 0.72 ± 0.12°C (3 µg), 0.95 ± 0.15°C (10 µg), and 0.65 ± 0.11°C (30 µg).

FOOT. Foot temperature did not change significantly across time, nor were measures significantly affected by melatonin injections at any dose.

Subjective sleepiness. There was a slight trend for subjective sleepiness to change with condition (P = 0.10), with the greatest differences occurring at 15 min after injection of melatonin or saline. Mean relative

Fig. 2. Saliva melatonin levels (means ± SE) for each dose group: ●, saline; □, 3 µg melatonin; ▲, 10 µg melatonin; ○, 30 µg melatonin. Limit of detection in assay was 8.6 pM. Saliva melatonin levels were significantly elevated above saline control levels for 60 min after injection of both 30 and 10 µg melatonin (P < 0.05).

Fig. 3. Time course of rectal core temperature (means ± SE) for all conditions: ●, saline; □, 3 µg melatonin; ▲, 10 µg melatonin; ○, 30 µg melatonin. Data are expressed relative to temperature at 1000 in each condition. Melatonin injected at both 30- and 10-µg doses significantly attenuated the normal daytime increase in core temperature for 300 min (P < 0.05).

Fig. 4. Group plot of highest observed plasma melatonin level in each condition against mean change in rectal core temperature relative to saline condition over the time period 1015–1500. Data are expressed for all dose groups as means, with SE bars on both x- and y-axes. A significant correlation between plasma melatonin levels and core temperature response was found (r = 0.95, P < 0.0001).
In the present study, significant effects on self-rated sleepiness were not apparent at any melatonin dose administered. This result represents the first demon-
stration that the effects of melatonin on subjective sleep propensity and body temperature may be dissociated. Previous studies reporting effects of melatonin on both sleepiness and body temperature have typically used supraphysiological oral doses (6, 14, 26), suggesting that the soporific effects of melatonin may appear only at circulating levels above those normally produced during the night. For example, one study reported significantly shortened latency to sleep onset 2–4 h after 0.3 mg oral melatonin at 2100, at which time physiological levels of melatonin in serum were detected (40). However, circulating melatonin levels in this previous study most likely reached supraphysiological levels shortly after administration. This group found similar effects on sleepiness with 0.3- and 1.0-mg oral melatonin doses given at 1800, 2000, and 2100 (39). Overall, it appears that the thermoregulatory and soporific effects of daytime melatonin may occur at physiological and supraphysiological doses, respectively. However, this evidence is drawn from studies in which the rate of melatonin onset may have varied significantly because of the different doses, preparations, and routes of administered melatonin. Also, given the subjective nature of the linear sleepiness rating, it is possible that a small increase in sleep propensity in the present study may have occurred after melatonin injection but was not detected (i.e., a type II error). As a nonsignificant trend for increased sleep propensity after melatonin was observed, it may be the case that a rapid onset of melatonin to physiological levels after injection may produce both soporific and thermoregulatory effects commonly associated with large daytime oral doses (>1 mg). Nevertheless, the current results may raise some doubt as to whether soporific effects always accompany thermoregulatory effects of melatonin administration and should be studied in more detail.

If the present results do preclude a direct relationship between physiological melatonin levels and increased sleep propensity, they may support the hypothesis that melatonin acts via its chronobiotic effects (11). For example, it has been previously suggested that melatonin may participate in the physiological regulation of sleep by determining the phase of circadian rhythms of sleep and sleepiness (22, 25, 30, 37). From this perspective, the acute effects of daytime oral melatonin administration may appear to be side effects of supraphysiological doses and unrelated to its role in the body.

Although the precise mechanism of action of melatonin remains unclear, the results of this and our previous study (32) suggest that the alteration in core temperature after daytime melatonin is most likely achieved by increased peripheral heat loss. According to current theoretical models of thermoregulation, temperature homeostasis is achieved by balancing heat production and heat loss. For melatonin to reduce core temperature, therefore, either heat loss at the periphery has to increase (as suggested by an increase in hand temperature) or heat production by metabolism must decrease. Alternatively, both of these may occur, and it is not clear from the present results what contribution changes in heat production may make to the response to melatonin administration. In addition, in the present study the increase in hand temperature suggests that heat loss was not equally distributed over the extremities (i.e., hands vs. feet). However, in this study at least, the hand temperature data were very variable, particularly in the saline control condition. It is possible that the injection process per se could exert thermoregulatory effects (as suggested by a decrease in hand temperature after 1000). Whether these functional temperature changes have any significance to the mechanism or site of effect of exogenous melatonin is unclear. However, the localization of melatonin receptors in rat vasculature associated with peripheral areas that dissipate heat (34) suggests that melatonin may have a specific role in thermoregulation mediated by changes in vascular tone.

The reported ratio of saliva melatonin to plasma melatonin (S:P) is ~0.30 (31, 35); however, this was generally lower than the range of values obtained in the present study. At the highest plasma concentrations of melatonin, S:P only reached as low as 0.50 (i.e., saliva was 50% of plasma levels). In general, however, this finding supports the results of Laakso and colleagues (20), who found that the proportion of endogenous melatonin found in saliva decreased with increasing plasma levels. It is possible that the rapid onset and elimination of melatonin after intravenous injection result in altered binding kinetics, as reflected by a higher than expected ratio of free melatonin in the saliva compared with melatonin in the plasma. On the other hand, because of a smaller difference between peak melatonin levels compared with those in the saline condition, the relatively high background in saliva melatonin levels may have artificially truncated measured S:P.

In conclusion, the present results suggest that raising the levels of melatonin into the nocturnal physiological range by intravenous bolus significantly suppressed the daytime increase in core temperature. This thermoregulatory effect appears to be maximal within a range of melatonin levels in plasma achieved during the night. A slight but nonsignificant trend for increased subjective sleepiness was observed at the highest dose. It may be that the typical soporific effects observed after daytime oral doses of ~0.3 mg melatonin reflect a pharmacological side effect of the hormone, rather than a mimicking of the normal physiological action. Melatonin administration studies should aim to control for the rate of increase as well as peak level and possibly duration when assessing its physiological effects. Furthermore, it is not yet clear whether and under which conditions, with measures that are more objective, the soporific effects of melatonin are dissociable from the thermoregulatory effects. Ideally, assessment of these factors should employ sleep propensity measures, such as the multiple sleep latency test or the ultrashort sleep/wake paradigm, to investigate the effects of dose, rate of onset, and time of day of melatonin administration.
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Address for reprint requests: D. Dawson, The Centre for Sleep Research, 5th Floor CDRC Bldg., The Queen Elizabeth Hospital, 11–23 Woodville Rd., Woodville, SA 5011, Australia.

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