Thermoregulatory and soporific effects of very low dose melatonin injection

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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.

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 491B, Cincinnati Sub-Zero Products, Cincinnati, OH). All thermistors were connected to a custom data collection and display and storage system comprising Workbench for Win-
E250
INJECTION OF VERY LOW DOSE MELATONIN

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
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

**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).

“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
The temperature effects of melatonin injection in the current study were similar to those in a recent study by our group using prolonged melatonin infusion at supra-physiological levels (32). Given that similar effects were seen at much lower achieved levels of melatonin, the suggestion would be that the rapid onset with bolus melatonin injection may be responsible for eliciting physiological responses at much lower doses than a steady-state infusion.

Interestingly, the maximum change in core temperature in the present study (−0.18 ± 0.04°C), achieved by mimicking physiological levels of melatonin, is similar to that observed in our previous study (−0.17 ± 0.01°C) during prolonged melatonin infusion (32). However, the corresponding plasma melatonin levels that produced the rectal temperature changes were up to 70-fold higher in the previous study (34.5 ± 12.4 nM), compared with the highest observed peak after a 30-µg injection (569.1 ± 110.8 pM). Thus the threshold melatonin level for physiological effects is not clear in our previous infusion study. It therefore appears that the rectal temperature effects of melatonin administration are maximal within the normal physiological range of endogenous melatonin production; however, this is yet to be conclusively demonstrated. It is also unclear whether the threshold for effects is dependent on the rate of onset of melatonin in the circulation. It would be useful to infuse melatonin at various doses and rates to precisely characterize how these factors alter the threshold for physiological effects of melatonin. Taken together with our previous study (32), it appears that the duration of elevated melatonin in the plasma has little bearing on body temperature effects. This is supported by the observation that both short (injection) and long melatonin durations (infusion) have a suppressive effect on daytime core temperature for at least 1–2 h after plasma melatonin levels return to normal daytime values.

It is interesting to note that injection of melatonin at very low doses appears to suppress the normal daytime increase in core temperature for only a very short period of ~30–90 min. After this time, a significant difference in core temperature still exists between the saline and the 10- to 30-µg melatonin conditions; however, the rate of temperature change in all conditions would appear to be equivalent. This short period is congruent with the time that melatonin levels are elevated above those in the saline condition after injection. It is possible that exogenous melatonin exerts an acute suppressive effect on core temperature when administered during the day but that a difference exists for a longer time because of a limitation on how quickly core temperature can increase. This would be determined by how much heat can be produced under basal conditions.

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 chronobiologic 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 temperature 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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