Melatonin attenuates the vestibulosympathetic but not vestibulocollic reflexes in humans: selective impairment of the utricles

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Cook JS, Ray CA. Melatonin attenuates the vestibulosympathetic but not vestibulocollic reflexes in humans: selective impairment of the utricles. J Appl Physiol 109: 1697–1701, 2010. First published September 9, 2010; doi:10.1152/japplphysiol.00698.2010.—Melatonin has been reported to decrease nerve activity of medial vestibular nuclei in the rat and is associated with attenuated muscle sympathetic nerve activity (MSNA) responses to baroreceptor unloading in humans. The purpose of this study was to determine if melatonin alters the vestibulosympathetic reflex (VSR) and vestibulocollic reflex (VCR) in humans. In study 1, MSNA, arterial blood pressure, and heart rate were measured in 12 healthy subjects (28 ± 1 yr; 6 men, 6 women) during head-down rotation (HDR) before and after ingestion of either melatonin (3 mg) or placebo (sucrose). Subjects returned at least 2 days later at the same time of day to repeat the trial after ingesting the opposite treatment (melatonin or placebo). Melatonin significantly attenuated MSNA responses during HDR compared with placebo (burst frequency Δ 4 ± 1 vs. Δ 7 ± 1 bursts/min, and total MSNA Δ 51 ± 20 and Δ 96 ± 15%, respectively; P < 0.02). In study 2, vestibular evoked myogenic potentials (VEMP) were measured in 10 healthy subjects (26 ± 1 yr; 4 men and 6 women) before and after ingestion of 3 mg melatonin. Melatonin did not alter the timing of the p13 and n23 peaks (pre-melatonin 13.2 ± 0.4 and 21.3 ± 0.6 ms vs. post-melatonin 13.5 ± 0.4 and 21.4 ± 0.7 ms, respectively) or the p13-n23 interpeak amplitudes (pre-melatonin 22.5 ± 4.6 arbitrary units [au] and post-melatonin 22.7 ± 4.6 au). In summary, melatonin attenuates the VSR and supports the concept that melatonin negatively affects orthostatic tolerance. However, melatonin does not alter the VCR in humans suggesting melatonin’s effect on the VSR appears to be mediated by the utricles.

sympathetic; otolith organs; vestibular

MELATONIN, which is synthesized in a circadian rhythm, increases during the night and decreases throughout the day (35). Endogenous melatonin has been demonstrated to impact sleep quality, sexual maturation, and tumor suppression (7). As a supplement, melatonin is commonly ingested by the general population as an over-the-counter sleep aid (4). Pharmacological levels of melatonin have been demonstrated to lower blood pressure, function as an antioxidant, and improve jet lag symptoms (22, 26, 40). Cagnacci et al. (9) and Arangino et al. (1) observed a reduction in arterial blood pressure at rest with acute melatonin ingestion in men and women. Additionally, Ray (30) demonstrated that melatonin attenuates muscle sympathetic nerve activity (MSNA) in response to lower-body negative pressure in humans. It was concluded that melatonin attenuates baroreflex-mediated increases in MSNA. The reduction in arterial blood pressure and decrease in MSNA response to orthostatic stress suggest that melatonin might affect orthostatic tolerance in humans. However, the effect of melatonin on other mechanisms affecting postural blood pressure is unknown.

Hypothesized as an orthostasis feedforward mechanism, the vestibulosympathetic reflex (VSR) has been demonstrated to affect blood pressure regulation in animals (12, 19, 21). Ray and colleagues (15, 24, 25, 29, 31, 33, 37, 39) demonstrated that head-down rotation (HDR), which activates the VSR, elicits increases in MSNA and calf and renal vascular resistance in humans. Importantly, the VSR is able to increase MSNA during baroreceptor unloading (15, 29). These findings suggest that the VSR contributes to orthostasis in humans.

The vestibulocollic reflex (VCR) functions as a head posture regulator (5) and is measured in part by vestibular evoked myogenic potentials (VEMP) (10, 44). VEMP have been used clinically to test for vestibular disorders such as superior semicircular canal dehiscence (6) and Ménière’s disease (28). The tone bursts that elicit VEMP stimulate only the sacculus, thereby representing a specific marker of otolithic function (10).

Afferent nerves from the otolith organs, which trigger the VSR and VCR, synapse at the vestibular nuclei (44, 47). Using an in vitro model, Podda et al. (27) demonstrated that melatonin attenuates nerve firing of the medial vestibular nuclei in the rat. Thus reductions in neuronal activity through the medial vestibular nuclei by melatonin could attenuate the VSR and VCR in humans.

The purpose of the present studies was to determine the effect of melatonin on the vestibulosympathetic and vestibulocollic reflexes. Study 1 investigated the effects of exogenous melatonin on the VSR in humans. Study 2 determined if the VCR is affected by melatonin and specifically addresses the effect of melatonin on the sacculus. On the basis of previous studies, it was hypothesized that increases in melatonin would attenuate MSNA responses during HDR and attenuate VEMP responses to auditory stimuli.

METHODS

Subjects

In study 1, 12 healthy subjects (6 men and 6 women; age: 28 ± 1 yr; height: 174 ± 3 cm; weight: 71 ± 5 kg) were tested. In study 2, 10 healthy subjects (4 men and 6 women; age: 26 ± 1 yr; height: 174 ± 3 cm; weight: 70 ± 5 kg) were in the experimental group. Nine of these subjects participated in study 1. Additionally, eight healthy subjects (1 man and 7 women; age: 25 ± 1 yr; height: 167 ± 2 cm; weight: 62 ± 3 kg) were in the placebo group for study 2. All subjects were tested at midday (11 AM–1 PM) when endogenous melatonin levels are lowest (2). All subjects were normotensive, nonsmokers, nonobese, and not taking any medications that would interfere with the measurements of the
protocol. All subjects received a physical examination before participation. Written informed consent was obtained from all subjects after verbal explanation of the experimental protocol. The Institutional Review Board of The Pennsylvania State University College of Medicine approved this study.

**Experimental Protocol**

**Study 1.** Each subject randomly ingested in a double-blind manner either melatonin (3 mg) or placebo (sucrose). Each subject and the investigator performing the microneurography measurements and MSNA analyses were blinded to the treatment. The experimental protocol began 45 min after ingestion of either melatonin or placebo. We have demonstrated that this is the time required for plasma melatonin levels to reach peak levels (30). Venous blood samples were obtained from eight subjects before ingestion and following the experimental protocol. Subjects returned at least 2 days later at the same time of day to repeat the trial after ingesting the opposite drug. MSNA, heart rate, and mean arterial blood pressure (MAP) were measured continuously in the prone position during 3 min of baseline, 3 min of HDR, and 3 min of recovery.

During baseline, the subject’s neck was extended with the chin supported to bring the head upright as close to the vertical plane as possible. This position approximates the gravitational orientation of the head when an individual is in the upright posture (39). For HDR of both trials, the head was maximally lowered in the vertical plane over the edge of the table. An investigator moved the head by supporting the forehead and chin, thus producing a passive head movement. Once the head became stationary, only afferent inputs from the otolith organs and not the semicircular canals were engaged.

**Study 2.** Subjects in the experimental group first performed a bout of VEMP testing (no treatment), ingested 3 mg of melatonin, waited 45 min, then performed a second bout of VEMP testing. Each bout included four separate trials of VEMP testing to ensure reproducibility. A description of the VEMP testing is presented below in Measurements. The time-control subjects performed the same protocol but ingested a placebo (sucrose) instead of melatonin.

**Measurements**

**Study 1.** Heart rate and arterial blood pressure were continuously recorded during all trials using a Finometer (FMS, Amsterdam, The Netherlands). Before each trial, brachial artery blood pressure was measured by an automated sphygmomanometer (Dinamap, General Electric, Waukesha, WI).

Multifiber recordings of MSNA were obtained from a tungsten microelectrode inserted in the peroneal nerve behind or lateral to the knee, as previously described (29). A reference electrode was placed subcutaneously 2–3 cm from the recording electrode. The criteria for an adequate MSNA signal included 1) tapping of the muscles or tendons innervated by the nerve produced afferent mechanoreceptor discharges; 2) apnea produced an increase in sympathetic nerve activity; 3) stroking of the skin did not produce any afferent activity; and 4) sudden, unexpected arousal stimulus (shout or clap) did not produce any increases in sympathetic activity (42). The nerve signal was amplified (20,000–50,000 times), fed through a band-pass filter, and subsequently passed through a loudspeaker for monitoring during the study. Sympathetic recordings that demonstrated possible electrode site shifts, altered respiratory patterns (e.g., breath holding, inspiratory gasp, and hyperventilation), or electromyographic artifact during experimental intervention were excluded from analysis.

An arm vein catheter was used to obtain venous blood samples for measurement of plasma melatonin. The blood sample was stored on ice and subsequently spun to separate the plasma. Plasma melatonin was measured in duplicate by radioimmunoassay (Buhlmann Laboratories). All samples were analyzed together after the completion of all tests. The lower limit of detection was 0.5 pg/ml, and the intra- and interassay coefficient of variation was 4.5 and 7.2%, respectively.

**Study 2.** Subjects were in the supine position for the duration of the experimental protocol. VEMP testing was performed with the Interacoustics Eclipse EP25 (Interacoustics). Surface EMG was recorded bilaterally using surface electrodes placed at half the distance between the mastoid tip and sternal notch on the muscle belly of the sternocleidomastoid muscle, and a reference and ground electrode were placed on the forehead. The subject’s nose was centered 3–4 cm under a stationary band suspended from the ceiling. During the VEMP trials, subjects were instructed to lift and turn their head to the left so that their cheek touched the band. Controlling the degree of head movement limited the variation in neck tension between trials. VEMP were evoked by a tone burst stimulus transmitted by an earphone inserted into the ear ipsilateral of muscle recording (right side). The stimulus rate was 5.3 Hz at 100 dB above normal adult hearing level, and 150 sweeps were averaged per trial. Each trial lasted ~30 s. To minimize startle from the tone burst onset, the noise level was ramped up to maximum intensity. The latency from stimulus (p13 and n23) and interpeak (p13-n23) amplitude were measured. p13 and n23 refer to the first nadir and peak, respectively, after the stimulus is applied. Four trials were performed to ensure reproducibility of VEMP measurements. Subjects rested 1–2 min between trials to minimize fatigue.

**Data Analysis**

**Study 1.** Sympathetic bursts were identified from individual inspection of the mean voltage neurograms and with computer assistance. The investigator who identified the sympathetic bursts was blinded to the drug condition. MSNA was expressed as burst frequency (bursts/min) and total MSNA (i.e., sum of bursts area). The area of the bursts was measured by a computer program (Peaks; ADInstruments). Absolute changes from baseline are reported for burst frequency. Relative changes (%) from baseline are reported for total MSNA. For all trials, the 3 min of baseline were averaged together and reported as the baseline value for the respective trial. Because MSNA has been demonstrated to significantly increase during the first minute of HDR and not change during prolonged HDR (20, 39), the first minute of HDR is reported and used for statistical tests for the VSR. Statistical analyses of the data during HDR trials were performed with a two-within-factor [drug × intervention (HDR)] repeated-measures ANOVA (n = 12). Plasma melatonin levels were analyzed with a two-within-factor (drug × time) repeated-measures ANOVA (n = 8). A paired r-test was performed to compare hemodynamic values at baseline between the two trials. Significance was set at P < 0.05. All data are presented as means ± SE.

**Study 2.** The four VEMP trials were averaged together to represent the pre- and posttreatment interventions. Latency from stimulus (p13 and n23) and interpeak amplitude (absolute value between p13 and n23 peaks) were calculated. Statistical analyses of the data during VEMP trials were performed with a one-within (time), one-between (group) ANOVA (n = 18; 10 experimental and 8 control subjects). Significance was set at P < 0.05. All data are presented as means ± SE.

**RESULTS**

**Study 1: VSR**

Ingestion of melatonin significantly increased plasma melatonin compared with placebo (865 ± 28 vs. 6 ± 1 pg/ml; P < 0.05; Table 1). Plasma melatonin did not differ before melatonin or placebo ingestion (6 ± 1 and 9 ± 3 pg/ml, respectively).

MAP and heart rate were not different between the two drug trials during baseline (Table 1). MSNA burst frequency was
slightly higher during the melatonin than the placebo trial (11 ± 2 vs. 9 ± 1 bursts/min; P = 0.026).

A representative neurogram of MSNA from one subject is presented in Fig. 1. Melatonin significantly attenuated MSNA responses during HDR compared with placebo (burst frequency Δ 4 ± 1 vs. Δ 7 ± 1 bursts/min and total MSNA Δ 51 ± 20 and Δ 96 ± 15%, respectively; P < 0.02; Fig. 2). Heart rate and MAP responses were not significantly altered by melatonin during HDR (Δ 2 ± 1 beats/min and Δ −1 ± 1 mmHg, respectively).

**Study 2: VEMP**

A representative averaged VEMP waveform from one subject is presented in Fig. 3. Melatonin did not alter the timing of the first VEMP nadir (p13) or the first VEMP peak (n23) (Table 2). VEMP p13-n23 interpeak amplitude was not altered by melatonin [pre-melatonin 22.5 ± 4.6 arbitrary units (au) and post-melatonin 22.7 ± 4.6 au; Fig. 4]. Comparable results for the p13-n23 interpeak amplitude were observed in the placebo group (bout 1, 26.1 ± 5.6 au; and bout 2, 26.1 ± 6.9 au; Fig. 4).

**DISCUSSION**

Two novel findings from this study are 1) exogenous melatonin attenuates MSNA increases during otolith organ activation, and 2) exogenous melatonin does not alter the VEMP response to saccule activation in humans, suggesting melatonin’s effect on the VSR appears to be mediated by the utricles. These results support the concept that exogenous melatonin contributes to reduced orthostatic tolerance in humans.

In animal models, melatonin has been associated with reduced neuronal activity in several areas of the brain (18, 27, 41). Importantly, Podda et al. (27) demonstrated that melatonin decreases neuronal activity of the medial vestibular nuclei. Therefore, it is possible that the decrease in the VSR observed in our study by exogenous melatonin is mediated by this mechanism. Additionally, decreases in vestibular nuclei activity by melatonin could alter the baroreflex. Neural interactions of vestibular and baroreceptor afferents have been demonstrated to occur in the nucleus tractus solitarius (NTS) (3, 13, 14, 36, 45, 46). Importantly, Xue et al. (45) demonstrated a decreased baroreflex response during baroreceptor loading and unloading in otococnia-deficient mice, suggesting a lack of otolith organ input alters the baroreflex. Furthermore, we demonstrated that baroreceptor loading can alter the VSR in humans (15). Together, these findings support the concept that melatonin

**Table 1. Baseline values for study 1**

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<th>Placebo</th>
<th>Melatonin</th>
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<tr>
<td>Plasma melatonin, pg/ml</td>
<td>6 ± 1</td>
<td>865 ± 28*</td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
<td>85 ± 1</td>
<td>86 ± 2</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>60 ± 3</td>
<td>61 ± 3</td>
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Values are expressed as means ± SE. For plasma melatonin, n = 8, and for mean arterial pressure and heart rate, n = 12. *Significantly different from placebo: P < 0.05.
attenuates both the VSR and baroreflex, which would negatively impact blood pressure regulation.

In contrast to the attenuated MSNA responses during HDR in study 1, decreases in VEMP interpeak amplitude were not observed in study 2. In air-conducted sound VEMP, as used in the present study, the tone burst stimulates the saccules (10, 38). HDR is a general stimulus of the otolith organs. These data suggest melatonin does not alter the saccules and might attenuate the VSR via the utricles.

The degree of contractile force in the sternocleidomastoid muscle positively relates to increases in p13-n23 interpeak amplitude (10). Therefore, neck tension needs to remain relatively constant between trials and bouts if an accurate comparison of the reflex is to be made. In the present study, a constant degree of neck flexion was maintained for each trial to minimize changes in neck tension between trials and bouts. Additionally, the effect of fatigue on VEMP activation was minimized in study 2 because no change was observed in p13-n23 interpeak amplitude between bout 1 and bout 2 in the time-control group.

What is the physiological implication of exogenous melatonin modulating the VSR? On movement from a supine to standing position, blood pooling in the lower limbs occurs, resulting in a blood volume shift of 25–30% (16). To maintain blood perfusion to the brain, contributing to syncopal symptoms.

In summary, melatonin attenuates the vestibulospinal reflex. However, melatonin does not alter the vestibulocollic reflex during VEMP stimulation of the saccules in humans, suggesting melatonin’s effect on the vestibulospinal reflex appears to be mediated by the utricles. The attenuation of MSNA responses during HDR with melatonin ingestion supports the concept that exogenous melatonin negatively affects orthostatic tolerance in the general population and could be deleterious to older adults and astronauts who are susceptible to orthostatic intolerance.

Table 2. p13 and n23 latencies in the melatonin and placebo groups for study 2

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<tr>
<th></th>
<th>Melatonin Pre</th>
<th>Melatonin Post</th>
<th>Placebo Pre</th>
<th>Placebo Post</th>
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<tr>
<td>p13, ms</td>
<td>13.2 ± 0.4</td>
<td>13.5 ± 0.4</td>
<td>13.2 ± 0.3</td>
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<tr>
<td>n23, ms</td>
<td>21.3 ± 0.6</td>
<td>21.4 ± 0.7</td>
<td>20.2 ± 0.6</td>
<td>20.5 ± 0.6</td>
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Values are expressed as means ± SE. Vestibular evoked myogenic potential (VEMP) peak latencies from tone burst onset. Melatonin did not alter the p13 (first nadir) and n23 (first peak) peak latencies.

ACKNOWLEDGMENTS

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GRANTS

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DISCLOSURES

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