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The effects of day-time exogenous melatonin administration on cardiac autonomic activity

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Abstract: Melatonin has a functional role in the nocturnal regulation of sleep and thermoregulation. In addition to its action on peripheral receptors, melatonin may act by altering autonomic activity. To determine the effect of melatonin on cardiac autonomic activity, 5 mg of melatonin or placebo was orally administered to 12 young subjects at 14:00 hr, in a repeated measures design. Melatonin decreased sleep onset latency to Stage 2 sleep by 4.92 ± 1.81 min (measured by Multiple Sleep Latency Tests), rectal temperature by 0.19 ± 0.05°C, and increased foot temperature by 0.74 ± 0.45°C (all $P < 0.05$). Melatonin decreased heart rate by 3.66 ± 1.68 beats/min ($P < 0.05$) and pre-ejection period (measure of cardiac sympathetic activity) by 16.48 ± 4.28 ms ($P < 0.05$), but had no effect on respiratory sinus arrhythmia (measure of cardiac parasympathetic activity) ($P > 0.05$). As the decrease in pre-ejection period is likely to have resulted from a decrease in blood pressure, these results do not confirm an effect of melatonin on cardiac sympathetic activity. However, the results do clearly indicate that melatonin is unlikely to drive the previously observed presleep increase in cardiac parasympathetic activity.

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Introduction

At night, sleep propensity and peripheral body temperature increase in humans as rectal temperature decreases. These changes are temporally related to the nocturnal increase in plasma melatonin levels (Lushington et al., 1996; Hughes and Badia, 1997). Exogenous day-time melatonin administration results in sleep and thermoregulatory changes similar to those observed at night. Specifically, day-time melatonin administration produces decreased sleep onset latency (Dollins et al., 1994; Tzischinsky and Lavie, 1994; Luboshizsky and Lavie, 1998; Gilbert et al., 1999), increased self reported sleepiness/decreased alertness (Dollins et al., 1994; Tzischinsky and Lavie, 1994; Cajochen et al., 1997; Luboshizsky and Lavie, 1998), decreased rectal and increased peripheral temperature (Krauchi et al., 1997; Gilbert et al., 1999). These findings suggest that melatonin has a functional role in the nocturnal regulation of sleep and thermoregulation. However, the mechanism by which this regulation occurs remains unclear.

The soporific and hypothermic changes observed at night when endogenous melatonin is high, and following exogenous day-time melatonin administration, are associated with alterations in autonomic activity. Thus, melatonin may promote sleep by producing changes via an alteration in autonomic tone. The effects of melatonin on human autonomic tone have yet to be determined. If melatonin acts partly as an autonomic regulator, then day-time melatonin administration should produce changes consistent with an increase in parasympathetic, and decrease in sympathetic activity. Of particular interest is melatonin's effects on cardiac parasympathetic tone. This is because cardiac parasympathetic tone progressively increases approximately 4 hr prior to normal sleep onset, but no changes are observed in cardiac sympathetic activity (Burgess et al., 1997). Thus, if melatonin influences cardiac autonomic tone, this is more likely to be reflected in an increase parasympathetic activity than a decrease sympathetic activity.

Currently, recording of autonomic activity in humans is problematic. Direct recording of efferent sympathetic nerve activity (microneurography) in humans is possible, but painful and not suitable for long recordings (e.g. Somers et al., 1993). Furthermore, parasympathetic nerve activity can not be similarly recorded. To date, the most

strongly validated and reliable non-invasive measure of cardiac parasympathetic activity is respiratory sinus arrhythmia (RSA; e.g. Berntson et al., 1993; Cacioppo et al., 1994; Berntson et al., 1997). RSA is a rhythm in heart rate; beat-to-beat intervals shorten during inspiration, and lengthen during expiration. RSA is mainly due to the excitation and inhibition of cardiac parasympathetic nerves. The most strongly validated and reliable non-invasive measure of cardiac sympathetic activity is pre-ejection period (PEP; e.g. Cacioppo et al., 1994; Berntson et al., 1997). PEP approximates the isovolumetric contraction time of the left ventricle – as sympathetic activity increases, PEP shortens. The ventricles are predominantly innervated by sympathetic nerves, with little vagal innervation. For this reason, PEP is believed to mainly reflect sympathetic activity.

The current study examined melatonin as a potential regulator of autonomic tone by observing the effects of day-time melatonin administration on salivary melatonin levels, sleep propensity, body temperature, and cardiac activity.

Methods

Subjects

Twelve (six male, six female), young (mean age 22.7 ± 2.3 years) healthy individuals with an average BMI (23.3 ± 1.2 kg/m^2) participated. Subjects were non-smokers, with no personal or family history of cardiovascular or respiratory disease. They did not regularly consume large caffeine ($<$ 350 mg/day) or alcohol doses (≤ 6 drinks/week), and participated in a moderate amount of exercise (≤ 10 hr/week). They were not taking any medication (currently or in the past week), except all females were taking an oral contraceptive. Subjects had not undertaken shift work or transmeridian travel in the past 3 months, and had no history of sleep problems. They were not experiencing any major life stress.

The procedures were approved by the Queen Elizabeth Hospital's Human Research Ethics Committee and the University of South Australia's Human Research Ethics Committee. All subjects gave written informed consent prior to participation. Subjects received financial reimbursement for their time.

Design

All sessions were conducted at The Centre for Sleep Research at the Queen Elizabeth Hospital. Participants abstained from caffeine and other stimulants for 24 hr before and during the study. They maintained a self-selected constant sleep–wake schedule for 1 week prior to and during the study (verified by sleep–wake diaries). Participants attended the laboratory for two 24 hr periods (21:00–21:00 hr) separated by at least 3, but no more than 6 days. The study consisted of two double blind, counterbalanced sessions.

Procedures

General laboratory procedures

In each session, participants went to sleep at their normal sleep onset time and were woken at their normal wake time (07:30–08:30 hr) and ate a light breakfast. They were then fitted with the polysomnographic montage, cardiac and temperature equipment. By 10:20 hr, they were supine on a bed in an individual, dimly lit ($<$ 100 lux) room with the ambient temperature maintained at 25 ± 1°C. Between multiple sleep latency tests (MSLTs, see below), participants remained awake and read or watched television. Subjects received snacks at 12:30 and 15:30 hr. At 14:00 hr, each subject ingested a placebo (0.15 μg glucose) or melatonin capsule (5 mg, Sigma Aldrich Pty. Ltd., St. Louis, MO).

Assessment of objective sleep propensity

Sleep propensity was assessed hourly from 11:00 to 20:20 hr, using MSLTs (adapted from Carskadon and Dement, 1982). At the start of every hour, the lights were turned off and for 20 min, subjects closed their eyes and attempted to sleep. Subjects were woken after three consecutive 30 s epochs of Stage 2 non-rapid eye movement (NREM) sleep occurred, according to standardized criteria (Rechtschaffen and Kales, 1968). Sleep–wake state was assessed by a central (C_3–A_2) and occipital (O_1–A_2) electroencephalogram, electro-oculogram and electromyogram. Electrodes were connected to a Medilog MPA-2 sleep analysis system (Oxford Medical Ltd, Oxton, England). Sleep onset latency (SOL) was determined as the time from lights out to three consecutive 30 s epochs of either Stage 1 non-REM sleep (SOL1), or Stage 2 non-REM sleep (SOL2). If a subject did not fall asleep, their SOL was recorded as 20 min.

Assessment of salivary melatonin

Saliva samples were taken hourly from 10:55 to 19:55 hr to determine salivary melatonin concentrations. Subjects chewed the cotton swab of

polyester Salivettes (Sarstedt, Numbrecht, Germany) for 1 min and the saliva samples were stored frozen. The samples were assayed in the Department of Obstectrics and Gynaecology, University of Adelaide. Samples were assayed (200 uL) in duplicate by direct radioimmunoassay (Voultsios et al., 1997) using reagents obtained from Buhlmann Laboratories (Alschwil, Switzerland). The sensitivity of the assay was 4.3 pM. All samples from individual subjects were analyzed in the same assay.

Assessment of heart rate and PEP

An electrocardiogram (ECG) was obtained from disposable pregelled Ag/AgCl ECG spot electrodes (Meditrace, Graphic Controls, NY) that were placed at the jugular notch of the sternum, 4 cm under the left nipple and the right lateral side (ground). The electrodes were connected to a VU-AMS device (v 4.6, TD-FPP, Vrije Universiteit, Amsterdam, The Netherlands). The ECG was recorded by the VU-AMS using an amplifier with a time constant of 0.3 s, 1 MOhm impedance, and a low pass software filter of 17 Hz. Each R-peak was detected with a level detector with automatic adjustment (Thakor et al., 1983). From the R-peak time series, an average value for heart rate was obtained for each 30 s.

The VU-AMS also determined PEP via impedance cardiography. A 350 μA current at 50 KHz passed through the body via 'current' electrodes on the base of the neck over vertebrae C3/C4 and on the back over vertebrae T8/T9. Two 'recording' electrodes, on the jugular notch and xiphoid process of the sternum, measured the impedance (Z_0), and change in impedance with time (dZ/dt). The dZ/dt signal was sampled at 250 Hz, and time locked to the R-wave to enable 30 s ensemble averaging of the dZ/dt signal. Thus, for each 30 s period, PEP was determined off-line as the time period between the R-wave on the ECG and the upstroke on the ensemble averaged dZ/dt signal. Reliability and validity of the VU-AMS is described elsewhere (de Geus et al., 1995; Willemsen et al., 1996).

Assessment of RSA

Two minute periods of cardiac beat-to-beat intervals (determined by the VU-AMS) were selected for spectral analysis (see below). Frequency domain analysis of the interbeat intervals was calculated by the CARSPAN program (ProGAMMA, The Netherlands) that is based on sparse discrete Fourier transformation and produces a spectrum from 0.01 to 0.50 Hz. The spectrum is based on a series of equidistant samples representing heart rate, obtained from low pass filtering of the R-wave series as unit pulses. RSA was calculated as the power in the range of 0.15–0.40 Hz divided by the total power (0.04–0.50 Hz) (e.g. Pagani et al., 1986).

Variations in respiratory frequency can alter the magnitude of RSA independently of alterations in vagal tone (Berntson et al., 1993). Thus, respiration was assessed using a thermistor (Pro-tech, Washington) taped under the nose. The thermistor was attached to the Oxford system and breaths/s were calculated off-line.

Assessment of temperature

Rectal temperature was recorded using indwelling rectal thermistors (Steri-Probe 491B, Cincinnati Sub-Zero Products, Ohio) that recorded at 30 s intervals. Peripheral temperature was measured using thermistors (Steri-Probe 499B, Cincinnati Sub-Zero Products, Ohio) attached on the arches of the soles of both feet. Thermistors were connected by cable to a 486 computer and the data was analyzed later with a purpose-built system (Strawberry Tree, California).

Data analysis

Prior to each MSLT, a common 2 min period was selected for the analysis of all autonomic, respiratory and temperature measures. In each period, there was minimal body movement. Additionally, the 2 min period occurred in the 15 min period prior to the start of each MSLT. Thus, the autonomic, respiratory and temperature variables were calculated hourly, but not confounded by sleep.

As baseline, cardiac activity and temperature vary widely between individuals, these variables for each subject were recalculated relative to the subject's overall session average and to the administration (14:00 hr). The 11:00 hr value was not included in any analysis.

Statistical analysis

The data were analyzed using a 2 within subjects Condition (melatonin, placebo) × Time (12:00–20:00 hr) repeated measures analysis of variance (ANOVA). As the 3 hr period prior to administration was included, the interaction was considered most relevant for determining an effect of melatonin. All P-values were based on the Huynh–Feldt correction, but original degrees of freedom are reported. Statistical significance was determined at $P < 0.05$.

Paired t-tests were planned to evaluate differences between the placebo/melatonin conditions post-administration when a significant interaction occurred. Due to the large number of comparisons, the significant P-value for each comparison was reduced to 0.0083 to minimize the probability of a Type I error.

Results

All variables were normally distributed. Rectal recordings from two subjects (thus n = 10), and respiration and foot temperature recordings from one subject were lost (thus n = 11).

The salivary melatonin levels in the two conditions are illustrated in Fig. 1. Significant effects of time ($F(8,88) = 11.31$, $P < 0.05$) and condition ($F(1,11) = 33.48$, $P < 0.05$) were observed, as was a significant interaction ($F(8,88) = 11.28$, $P < 0.05$). The t-tests revealed that salivary melatonin concentrations were significantly higher after melatonin administration from 15:00–16:00 hr. On average, the melatonin concentration was maximally increased by 5500.08 ± 1547.30 pM 1 hr after administration.

SOL1 and SOL2 decreased following melatonin administration (Fig. 2). For both, there was a significant effect of time ($F(8,88) = 12.02$, $P < 0.05$; $F(8,88) = 6.00$, $P < 0.05$, respectively), condition ($F(1,11) = 13.25$, $P < 0.05$; $F(1,11) = 8.73$, $P < 0.05$, respectively), and a significant interaction ($F(8,88) = 3.17$, $P < 0.05$; $F(8,88) = 3.35$, $P < 0.05$, respectively). The planned tests revealed that on average, SOL1 significantly and maximally decreased with melatonin administration at 17:00 hr, by 8.00 ± 1.96 min. Similarly, SOL2 maximally decreased (non-significant) at 17:00 hr by 4.92 ± 1.81 min.

Rectal temperature decreased with melatonin administration (Fig. 3). A significant effect of time ($F(8,72) = 9.07$, $P < 0.05$), but no significant effect

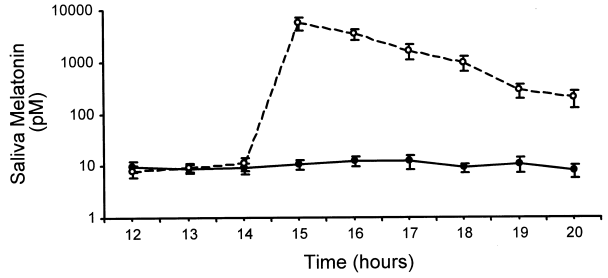

Fig. 1. Salivary melatonin concentrations in the placebo (solid line) and melatonin (dashed line) conditions from 12:00 to 20:00 hr. Drug administration was at 14:00 hr. The values are means ± S.E. for 12 young healthy subjects, plotted on a logarithmic scale.

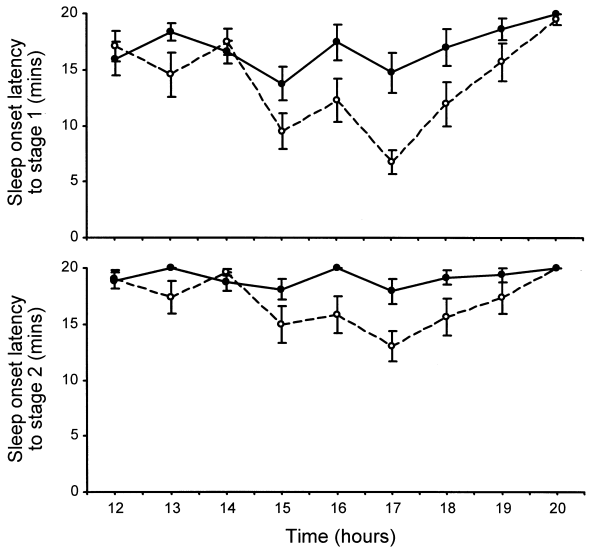

Fig. 2. Sleep onset latency to Stage 1 non-REM sleep (top) and to Stage 2 non-REM sleep (bottom) in the placebo (solid line) and melatonin (dashed line) conditions from 12:00 to 20:00 hr. Drug administration was at 14:00 hr. The values are means ± S.E. for 12 young healthy subjects.

of condition ($F(1,9) = 3.86$, $P > 0.05$) occurred. There was a significant interaction ($F(8,72) = 5.18$, $P < 0.05$). The average maximal but non-significant decrease in rectal temperature was 0.19 ± 0.05°C at 17:00 hr.

Foot temperature (mean of feet) increased after melatonin administration (Fig. 3). There was a significant effect of time ($F(8,80) = 9.16$, $P < 0.05$),

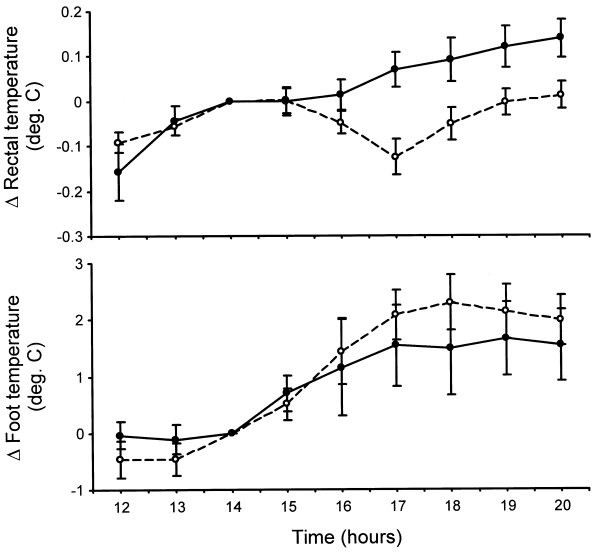

Fig. 3. Change in rectal (top) and foot (bottom) temperature in the placebo (solid line) and melatonin (dashed line) conditions from 12:00 to 20:00 hr. Drug administration was at 14:00 hr. The values are means of change ± S.E. for 10 (rectal temperature, top) and 11 young healthy subjects (foot temperature, bottom).

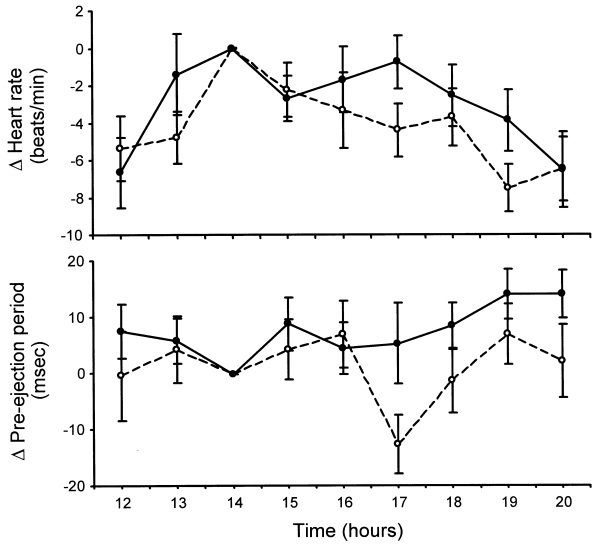

Fig. 4. Change in heart rate (top) and PEP (bottom) in the placebo (solid line) and melatonin (dashed line) conditions from 12:00 to 20:00 hr. Drug administration was at 14:00 hr. The values are means of change ± S.E. for 12 young healthy subjects.

but no significant effect of condition ($F(1,10) = 0.48$, $P > 0.05$). A significant interaction occurred ($F(8,80) = 2.28$, $P < 0.05$). After melatonin administration, the average foot temperature increased (non-significant) maximally by 0.74 ± 0.45°C at 18:00 hr.

Heart rate decreased following melatonin administration (see Fig. 4). There was a significant effect of time ($F(8,88) = 6.58$, $P < 0.05$), but no significant effect of condition ($F(1,11) = 1.33$, $P > 0.05$). There was a significant interaction ($F(8,88) = 2.18$, $P < 0.05$). The average maximal decrease (non-significant) in heart rate was 3.66 ± 1.68 beats/min at 17:00 hr.

Melatonin administration did not alter cardiac parasympathetic activity (RSA, Fig. 5). There were no significant effects of time ($F(8,88) = 0.44$, $P > 0.05$), or condition ($F(1,11) = 0.01$, $P > 0.05$), nor a significant interaction ($F(8,88) = 0.76$, $P > 0.05$). Respiratory rate also showed no significant effects (time: $F(8,80) = 1.36$, $P > 0.05$; condition: $F(1,10) = 0.69$, $P > 0.05$; time × condition: $F(8,80) = 1.21$, $P > 0.05$; Fig. 5). The average Pearson correlation between RSA and respiratory rate was 0.21 for the melatonin, and 0.04 for the placebo condition.

PEP decreased (inversely representing cardiac sympathetic activity) following melatonin administration (Fig. 4). There were significant effects of time ($F(8,88) = 2.29$, $P < 0.05$) and Condition ($F(1,11) = 10.42$, $P < 0.05$), and there was a significant interaction ($F(8,88) = 2.15$, $P < 0.05$). The average maximal and significant decrease in PEP was 16.48 ± 4.28 msec at 17:00 hr.

Discussion

The results indicate that day-time administration of 5 mg melatonin increases salivary melatonin levels, sleep propensity and peripheral temperature and decreases rectal temperature, heart rate and PEP (a measure of cardiac sympathetic activity). In contrast, RSA (a measure of cardiac parasympathetic activity) was not altered by melatonin. As discussed below, these findings suggest that melatonin does not directly alter cardiac autonomic activity.

The observed effects of day-time melatonin administration on salivary melatonin concentration, sleep propensity, and body temperature are consistent with previous research. The same dose of melatonin has been reported to increase salivary melatonin levels (Reid et al., 1996), decrease SOL (Dollins et al., 1994; Reid et al., 1996; Luboshizsky and Lavie, 1998; Gilbert et al., 1999) and decrease rectal temperature (Reid et al., 1996; Hughes and Badia, 1997; Krauchi et al., 1997; Gilbert et al., 1999). The decreases in SOL and rectal temperature observed here are similar in timing and magnitude to previous reports (e.g. Dollins et al., 1994; Gilbert et al., 1999). However, previous studies have found larger increases in peripheral temperature (2.4°C, Krauchi et al., 1997; 1.2°C, Gilbert et al., 1999) than observed here (0.7°C).

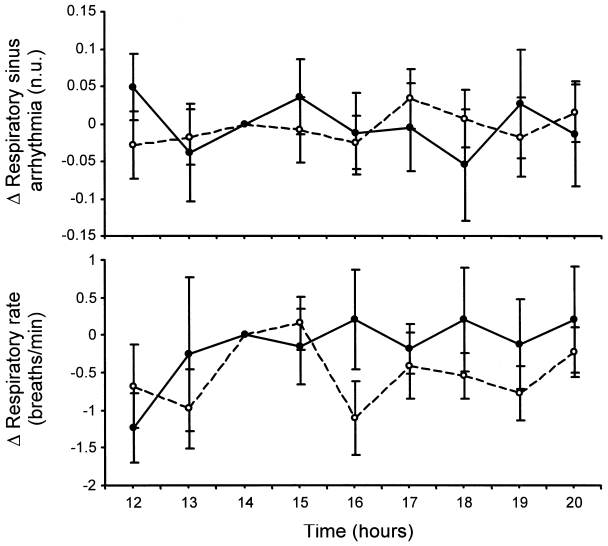

Fig. 5. Change in respiratory sinus arrhythmia (top) and respiratory rate (bottom) in the placebo (solid line) and melatonin (dashed line) conditions from 12:00 to 20:00 hr. Drug administration was at 14:00 hr. The values are means of change ± S.E. for 12 (respiratory sinus arrhythmia, top) and 11 young healthy subjects (respiratory rate, bottom).

Previous research into the effect of melatonin (1–5 mg) on cardiac activity in humans during the day found no change, or a decrease in heart rate (Krauchi et al., 1997; Cagnacci et al., 1998; Gilbert et al., 1999), a decrease in systolic and diastolic blood pressure, and no change in supine catecholamine levels (Cagnacci et al., 1997). Animal studies report that melatonin decreases heart rate and blood pressure (Bosman et al., 1991; Chuang et al., 1993). When found in humans, the magnitude of heart rate decrease (3.3 ± 1.2 beats/min, Gilbert et al., 1999) is comparable to the decrease observed here (3.7 ± 1.7 beats/min) and the decrease observed after sleep onset (Burgess et al., 1997).

The effects of melatonin on cardiac autonomic tone in humans has not been previously investigated. Here, we utilized RSA and PEP as indices of cardiac parasympathetic and sympathetic activity, respectively. Melatonin administration did not significantly alter RSA. Measurement of RSA can be confounded by variations in respiratory activity (e.g. Berntson et al., 1993). However, as evident in the minimal correlations, it is unlikely that the magnitude of RSA was confounded by respiratory activity. Thus, the results suggest that melatonin has no direct effect on cardiac parasympathetic activity. Previous research indicates that cardiac parasympathetic activity (also assessed with RSA), increases approximately 4 hr prior to normal sleep onset, even after food intake, posture and physical activity are controlled for (Burgess et al., 1997). The current findings suggest that this increase is not a result of the nocturnal increase in endogenous melatonin, but rather is directly mediated by the circadian pacemaker.

Exogenous melatonin produced a decrease in PEP, suggesting an increase in cardiac sympathetic activity. However, PEP can be confounded by alterations in blood pressure (e.g. Sherwood et al., 1990). For example, if diastolic blood pressure (afterload) decreases, PEP shortens. It is possible that the changes in PEP here were confounded by changes in blood pressure. This is supported by the observation that the shortening of PEP did not correspond to a heart rate increase. Furthermore, previous studies report that melatonin significantly decreases blood pressure in humans (Cagnacci et al., 1997) and animals (Chuang et al., 1993). Therefore, the effect of melatonin on cardiac sympathetic activity remains undetermined.

Melatonin receptors have been identified in the suprachiasmatic nucleus and hypothalamus (see Cardinali and Pevet, 1998), suggesting that melatonin may act centrally. The current results do not support the proposal that melatonin may centrally modulate autonomic activity. Autoradiographic studies have identified melatonin receptors in the peripheral vasculature (Cardinali and Pevet, 1998). Thus, melatonin may produce its physiological effects by stimulating these peripheral receptors. Here, the greatest alteration in sleep propensity and body temperature coincided with the maximal changes in heart rate and PEP – approximately 2 hr after the peak in salivary melatonin. All of these effects may be due to a melatonin-induced increase in peripheral vasodilatation. For example, increased peripheral vasodilatation typically increases heat loss, thereby decreasing rectal temperature. These temperature changes may be the cause of the alterations in sleep propensity (Gilbert et al., 1999; Krauchi et al., 1999). Increased peripheral vasodilatation typically decreases arterial blood pressure, thereby accounting for the shortening of PEP. A reduction in arterial blood pressure should however produce a heart rate increase due to the baroreceptor reflex (e.g. Berne and Levy, 1997). Thus, it remains undetermined how the decrease in heart rate was produced. Autoradiographic studies have identified melatonin receptors in the heart of bird species, evenly distributed across the atria, ventricles and septum (Pang et al., 1996). Melatonin also relaxes rat aortic smooth muscle, independent of any autonomic influences (Weekley, 1991). Thus, one possibility is that melatonin decreased heart rate (here in humans), via its direct action on receptors, than via the autonomic system.

There was a large interindividual variability in the increase in salivary melatonin concentrations following melatonin administration. There may also be such variability in the response of cardiac activity to melatonin, accounting for the mixed findings on heart rate. If so, this large variability may also account for the lack of change in cardiac parasympathetic activity. It is also possible that the effect of melatonin on cardiac activity varies across the day. This could be due to variations in receptor sensitivity perhaps regulated by melatonin itself (Gauer et al., 1993; Masana et al., 2000), or due to a variation in the capacity of the cardiac system to respond to melatonin. For these reasons, studies should consider larger sample sizes and examine the effect of night-time melatonin administration on autonomic activity. Microneurographic recordings following melatonin administration would more clearly determine the effects of melatonin on sympathetic activity.

Acknowledgments

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