Melatonin and Zopiclone: The Relationship Between Sleep Propensity and Body Temperature

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Study Objectives: The sleep promoting effects of the sedative-hypnotics, melatonin and temazepam, have been associated with a decline in core body temperature ($T_C$). To determine whether changes in body temperature are a general feature of sedative-hypnotics, the present study compared the sleep inducing, core and peripheral temperature effects of melatonin, with those of zopiclone.

Design: Subjects were supine from 08:00-21:30 h and received melatonin, zopiclone or placebo at 14:00 h.

Setting: Individual, light and temperature controlled bedrooms.

Participants: 12 healthy, young, adults (7m, 5f; 20.3±0.6 years).

Interventions: Melatonin (5mg), zopiclone (Imovane™; 7.5mg) and placebo were administered in a double-blind, crossover design.

Measurements and Results: From 11:00-20:00 h, modified hourly multiple sleep onset latency tests (MSLT) of a 20-min duration were conducted and heart rate (HR) was recorded. $T_C$ and foot temperature ($T_{Ft}$) were recorded continuously using thermistors. Compared with placebo, melatonin and zopiclone significantly reduced sleep onset latency (SOL) to stage 1 (by 3.50±0.73 min and 6.80±0.61 min, respectively) and reduced $T_C$ (by 0.22±0.02°C and 0.14±0.02°C, respectively). For melatonin, $T_C$ declined as the result of an increase in peripheral heat loss (increase in $T_{Ft}$ of 1.65±0.43°C), and possibly a reduction in heat production as indicated by a decrease in HR (4.56±0.94 bpm). Zopiclone increased heat loss (increase in $T_{Ft}$ of 1.43±0.68°C) and had no cardiac effects. For melatonin, a negative association was found between $T_C$ (mean r=-0.43), however, this association was only weak for zopiclone (mean r=-0.23).

Conclusions: These results suggest that body temperature changes may be a general feature of sedative-hypnotics. The potential role of this effect in the promotion of sleep appears to vary between agents.

Key words: Melatonin; zopiclone; thermoregulation; core temperature; peripheral temperature; sleep; sedative-hypnotics

INTRODUCTION

THERMOREGULATION AND SLEEP ARE THOUGHT TO BE INTERRELATED PROCESSES. UNDER CONSTANT CONDITIONS, THE CIRCADIAN RHYTHMS OF CORE BODY TEMPERATURE ($T_C$) and sleep propensity vary inversely across the day and night in healthy young human subjects.1-3 Changes in body temperature are also apparent in humans following the administration of agents known to reduce alertness. For example, hypothermia is a common problem during anaesthesia4 and ethanol both decreases $T_C$ and alertness.5 Conversely, stimulants such as caffeine and amphetamines are known to increase $T_C$ and promote wakefulness.6 Thus, it appears that reductions in $T_C$ and increases in sleepiness are often associated.

Concomitant soporific and temperature effects have been reported following the daytime oral administration of melatonin.7-12 Changes in body temperature are also evident in the actions of another sedative-hypnotic, the benzodiazepine temazepam. The daytime administration of temazepam (20 mg) and melatonin (5 mg) is reported to increase sleep propensity and concomitantly reduce $T_C$ in young healthy subjects.11 Furthermore, following the evening administration of temazepam (30 mg) for seven consecutive days, tolerance to the sleep promoting properties of this agent is accompanied by a concomitant attenuation in its effects on both $T_C$ and $T_{Ft}$.13 These findings suggest that body temperature changes may be a general feature of sedative-hypnotics, and that, in turn, these changes may play a role in the sleep promoting effects of the agents.

It was the aim of the current study to explore this hypothesis, by comparing the changes in body temperature and sleep propensity evoked by a standard dose of melatonin (5mg) with those of another sedative hypnotic, zopiclone (Imovane™; 7.5mg). Melatonin was selected as a control because although the pharmokinetic of exogenous melatonin are time dependent and highly variable among individuals,14 its effects on body temperature in relation to sleep have already been well documented. Additionally, reports have consistently shown that when administered at pharmacological levels (5 mg) during the day (14:00 h) in healthy young subjects, the effects of melatonin on sleep propensity are negligible after three to four hours.9,11,12 The physiological and pharmacological mechanisms underlying the sleep-promoting effects of melatonin have not yet been unequivocally defined.14 A cyclopyrrolone, zopiclone was chosen because it is chemically unrelated to temazepam, but has a similar pharmacological profile to benzodiazepines in that it allosterically modulates the GABA-A receptor.15 Zopiclone reaches a maximum bioavailability of 90% one to one and a half hours after administration.16

METHODS

Subjects

Twelve healthy young subjects (seven male and five female)
aged between 19 and 25 (mean ± S.E.M = 20.3±0.6 years) participated in this study. Potential subjects were screened using a general health questionnaire (General Practice Research Unit, Windsor, UK) and a two-week sleep diary. Potential subjects were excluded if they were smokers, had any current health or sleep problems or were taking medications known to affect thermoregulation, sleep or melatonin production. No subjects sleeping less than eight hours a night or with an irregular sleep-wake schedule were included in the study. Female subjects participated only during the follicular phase of their menstrual cycle (4-14 days after menstruation) as women are less responsive to daytime melatonin administration during the luteal phase.17 Subjects gave written informed consent to participate in the study and were compensated for any inconvenience. This study was approved by The Queen Elizabeth Hospital Research Ethics Committee and was performed according to the Declaration of Helsinki.

**Experimental Protocol**

Subjects were required to attend the laboratory for three 24-hour (21:00-21:00 h) experimental sessions that were not less than five days apart. Subjects abstained from caffeine, alcohol and medications for 24 hours prior to, and during each session. Throughout the experimental procedure, ambient temperature was maintained at 25°C.

Upon arrival at the laboratory, subjects were fitted with a conventional montage of polysomnographic (PSG) electrodes18 to their face and scalp. Standard electrocardiogram (ECG) electrodes (Oxford disposable electrodes, Oxford Medical Limited, England) were attached to the right upper chest and left lower rib cage. All electrodes were connected to a Medilog MPA-2 patient junction box (Oxford Medical Limited, England). PSG and heart rate (HR) recordings were collected using a 847 Sleep Analysing Computer (SAC; Oxford Instruments, Oxford, UK).

Foot temperature (T\textsubscript{Ft}) was measured via skin temperature thermistors (YSI-4499E, Yellow Springs Instruments, OH) attached to the instep of both feet using Micropore surgical tape (3M Health Care, St. Paul, USA). Core body temperature (T\textsubscript{C}) thermistors were self-inserted approximately 10 cm into the rectum (Steri-Probe 491B, Cincinnati Sub-Zero Products Inc., Cincinnati, OH). An additional thermistor was placed on the bedhead to record ambient temperature. All thermistors were accurate to 0.05°C and connected to a custom temperature system (Strawberry Tree, CA, USA) that sampled temperature every second and calculated averages at 30-second intervals. For the entirety of the experiment, the laboratory was thermostatically maintained at 25°C by a Daiken FDY100B/RY100 air conditioner (Raymol, Woodville, Australia). All subjects wore shorts and a t-shirt when participating in the experiment and when in bed, their feet remained covered by a cotton sheet.

During each experimental session subjects were housed in individual bedrooms and lights out time was self-selected (prior to 01:00 h for all subjects). Following a normal night sleep in the laboratory, subjects were woken at 08:00 h and received a light breakfast. From 09:00 h onwards, subjects were required to remain supine, in dim lighting (<150 lux) and were permitted to read or watch television. At 14:00 h, standard dosages of melatonin (5mg; Sigma Aldrich Pty Ltd, Castle Hill, NSW, Australia), zopiclone (7.5mg; 27267 RP, Imovane\textsuperscript{TM}) or placebo (10mg lactose) were administered orally in a capsule in a double blind, counterbalanced design. A daytime protocol ensured that the confounding effects of endogenous nighttime melatonin were controlled for. An administration time of 14:00 h was selected to allow direct comparisons between our results and similar earlier studies.9,11,12 Subjects received a snack at 12:30 h, a cold lunch at 15:30 h and were allowed to drink water freely throughout the experiment.

**Measurement of Sleep Onset Latency**

Sleep onset latency (SOL) was assessed hourly from 11:00 hour to 20:00 h using a modified Multiple Sleep Latency Test18 (MSLT; employed in previous similar studies).9,11,12 In contrast to the standard MSLT protocol, which assesses sleep propensity at two-hourly intervals, we tracked the likelihood of sleep more closely by conducting an MSLT at the start of each hour. Additionally, SOL is traditionally referred to as the first epoch of sleep, but we utilized the definition of SOL to stage 1 (SOL) and stage 2 (SOL 2) as the time from lights out until three consecutive epochs in the respective sleep stage. At the start of each MSLT, in an environment conducive to sleep [i.e., dark (>1 lux) and quiet], subjects were instructed to lie still on their backs, close their eyes, and attempt to fall asleep. Subjects were woken if they remained in stage 2 sleep for three consecutive 30-second epochs (determined from the PSG recording). If a subject did not fall asleep in an MSLT, his/her SOL for that test was recorded as 20 minutes Each 30-second epoch of sleep was manually scored using standard sleep-scoring criteria.19

**Statistical Analysis**

Treatment effects. T\textsubscript{C} and T\textsubscript{Ft} data were averaged into 30-minute and hourly bins. All data were expressed relative to the time of drug administration (1400 h) in order to minimise random variability and inter-conditional differences in raw scores. The effects of placebo, melatonin and zopiclone on each of the variables measured were compared using repeated-measures analyses of variance (ANOVA). To determine the time at which significant differences occurred, planned comparisons were conducted between each condition at every time bin. To account for possible violations in the covariance matrix resulting from large numbers of repeated measures, adjusted Greenhouse-Geisser (G-G) significance values were used to determine significance in the ANOVAs as well as the planned comparisons (α=0.05). Data are expressed as mean ± S.E.M.

Correlational analyses. Due to the complexity of comparing two non-stationary time courses, the relationships between changes in body temperature (T\textsubscript{C} and T\textsubscript{Ft}) and SOL were calculated intra-individually. For each subject, the hour to hour (15:00–20:00 h) changes in T\textsubscript{C} and T\textsubscript{Ft} (placebo—treatment) were correlated with the hourly changes in SOL (placebo—treatment) using Pearson’s r correlation coefficient. This yielded intra-individual correlation coefficients for each subject in each treatment condition. To give an average indicator of how well the variables correlated, the correlation coefficients were transformed to Fisher z scores, which were then averaged and reconverted back to a Pearson’s r-value. For each treatment, data are expressed as a mean of all the subjects’ correlation coefficients ± SEM.

The magnitude to which both agents influenced T\textsubscript{C} was inves-
tigated using Pearson’s r correlation coefficient. Each individual’s maximum reduction in $T_c$ in the melatonin condition was correlated with that for the zopiclone condition.

**RESULTS**

No significant differences were detected between conditions during the pre-drug administration period (11:00-14:00 h). As such, post-administration differences can be attributed to drug effects.

**Sleep Onset Latency**

The mean changes in SOL relative to the time of drug administration (14:00 h) for the melatonin, zopiclone and placebo conditions are illustrated in Figure 1. Significant main effects of both “condition” $[F(2,22)=61.4; \text{G-G}<0.0]$ and of “time” $[F(9,99)=10.7; \text{G-G}<0.05]$ were detected, as was a significant interaction effect $[F(18,198)=5.2; \text{G-G}<0.05]$.

Planned comparisons revealed that relative to placebo, zopiclone administration significantly reduced SOL at all time points ($p<0.05$) by a mean of $6.80\pm0.61$ min. In contrast, melatonin reduced SOL at 16:00 h and 17:00 h and by a mean of $3.50\pm0.73$ min. In addition, zopiclone maximally reduced SOL by $8.79\pm1.56$ min (19:00 h) while melatonin induced a maximum reduction of $7.79\pm1.23$ min (16:00 h). Planned comparisons indicated that zopiclone had a significantly greater effect on SOL compared to melatonin at 15:00 h and from 17:00 h to 19:00 h.

**Core Body Temperature**

The mean changes in $T_c$ for the three conditions are illustrated relative to 14:00 hour in Figure 2. A repeated measures ANOVA revealed significant main effects of “condition” $[F(2,22)=34.0; \text{G-G}<0.05]$ and “time” $[F(18,198)=18.1; \text{G-G}<0.05]$. In addition, a significant interaction effect $[F(36,396)=7.3; \text{G-G}<0.05]$ was observed.

Planned comparisons revealed that, relative to placebo, melatonin and zopiclone significantly reduced $T_c$ from 15:00 h onwards ($p<0.05$). Additionally, between 14:00 h and 17:00 h $T_c$ was significantly ($p<0.05$) lower in the melatonin condition in comparison with the zopiclone condition. Furthermore, relative to placebo, melatonin evoked an average $0.22\pm0.02$C decline in $T_c$ while zopiclone reduced $T_c$ by a mean of $0.14\pm0.02$C. The maximum hypothermic effect of melatonin relative to placebo was $0.26\pm0.04$C at 18:00 h, while zopiclone evoked a maximum drop of only $0.18\pm0.03$C at 19:00 h.

When each individual’s maximum hypothermic response to melatonin was correlated with that for zopiclone no significant linear relationship was observed (mean $r=0.19; p>0.05$).

**Foot Temperature**

The mean changes in $T_{ft}$ for each treatment relative to 14:00 h are displayed in Figure 3. Significant main effects were obtained for both “condition” $[F(2,22)=2.2; \text{G-G}<0.05]$ and “time” $[F(18,198)=5.3; \text{G-G}<0.05]$. Additionally, a significant interaction effect $[F(36,396)=1.7; \text{G-G}<0.05]$ was observed.

Compared with placebo, melatonin significantly increased $T_{ft}$ from 14:30-16:30 hour and at 17:30 hour, while zopiclone only significantly increased $T_{ft}$ at 19:30 hour and 20:00 hour. Relative to placebo, the average and maximum increase in $T_{ft}$ following melatonin administration (1.37±0.32C and 1.65±0.43C at 15:00 hour, respectively) were greater than that following zopiclone administration (0.78±0.42C and 1.43±0.68C at 20:00 hour, respectively). Nonetheless, the effects of melatonin and zopiclone on $T_{ft}$ were only significantly different for one hour (15:30-16:30 hour; $p<0.05$).

**Heart Rate**

The average changes in HR relative to 14:00 h for each condition are presented in Figure 4. A significant main effect of “condition” $[F(2,22)=6.2; \text{G-G}<0.05]$ and “time” $[F(9,99)=4.4; \text{G-G}<0.05]$ was observed, as well as a significant interaction $[F(18,198)=3.4; \text{G-G}<0.05]$ effect.
Melatonin significantly reduced HR relative to placebo at all time points (1500-2000 h; p<0.05) by a mean of 4.56±0.94 bpm. In contrast, zopiclone only significantly reduced HR at 16:00 h and evoked a mean reduction in HR of 1.09±0.86 bpm. The reduction in HR evoked by melatonin was significantly more than that of zopiclone at 15:00 h and from 17:00 h to 19:00 h. Melatonin also had a greater maximal effect on HR (6.08±0.92 bpm at 16:00 h) compared with zopiclone (2.50±1.52 bpm at 16:00 h).

Association Between Sleep Onset Latency, Core and Peripheral Temperature

The temporal relationship between SOL and TC is displayed in Figure 5 for both the melatonin and zopiclone conditions (A and B respectively). Mean (±S.E.M.) intra-individual correlations of -0.43±0.10 and -0.23±0.09 (for the melatonin and zopiclone conditions respectively) were obtained between SOL and TC. Mean (±S.E.M.) intra-individual correlations of 0.34±0.07 and 0.21±0.08 were obtained between SOL and TFt (for the melatonin and zopiclone conditions, respectively). No significant linear relationship was found between the maximum effects of melatonin and zopiclone on TC.

DISCUSSION

The present study investigated the effects of the sedative-hypnotic zopiclone on sleep propensity and body temperature, and compared them with those of melatonin. The administration of both agents significantly reduced SOL and TC. While the sleep-promoting efficacy of melatonin was temporally related to its effects on TC, this relationship was only weak following the administration of zopiclone. These results suggest that while changes in TC may be a general feature of sedative-hypnotics, the functional role of this effect in the promotion of sleep may vary between agents.

Melatonin maximally reduced SOL (7.79±1.23 min at 16:00 h; Figure 1) in a manner similar in timing and magnitude to that reported previously (e.g., 7±3 min at 15:00 h9 and 8.00±1.96 min at 17:00 h12). In contrast, mean SOL was approximately 50% faster following zopiclone administration. The effects of zopiclone have not been previously assessed during the day, but the mean reduction in SOL evoked by zopiclone here, is comparable to that reported following the daytime administration of temazepam (6.5±1.62 min).11 Additionally, during the night, the efficacy of zopiclone (7.5mg) in patients suffering from insomnia is not different to that of temazepam (20mg),20,21 flurazepam (30mg),21 and nitrazepam (5mg).21 The contrasting effects of melatonin and zopiclone on sleep propensity, highlight the superior sedative properties pharmacological sedative-hypnotics have in comparison to melatonin.

In addition to promoting sleep, compared with placebo, both melatonin and zopiclone significantly reduced TC at all time points (Figure 2). Coupled with previous reports of reductions in TC following the administration of sedative-hypnotics, such as the benzodiazepines temazepam,11,13 triazolam,22 and midazolam,23 these results suggest that a TC effect may be a general feature of at least some sedative-hypnotics. Melatonin evoked a greater decline in TC in comparison to zopiclone (from 14:00-17:00 hour; Figure 2). Specifically, while melatonin evoked a distinct reduction in TC (maximum magnitude of 0.26±0.04C within the previously reported range9,11,12,24), zopiclone simply prevented the normal circadian afternoon increase in TC, thereby maintaining TC at a value similar to that at the time of administration.

In order to determine what mediated the different effects of the two agents on TC, heat loss was assessed.25 Melatonin and zopiclone increased heat loss, as measured via TFt,26,27 in a manner that was only significantly different for one hour (15:30-16:30 h; Figure 3). Heat loss is well known to be associated with the actions of melatonin9-12 and comparable increases in TFt have been attributed to temazepam.11 These findings suggest that an increase in heat loss of a relatively stable magnitude, could be a general feature of sedative-hypnotics.

In contrast, while melatonin significantly slowed HR throughout the experiment by a mean (4.56±0.94 bpm; Figure 4) compa-
account for the greater effects of this agent on Tc. As such, it is not surprising that no significant linear relationship was found between the maximum hypothermic effects of the two agents. Moreover, this finding suggests that it is unlikely that sedative-hypnotics activate a common thermoregulatory mechanism. In support of this suggestion, the actions of zopiclone are probably mediated by its action at GABA receptors, while melatonin has been proposed to act on peripheral receptors to adjust blood flow and thus heat loss in the periphery.29-30

Irrespective of the mechanisms involved, both agents reduced Tc and promoted sleep (Figures 5A and 5B). Moreover, following melatonin administration, an association between SOL and Tc (mean r=-0.43; Table 1) was found consistent with the suggestion that the sedative-hypnotic properties of melatonin may be mediated by its effects on body temperature.9-11 The effects of zopiclone on sleep propensity and temperature do not appear to be as strongly linked. A small correlation of -0.23 was identified between SOL and Tc, suggesting that for sedative-hypnotics, the role of changes in Tc in the promotion of sleep may vary between agents.

It has been suggested that warm feet promote the rapid onset of sleep31 and that distal-proximal skin gradient (DPG), a measure of blood flow in distal skin regions, is a better predictor of SOL than Tc.32 However, in the current study, TPr correlated to a similar degree with SOL as did Tc (mean=0.34 and mean r=0.21 for melatonin and zopiclone, respectively). A stronger association may not have been found between heat loss and sleep propensity because sleep onset may be promoted by receptors that specifically respond to changes in temperature between proximal and distal skin temperature (measured via the DPG) as opposed to absolute temperature (measured via TPr). Although the exact mechanisms by which body temperature influences sleep have not been defined, it has been postulated that the PoAH may be involved in the control of sleep as well as temperature regulation.33-35

It is not surprising to find that the temperature effects of sedative-hypnotics may facilitate sleep to distinct extents, as temperature changes are only one facet of a more general mechanism by which these agents promote sleep. In order to more comprehensively understand the role that changes in body temperature may play in the promotion sleep, we need to assess the dynamic changes in body temperature that accompany sleep onset, as opposed to investigating temperature at specific body sites (Tc and TPr). Prospective research in our laboratory aims to do this using infrared thermal imaging technology. Future investigations should aim to better control for the potentially confounding influences of circadian phase and sleep history. Although we found no significant differences in any variable during the four hours prior to drug administration time, this time period may not have been long enough to identify the effects of any changes in sleep or circadian phase that may have occurred between experimental conditions. Additionally, in the present study, food intake was constant between conditions (12:30 h and 15:30 h), but digestion may have interacted with the drug effects and could have been better controlled for.

In summary, a reduction in Tc following the daytime administration of two disparate sedative-hypnotics, melatonin and zopiclone, suggests that a Tc effect may be a general feature of some sedative-hypnotics. Moreover, following melatonin administr-
lation of Tc may play a role in the sleep promoting properties of sleep. As such, it is possible that the modulation of Tc may play a role in the sleep promoting properties of only some sedative-hypnotics. Further clarification of this potential role, perhaps attainable via administering sedative-hypnotics to people with impaired thermoregulatory systems, could improve the understanding and usage of sedative-hypnotics. Such information would be helpful in unravelling the relationship between body temperature and normal sleep onset and could thus potentially improve our understanding of some sleep disorders.

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