Modification of cutaneous vasodilator response to heat stress by daytime exogenous melatonin administration

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Aoki, Ken, Dan P. Stephens, Kun Zhao, Wojciech A. Kosiba, and John M. Johnson. Modification of cutaneous vasodilator response to heat stress by daytime exogenous melatonin administration. Am J Physiol Regul Integr Comp Physiol 291: R619–R624, 2006.—In humans, the nocturnal fall in internal temperature is associated with increased endogenous melatonin and with a shift in the thermoregulatory control of skin blood flow (SkBF), suggesting a role for melatonin in the control of SkBF. The purpose of this study was to test whether daytime exogenous melatonin would shift control of SkBF to lower internal temperatures during heat stress, as is seen at night. Healthy male subjects (n = 8) underwent body heating with melatonin administration (Mel) or without (control), in random order at least 1 wk apart. SkBF was monitored at sites pretreated with bretylium to block vasoconstrictor nerve function and at untreated sites. Cutaneous vascular conductance, calculated from SkBF and arterial pressure, sweating rate (SR), and heart rate (HR) were monitored. Skin temperature was elevated to 38°C for 35–50 min. Baseline esophageal temperature (Tes) was lower in Mel than in control (P < 0.01). The Tes threshold for cutaneous vasodilation and the slope of cutaneous vascular conductance with respect to Tes were also lower in Mel at both untreated and bretylium-treated sites (P < 0.05). The Tes threshold for the onset of sweating and the Tes for a standard HR were reduced in Mel. The slope of the relationship of HR, but not SR, to Tes was lower in Mel (P < 0.05). These findings suggest that melatonin affects the thermoregulatory control of SkBF during hyperthermia via the cutaneous active vasodilator system. Because control of SR and HR are also modified, a central action of melatonin is suggested. Circadian rhythm; skin blood flow; sweating; thermoregulation

IN HUMANS, THERE IS A NOCTURNAL increase in plasma melatonin levels. At the same time, there is a decrease in internal temperature and an increase in peripheral body temperature (6, 23, 24). Administration of melatonin in the daytime leads to a fall in internal temperature (11, 14, 16). These findings suggest the possibility that melatonin may play an important role in the nocturnal variation in thermoregulatory control of body temperature.

There are also nocturnal patterns in thermoregulatory effectors. Skin blood flow (SkBF), which has an important role in heat dissipation, shows a peak around midnight (29). SkBF responses to hyperthermic challenges also show a nocturnal change. For example, the nocturnal lowering of internal temperature is correlated with a lower internal temperature threshold for the onset of cutaneous vasodilation during heat stress (32, 37). Although suggested by these observations, it is not clear whether there is a role for melatonin secretion in this shift in the control of SkBF. There is evidence to support the hypothesis of such a role, however. It is well known that the circadian rhythms in both endogenous melatonin and resting internal temperature can be entrained or phase-shifted by artificial light exposure (3, 9, 12, 17, 31, 39). For example, bright light exposure (>2,000 lx) for a single night leads to both suppressed secretion of melatonin and to an attenuation in the usual fall in internal temperature the next morning (3, 31, 39). Recently, we found that the usual thermoregulatory changes in the control of SkBF were similarly suppressed by bright light exposure at night (3). These results suggest that melatonin may be functionally important in the nocturnal regulation of the thermoregulatory control of the cutaneous circulation. If melatonin contributes to the nocturnal variation of the control of SkBF, then daytime melatonin administration should modify the control of SkBF in a pattern directionally opposite to that observed with sleep deprivation, as described above. Because increased melatonin levels are associated with the nocturnal lowering of internal temperature (11) and that there is also a nocturnal reduction in the internal temperature threshold for cutaneous vasodilation (32, 37), we hypothesized that exogenous melatonin in the daytime would cause a shift to lower internal temperatures in the control of SkBF during a hyperthermic challenge.

Efferent neural control of SkBF is accomplished through the sympathetic noradrenergic vasoconstrictor system and the nonadrenergic vasodilator system (19). The active vasodilator system can be studied in the absence of a functional vasoconstrictor system through the use of bretylium (BT) (21), which blocks norepinephrine and cotransmitter release from noradrenergic terminals (15), effectively removing vasoconstrictor nerve function from that area of skin. In a previous study (1), we used the technique of iontophoretic application of BT (21) to a small area of skin and found that the diurnal shift of the internal temperature threshold between the morning and evening is dependent on the active vasodilator system. We also found that the sensitivity of the response in cutaneous vascular conductance (CVC) to internal temperature is lower in the early morning, and that difference is dependent on a diurnal
variation in sympathetic noradrenergic vasoconstrictor function (1). How these separate pathways might contribute to any effect of exogenous melatonin on the control of SkBF is not known. However, given the above findings, we hypothesize that exogenous melatonin would shift the thermoregulatory control of the cutaneous active vasodilator system to lower temperatures and would also cause a reduced sensitivity of the increase in SkBF relation to internal temperature, this latter effect through the vasoconstrictor system.

To test the above hypotheses, we measured cutaneous vascular responses relative to internal temperature during whole body heating, with and without daytime administration of oral melatonin. Furthermore, to discriminate between the roles of the active vasodilator system and the noradrenergic vasoconstrictor system, responses were measured at control sites and at sites at which cutaneous vasoconstrictor system function had been inhibited by pretreatment with BT.

**MATERIALS AND METHODS**

The protocol for this study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. Eight male subjects (19–33 yr) were recruited for this study and provided written, informed consent before participation. All subjects were nonsmokers and in good health. Each subject was tested on two occasions: melatonin (Mel) and control (Cont) sessions. The order of the two experiments was randomized, with at least 1 wk between them. The subjects did not consume caffeine within 12 h before the beginning of any experiment or take any medication, including pain medication, within 24 h of the experiments. Furthermore, subjects were prohibited from eating within 3 h before each session.

**Protocol.** All experiments were conducted in a laboratory at a constant ambient temperature and light intensity. On arriving at the laboratory at 1230, BT was applied by iontophoresis to two 0.6-cm² spots on the dorsal forearm of subjects by a weak current (240 μA; 400 μA/cm²) for 10 min to block all neurotransmission from sympathetic noradrenergic nerve terminals (1, 15, 21, 28). After the application of BT, the subject dressed in a water-perfused suit designed to control whole body skin temperature (33). The suit covered the entire body surface with the exception of the head, feet, and arm where SkBF was measured. At 1320, the subject lay down on the bed and maintained the supine posture until the end of the experiment. In the Mel sessions, immediately after measurement of preingestion data, the subject took a 3-mg tablet of melatonin. Mean skin temperature (Tsk) was held at 35°C throughout the resting period. In addition, the local skin temperature at each of the sites of SkBF measurement was set to 34°C throughout the resting period and whole body heating portions of the protocol. Two hours later at 1520, preheating baseline data were measured. Whole body cold stress was then performed. The threshold for cutaneous vasoconstriction was defined as the Tsk at which CVC began to increase steadily. The threshold for sweating was identified as the Tsk at which active sweating began. CVC and SR were plotted as functions of the sensitivity of their responses during whole body heating. The sensitivity of CVC or SR to Tsk for each experiment was determined by calculating the regression from the beginning of cutaneous vasodilation or onset of sweating to the time of achieving a steady state. The data from the two BT-treated sites were averaged when there was adequate blockade of the vasoconstrictor system at both sites. Linear regression analysis was also conducted to find the sensitivity of the HR-Tsk relationship for each subject. In addition, Tsk for a standard HR of 70 beats/min during whole body heating was calculated from the regression analysis.

Tsk, MAP, and HR from the preingestion period and from the preheating baseline were analyzed by two-way analysis of variance (ANOVA) with repeated measures (melatonin administration and time) and a Bonferroni post hoc test when a significant difference was identified. Also, a paired t-test was used to test for any effect of melatonin on Tsk or on MAP between the preingestion period and the preheating baseline. The threshold for cutaneous vasodilation and the sensitivity of CVC with respect to Tsk were analyzed by two-way ANOVA with repeated measures (melatonin administration and BT treatment), and a Bonferroni post hoc test was applied when a significant difference was identified. The Tsk threshold for the onset of sweating, the Tsk for a standard HR of 70 beats/min, and the slopes of SR and HR with respect to Tsk were compared between Mel and Cont sessions by paired t-test. The level of significance was set at $P < 0.05$. All data are expressed as means ± SE.

**RESULTS**

**Preheating data.** Tsk in the preingestion period was not significantly different between Mel and Cont sessions. Oral melatonin induced a decrease in Tsk during the 120 min following ingestion. Tsk at preheating baseline was significantly lower in Mel than in Cont ($P < 0.01$, Table 1). The decrease in Tsk from the preingestion period to preheating baseline was significantly larger in Mel than in Cont sessions [$−0.35 ± 0.04$ (Mel) compared with $−0.17 ± 0.04°C$ (Cont), $P < 0.01$]. On the other hand, neither HR nor MAP differed...
Table 1. Esophageal temperature, heart rate, and mean arterial pressure for the preingestion period and the preheating baseline (2 h following melatonin ingestion) in melatonin and control sessions

<table>
<thead>
<tr>
<th></th>
<th>Preingestion Period (1320)</th>
<th>Preheating Baseline (1520)</th>
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<tbody>
<tr>
<td></td>
<td>Mel</td>
<td>Cont</td>
</tr>
<tr>
<td>T_e, °C</td>
<td>36.71±0.07</td>
<td>36.69±0.07</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>59.0±3.3</td>
<td>59.0±2.6</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>85.3±3.4</td>
<td>82.3±2.6</td>
</tr>
</tbody>
</table>

Values are means ± SE from 8 subjects. T_e, esophageal temperature; HR, heart rate; MAP, mean arterial pressure; Mel melatonin session; Cont, control session. *Significantly different from preingestion period for same session (P < 0.01). †Significantly different from Cont for the same state (P < 0.05).

significantly between Mel and Cont in the preingestion period or at the preheating baseline (P > 0.1, Table 1). However, changes in MAP from the preingestion period to the preheating baseline showed a small but statistically significant difference between Mel and Cont sessions [−3.29 ± 0.45 (Mel)] compared with 1.03 ± 0.63 mmHg (Cont), P < 0.01]. Whole body cold stress induced significant vasoconstriction at control sites [39.4 ± 4.5% (Mel) and 34.8 ± 4.2% (Cont)] decreases in CVC, P < 0.01], but CVC at BT-treated sites did not significantly change with cold stress [1.2 ± 3.5% (Mel) and 4.4 ± 3.5% (Cont) decrease in CVC, P < 0.01]. This result indicated that the noradrenergic vasconstrictor function at BT-treated sites was effectively abolished (21, 28).

Physiological responses to whole body heating. Figure 1 shows responses in T_e, HR, %CVC_{max} (untreated sites), and SR during whole body heating from Cont and Mel sessions averaged for the entire group. Figure 2 shows group averages for the relationship of CVC to T_e. Overall results for the T_e thresholds for cutaneous vasodilation and the slopes of the %CVC_{max}-T_e regressions from both untreated and BT-treated sites are shown in Table 2. The T_e thresholds for cutaneous vasodilation at both untreated and BT-treated sites were significantly lower in Mel than in Cont (P < 0.05). We did not detect a statistically significant difference in this shift between untreated (0.20 ± 0.07°C) and BT-treated (0.21 ± 0.07°C) sites (P > 0.1). As shown by the individual data in Fig. 3 and by the group averages in Fig. 2 and Table 2, at both untreated and BT-treated sites, the sensitivity of the cutaneous vasodilator response with respect to T_e in Mel was significantly lower than that in Cont (P < 0.05).

Although the T_e threshold for the onset of sweating in Mel was significantly lower than that in Cont [36.59 ± 0.08 (Mel) compared with 36.81 ± 0.04°C (Cont), P < 0.05], the sensitivity of the sweating response to T_e did not show a statistically identifiable effect of oral melatonin administration [1.52 ± 0.53 (Mel) compared with 1.78 ± 0.36 mg·cm⁻²·min⁻¹·°C⁻¹ (Cont), P > 0.1].

Figure 4 illustrates the relationship of HR to T_e in the Mel and Cont sessions. The T_e for a standard HR of 70 beats/min was significantly lower in Mel than in Cont [36.74 ± 0.05 (Mel) compared with 36.85 ± 0.05°C (Cont), P < 0.01]. Moreover, the slope of this relationship was significantly reduced by exogenous melatonin administration [36.20 ± 4.61 (Mel) compared with 63.13 ± 11.71 beats·min⁻¹·°C⁻¹ (Cont), P < 0.05].

DISCUSSION

The major findings from this study are 1) melatonin administration shifts the control of SkBF during whole body heating to lower internal temperatures and a lower sensitivity to internal temperature changes; 2) the mechanism for this shift in the internal temperature threshold and sensitivity for cutaneous vasodilation is dependent on a similar shift in the function of the cutaneous active vasodilator system; and 3) HR and SR relationships to internal temperature are both shifted to lower temperatures by melatonin, with the HR relationship showing a reduced sensitivity.

In several earlier studies, a fall in internal temperature was noted following the ingestion of melatonin (11, 14, 16). Unique to the present study is the finding that not only is baseline body temperature reduced by exogenous melatonin, but the regulation of body temperature is also changed such that the thresholds for SkBF and SR are reduced in parallel to the baseline temperature. This finding is quite similar to those in which day-night shifts in internal temperature at rest are accompanied by parallel changes in thermoregulatory thresholds (30, 37).

**Fig. 1.** Standard protocol for whole body heating showing time courses of esophageal temperature (T_e), heart rate (HR), and cutaneous vascular conductance (CVC) at untreated sites, expressed as a percentage of maximal CVC (%CVC_{max}), and sweating rate (SR) in melatonin (Mel; ○) and control (Cont; ●) sessions. Whole body skin temperature was elevated to 38°C for 35–50 min, beginning at time 0. Values are means ± SE from all subjects.
Thus, not only is internal temperature in a neutral environment affected, but also that change is defended when faced with the thermal challenge of heat stress.

The internal temperature threshold for cutaneous vasodilation during passive heat stress in the early morning is shifted upward, and melatonin secretion is suppressed by bright light exposure during the prior night (3). In the present study, a reduction in the internal temperature threshold for the onset of cutaneous vasodilation at untreated sites by daytime melatonin administration was seen. Both of these observations clearly indicate a relationship of the internal temperature threshold for cutaneous vasodilation and resting internal temperatures to melatonin levels. In the present study, the threshold for cutaneous vasodilation at BT-treated sites also had a similar shift as that seen at untreated sites with melatonin administration. This indicates that the shift of the internal temperature threshold for the onset of cutaneous vasodilation was caused by modulation in the function of the cutaneous active vasodilator system. In a previous study using BT to inhibit vasoconstrictor nerve function, we found that the mechanism for the diurnal shift in the thermoregulatory threshold for cutaneous vasodilation is also due to modulation of the active vasodilator system (1).

The slope of the cutaneous vasodilator response with respect to internal temperature was significantly lower in the early morning and was found to be dependent on a diurnal variation in the activity of the sympathetic vasoconstrictor system (1). In the present study, in contrast, the sensitivity of cutaneous vasodilation declined significantly with melatonin at both untreated and BT-treated sites (see Fig. 3). This indicates that, in the present study, the lower sensitivity of the cutaneous vasodilator response to increased internal temperature by exogenous melatonin was due to an attenuation of cutaneous active vasodilator system function, which is not consistent with the previously observed mechanism for the diurnal changes in sensitivity. Interestingly, the slope of $T_{es}$-HR relationship was blunted by oral melatonin, similar to the sensitivity of cutaneous vasodilation, whereas the slope of the relationship of HR to $T_{es}$ did not show diurnal changes (1). The present data indicate daytime melatonin affects the autonomic control of HR at a given internal temperature during hyperthermic conditions.

The results of the current study of the effects of exogenous melatonin support the notion that endogenous melatonin plays a role in the nocturnal changes in the control of SkBF. The pattern of a reduced threshold with melatonin administration is similar to the shift in active vasodilator threshold between early morning and evening (1). Similarly, shifts in the thermoregulatory control of HR and SR show similar patterns between melatonin administration and the early morning. These

### Table 2. Esophageal temperature thresholds for the onset of vasodilation and the sensitivity of the vasodilator response to esophageal temperature in melatonin and control sessions at both untreated sites and at BT-treated sites

<table>
<thead>
<tr>
<th>Threshold, °C</th>
<th>Sensitivity, %CVC$_{\text{max}}$°C</th>
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<tbody>
<tr>
<td></td>
<td>Untreated sites</td>
</tr>
<tr>
<td></td>
<td>Mel</td>
</tr>
<tr>
<td>36.52±0.07*</td>
<td>36.72±0.06</td>
</tr>
<tr>
<td>36.61±0.08*</td>
<td>36.81±0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE from 8 subjects. BT, bretylium; CVC$_{\text{max}}$, maximum cutaneous vascular conductance. *Significantly different from Cont for the same treatment group ($P < 0.05$).
changes, plus the shift in active vasodilator function, suggest a central shift in thermoregulatory control, rather than a peripheral effect on the cutaneous vasculature. However, there are also reasons to question the conclusion that the nocturnal pattern of plasma melatonin concentration is the sole mechanism leading to these shifts in thermoregulatory control. First, we did not measure plasma levels in the present study but chose a dose of melatonin that causes a greater change in plasma levels than would be expected from endogenous production. Indeed, it is estimated that a dosage of 10% of the melatonin intake used in the present study would reproduce normal nighttime plasma levels (8). Second, although the increase in plasma melatonin in the late evening partially contributes to the nocturnal reduction in internal temperature (11), the subsequent early morning reduction in melatonin is not accompanied as closely with an increase in internal temperature. Third, even marked suppression of melatonin secretion by bright light exposure at night only partially inhibits the usual nocturnal decline in internal temperature (3). This partial correspondence suggests a more important role for melatonin in nocturnal thermoregulatory changes than in daytime changes. These observations, coupled with the present findings, suggest participation by melatonin in the nocturnal pattern of thermoregulatory control, but also suggest melatonin does not account for the entire pattern.

The internal temperature threshold for the onset of sweating showed a reduction similar to that seen for cutaneous vasodilation in the Mel session. On the other hand, a significant effect of melatonin administration was not detected on the slope of the SR-Tes regression. These results on the threshold and sensitivity of thermoregulatory sudomotor responses during hyperthermia are consistent with earlier reports on diurnal or nocturnal variations and the effect of bright light exposure at night on the control of thermoregulatory sweating (1, 30, 32, 37).

When thermal challenges are severe, as in uncompensable heat stress, the hypothermic effects of melatonin can be overwhelmed. For example, McLellan et al. (25, 26) investigated the hypothermic effect of two oral doses of melatonin (1 and 5 mg) on thermoregulatory responses to uncompensable heat stress. Oral melatonin administration had no significant effect on exercise tolerance or on the sweating response to exercise in the heat, although rectal temperature following the ingestion of 5 mg of melatonin in a cool environment showed only a small reduction at rest. These authors mentioned the possibility that the seated position used in their studies may have been sufficient to negate the hypothermic effect of exogenous melatonin.

Tes at preheating baseline in both Cont and Mel sessions fell significantly compared with the respective preingestion periods. The change in posture from upright to supine has been seen to be associated with an increased skin temperature of distal regions and a decreased rectal temperature (22). In the present study, the decrease in Tes in Cont and part of the decrease in Mel can be explained by such an effect of the postural change to the supine position. Nevertheless, 3-mg daytime melatonin administration induced a further decrease in Tes (∼0.18°C) during the 120 min following ingestion. This result from the present study is in keeping with earlier observations of the hypothermic effects of oral melatonin, in which doses ranging from 2.5 to 5 mg were reported to reduce internal temperature by 0.15–0.3°C (11, 14, 16).

Previous studies of daytime melatonin administration from 1 to 5 mg in humans found either no change in HR (22), or a reduction in HR (14, 16), and a decrease in MAP (5, 10). Although HR and MAP at preheating baseline in the present study did not show significant differences between Mel and Cont sessions, the decrease in MAP from the preingestion period to the preheating baseline in Mel was significantly larger than in the Cont session. Also, in the present study, the level of HR at a given level of Tes was higher following Mel than in Cont.

The data from the present study do not show, specifically, where melatonin acts to have the observed effects on thermoregulatory function seen here. Melatonin has been shown to potentiate the vasoconstrictor actions of norepinephrine on rat caudal arteries in vitro (35, 36), but such an influence would oppose our results rather than explain them. Indeed, in vivo studies, melatonin has been shown to suppress sympathetic activity as indexed by plasma catecholamine levels (5). In the present study of responses to hyperthermia, neither of these actions would appear to play a significant role, because the responses in CVC in areas of skin lacking functional vasoconstrictor control (BT treated) were virtually identical to those in untreated skin. However, there exists the possibility for such actions of melatonin to be important during cooling, when cutaneous vasoconstriction is enhanced by noradrenergic function. Our limited knowledge of the mechanisms of cutaneous active vasodilation (7, 38) prevents a clear dissection of the possible sites where melatonin would act to stimulate that system. However, the similar leftward shifts in the relationship of sweat rate, HR, and CVC to Tes suggests an important central action.

Finally, although this study was not placebo controlled, we do not feel this affects our results or conclusions in any important way. The analysis of the data was blinded, so the investigator choosing the thresholds was not aware of whether the data came from Mel or Cont sessions. Furthermore, a subsequent study of the influence of exogenous melatonin on the responses to whole body skin cooling was placebo controlled (4), and the initial differences in the internal temperature between Mel and placebo sessions were virtually identical to these seen here.

In summary, we found that the reflex thermoregulatory control of SkBF during hyperthermia is modified by daytime exogenous melatonin. Both the smaller shift of threshold for cutaneous vasodilation and the decline in sensitivity of the cutaneous vasodilator response depend on the function of the active vasodilator system. Thermoregulatory control of sweating activity and HR was also modified by exogenous melatonin, suggesting a central action of melatonin. Thus the secretion of melatonin at night would, at least in part, play an important role in nocturnal variation in thermoregulatory control.

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REFERENCES


