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Neuroscience Letters 207 (1996) 209–213

Daytime melatonin administration enhances sleepiness and theta/alpha activity in the waking EEG

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Received 25 January 1996; revised version received 7 March 1996; accepted 7 March 1996

Abstract

It is still controversial whether the pineal hormone melatonin can be characterized as a hypnotic. We therefore measured subjective sleepiness and waking EEG power density in the range of 0.25–20 Hz after a single dose of melatonin (5 mg). During an 8 h mini-constant routine protocol, melatonin administered in a double blind cross-over design to healthy young men at 1300 h or 1800 h increased subjective sleepiness, as rated half-hourly on three different scales (Visual Analogue Scale, Åkerstedt Sleepiness Symptoms Check List, Åkerstedt Sleepiness Scale) and objective fatigue as evidenced by augmented waking EEG power density in the theta/alpha range (5.25–9 Hz). The increase in subjective sleepiness reached significance 40 min and 90 min after melatonin administration (at 1300 h and 1800 h, respectively) and lasted for 3 h (at 1300 h) and 5 h (at 1800 h). The increase in the theta/alpha frequencies of the waking EEG occurred immediately after melatonin ingestion and stayed significantly higher parallel to the higher sleepiness ratings. However, the EEG changes appeared before the subjective symptoms of sleepiness became manifest. There was a significant correlation between salivary melatonin levels and the timing of increased subjective sleepiness. Melatonin had no effects on mood.

Keywords: Melatonin; Waking EEG power density; Subjective sleepiness; Mood

In humans, the pineal hormone melatonin is secreted nocturnally and is therefore a 'natural' candidate for a sleep promoting factor. Early human studies showed a clear hypnotic effect of melatonin when administered both day and night in dosages far beyond the normal physiological range [2,7]. More recent studies suggest that even very low doses of exogenous melatonin can induce sleep when ingested before the endogenous melatonin onset [12,20,22,24]. Thus, nocturnal melatonin secretion may be involved in gating physiologic sleep onset in humans. In addition, melatonin is a chronobiotic, that is, it is able to 'reset' the biological clock [17,23]. Its phase-shifting characteristics have been applied in treating jet lag [3], maladaptation to night shift work [14], delayed sleep phase syndrome [9] and recurrent insomnia in the blind [18].

Since there is growing interest in the clinical use of melatonin, investigation of its acute hypnotic action when administered in the daytime is of importance. Qualitative analyses of the constituent frequency components of the waking EEG provide useful EEG parameters for objective assessment of fatigue [6,19]. Timed recordings of the waking EEG during a constant routine protocol (CR), an experimental setting in which subjects are kept awake under controlled conditions, allows accurate assessment of the effects of exogenous drug application. In contrast to other experimental settings that use the multiple sleep latency test (MSLT), the recording of the waking EEG permits repeated estimation of the subjects' sleepiness without enforcing them to sleep. In contrast to previous studies in which sleep EEG parameters were used to measure the hypnotic action of melatonin (e.g. sleep latency, sleep efficiency), we hypothesized that the acute effect of melatonin would be detectable in changes of EEG power density during waking.

The study comprised two separate experiments, during which melatonin was either administered at 1800 h (exp. 1) or at 1300 h (exp. 2). In both experiments eight male students (exp. 1: age range 23–32 years, mean ± SD 27 ± 4 years; exp. 2: age range 21–31 years, mean ± SD 24.8 ± 3.5 years) were paid to participate in the study to which

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0304-3940/96/$12.00
PII: S0304-3940(96)12517-7

they gave their informed consent. The experimental protocol was approved by the local ethical committee. Medical, sleep or psychiatric disorders were screened by history, questionnaires and physical examination. Prior to the study the subjects were asked to maintain a regular sleep-wake cycle for at least 7 days. This was verified by a daily sleep diary and ambulatory monitoring of the subjects' motor activity. During the study the amount of caffeine was limited to one morning cup of coffee per day, and no alcohol consumption was allowed.

Both experiments 1 and 2 were double blind placebo-controlled. Each subject participated in two consecutive treatment periods which comprised 1 day with placebo and 1 day of treatment with melatonin (5 mg), in randomized order. The volunteers reported at 1500 h (exp. 1) or 0900 h (exp. 2) each day to the chronobiology laboratory where the electrodes were attached. They remained supine and awake in bed in a sound-attenuated chronobiology room (temperature 22°C, humidity 60%, light <10 lux) from 1600 h to 2300 h (exp. 1) or 1000 h to 1700 h (exp. 2). During these periods a 'mini'-constant routine (mini-CR) was carried out, with protocol and procedure adapted from others [8]. Isocaloric meals and isotonic drinks were given every hour in order to meet energy requirements. To ensure wakefulness the subjects were not allowed to close their eyes at any time during the mini-CR.

Throughout the mini-CR, half-hourly self-ratings of fatigue and mood were obtained on 100 mm visual analogue scales (VAS). Subjects completed the Åkerstedt Sleepiness Symptoms Check List (ASSC), described in [15] as the accumulated Time with Sleepiness Scale, and the Åkerstedt Sleepiness Scale (ASS) which corresponds to the Karolinska Sleepiness Scale [15]. Saliva was collected in parallel for analysis of melatonin. The melatonin levels in exp. 1 were determined by a GC-MS method developed by Servier Dept. of Technology, whereas in exp. 2 the analysis of the melatonin levels were performed by RIA [13].

In exp. 1, the waking EEG signal was recorded 1 h before (1700 h) and 1 h after (1900 h) melatonin intake. The waking EEG recordings of exp. 2 were scheduled every 45 min throughout the mini-CR from 1000 h to 1700 h; the first two recordings served as adaptation and were excluded from further analysis. Each recording lasted for 6 min, during which the subjects were instructed to relax, to watch a small picture on the wall, to keep their eyes open and to avoid movement. These instructions were intended to maximize signal quality. During these 6-min periods, two EEG signals (C3/A2, C4/A1), two EOG signals, and one EMG and ECG signal were recorded on polygraph paper (Nihon Kohden; 10 mm/s paper speed). The EEG signals were high-pass filtered with a time constant of 1.0 s and low pass filtered at 35 Hz (12 dB/octave), on-line digitized at a sampling rate of 128 Hz, and subjected to spectral analysis by a fast Fourier routine. Power spectra were computed for consecutive 4-s epochs and 0.25 Hz frequency bins by applying a Kaiser–Bessel window.

By computing the mean values over adjacent frequencies, the data were reduced to 0.5 Hz bin width for frequencies between 0.25 and 5.0 Hz, and to 1 Hz bin width for frequencies between 5.25 and 25.0 Hz. All waking EEG recordings (C3/A2 or C4/A1) were visually inspected on a 4-s basis. Four-second epochs with artifacts due to body movements, slow eye movements, and sweating were excluded from subsequent analyses. All P values derived from repeated measure ANOVAs were based on Huynh-Feldt's corrected degrees of freedom, but the original degrees of freedom are reported.

In exp. 1, the 5 mg dose of melatonin yielded high levels peaking 1–1.5 h after administration and declining exponentially thereafter (Fig. 1, top panel). Even 5 h after melatonin intake, the melatonin level was still seven times higher than the physiological range. The endogenous melatonin onset in the placebo condition occurred at 2130 h (Fig. 1, top panel).

Melatonin induced a significant increase in subjective sleepiness as rated on the VAS, ASSC and ASS. The ANOVAs for all three scales separately revealed a significant interaction term 'treatment * time' (VAS, $P < 0.02$; ASSC, $P < 0.04$; ASS, $P < 0.001$). After melatonin, self

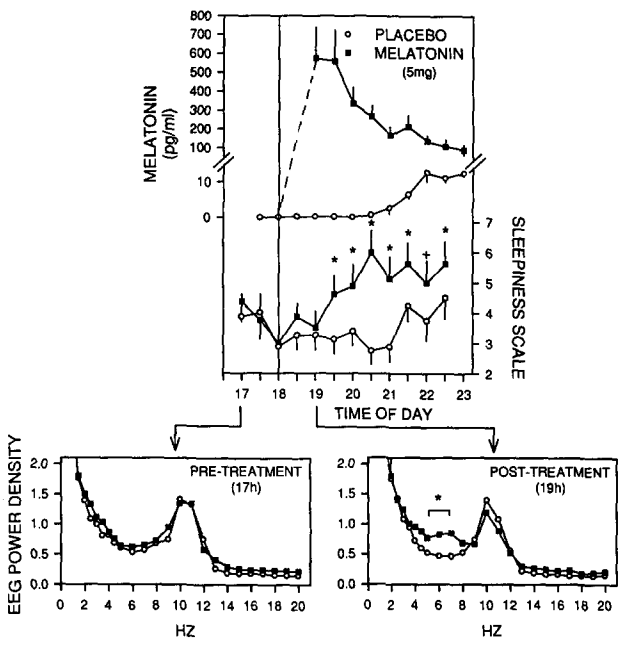

Fig. 1. (Top) Time course of salivary melatonin levels and subjective sleepiness as rated on the Åkerstedt Sleepiness Scale after the intake of a single dose of 5 mg melatonin (■) and placebo (○) during the mini-constant routine of exp. 1 (mean half-hourly values ± SEM, $n = 8$). The vertical reference line at 1800 h indicates the time of melatonin or placebo administration. Asterisks indicate significant differences melatonin versus placebo (*$P < 0.05$; + indicates tendencies $P < 0.1$, paired t-test). (Bottom) EEG power density (μV^2/HZ) in single frequency bins (0.25–20 Hz) before melatonin intake at 1700 h (left panel) and after melatonin intake at 1900 h (right panel; mean values, $n = 8$). The asterisk indicates a significant interaction term 'time * treatment' for the averaged frequency band 5.25–7 Hz (ANOVA for repeated measures).

rated sleepiness on the ASS remained higher than after placebo from 1930 h for the remainder of the evening ($P < 0.05$, paired t-test; Fig. 1, lower part, top panel). All subjects documented good and stable self-rated mood throughout the study, without any influence of melatonin (data not shown).

The ANOVA comparing absolute EEG power density in the theta range (5.25–7 Hz) 60 min prior to melatonin intake with 60 min after, revealed a significant effect of the interaction term 'treatment * time' (ANOVA for repeated measures: $F_{1,7} = 5.1$, $P < 0.05$; Fig. 1, right bottom panel). Post-hoc comparisons revealed a significant increase in the theta activity after melatonin administration ($P < 0.05$, paired t-test). Other frequency ranges, i.e. slow-wave activity (SWA; power density 0.75–4.5 Hz), spindle activity (12.25–15 Hz) and the beta band (15.25–20 Hz) were not significantly different from placebo.

In exp. 2, melatonin peak levels occurred within 30 min (Fig. 2, top panel) and declined exponentially thereafter. Even 4 h after melatonin intake, at 1700 h when the endogenous melatonin levels are normally around zero, the mean level was still greater than 100 pg/ml.

Compared to placebo, melatonin administration at 1300 h significantly increased subjective sleepiness as rated on the ASS (Fig. 2, middle panel; ANOVA for repeated measures, interaction term 'treatment * time': $F_{13,91} = 2.00$, $P < 0.05$). Post-hoc comparisons revealed a significant increase 40 min after drug intake which remained higher in the following 3 h. Although the mean time course of self rated sleepiness on the other two scales (VAS and ASSC) was similar as on the ASS, the interaction term 'treatment * time' did not reach significance (data not shown). Mood was not affected by melatonin administration.

During exp. 2 four EEGs were recorded prior to and six EEGs after melatonin administration. For statistical analysis, each frequency bin in the range of 0.25–20 Hz was expressed as percentage of the mean power density in the corresponding frequency bin of the first two EEG recordings before melatonin intake. The ANOVA for repeated measures revealed a significant factor 'treatment' for the following frequency bins: 5.25–6 Hz; 6.25–7 Hz and 8.25–9 Hz ($P < 0.05$ for each band separately). Therefore, the frequency bins of a theta/alpha band (5.25–9 Hz) were averaged and plotted against time (Fig. 2, bottom panel). Calculating the ANOVA for this band separately, the factor 'treatment' was significant ($F_{1,7} = 4.78$, $P < 0.05$). EEG power density in this band was enhanced immediately (15 min) after melatonin administration and remained higher compared to placebo in the first 3 h of the post-treatment period (paired t-test).

To analyze the temporal relationships between melatonin concentrations, subjective sleepiness and theta/alpha activity in the waking EEG, the timing of the center point of gravity was determined for each variable separately. Both the subjective sleepiness and the EEG power center point of gravity were significantly different from the melatonin center point ($P < 0.002$, paired t-test). In addition, the subjective sleepiness center point occurred 23.4 min later than the EEG power center point ($P < 0.002$, paired t-test; Table 1).

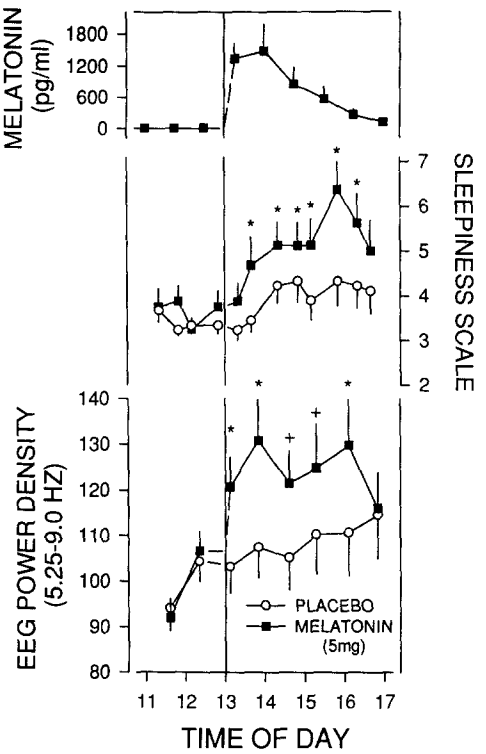

Fig. 2. (Top) Time course of salivary melatonin levels after a single dose of 5 mg melatonin at 1300 h (mean half-hourly values ± SEM, $n = 8$) during the mini-constant routine of exp. 2. (Middle) Subjective sleepiness on the Åkerstedt Sleepiness Scale after melatonin or placebo at 1300 h rated half-hourly. Asterisks indicate significant differences melatonin versus placebo (*$P < 0.05$, paired t-test). (Bottom) Time course of averaged EEG power density in the range of 5.25–9.0 Hz in exp. 2. The values are expressed as percentage of the mean power density in the corresponding frequency bins of the first two EEGs before melatonin intake. Asterisks indicate significant differences melatonin versus placebo (*P < 0.05, paired t-test), whereas the symbol + indicates tendencies (+$P < 0.1$).

To test the relationship between melatonin levels and the amount of increase in subjective and objective fatigue as measured in the waking EEG, the area under the melatonin curve (AUC) was calculated. There were no significant correlations between the increase in subjective fatigue and the melatonin AUC (data not shown). However, the position of the center point of subjective sleepiness was significantly negatively correlated with the melatonin AUC ($r = 0.72$; $P < 0.05$; $n = 8$). The more exogenous melatonin was absorbed, the earlier the increase in subjective sleepiness.

The present study demonstrates that daytime administration of melatonin exerts a powerful acute effect on physiology and behavior. Melatonin at both times of day increased self-reported sleepiness. These findings corroborate prior reports of increased fatigue after different doses

Table 1
Position of the center point (h, time of day) in saliva melatonin, fatigue and EEG power density in the range of 5.25–9 Hz and the AUC of saliva melatonin (log) for each subject, and the mean values ± SEM ($n = 8$)

Subj.	Timing of the center point (h)			AUC (pg/ml × h)
	Melatonin	Fatigue	EEG power	Melatonin
1	14.60	15.04	14.73	8.42
2	13.51	15.08	14.75	7.96
3	14.48	14.98	14.63	8.89
4	14.64	15.14	14.80	8.69
5	13.74	15.44	14.65	6.86
6	14.36	14.87	14.70	8.16
7	13.42	15.22	14.84	6.71
8	13.46	15.19	14.72	7.35
Mean	14.03 ± 0.19	15.12 ± 0.06	14.73 ± 0.03	7.88 ± 0.29

Subj., subject.

of melatonin [2,7,12,20,22,24]. In contrast to other studies using sleep EEG parameters to identify the acute effect of melatonin [2,7,11,20,22,24], our data show that the soporific impact of melatonin is also manifested in spectral EEG changes during waking. Waking EEG power density in the theta/alpha range can be considered as an objective assessment of fatigue [1,6,20], that was increased after melatonin administration. Interestingly, we had previously found that bright light in the evening tended to lower values in the theta/alpha band of the waking EEG [5], which may reflect the alerting effect of bright light. The present results confirm that waking EEG power densities can provide a sensitive measure to detect soporific as well as alerting influences.

Since melatonin is one of the few lipid-soluble hormones [4] and readily crosses from the circulatory system into all areas of the brain, it is likely that its action on the EEG is very rapid. Comparing the kinetics of the salivary melatonin levels, the subjective sleepiness ratings and the changes in EEG power density, it appears that the increase in the theta/alpha frequencies occurred together with the rapid increase of melatonin levels in saliva, whereas the increase in subjective sleepiness occurred later. Significant EEG changes occurred very rapidly after melatonin administration (15 min), even though the subjects still rated themselves as 'alert'. Thus, the EEG changes appeared before the subjective symptoms of fatigue became manifest. This is in contrast to Åkerstedt's own study [1] under naturalistic conditions, where EEG changes only appeared after subjective fatigue had reached considerable heights i.e. the subject rated himself as 'sleepy' or close to 'very sleepy, fighting sleep'. The fact that exogenous melatonin acts immediately on the EEG before it is subjectively registered should be taken as a warning for potential dangers in reduced vigilance after melatonin administration.

The peak levels of salivary melatonin were more than twice as high after ingestion of 5 mg at 1300 h than at 1800 h. There are several possible explanations for this: melatonin analyses were performed by two different methods; there is large interindividual variation in absorption of orally administered melatonin [21]; and the pharmacokinetics of melatonin may show time-dependent differences. After the higher melatonin peak following administration at 1300 h, subjective fatigue increased faster (at 40 min post-ingestion) than at 1800 h (at 90 min post-ingestion). This favors the idea that the higher levels attained also resulted in a dose-dependent action of melatonin. The endogenous melatonin onset after placebo in exp. 1 occurred at 2130 h in parallel with a significant rise in subjective fatigue (Fig. 1, top panel). It should be noted that this naturalistic time course of covariance occurs at very low physiological values of the hormone. This favors the idea of a low threshold-like soporific action of melatonin.

The mechanisms involved in the fatigue-inducing effects of melatonin are still not known. Whether they are similar or independent from the phase shifting and hypothermic capacity of melatonin is an important question [10]. In these studies, we additionally recorded physiological variables such as rectal and peripheral skin temperatures. On the basis of these data it appears that the soporific action of melatonin may result from a direct hypothermic effect mediated by vasodilation [16].

We thank Drs. R. Defrance and G. Lapeyre (Institute de Recherches Internationales Servier, Paris, France) for their help in designing and supporting exp. 1; also C. Hetsch and G. Balestrieri for their assistance in data acquisition. Melatonin was assayed by Dr. E Mocaer and the Department of Technology, Servier (GC-MS), and J. English (RIA), Stockgrand, University of Surrey, UK.

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