Melatonin attenuates the skin sympathetic nerve response to mental stress

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Muller MD, Sauder CL, Ray CA. Melatonin attenuates the skin sympathetic nerve response to mental stress. Am J Physiol Heart Circ Physiol 305: H1382–H1386, 2013. First published August 30, 2013; doi:10.1152/ajpheart.00470.2013.—Melatonin attenuates muscle sympathetic nerve responses to sympathoexcitatory stimuli, but it is unknown whether melatonin similarly attenuates reflex changes in skin sympathetic nerve activity (SSNA). In this double-blind, placebo-controlled, crossover study, we tested the hypothesis that melatonin (3 mg) would attenuate the SSNA response to mental stress (mental arithmetic). Twelve healthy subjects underwent experimental testing on two separate days. Three minutes of mental stress occurred before and 45 min after ingestion of melatonin (3 mg) or placebo. Skin temperature was maintained at 34°C. Reflex increases in SSNA (peroneal nerve), mean arterial pressure, and heart rate (HR) to mental stress before and after melatonin were determined. Melatonin lowered HR (pre, 66 ± 3 beats/min; and post, 62 ± 3 beats/min, P = 0.046) and SSNA (pre, 14,282 ± 3,706 arbitrary units; and post, 9,571 ± 2,609 arbitrary units, P = 0.034) at rest. In response to mental stress, SSNA increases were significantly attenuated following melatonin ingestion (second minute, 114 ± 30 vs. 74 ± 14%; and third minute, 111 ± 29 vs. 54 ± 12%, both P < 0.05). The mean arterial pressure increase to mental stress was blunted in the third minute (20 ± 2 vs. 17 ± 2 mmHg, P = 0.032), and the HR increase was blunted in the first minute (33 ± 3 vs. 29 ± 3 beats/min, P = 0.034) after melatonin. In summary, exogenous melatonin attenuates the SSNA response to mental stress.

Melatonin is an endogenous hormone secreted by the pineal gland in a circadian rhythm. This hormone is mechanistically linked to the sleep-wake cycle, and peak plasma levels are evident in the early morning (1:00 to 5:00 AM) (19, 27). For this reason, melatonin is commonly ingested in high doses to improve sleep (i.e., 3 to 5 mg, which leads to >50 times above physiological levels). In addition to its effectiveness as a sleep aid, mounting evidence suggests that exogenous melatonin has widespread effects on immune response, aging, and cancer (c.f., 7, 37). Furthermore, melatonin supplementation has been reported to reduce blood pressure in patients with metabolic syndrome (20) and essential hypertension (30). One mechanism by which melatonin decreases blood pressure could be its effect on cardiovascular reflexes.

In previous studies, our laboratory demonstrated that melatonin attenuated increases in muscle sympathetic nerve activity (MSNA) to both lower body negative pressure (24) and head-down rotation (14). These findings suggest that melatonin attenuates reflex changes in sympathetic outflow during postural change. However, the effect of melatonin on reflex changes in skin sympathetic nerve activity (SSNA) is unknown. The interaction of melatonin on SSNA is important to understand because both participate in thermoregulation (13). For example, Aoki et al. (2) reported that ingestion of melatonin attenuated the reflex cutaneous vasoconstriction in response to whole body cooling. However, this study did not measure reflex changes in SSNA to ascertain whether the reduced vasoconstriction was neurally mediated or due to direct effects of melatonin on vascular smooth muscle. Therefore, we tested the hypothesis that reflex changes in SSNA would be attenuated by melatonin (3 mg oral dose). We used an established laboratory model of mental stress to elicit marked increases in SSNA (23). Our results further support the concept that melatonin has a significant effect on reflex changes in sympathetic nerve activity in humans.

METHODS

Subjects. Twelve subjects (8 men, and 4 women) with a mean age of 26 ± 1 yr, height of 1.76 ± 0.04 m, and weight of 76.4 ± 4.2 kg participated in this study. Subjects were determined to be healthy via medical history and physical examination and were not taking medication. All subjects refrained from caffeine, alcohol, and exercise for 24 h before the study and arrived to the laboratory in a semifasted state (i.e., at least 4–6 h after their last meal). The study protocols were approved in advance by the Institutional Review Board of the Penn State Milton S. Hershey Medical Center and conformed to the Declaration of Helsinki. Each participant provided written, informed consent.

Design. This study employed a randomized, double-blind, placebo-controlled, crossover design. On separate days, subjects underwent mental stress before and after ingestion of either 3 mg melatonin or placebo (cellulose). Two trials occurred each day. Neither the investigators nor the subject was aware of the treatment, and the code was broken when all data analysis was completed. Because SSNA recordings cannot be obtained from the exact same anatomical location between days, responses within the same day (pre-melatonin, post-melatonin) were considered to be the primary outcome measure in this study. Our laboratory recently reported that under control conditions (without drug), the reflex increases in SSNA, mean arterial pressure (MAP), and heart rate (HR) in response to mental stress are reproducible within and between days (23). Thus, in the current study, any alterations in the SSNA response to mental stress were attributed to melatonin and not time/order effects. It is also important to emphasize that 3 mg of melatonin is a common dose that people take to improve sleep. Twenty-five to 50 min after ingestion of 3 mg melatonin, plasma melatonin peaks levels (i.e., >50 times above baseline), and plasma levels remain in the supraphysiological range for several hours (14, 24).

Protocol. All experiments were conducted in the supine posture during the morning hours (8:00 to 11:30 AM) in a dimly lit, quiet, thermonneutral laboratory (22–25°C). To maintain neutral skin temperature, each subject wore a custom-designed tube-lined suit (Med-Eng Systems, Ottawa, ON, Canada). The suit covered the entire body except for the head, hands, feet, and the left lower leg (i.e., where SSNA was measured). Water was perfused at 34 to 35°C for the entire study.
Table 1. Resting baseline parameters before and after ingestion of melatonin or placebo

<table>
<thead>
<tr>
<th></th>
<th>Pre-Melatonin</th>
<th>Post-Melatonin</th>
</tr>
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<tbody>
<tr>
<td>SBP, mmHg</td>
<td>116 ± 2</td>
<td>116 ± 2</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>66 ± 2</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>86 ± 1</td>
<td>85 ± 1</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>66 ± 3</td>
<td>62 ± 3*</td>
</tr>
<tr>
<td>°C</td>
<td>36.6 ± 0.2</td>
<td>36.8 ± 0.1</td>
</tr>
<tr>
<td>°C</td>
<td>27.1 ± 0.3</td>
<td>26.5 ± 0.3</td>
</tr>
<tr>
<td>°C</td>
<td>34.1 ± 0.3</td>
<td>34.5 ± 0.3</td>
</tr>
<tr>
<td>AU</td>
<td>0.29 ± 0.03</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>mg·cm⁻²·min⁻¹</td>
<td>0.18 ± 0.02</td>
<td>0.16 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; for the melatonin trials, n = 11, and for placebo, n = 8. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; °C, tympanic membrane temperature; °C, mean skin temperature; AU, arbitrary units; SR, sweat rate. *P < 0.05, significant difference from the preingestion value.

Upon arrival at the laboratory, subjects were outfitted with several hemodynamic and thermal monitoring devices (see below). They were familiarized to the procedures and encouraged to respond as quickly and correctly as possible during the upcoming mental stress trial. Following successful nerve recording and instrumentation of the subject, resting blood pressure was obtained. Next, a 5-min baseline occurred to quantify resting SSNA. The total duration from when the subject was placed in the thermoneutral suit until the start of baseline was always resting SSNA. The total duration from when the subject was placed in the thermoneutral suit until the start of baseline was always resting SSNA. The total duration from when the subject was placed in the thermoneutral suit until the start of baseline was always resting SSNA.

Resting baseline parameters before and after ingestion of melatonin or placebo

Data analysis and statistics. Sympathetic nerve recordings that demonstrated possible electrode site shifts or electroganographic artifact were excluded from analysis (n = 1; leaving 11 full SSNA data sets for the melatonin trial). Consistent with previous experiments in our laboratory (23, 26, 35, 36) and others (18, 32, 34), SSNA was expressed as a percent change in total area under the mean voltage neurogram relative to the preceding baseline (5 min average). This analysis was achieved using computer software (Chart 5, ADInstruments), and the focus was on the first 10 s of mental stress.

**Instrumentation and measurements.** Mean skin temperature was measured via weighted average of three thermocouples (model TC-1000, Sable Systems) attached to the skin (8, 29). These thermocouples were underneath the suit but insulated from contacting the suit itself. Tympanic temperature, an index of core temperature (6), was measured before and after testing with an automated device (Genius 3, Accusystem). HR was measured via three-lead EKG, and beat-by-beat MAP was determined by photoplethysmography (Finometer, FMS, The Netherlands). Blood pressures at rest were obtained by automated oscillometry (Dinamap XL, Critikon/GE).

Multifiber recordings of SSNA were made with a tungsten microelectrode inserted in the peroneal nerve at the fibular head. A reference electrode was placed 2 to 3 cm from the recording electrode. The recording electrode was adjusted until a site was found in which SSNA bursts were clearly identified using previously established criteria (15, 33). In brief, these included 1 light stroking of the skin within the innervated region resulted in afferent discharge and 2 deep inspiration and arousal stimuli resulted in activity not synchronous with the cardiac cycle. The nerve signal was amplified, passed through a band-pass filter with a bandwidth of 700–2,000 Hz, and integrated with a time constant of 0.1 s (Iowa Bioengineering, Iowa City, IA). Mean voltage neurograms were visually displayed and recorded on a data acquisition system (16SP PowerLab, ADInstruments, New Castle, Australia) and routed to a loudspeaker for monitoring throughout the study. SSNA responsiveness to auditory stimuli and deep inspiration was confirmed at the very end of experiments to ensure a consistent recording site.

Once the recording nerve site was established, two skin blood flow lasers (Moor Instruments, local heater set at 34°C) and one thermocouple were carefully attached to the dorsal foot (within the region of innervation on the foot) (28, 31, 36). Sweat rate was measured on the contralateral dorsal foot via capacitance hygrometry (Vaisala, Woburn, MA) by perfusing 100% nitrogen at a flow rate of 150 ml/min through a ventilated capsule (surface area, 2.0 cm²). Perception of stress was quantified after the bout of mental arithmetic (0 = not stressful, 1 = somewhat stressful, 2 = stressful, 3 = very stressful, and 4 = very very stressful) (9). Thermal sensation and thermal comfort were also determined before and after testing (16).

For all trials, beat-by-beat physiological measurements were recorded electronically and analyzed offline (16SP, PowerLab, ADInstruments). Perceptual variables were obtained by verbal report.

**Fig. 1.** Representative recordings of skin sympathetic nerve activity (SSNA) at rest (left) and during mental stress (right) in the same subject. All recordings are 60 s in duration. The mental stress recordings were both taken during the third minute. AU, arbitrary units.
(arousal response), as well as averages of minutes 1–3. Repeated-measures ANOVA (pre, post × minute 1, minute 2, minute 3) was used to determine if melatonin attenuated reflex responses. Cutaneous vascular conductance (CVC) was calculated as the quotient of skin blood flow flux and MAP. Changes in CVC due to mental stress were expressed as a percent change from the preceding 5-min baseline. Absolute changes in HR, MAP, sweat rate, and skin temperature were also determined. All values are reported as means ± SE, and P values < 0.05 were considered statistically significant.

RESULTS

In response to mental stress, perceived stress level was comparable before (2.8 ± 0.2) and after (2.8 ± 0.2) melatonin. As displayed in Table 1, melatonin ingestion lowered HR at rest (P = 0.05) but did not have a significant effect on blood pressure or body temperature. In a subset of subjects, where we were confident no electrode shift occurred during the entire trial (n = 6), resting SSNA was also lower following melatonin ingestion [pre, 14,282 ± 3,706 arbitrary units (AU); and post, 9,571 ± 2,609 AU, P = 0.03]. Representative recordings of SSNA at baseline and during mental stress are displayed in Fig. 1. Depicted in Fig. 2 are the reflex changes in SSNA, MAP, and HR in response to mental stress. For SSNA, repeated-measures ANOVA revealed a treatment by time interaction (P = 0.02) such that melatonin attenuated the reflex increase in SSNA in response to mental stress. During the second (P = 0.03) and third minute (P = 0.04) of mental stress, SSNA responses were significantly lower after melatonin ingestion. Likewise, the MAP response was blunted in the third minute (P = 0.03) and HR was blunted in the first minute (P = 0.034). In the first 10 s of mental stress (arousal response), there was no observed effect of melatonin (pre, 173 ± 47 AU; and post, 160 ± 34 AU, P = 0.61). The end-organ responses to mental stress measured on the dorsal foot were also not significantly impacted by melatonin (Table 2). Before and after mental stress, individuals consistently reported that they felt “neutral” and “comfortable.” During the third minute of mental stress, the rate pressure product (an index of myocardial oxygen demand) was ~5% lower with melatonin (12,450 ± 760 vs. 11,767 ± 805, P = 0.04).

Eight of the 12 subjects who completed the melatonin trial also completed the placebo trial (Table 1). SSNA at rest was not changed in response to placebo ingestion (pre, 8,945 ± 1,955 AU; and post, 11,414 ± 1,597 AU, P = 0.38). Responses of SSNA, MAP, and HR to mental stress were also not altered by placebo ingestion. Specifically, the change in SSNA in the first 10 s (198 ± 44 vs. 259 ± 61%), first minute (119 ± 25 vs. 154 ± 29%), second minute (63 ± 18 vs. 118 ± 28%), and third minute (93 ± 18 vs. 144 ± 44%) of mental stress were not different before and after placebo ingestion (drug × time interaction, P = 0.64). The changes in MAP during mental stress were not different before and after placebo ingestion (first minute, 9 ± 2 vs. 6 ± 2 mmHg; second minute, 17 ± 4 vs. 15 ± 3 mmHg; and third minute, 12 ± 4 vs. 8 ± 3 mmHg; drug × time interaction, P = 0.43). The changes in HR to mental stress were not different before and after placebo ingestion (first minute, 32 ± 5 vs. 32 ± 5 beats/min; second minute, 30 ± 7 vs. 27 ± 6 beats/min; and third minute, 29 ± 7 vs. 24 ± 6 beats/min; drug × time interaction, P = 0.30).

DISCUSSION

Our previous studies demonstrated that melatonin attenuates MSNA responses to postural changes (14, 24). These studies indicated that melatonin directly affects neural control of reflex changes in MSNA. In the current study, we tested the hypothesis that melatonin would alter reflex changes in SSNA in a similar manner to that observed with MSNA. Consistent with our hypothesis, melatonin attenuated the SSNA response to mental stress. This finding might provide an important mechanistic explanation of the interaction that melatonin has on skin blood flow and thermoregulation in humans.

In response to sympathoexcitatory stress, the human body activates several reflex pathways to maintain cardiovascular homeostasis. Studies from our laboratory have demonstrated that melatonin attenuates the reflex increases in MSNA to
lower body negative pressure and head-down rotation (14, 24). Along the same lines, Arangino et al. (3) reported that plasma norepinephrine in response to standing was reduced following melatonin ingestion. In the current study, we observed that reflex SSNA responses to mental stress were attenuated following melatonin. We chose mental stress because it is a robust and reproducible activator of SSNA and it does not impact body temperature (15, 17, 23). The use of mental stress is an important strength in this study because both melatonin and SSNA contribute to thermoregulation. If we heated or cooled the body in this study, we would have been unable to discern whether the effects of melatonin were due to altered autonomic control or due to a shift in hypothalamic thermoregulatory set point (1, 5). Because we used a short-duration, nonthermal stimulus, it is not surprising that CVC, sweat rate, and skin temperature were not altered by melatonin (Table 2). Aoki et al. (2) observed that the cutaneous vasconstrictor response to whole body cooling was blunted following melatonin. This study was not able to determine if the effect of melatonin on skin blood flow was because of its local vasodilator effect on smooth muscle or inhibition of skin vasconstrictor activity by reduced SSNA. The current study suggests that reduced SSNA by melatonin contributed to the decrease in cutaneous vascular resistance. Our past and current studies clearly indicate melatonin is capable of attenuating reflex responses to both physiological and psychological stress. Mental and social stress can trigger myocardial ischemia and adverse cardiovascular events (4, 21). Our current data indicate that HR and MAP responses to mental stress are blunted after melatonin ingestion, which would presumably reduce myocardial oxygen demand, thereby lessening episodes of angina. During the third minute of mental stress, the rate pressure product, an index of myocardial oxygen demand, was ~5% lower with melatonin. Whether this translates into a clinically relevant cardioprotective effect on the heart requires further study.

Conclusions. In the current study, exogenous melatonin attenuated the SSNA response to mental stress. These data in healthy humans, along with previously published work from our laboratory (14, 24), support the concept that acute ingestion of melatonin attenuates reflex changes in sympathetic outflow to skin and muscle. Therefore, melatonin can have a profound effect on autonomic regulation in humans.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES

Table 2. Thermal response to mental arithmetic

<table>
<thead>
<tr>
<th>Minute</th>
<th>Δ1'</th>
<th>Δ2'</th>
<th>Δ3'</th>
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<th>Time</th>
<th>Trial × Time</th>
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</thead>
<tbody>
<tr>
<td>Tfoot, ºC</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pre</td>
<td>0.01 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.10 ± 0.04</td>
<td>0.082</td>
<td>0.029</td>
<td>0.150</td>
</tr>
<tr>
<td>Post</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.03 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVC, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>−9 ± 8</td>
<td>9 ± 13</td>
<td>8 ± 13</td>
<td>0.350</td>
<td>0.147</td>
<td>0.695</td>
</tr>
<tr>
<td>Post</td>
<td>−12 ± 12</td>
<td>1 ± 13</td>
<td>5 ± 14</td>
<td></td>
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</tr>
<tr>
<td>SR, mg-cm⁻²·min⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pre</td>
<td>0.00 ± 0.00</td>
<td>0.01 ± 0.01</td>
<td>0.03 ± 0.02</td>
<td>0.342</td>
<td>0.074</td>
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<tr>
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<td>0.01 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
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</tbody>
</table>

Subjects underwent 3 min of fast-paced, verbal, mental arithmetic in the supine posture. Tfoot, CVC, and SR were measured, and changes (Δ) from baseline were calculated. Trials occurred before (pre) and after (post) blinded ingestion of melatonin (n = 11). Values are means ± SE.

GRANTS
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