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Effects of bright light and melatonin on sleep propensity, temperature, and cardiac activity at night

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Burgess, Helen J., Tracey Sletten, Natasha Savic, Saul S. Gilbert, and Drew Dawson. Effects of bright light and melatonin on sleep propensity, temperature, and cardiac activity at night. *J Appl Physiol* 91: 1214–1222, 2001.—Melatonin increases sleepiness, decreases core temperature, and increases peripheral temperature in humans. Melatonin may produce these effects by activating peripheral receptors or altering autonomic activity. The latter hypothesis was investigated in 16 supine subjects. Three conditions were created by using bright light and exogenous melatonin: normal endogenous, suppressed, and pharmacological melatonin levels. Data during wakefulness from 1.5 h before to 2.5 h after each subject's estimated melatonin onset (wake time + 14 h) were analyzed. Respiratory sinus arrhythmia (cardiac parasympathetic activity) and preejection period (cardiac sympathetic activity) did not vary among conditions. Pharmacological melatonin levels significantly decreased systolic blood pressure [5.75 ± 1.65 (SE) mmHg] but did not significantly change heart rate. Suppressed melatonin significantly increased rectal temperature (0.27 ± 0.06°C), decreased foot temperature (1.98 ± 0.70°C), and increased sleep onset latency (5.53 ± 1.87 min). Thus melatonin does not significantly alter cardiac autonomic activity and instead may bind to peripheral receptors in the vasculature and heart. Furthermore, increases in cardiac parasympathetic activity before normal nighttime sleep cannot be attributed to the concomitant increase in endogenous melatonin.

heart rate variability; respiratory; sympathetic

THE HORMONE MELATONIN IS SECRETED at night from the pineal gland in a pattern generated by the central circadian clock, the suprachiasmatic nuclei (SCN) located in the hypothalamus (9). In humans, the nocturnal increase in endogenous melatonin is associated with an increase in sleep propensity, a decrease in rectal temperature, and an increase in peripheral body temperature (18). The daytime administration of exogenous melatonin produces similar physiological changes (14, 15, 27). From these results, it has been suggested that melatonin may be functionally important in the nocturnal regulation of sleep and thermoregulation (14, 18). As melatonin receptors have been identified in the periphery (see Ref. 9 for review), it is possible that melatonin may produce its physiological effects by directly stimulating these peripheral receptors. Alternatively, melatonin may produce its effects indirectly through central modulation of autonomic activity.

There are two lines of evidence that support the hypothesis that melatonin directly alters cardiac autonomic activity. First, the soporific and hypothermic changes resulting in part from high levels of endogenous melatonin at night are associated with alterations in cardiac autonomic activity. Cardiac parasympathetic activity has been found to increase several hours before normal sleep onset (5, 33), in close parallel to the increase in endogenous melatonin. The increase in cardiac parasympathetic activity was found after posture, physical activity, and sleep were controlled for. Second, the daytime administration of exogenous melatonin has been found to decrease blood pressure (BP) and heart rate (HR) in humans and animals (4, 7, 10, 14). These findings point to a direct effect of melatonin on cardiac autonomic activity, thereby downregulating cardiac activity in preparation for and during sleep.

To date, two studies have investigated the effects of exogenous melatonin on cardiac autonomic activity (15, 19). In the first of these, 5 mg of melatonin were orally administered at 1400 to 12 young healthy subjects (15). In addition to the expected increase in sleep propensity, decrease in rectal temperature, and increase in peripheral body temperature, a small decrease in HR was reported (15). However, there were no conclusive changes in cardiac autonomic activity. The second study was similar. Melatonin was administered at 0700 to nine subjects (19). There was no subsequent alteration in the estimate of cardiac parasympathetic activity or in HR (19). The results from these studies suggest that melatonin does not influence cardiac autonomic activity. However, in these studies, melatonin was administered during the day. The effects of nighttime melatonin on human autonomic tone may be quite different because of circadian alterations in melatonin receptor sensitivity (13, 23) or potential circadian variations in the responsiveness of the cardiac system to melatonin.

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The present study aims to measure the changes in cardiac and autonomic variables before normal sleep onset in response to three different circulating melatonin levels. In doing so, the present experiment will substantially add to our understanding of melatonin's effect on cardiac autonomic tone by observing for the first time the effects of variations in melatonin levels on autonomic activity at night rather than during the day. Respiratory sinus arrhythmia (RSA), a measure of cardiac parasympathetic activity, was derived from the spectral analysis of interbeat intervals. Preejection period (PEP), derived from impedance cardiography, was used as a measure of cardiac sympathetic activity. BP was also measured to examine potential effects on the peripheral vasculature. Salivary melatonin levels, sleep onset latency (SOL), and rectal and foot temperature were also assessed.

METHODS

Subjects

Sixteen (8 men, 8 women) young (mean age ± SE = 21.3 ± 2.7 yr), healthy individuals with normal body mass indexes (21.7 ± 2.0 kg/m^2) participated. Subjects did not regularly consume large caffeine (<350 mg/day) or alcohol doses (≤6 drinks/wk) and participated in a moderate amount of exercise (≤10 h/wk). All were nonsmokers, with no personal or family history of cardiovascular or respiratory disease. They were not taking medications (presently or in the past week), except that all women but one were taking an oral contraceptive. The menstrual phase of the female subjects during the study was randomly distributed. Subjects had no history of sleep problems and had not undertaken shift work or transmeridian travel in the past 3 mo. They also reported experiencing no major life stress at the time of the study.

The procedures were approved by the Queen Elizabeth Hospital, University of Adelaide, and University of South Australia Human Research Ethics Committee. All subjects gave written, informed consent before participation. Subjects received a financial gratuity for any inconvenience associated with participation.

Design

All sessions were conducted at The Centre for Sleep Research at the Queen Elizabeth Hospital. In an attempt to stabilize circadian phase, subjects maintained a self-selected constant sleep-wake schedule for 6 days before and during the study (verified by sleep-wake diaries and wrist actigraphs), although light exposure was self-selected outside the Centre. Participants abstained from caffeine and other stimulants for 24 h before and during the study. After an adaptation night, subjects attended the laboratory for three conditions (dim light, bright light, and melatonin + bright light; see Fig. 1). In the dim-light condition, subjects lay supine in bed in dim light (<10 lx) to facilitate the normal nocturnal rise in endogenous melatonin. In the other two conditions, subjects were also supine but were exposed to bright light (>3,000 lx), which is known to suppress the onset of endogenous melatonin production (21). In the bright-light condition, the subjects consumed a placebo capsule (0.15 g glucose), whereas in the melatonin + bright-light condition, a 5-mg oral dose of melatonin (Sigma Aldrich) was consumed (both conditions were double blind). Both melatonin and placebo were administered 14 h after each subject's habitual waking time, such that the pill administration occurred approximately at each subject's individual dim-light melatonin onset (DLMO) (20, 22). The dim-light condition was always first, with the other two conditions counterbalanced in order after that. Two to three days separated the dim-light condition from the next, whereas the bright-light and melatonin + bright-light conditions were separated by 3–5 days to control for possible phase shifting due to the bright-light exposure. The dim-light condition was always first, to avoid the subjects having to maintain their constant sleep-wake schedule for >2 wk.

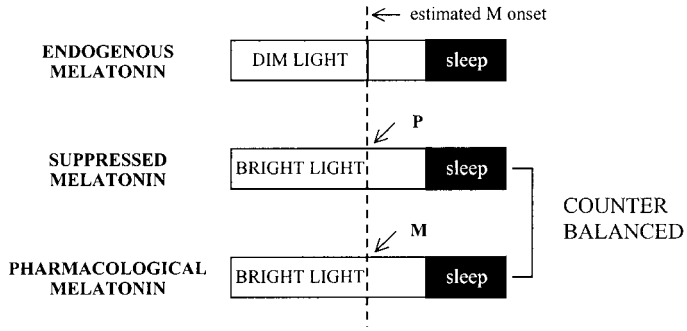

Fig. 1. Schematic representation of the experimental protocol. There were 3 conditions: dim light, bright light, and melatonin + bright light (resulting melatonin levels are shown on the *left*). All subjects were supine for at least 2.5 h before their estimated dim-light melatonin onset (DLMO). Light levels were low (<10 lx) in the dim-light condition. The bright-light and melatonin + bright-light conditions were counterbalanced in order after the dim-light condition. In these conditions, the light was bright (>3,000 lx). In the bright-light condition, subjects received a placebo (P), whereas in the melatonin + bright-light condition they received 5 mg of melatonin (M) orally. Drug administration occurred at each subject's individual estimated DLMO. Sleep was permitted 2 h after normal sleep onset time, ~3.5 h after each subject's estimated DLMO.

Procedures

General laboratory procedures. In all three experimental conditions, data were analyzed from 90 min before to 150 min after the estimated DLMO for each subject. Subjects arrived at the laboratory 2 h before the initiation of data collection to have the monitoring equipment attached (see below). They adopted the supine position at least 1 h before the onset of data collection to permit stabilization of the dependent variables. Subjects were only permitted to roll slowly along their longitudinal axis in a temperature-controlled room (22 ± 1°C).

Experimenters constantly monitored subjects to ensure that they did not fall asleep. Two-hundred-calorie snacks were provided at two hourly intervals during the waking period, and water was available ad libitum. In each session, subjects were asked to go to sleep 2 h later than their regular sleep onset time so that more saliva samples for melatonin analysis could be taken. At this time, the lights were switched off, and subjects were permitted to sleep. They were woken at their normal wake time the next morning.

Light exposure began 30 min before data collection. Bright light was produced by two light boxes (31 × 59 cm), each placed on one side of a 34-cm-wide television on a table positioned over the bed, 1.1 m from the bedhead. Subjects were permitted to watch television so that their gaze was directed toward the light boxes and were requested to stare directly into the light for at least 2–3 min of every 30 min. The purpose of the bright light in the melatonin + bright-

light condition was to control for the effects of bright light on the dependent variables.

Assessment of objective sleep propensity. Sleep-wake state was assessed by a central (C_3-A_2) and occipital (O_1-A_2) electroencephalogram, electrooculogram, and electromyogram according to standardized procedures (26). Electrodes were connected to a Medilog MPA-2 sleep analysis system (Oxford Medical, Oxton, UK). SOL was determined as the time from lights out to three consecutive 30-s epochs of either stage 1 non-rapid eye movement sleep (SOL1) or stage 2 non-rapid eye movement sleep (SOL2).

Assessment of salivary melatonin. Saliva samples were collected every 30 min during the data collection period for later melatonin measurement. Subjects chewed a polyester swab (Salivettes, Sarstedt, Numbrecht, Germany) for 3 min, and the saliva samples were stored frozen. The samples were assayed in the Department of Obstetrics and Gynaecology, University of Adelaide. Samples were assayed (200 μl) in duplicate by direct radioimmunoassay (34) with the use of reagents obtained from Bühlmann Laboratories (Allschwil, Switzerland). The sensitivity of the assay was 4.3 pM. All samples from individual subjects were analyzed in the same assay.

Assessment of HR and PEP. An electrocardiogram (ECG) was obtained from disposable pregelled Ag-AgCl ECG spot electrodes (Meditrace, Graphic Controls) 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 Vrije Universiteit ambulatory monitoring system (VU-AMS) device (version 4.6, TD-FPP, Vrije Universiteit, Amsterdam, The Netherlands). The ECG was recorded by the VU-AMS with the use of an amplifier with a time constant of 0.3 s, 1-MΩ impedance, and a low-pass software filter of 17 Hz. Each R peak was detected with a level detector with automatic adjustment (32). From the R-peak time series, an average value for HR was obtained for each 30 s.

The VU-AMS also used impedance cardiography to determine PEP. PEP is the most valid and reliable noninvasive measure of cardiac sympathetic activity (2, 6). 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. To measure PEP, a 350-μA alternating current at 50 kHz passed through the body via "current" electrodes on the base of the neck over vertebrae C_3-C_4 and on the back over vertebrae T_8-T_9. Two "recording" electrodes, on the jugular notch and xiphoid process of the sternum, measured the impedance 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 (11, 36).

Assessment of RSA. The most valid and reliable noninvasive measure of cardiac parasympathetic activity is RSA, a specific index of HR variability (2, 3, 6). RSA is a rhythm in HR; beat-to-beat intervals shorten during inspiration and lengthen during expiration. RSA is mainly due to the excitation and inhibition of cardiac parasympathetic nerves. To measure RSA, 5-min 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), which 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 HR, 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) (24).

Variations in respiratory frequency can alter the magnitude of RSA independently of alterations in vagal tone (3). Thus respiration was assessed from the thoracic impedance signals obtained as above. The dZ/dt signal was band-pass filtered to obtain the frequency containing thoracic impedance changes due to respiration. From the resulting respiration signal, respiratory rate (RR) was calculated (11, 12).

Assessment of BP. BP was measured indirectly by using standard sphygmomanometry. Three readings were obtained at each 30-min time point, and the average of these values was calculated.

Assessment of temperature. Rectal temperature was recorded by using indwelling rectal thermistors (Steri-Probe 491B, Cincinnati Sub-Zero Products, Cincinnati, OH). Peripheral temperature was measured by using thermistors (Steri-Probe 499B, Cincinnati Sub-Zero Products) attached on the arches of the soles of both feet. Thermistors were connected by cable to a 486 computer, and the data were recorded as text files by a purpose-built temperature program (Strawberry Tree). The data were recorded at 1-s intervals but averaged into 30-s intervals for later data analysis.

Data Analysis

Five-minute epochs were selected from within 10 min of either side of each 0.5-h sample point. The criteria for the period were minimal body movement and no abrupt changes in HR. RSA and RR were calculated for this time period, and the corresponding 30-s epochs of temperature and PEP were averaged to a representative 5-min mean. Therefore, a value for each dependent variable was obtained for every 30-min period of wakefulness, before lights out.

Statistical analysis. Data for each subject were aligned with each subject's estimated DLMO. Group data averages were calculated for each dependent variable across all three conditions. To assess the effect of melatonin on each variable, data were analyzed by using a two-way within-subjects factor repeated-measures ANOVA. The two factors were condition (3 levels: dim light, bright light, and melatonin + bright light) and time (9 levels: every 30 min from −90 to 150 min from estimated DLMO). As the first three time points (−90, −60, and −30 min) were before the estimated DLMO and administration time, the interaction was considered most relevant for determining an effect of melatonin. To account for the large number of repeated measures, all P values were based on the Huynh-Feldt corrected degrees of freedom, but the original degrees of freedom are reported. Statistical significance was determined at $P < 0.05$.

Paired t-tests were planned to evaluate differences among the three conditions at the postadministration times when a significant interaction occurred. Because of the large number of comparisons, the α-level for each comparison was corrected to minimize the probability of a type I error [$\alpha = 0.05/(\text{number of tests} - 1)$].

RESULTS

Because of technical difficulties, sleep onset latencies from two subjects, cardiac data from one subject, and diastolic BP (DBP) recordings from four subjects were

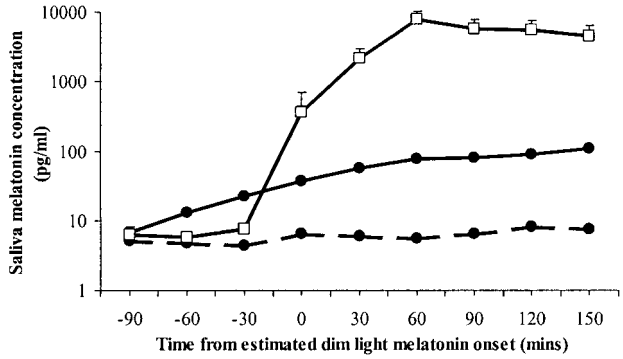

Fig. 2. Salivary melatonin concentrations in the bright-light (dashed line, ●), dim-light (solid line, ●), and melatonin + bright-light (solid line, □) conditions from −90 to 150 min from estimated DLMO (also drug administration time). Values are means for 16 young healthy subjects, plotted on a logarithmic scale. To improve clarity, the SEs (error bars) are only shown for 1 condition but were similar in magnitude in all 3 conditions.

lost. All variables, except for the salivary melatonin concentrations, were normally distributed.

Saliva Melatonin Concentrations

The melatonin data were log transformed (see Fig. 2). The main effect of time [$F(8,120) = 131.69$, $P < 0.001$] and condition [$F(2,30) = 91.77$, $P < 0.001$] and the time-by-condition interaction [$F(16,240) = 48.30$, $P < 0.001$] were all highly significant. The planned comparisons revealed that the average melatonin concentration in the dim-light condition was significantly higher than that in the bright-light condition for the first time at 30 min before the estimated onset time [$t(15) = 4.10$, $P < 0.001$]. This result indicates that, on average, the endogenous melatonin onset was 30 min to 1 h earlier than estimated. Further tests indicated that melatonin levels in the melatonin + bright-light condition increased significantly above those present in the bright-light condition starting 30 min after the pill administration [$t(15) = -18.06$, $P < 0.001$]. On average, the melatonin concentration was maximally increased by 7,970 ± 2,352 pM 1 h after administration.

SOL1 and SOL2

SOL1 and SOL2 were significantly affected by the three conditions (see Fig. 3). The analysis revealed a significant effect of condition for both SOL1 [$F(2,26) = 4.59$, $P = 0.04$] and SOL2 [$F(2,26) = 8.21$, $P = 0.002$; see Fig. 3]. Post hoc tests indicated that both SOL1 (mean difference, 2.98 ± 0.99 min) and SOL2 (mean difference, 5.25 ± 0.96 min) were significantly longer in the bright-light compared with the dim-light condition [$t(13) = -2.27$, $P = 0.04$; $t(13) = -3.70$, $P = 0.003$, respectively] and melatonin + bright-light condition [$t(13) = 2.22$, $P = 0.045$; $t(13) = 3.25$, $P = 0.006$, respectively].

Rectal and Foot Temperatures

Rectal temperature was also significantly affected by the manipulation of melatonin levels (Fig. 4). Bright light alone (melatonin suppression) attenuated the

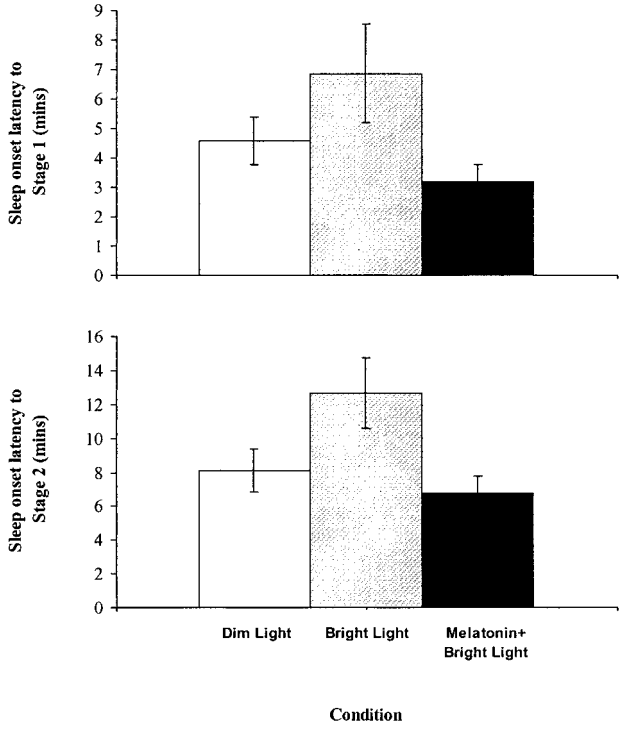

Fig. 3. Sleep onset latency to stage 1 (*top*) and stage 2 (*bottom*) non-rapid eye movement sleep in the dim-light (open bars), bright-light (shaded bars), and melatonin + bright-light (solid bars) conditions. Lights out was 2 h after each subject's normal sleep onset time. Values are means ± SE for 14 young healthy subjects.

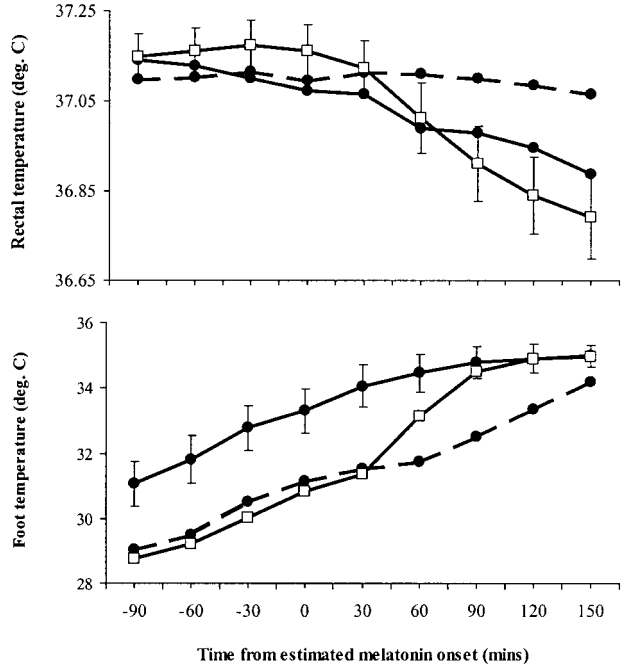

Fig. 4. Rectal (*top*) and foot (*bottom*) temperatures in the bright-light, dim-light, and melatonin + bright-light conditions from −90 to 150 min from estimated melatonin onset (also drug administration time). Lines and symbols are as defined in Fig. 2 legend. deg, Degrees. Values are means for 16 young healthy subjects. To improve clarity, the SEs (error bars) are only shown for 1 condition but were similar in magnitude in all 3 conditions.

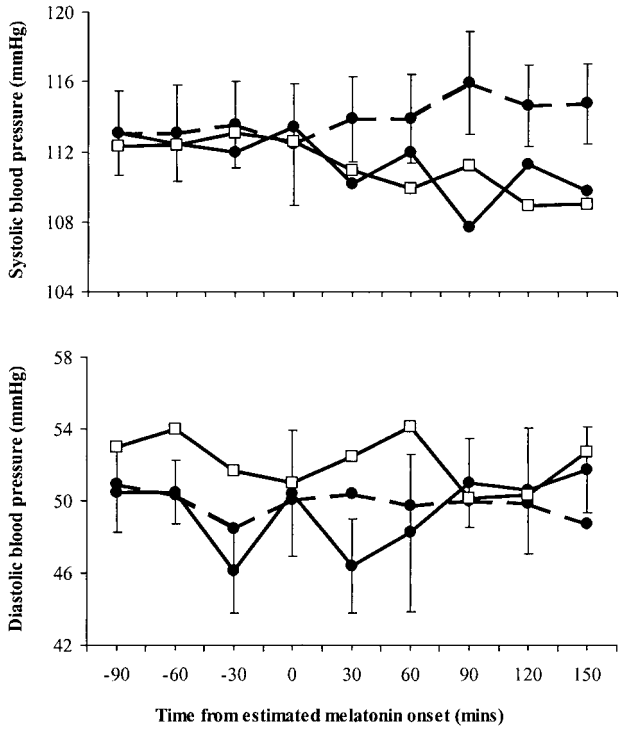

Fig. 5. Systolic (*top*) and diastolic (*bottom*) blood pressures in the bright-light, dim-light, and melatonin + bright-light conditions from −90 to 150 min from estimated melatonin onset (also drug administration time). Lines and symbols are as defined in Fig. 2 legend. Values are means for 16 and 12 young healthy subjects, respectively. To improve clarity, the SEs (error bars) are only shown for 1 condition but were similar in magnitude in all 3 conditions.

nighttime decrease in rectal temperature observed in the dim-light condition. In the melatonin + bright-light condition, rectal temperature was even lower than in the dim-light condition. The mean maximum difference between the bright-light and melatonin + bright-light conditions was 0.27 ± 0.06°C at 150 min after the administration. The main effect of time [$F(8,120) = 26.60$, $P < 0.001$] was significant, but the effect of condition [$F(2,30) = 0.85$, $P = 0.44$] was not. The interaction [$F(16,240) = 8.79$, $P < 0.001$] was significant. Rectal temperature significantly decreased 90 min after administration in the melatonin + bright-light condition compared with the bright-light condition [$t(15) = 2.78$, $P = 0.014$].

The temperatures from both feet were averaged into one mean foot temperature (Fig. 4). Here, bright light clearly lowered foot temperature before treatment, until melatonin significantly increased foot temperature 1 h after administration [$t(15) = -2.23$, $P = 0.042$]. The mean maximum increase in foot temperature after the melatonin administration was at 90 min after administration and was 1.98 ± 0.70°C. The effect of time [$F(8,120) = 52.62$, $P < 0.001$] and condition [$F(2,30) = 7.61$, $P = 0.004$] and the interaction [$F(16,240) = 5.78$, $P < 0.001$] were all significant.

BPs

Melatonin did not affect DBP (Fig. 5, *bottom*). There was no significant effect of time [$F(8,88) = 1.21$, $P = 0.31$], condition [$F(2,22) = 0.74$, $P = 0.48$], or interaction [$F(16,176) = 1.21$, $P = 0.29$]. In contrast, systolic BP (SBP) was affected by melatonin (Fig. 5, *top*). SBP showed no effects of time [$F(8,120) = 1.09$, $P = 0.38$] or condition [$F(2,30) = 2.53$, $P = 0.10$], but there was a significant interaction [$F(16,240) = 2.53$, $P = 0.006$]. Planned comparisons revealed that SBP in the bright-light condition was significantly greater than in the dim-light condition at 90 min after the estimated DLMO [$t(15) = -4.09$, $P = 0.001$]. SBP was significantly greater in the bright-light vs. melatonin + bright-light condition at 120 and 150 min after pill administration [$t(15) = 3.05$, $P = 0.008$; and $t(15) = 3.60$, $P = 0.003$, respectively]. Thus the exogenous melatonin dose began to significantly decrease SBP, overcoming the effects of bright light 120 min after melatonin administration. The mean maximum decrease in SBP after the melatonin administration was 5.75 ± 1.65 mmHg.

HR

HR showed a significant effect of time [$F(8,112) = 5.46$, $P = 0.002$] but not of condition [$F(2,28) = 2.01$, $P = 0.15$]. The interaction was close to significance [$F(16,224) = 1.80$, $P = 0.052$], suggesting a slight decrease in HR after exogenous melatonin administration (see Fig. 6). The mean maximal difference in HR between the melatonin + bright-light condition and

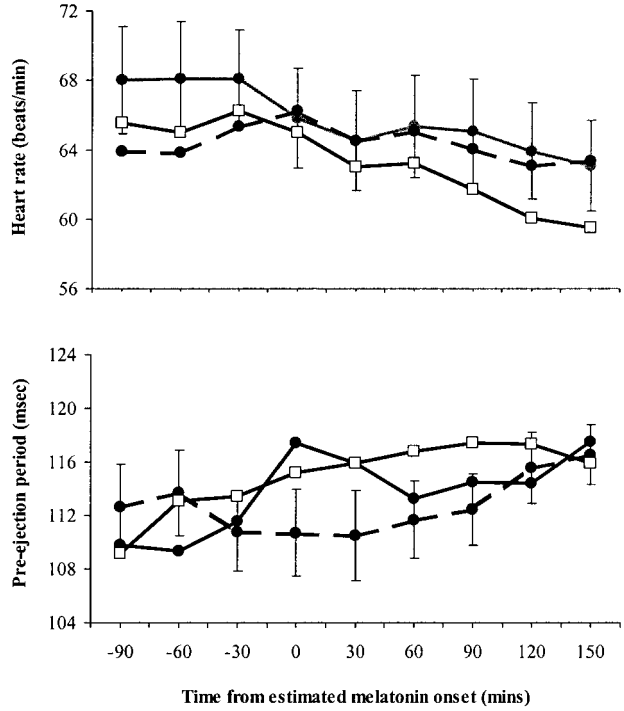

Fig. 6. Heart rate (*top*) and preejection period (*bottom*) in the bright-light, dim-light, and melatonin + bright-light conditions from −90 to 150 min from estimated melatonin onset (also drug administration time). Lines and symbols are as defined in Fig. 2 legend. Values are means for 15 young healthy subjects. To improve clarity, the SEs (error bars) are only shown for 1 condition but were similar in magnitude in all 3 conditions.

bright-light condition was 3.80 ± 1.95 beats/min 150 min after pill administration.

PEP

PEP was not significantly affected by melatonin (Fig. 6). PEP showed a significant main effect of time [$F(8,112) = 3.71$, $P = 0.007$] due to a general lengthening in PEP (decrease in cardiac sympathetic activity) in all conditions with increasing time. There was no significant main effect of condition [$F(2,28) = 0.49$, $P = 0.57$] or a time-by-condition interaction [$F(16,224) = 1.53$, $P = 0.17$].

PEP can be influenced by afterload (DBP), such that, as afterload decreases, PEP shortens in length, independent of any changes in cardiac sympathetic activity (31). Thus PEP and DBP were correlated within each subject, and each individual Pearson's product-moment correlation coefficient was transformed to Fisher's Z score, and the scores were averaged. This average was then transformed back to a correlation to obtain the group average correlation, which was 0.26. As this correlation was positive and moderate, a further repeated-measures analysis was conducted on PEP, with DBP included as a covariate. This analysis yielded the same results as the first analysis.

RSA and RR

Melatonin did not significantly affect RSA (measure of cardiac parasympathetic activity, see Fig. 7). RSA showed no significant main effect of time [$F(8,112) = 0.35$, $P = 0.95$] or condition [$F(2,28) = 3.49$, $P = 0.06$] or a significant interaction [$F(16,224) = 0.79$, $P = 0.65$]. RR also showed no significant effect of time [$F(8,112) = 0.40$, $P = 0.89$] or condition [$F(2,28) = 1.21$, $P = 0.31$] or a significant interaction effect [$F(16,224) = 0.99$, $P = 0.46$; see Fig. 7].

RSA can be confounded by alterations in RR. Specifically, as RR increases, RSA can decrease, independent of alterations in vagal tone (3). Thus RR was calculated and correlated with RSA (see *PEP* above for description of analyses). The group average correlation between RR and RSA was 0.01, and so it was considered unlikely that respiratory variations confounded RSA.

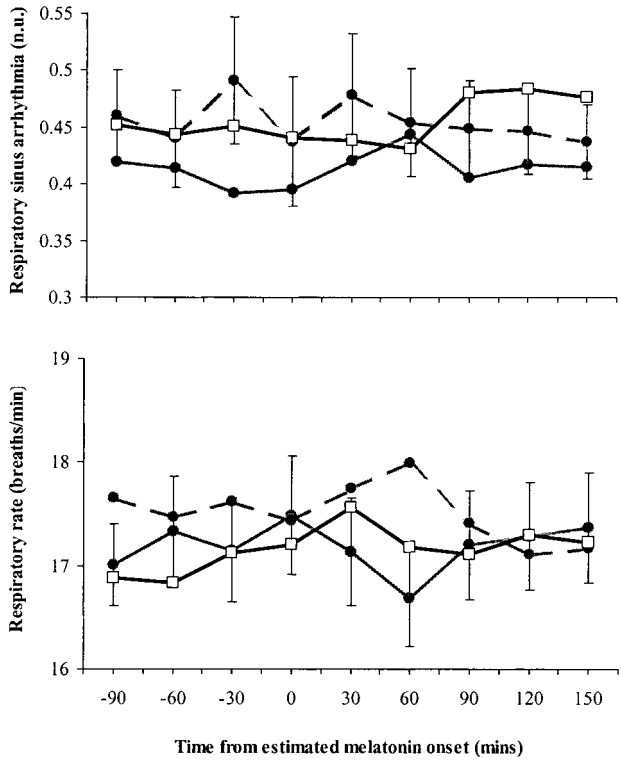

Fig. 7. Respiratory sinus arrhythmia (*top*) and respiratory rate (*bottom*) in the bright-light, dim-light, and melatonin + bright-light conditions from −90 to 150 min from estimated melatonin onset (also drug administration time). Lines and symbols are as defined in Fig. 2 legend. Values are means for 15 young healthy subjects. To improve clarity, the SEs (error bars) are only shown for 1 condition but were similar in magnitude in all 3 conditions. n.u., Normalized units.

DISCUSSION

In this study, nighttime melatonin concentrations were successfully manipulated by using bright light and exogenous melatonin. Bright-light exposure suppressed melatonin to very low levels, whereas a 5-mg exogenous melatonin dose reversed this suppression and significantly increased melatonin levels above normal nighttime endogenous melatonin levels. Therefore, as expected, three significantly different levels of melatonin were achieved: suppressed, endogenous, and pharmacological levels (Fig. 2). Furthermore, the experimentally induced changes in melatonin concentrations were made at approximately the same time as the rise in endogenous melatonin. Sleep propensity and rectal and peripheral temperature all changed in association with the different levels of melatonin (Figs. 3 and 4). However, RSA, a measure of HR variability (specifically reflecting cardiac parasympathetic activity), PEP (measure of cardiac sympathetic activity), and DBP remained unchanged (Figs. 5–7). There was a trend toward a significant decrease in HR, and SBP was significantly lower with high levels of melatonin (Figs. 5 and 6). As discussed below, these findings indicate that melatonin is unlikely to alter cardiac autonomic activity but, rather, may reflect a direct effect of melatonin on the heart itself.

The observed effects of the three levels of salivary melatonin on SOL and rectal temperature are consistent with previous nighttime studies (8, 16). Compared with a dim-light condition, SOL2 increased with bright light (Ref. 8, mean increase = 6.3 min; present study, mean increase = 4.5 min) and decreased with the addition of 5 mg of melatonin (Ref. 8, mean decrease = 5.6 min; present study, mean decrease = 6.1 min). Similarly, rectal temperature maximally increased with bright light (Ref. 16, mean increase = 0.17°C; present study, mean increase = 0.17°C) and decreased with melatonin (Ref. 16, mean decrease = 0.11°C; present study, mean decrease = 0.10°C).

Previous studies examining the effect of melatonin (1–5 mg) on cardiac activity in supine humans during the day have reported no change or a decrease in HR (7, 14, 15), as well as a decrease in SBP and DBP (7).

Animal studies have similarly reported that melatonin decreases HR and BP (4, 10). The magnitude of previous reductions in HR after daytime melatonin administration (3–4 beats/min; Refs. 14, 15) is comparable to the decrease observed in this study (3.8 beats/min) after nighttime administration of melatonin. The timing of the maximal decrease in HR after the administration of melatonin is also similar (2.5 h in this study vs. 3 h in Ref. 15).

The effects of melatonin on cardiac autonomic tone in humans at night have not been previously investigated. Here, we utilized RSA and PEP as indexes 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 (3). However, it is unlikely that the magnitude of RSA was confounded by respiratory activity in the present study, as the average correlation obtained between RSA and RR was $r = 0.01$. Therefore, it appears that melatonin had no direct effect on cardiac parasympathetic activity at night. In contrast, previous research indicated that cardiac parasympathetic activity (also assessed with RSA) increased several hours before normal sleep onset, even after the effects of food intake, posture, and physical activity were controlled for (5, 33). Thus the present findings suggest that the presleep increase may not be the result of the nocturnal rise in endogenous melatonin but, rather, may be directly mediated by the circadian pacemaker. Indeed, multisynaptic neural connections from the SCN (via the paraventricular nucleus) to the heart have been identified in rats (29), and there is evidence that the same may be true in humans (30). Exogenous melatonin did not produce a significant change in PEP, even when the potential confounding influence of DBP (afterload) was controlled for. This indicates that melatonin also does not directly alter cardiac sympathetic activity.

It is possible that the administration of bright light in the present study delayed the time course of the core temperature rhythm. Indeed, in a study most similar to the present study, bright light (5,000 lx) administered from 2100 to 2400 resulted in an average 46-min delay in the timing of the core body temperature minimum (16). Interestingly, this delay was reduced to 27 min when exogenous melatonin (5 mg at 2040) was administered before the light pulse (16). It is possible that our protocol produced a greater phase delay than that of the above study as the bright light was administered later in the night (on average until 0220). However, to influence the results, this phase delay would have to be close to immediate, thus making it difficult to determine the relative contributions that the immediate effects of melatonin and bright light, as well as any potential phase-shifting effects, may have had on the changes observed in body temperatures and sleep onset latencies. To confirm that the absence of an effect of melatonin on the cardiac variables was not due to a phase delay, we simulated a phase delay for each cardiac variable in the bright-light and melatonin + bright-light conditions by shifting each by 120 min later in time (assuming this to be the greatest shift possible). Reanalysis of this altered data set did not indicate any significant differences among the three conditions. In any case, ensuring that subjects maintained their prior sleep-wake schedule for at least 3 days before the following counterbalanced condition should have controlled any systematic effects of phase delays between the two bright-light conditions.

As mentioned previously, melatonin receptors have been identified in the SCN, highlighting a potential central mechanism of action of melatonin. However, the absence of a direct effect of melatonin on measures of cardiac autonomic activity does not support the proposal that melatonin centrally modulates autonomic activity. Autoradiographic studies have identified melatonin receptors in the peripheral vasculature (see Ref. 9 for review), which has led to the suggestion that melatonin may produce its physiological effects by stimulating peripheral receptors (18). The present results are consistent with this suggestion and can be illustrated by examining the temporal relationships among each of the measured variables. The order of effects was as follows: melatonin salivary concentrations peaked 1 h after melatonin administration; foot temperature was maximally increased 0.5 h after this peak (indicative of peripheral vasodilatation); and rectal temperature, HR, and SBP decreased about a further hour later (although the decrease in SBP may have leveled off before the decrease in HR). This time course of physiological changes suggests that the vasodilatation due to melatonin then produced further thermoregulatory and cardiovascular changes. It is now well established that vasodilatation of blood vessels in peripheral skin increases blood flow to these areas, resulting in the diffusion of heat from the blood to the environment, thereby decreasing core body temperature. Interestingly, it has been recently suggested that such alterations in thermoregulation (namely an increase in peripheral heat loss and a rapid decline in core temperature) are largely responsible for increasing sleep propensity (14, 18). Indeed, the temporal association observed here between SOL and the aforementioned thermoregulatory changes supports this proposal.

It would be expected that the increased peripheral vasodilatation induced by melatonin would also decrease arterial BP. In the present study, a decrease in SBP, but not DBP, was observed. It is possible that the nonsignificant decrease in DBP may have resulted from a reduction in statistical power caused by lost data. However, a reduction in SBP as well as a nonsignificant decrease in HR were observed. Normally, a reduction in arterial BP, induced by peripheral vasodilatation, should produce an increase in HR due to the baroreceptor reflex (1). Instead, we and others observed a slight decrease in HR in response to melatonin. These findings point to a direct effect of melatonin on the heart via melatonin receptors. Recent autoradiographic studies have identified melatonin receptors in the heart of bird species, evenly distributed across

the atria, ventricles, and septum (25). In addition, melatonin administration in rats has resulted in a relaxation of the aortic smooth muscle, independent of any autonomic influences (35). Therefore, it could be argued that melatonin decreases HR (here in humans) via a direct action on receptors rather than via the autonomic system.

The magnitude of the effect of melatonin on HR, during the day or at night, is similar to the decrease in HR observed after sleep onset (5). Although it appears small, a decrease of 3–4 beats/min is equal if not greater in magnitude to the decrease in HR observed in hypertensive subjects during rest after the chronic use of beta-blockers (28). Thus perhaps the potential antihypertensive use of exogenous melatonin should be further investigated. The effect of posture on melatonin's effect on the heart should also be further explored, as a preliminary report (17) suggests that melatonin may decrease HR beyond the normal decrease observed after a change from an upright to a supine posture.

In summary, although the effects of nighttime melatonin administration on SOL and core and peripheral body temperature were consistent with previous research, melatonin had no effect on well-validated measures of cardiac parasympathetic or sympathetic activity. Whereas DBP also showed no change, SBP significantly decreased. We interpret these findings to indicate that melatonin acts via receptors in the peripheral vasculature, and possibly in the heart itself, rather than by altering autonomic activity.

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