Effects of Low Doses of Melatonin on Late Afternoon Napping and Mood

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ABSTRACT

The effects of low doses of melatonin (0.1, 0.5 and 1 mg) given at 16:00 h on induction and quality of sleep in the late afternoon (17:00-21:00 h), as well as on subjective fatigue and mood ratings before and after sleep were studied. Ten healthy male volunteers (age 26-30 years) were given on a double-blind crossover basis, tablets containing melatonin, or placebo, with one day washout between treatments. Mood and fatigue were assessed before and after bedtime. Sleep quality was objectively monitored using wrist-worn actigraphs and subjectively by using sleep logs. Data were analysed by means of analysis of variance for repeated measures with a factor of group (placebo and the three melatonin doses).

The analysis revealed dose-dependent increase by melatonin in subjective evaluation of fatigue and sleepiness, and decrease in alertness, efficiency, vigor and concentration before the nap. Melatonin did not significantly affect actigraph-measured nap sleep latency and efficiency but reduced wake time after sleep onset and delayed sleep offset time compared to placebo. Melatonin did not significantly affect sleep latency and sleep efficiency in the night following the treatment.

These data indicate acute effects of low doses of melatonin given at 16:00h on sleepiness and fatigue but not on sleep efficiency or latency in healthy young individuals.

Abstracting keywords: Melatonin, sleep, dose, actigraph, mood.

INTRODUCTION

Melatonin (5-methoxy N-acetyltryptamine) is secreted by the pineal gland during the dark-phase of the day in all species studied to date. The nocturnal melatonin signal is thought to be a chemical transducer of information about the light-dark cycle in the organism (Reiter, 1991).

Human studies have shown that exogenous melatonin can affect the circadian system, thus mediating the following responses: a) Resynchronize the sleep/wake cycle in blind men (Folkard et al., 1990; Sack et al., 1991); b) Shift the phase response curve of rhythmic melatonin secretion (Lewy et al., 1992); c) Re-entrain circadian rhythms following a time zone shift (Arendt et al., 1986; Petrie

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et al., 1989; Claustrat et al., 1992); d) Remedy delayed sleep-phase insomnia (Dahlitz et al., 1991); e) Improve sleep maintenance in melatonin-deficient elderly subjects (Haimov et al., 1995; Garfinkel et al., unpublished). At pharmacological doses, melatonin has a hypnotic effect (McFarlane et al., 1991; Arendt et al., 1984; Lieberman et al., 1984; Dawson and Encel 1993; Dollins et al., 1993), which may be related to the hypothermia induced by melatonin administration (Cagnacci et al., 1992). Electroencephalographic studies have shown that sleep induced by melatonin resembles natural sleep, in sleep stage architecture, and in that subjects can be easily aroused (Cramer et al., 1974; Hughes et al., 1994).

Nickelsen et al. (1989) found that an acute dose of melatonin (50mg, PO) increased subjective feelings of fatigue when given in the morning (09:00 hours), but not in the evening (19:00 hours). This could indicate that the hypnotic effects of melatonin were dependent on, or mediated by, the circadian clock. In contrast, Naveh et al. (1995) have recently observed sleep-inducing effects of melatonin (3-6 mg) given in the afternoon (16:00-17:30 h), which resemble those found by Hughes et al. (1994) with melatonin (1-40 mg) given at noon.

Recent studies have described sleep-inducing effects of low doses of melatonin given at noon; these doses (0.1, 0.3 mg) produce blood levels similar to those found endogenously in the blood at night (Dollins et al., 1994; Zhdanova et al., 1995). The hypnotic potency of melatonin and its short half-life, make it potentially useful for night shift workers to induce evening nap and consequently reduce sleepiness during the shift.

The present study was conducted to determine: a) whether low doses of melatonin affect mood and sleep in the late afternoon. b) whether the melatonin treatment has residual effects on mood and sleep in the night following the nap.

We have studied the effects of low doses of melatonin (0.1-1 mg) or placebo ingested in the afternoon on urinary 6-sulfatoxy melatonin excretion, on sleep quality during a four hour nap in the late afternoon and the night following the nap, and on subjective mood ratings before and after sleep.

METHODS

Ten young volunteers (all male, age 28 ± 2 years) in healthy bodily and mental states participated. All were individually interviewed to exclude sleep disturbances, snoring and/or extreme morning or evening types. None used any kind of drugs or medications on a regular basis.

Protocol

This particular project was carried out between January and June 1994 in Tel Aviv, Israel (under ambient short as well as long days).
The study protocol was approved by the local ethics committee and health authorities. The aims, methods, anticipated benefits and potential side effects of the treatment were explained in non-technical terms by the physician and all volunteers gave their written consent.

Subjects entered the laboratory during the afternoon preceding the first day of the study and were in bed between 24:30 hr and 07:30 hr the following morning. Throughout the nine days’ study period meals were served at 08:00, 13:00 and 21:30 hr. Beverages containing caffeine were prohibited.

On a double-blind crossover basis, each subject received one of 3 melatonin doses or identical-looking placebo on four non-consecutive days with 1 day washout periods between doses. As indicated in the results section, we found no treatment-by-order effects indicating that the 1 day washout period was sufficient for the subjects to recover from any modification of the sleep-wake cycle. The tablets containing either 0 (placebo), 0.1, 0.5 or 1 mg of melatonin were taken orally at 16:00 hr. At 17:00 hr the subjects went to bed for a nap of four hours to enable spontaneous awakening and if not awake, were awakened at 21:00 hr. On rising, subjects washed, had dinner between 21:30-22:30 hr and then spent the evening (22:30-24:30 hr) at leisure. Subjects retired at 24:30 hr because of difficulty to retire to bed earlier due to the nap and were required to remain in bed until 07:30 hr the next morning. On awakening, subjects had breakfast and were permitted free time until 23:00 hr (washout day); During this time they were allowed to go outside. Subjects were in bed between 24:30 hr and 07:30 hr the next morning (treatment day).

**Measures**

**6-sulfatoxy melatonin excretion**

To assess bioavailability of the orally administered melatonin, urine was collected during each of the four treatment days: before lunch (14:00 hr), before taking the tablets (16:00 hr), after the nap (21:30 hr), before retiring to night sleep (23:00 hr) and after awakening the next morning (07:30 hr). The amount of the major melatonin metabolite 6-sulfatoxy melatonin was determined in the urine samples by radioimmunoassay (Stockgrand, Surrey, England) and remained blinded until all data was analyzed.

**Sleep quality**

Sleep quality during the naps and nights was estimated from activity data recorded using actigraphs (Somnitor, Neurim Pharmaceuticals, Israel) worn on the wrist of the non-dominant hand. Actigraphs were set to run in a zero-crossing event mode. Rest-activity data were collected in 30-second epochs. The amount of activity was defined as the number of zero crossings per epoch. From the actigraphic records of the nap and night sleep of each subject we have calculated
the movement index (i.e. the mean number of zero crossing events per minute) and the longest quiet and active episodes (i.e. time spent without or with continuous activity, respectively). Rest-activity data for all groups were then transformed into alternating periods of arousal and nonarousal, using an algorithm that determined whether continuous non-zero epochs of activity were above or below a preset threshold. This algorithm has been validated against polysomnographic (EEG) records and showed average percent correct sleep/wake identification of 86(6% for actigraphic compared to EEG measures (Zisapel et al., 1995).

**Sleep parameters**

Sleep parameters were derived from actigraphic data using this algorithm. The parameters measured were: sleep onset latency (time spent awake from bedtime to sleep onset), sleep efficiency (% time scored as sleep from total time in bed), wake time after sleep onset (WASO - time spent awake after sleep onset) and sleep onset and offset times. For sleep onset we used the criterion of persistent sleep, i.e. the beginning of the first 10 minute period scored as sleep. For sleep offset we used the criterion of persistent awakening, i.e. the beginning of the first 10 minute period scored as awake.

**Mood and sleepiness measures**

The Lader-Bond and Stanford Sleepiness Scale (SSS; Hoddes et al., 1973) were used. The SSS is a self rated 7 point scale designed to quantify the progressive stages of the sleep-alertness continuum. The Lader-Bond is a self report form that consists of 16 adjectives, each of which is rated on a 5 cm scale. Analysis yields the following emotional ratings:

1. Sleepy / Alert
2. Excited / Calm
3. Weak / Vigor
4. Lucid / Confused
5. Clumsy / Well-coordinated
6. Energetic / Fatigued
7. Satisfied / Dissatisfied
8. Calm / Worried
9. Quick-thinking / Slow thinking
10. Relaxed / Tense
11. Dreamy / Concentrated
12. Very efficient / Inefficient
13. Sad / Happy
14. Friendly / Hostile
15. Bored / Interested
16. Sociable / Reclusive

The questionnaires were filled by the volunteers before and following afternoon nap, 1 hour after awakening from nap, before night sleep and on awakening in the morning following each day of treatment.

**Sleep logs**

Sleep quality during napping and night was subjectively assessed by sleep logs. These measures included self-estimated sleep latency, number of awakenings
and sleep efficiency. Sleep logs were filled by the subjects on awakenings from the nap and night sleep.

Data analysis
At the end of the experiment, the results of the questionnaires, actigraphs and urine assays were analysed. At this stage the experiment code for tablet identification was revealed. After the analyses were completed, the types of tablets ingested were verified by the levels of 6S-MT in the urine samples.

After-treatment measures were analysed by means of analysis of variance (ANOVA) for repeated measures with a factor of group (placebo and the three melatonin doses). A separate analysis was performed for each mood rating and actigraph parameter. In addition, t-tests were performed for dependent samples in order to examine between which conditions there exists a significant difference.

Treatment effects for which there were significant contrasts of placebo vs. the various melatonin doses are reported. The treatment main effect for each of the reported contrasts is significant at the P < 0.05 level.

To simplify the demonstration of various parameters and enable to present several of them on the same scale, we have calculated the data presented in the Figures as percent of the corresponding values in placebo treatment. The placebo values used as 100% in the actigraph derived measures are presented in the Legends to Figures. In the analyses and in the text we have used the actual values of the parameters.

RESULTS

Urinary 6-sulfatoxy melatonin
The mean excretion rates of 6-sulfatoxy melatonin in the urine are illustrated in Fig. 1. The respective amounts of the melatonin metabolite excreted between 16:00 hr and midnight for the placebo, 0.1, 0.5 and 1 mg treatment conditions were 9.6±5.9, 110±31, 588±155 and 1413±365 µg, respectively. The total amounts excreted did not differ significantly from those expected from the ingested amounts. Maximal excretion rates (Fig. 1) were roughly proportional to the dose administered and differed significantly among the four treatment conditions. The treatment by order interactions were not significant.

Treatment effects on nap sleep
Actigraphic recordings of the late afternoon naps indicated that melatonin treatment did not significantly improve sleep efficiency (Fig. 2; F(3,24)=2.46 p=0.087) or latency (not shown). Melatonin significantly improved some measures of sleep
Fig. 1. Mean (±SD) urinary 6-S-MT excretion rates of 10 subjects sampled at various times after ingesting 0.1 (■) 0.5 (▲) and 1.0 (●) mg of melatonin or placebo (●) at 16:00 h.

Fig. 2. Actigraph-derived nap sleep parameters. Melatonin (0.1-1mg) or placebo was ingested at 16:00 h and the subjects retired to a 4 hr nap between 17:00-21:00 h. The mean (± SD) sleep efficiency (■), wake time after sleep onset (WASO) (▲), movement index (●) and longest active episode (▼) are indicated (n = 10). Placebo results were: sleep efficiency (76(12%), WASO (43(26 min.), movement index (1048(710) and longest active episode (7(5 min.). Asterisks denote statistically significant differences compared to placebo (n = 10).
Fig. 3. Actigraph-derived sleep offset.
Melatonin (0.1-1mg) or placebo was ingested at 16:00 h and the subjects’ nap took place between 17:00-21:00 h. The mean (± SD) hour of sleep offset is presented (n = 10). Asterisks denote statistically significant differences compared to placebo (n = 10).

Fig. 4. Mean response scores on the SSS Scale before and after the 4 hr nap. Melatonin (0.1-1mg) or placebo was ingested at 16:00 hr. Nap took place between 17:00-21:00 hr. The SSS assessments were performed before nap, at 16:45 h (▼) and immediately after the wake-up, at 21:15 hr (■). Asterisks denote statistically significant differences compared to placebo (n = 10).
Fig. 5. Mean response scores on the Lader-Bond questionnaires before the nap. Melatonin (0.1-1mg) or placebo (0 mg) was ingested at 16:00 h. Lader-Bond assessment was performed at 16:50 h. Increased feelings of: (Top diagram) alertness (■), vigor (▲), concentration (▲). (Bottom diagram) inefficiency (■) and fatigue (▲) are indicated by higher scores. Asterisks denote statistically significant differences compared to placebo (n = 10).
relative to placebo (Fig. 2): the longest active episode decreased significantly from a mean of 8 min. with placebo to 4 min. and less with all melatonin doses used (F(3,24) = 4.39 p = 0.013): movement index decreased significantly with 0.1 and 1mg melatonin compared with placebo treatment (F(3.24) = 3.26 p = 0.039). Wake after sleep onset was significantly lower with 1 mg melatonin than placebo (F(3,24) = 3.06 p = 0.048). Melatonin (0.5 and 1 mg) significantly delayed sleep offset time compared to placebo (F(3.24) = 3.37 p = 0.035) (Fig. 3).

No main effect was found for the subjective assessment of nap sleep efficiency (F(3.27) = 2.43 p = 0.087).

After treatment night sleep
No main effects were found in the night following the nap. However, the longest quiet episode after 0.5 mg melatonin (41 min.) tended to be longer than after placebo (33 min.) (F(3,27) = 2.192 p = 0.052).

Stanford sleepiness scale responses
Before napping, subjects felt significantly more fatigued after taking melatonin (1 mg) than after placebo or 0.1 mg melatonin (F(3.27) = 4.32 p = 0.013) (Fig. 4).

Fig. 6. Mean response scores of 10 subjects on the Lader-Bond questionnaire after the nap. Melatonin (0.1-1mg) or placebo was ingested at 16:00 hr. Nap took place between 17:00-21:00 hr. Lader-Bond assessment was performed at 21:10 h. Increased feelings of non-efficiency (▼), slow thinking (●) and sleepiness (■) are indicated by higher scores. Asterisks denote statistically significant differences compared to placebo (n = 10).
Immediately after the four hour nap, subjects still felt more fatigue after taking melatonin (1 mg) than after placebo (F(3,27) = 3.05 p = 0.046 t(8) = 2.24 p = 0.052) (Fig. 4). However, 1 hr after awakening from the nap, differences in fatigue between placebo and melatonin treatments were no longer significant.

Lader-Bond mood rating responses
Before napping, melatonin treatment caused significant differences in feeling of fatigue, vigor, alertness, concentration and efficiency (F(3.27) = 3.34 p = 0.034; F(3.27) = 3.85 p = 0.02; F(3.27) = 3.08 p = 0.044; F(3.27) = 3.78 p = 0.022 and F(3.27) = 3.34 p = 0.034 respectively). The analyses indicate that subjects felt more vigorous, efficient, concentrated and alert, and less fatigued, when taking placebo than when taking 0.5 and 1 mg melatonin (Fig. 5).

Immediately after napping, subjects still felt more sleepy and inefficient after melatonin (0.5 and 1 mg) than after placebo (F(3.27) = 3.03 p = 0.047 and F(3.27) = 3.72 p = 0.023 respectively). In addition, speed of thought was slower after the highest melatonin dose (1 mg) compared to placebo (F(3.27 = 3.27 p =

![Fig. 7. Actigraph-derived sleep efficiency during the nap (■) and the following night (●). Melatonin (0.1-1mg) or placebo was ingested at 16:00 hr. Nap took place between 17:00-21:00hr and night sleep between 24:30-07:30 hr the next morning. The mean sleep efficiency values of responders (solid line n = 7) and non-responders (striated line n = 3) during the nap and night sleep (n = 6 and n = 3, respectively) are presented. Placebo values of sleep efficiency during the nap and night sleep were: 72±9.8 and 77±6.5 respectively, for responders, 86±5.5 and 77±4.2 respectively, for non-responders.](image)
The lowest melatonin dose (0.1 mg) did not produce significant responses (Figs. 5, 6).

One hour after awakening from the nap, no main effects were found between melatonin and placebo treatments.

No significant differences were found between melatonin and placebo treatment in mood ratings before or after night sleep.

No significant order effects were found for any of the measures. All treatment by order interactions were non-significant.

Variability in response of individual subjects
The effects of melatonin treatment on actigraph-monitored sleep efficiency in the afternoon nap and the following night sleep are shown in Fig. 7. As mentioned above (Fig 2), the effects of the treatment on the group means were not significant.

Nevertheless it was interesting to note individual differences in the responses: in 7 out of the 10 volunteers, nap sleep efficiency increased with increasing dose of melatonin. In addition, in 6 out of these 7 subjects, sleep efficiency during the night following the treatment decreased after the higher melatonin doses. The other subjects did not show such responses (Fig. 7).

DISCUSSION

The melatonin delivered in tablet form was absorbed without problems in the digestive system and largely or wholly excreted in urine. No accumulation and/or change in the endogenous levels of the hormone’s excretion was seen after short-term use of melatonin. Similarly, the level of melatonin absorption was not seen to be lower in those subjects in whom the reaction to the influence of melatonin on sleep was smaller.

The data indicate that melatonin ingestion significantly increased feelings of sleepiness, fatigue and confusion, and decreased feelings of vigor and concentration compared to placebo treatment. The effects of melatonin given in the afternoon on subjective measures were similar to those observed previously with comparable melatonin doses given at noon and evening (Dollins et al., 1994; Zhdanova et al., 1995). Hence, the responses to melatonin do not appear to be dependent upon the time of administration and may not be mediated by the circadian clock. Alternatively, melatonin may directly affect somnogenic mechanisms in the brain. Indeed, it has been demonstrated that melatonin synchronized the sleep-wake cycle but not the cortisol and temperature rhythms in a blind man (Folkard et al., 1990).

The objective measures of sleep indicated some improvements in nap sleep
quality (decreases in longest active episode, WASO, and movement index, and increase in nap offset time) with melatonin, especially with the highest dose used (1 mg). However, improvement in sleep latency and efficiency was not significant. These results are compatible with those of Hughes et al. (1994), that melatonin given at noon was efficacious in both initiating and sustaining daytime sleep at 10 and 40 mg doses but was less effective at 1 mg dose. Yet, in recent studies, low doses of melatonin ((0.3 mg) were found to effectively shorten sleep latency when given at noon and evening (Dollins et al., 1994; Zhdanova et al., 1995). The discrepancy between results obtained from different laboratories could be explained by the relatively small size of study populations used in the various studies and the fact that our study population had apparently less difficulties to fall asleep than that included in the above mentioned studies.

Although the experimental group in our study consisted of a small number of volunteers, it can be discerned that the improvement in nap sleep quality occurs in a portion of the population (70%), and that the rest 30% do not respond to melatonin treatment. This value is compatible with previous reports on individual differences in improvement of jet lag symptoms after melatonin ingestion (Arendt et al., 1986).

The hypnotic effects of melatonin increased with increasing dose, without reaching saturation at the highest dose used (1 mg). The doses needed to induce the hypnotic activity of melatonin are similar to those used to phase reset the human biological clock (Lewy et al., 1992). These doses may yield blood concentrations higher than those normally present at night (Dollins et al., 1994). Interestingly, the minimal dose of melatonin used in our study (0.1 mg), which reportedly yields near to physiological concentrations of melatonin (Dollins et al., 1994), had weak effects on objective sleep measures. These results may indicate that the biochemical mechanisms mediating melatonin’s effects were not saturated at physiological melatonin levels.

It is not known what receptor(s) mediates the hypnotic effects of melatonin. Specific high affinity melatonin binding sites have been identified in discrete locations within mammalian brain. Among these are the human suprachiasmatic nuclei thought to function as a circadian clock. (Reppert et al., 1988). These sites are most likely saturated at physiological melatonin concentrations such as those present at night in blood. However, our observations that the hypnotic effects of melatonin seem to saturate only at supraphysiological concentrations suggest that this may not be the case. Indeed, a recent study has suggested that at least in animals melatonin’s sedative effects may be mediated by a receptor type different from that described in the biological clock (Sugden, 1995).

Some of the effects of melatonin given in the afternoon on mood, were still evident upon awakening from the four hours nap after the highest melatonin dose, but disappeared later on. This could indicate that effective melatonin con-
centrations were still present at the time of awakening. The residual effects might also be related to the occasionally forced wake up from the nap.

REFERENCES


