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Melatonin in Patients with Reduced REM Sleep Duration: Two Randomized Controlled Trials

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Recent data suggest that melatonin may influence human physiology, including the sleep-wake cycle, in a time-dependent manner via the body's internal clock. Rapid-eye-movement (REM) sleep expression is strongly circadian modulated, and the impact of REM sleep on primary brain functions, metabolic processes, and immune system function has become increasingly clear over the past decade. In our study, we evaluated the effects of exogenous melatonin on disturbed REM sleep in humans. Fourteen consecutive outpatients (five women, nine men; mean age, 50 yr) with unselected neuropsychiatric sleep disorders and reduced REM sleep duration (25% or more below age norm according to diagnostic polysomnography) were included in two consecutive, randomized, double-blind, placebo-controlled, parallel design clinical trials. Patients received 3 mg melatonin daily, administered between 2200 and 2300 h for 4 wk. The results of the study show that melatonin was significantly more effective than placebo: patients on melatonin experienced significant increases in REM sleep percentage (baseline/melatonin, 14.7/17.8 *vs.* baseline/placebo, 14.3/12.0) and improvements in subjective measures of daytime dysfunction as well as clinical global impression score. Melatonin did not shift circadian phase or suppress temperature but did increase REM sleep continuity and promote decline in rectal temperature during sleep. These results were confirmed in patients who received melatonin in the second study (REM sleep percentage baseline/placebo/melatonin, 14.3/12.0/17.9). In patients who received melatonin in the first study and placebo in the second, the above mentioned effects outlasted the period of melatonin administration and diminished only slowly over time (REM sleep percentage baseline/melatonin/placebo, 14.7/17.8/16.2). Our findings show that exogenous melatonin, when administered at the appropriate time, seems to normalize circadian variation in human physiology. It may, therefore, have a strong impact on general health, especially in the elderly and in shift workers. (*J Clin Endocrinol Metab* 89: 128–134, 2004)

SINCE 1994, MELATONIN has been classified as a nutritional supplement in the United States and may be purchased there without a prescription, resulting in perhaps the largest uncontrolled drug trial in medical history. Nearly a decade after this so-called melatonin madness (1, 2), however, the precise role of melatonin in human physiology—and particularly in the sleep-wake cycle—is still poorly understood.

There is much evidence that melatonin exerts highly time-dependent actions via the internal clock located in the suprachiasmatic nucleus (SCN) (3, 4). The SCN transmits signals to the brain, organizing circadian rhythms throughout the body (5–8). In mammals, the daily variation of almost any physiological or psychological variable evaluated thus far has been shown to be driven or modulated in a circadian fashion (9–13). The amplitude of this daily variation depends predominantly on the strength of the SCN output signal. This signal may be weakened by a variety of factors, including shift work, many aspects of modern lifestyle, and the aging process (14–16). However, it has been proposed that the reduced amplitude of the clock in neuronal activity levels might be reversed by well-timed stimulation or deactivation using the clock's two primary *zeitgebers* (time cues): light and melatonin (3, 14, 15).

Melatonin has been called a chronobiotic. A chronobiotic is defined as a substance capable of shifting the phase of the circadian timing system (CTS) and synchronizing circadian rhythms that have been dissociated on the short-term or desynchronized on the long-term (17). The beneficial effects of melatonin have been well documented in patients suffering from delayed sleep phase syndrome (18), jet lag (short-term dissociation) (19), or sleep disturbances associated with free running rhythms due to blindness (long-term external desynchronization) (20).

However, even though the phase-shifting effects of exogenous melatonin may be welcomed in times of transcontinental flights, they are of minor importance when compared with the role played by endogenous melatonin. It has been proposed that the melatonin secreted during nighttime darkness serves as a kind of so-called circadian cement, which provides enough inertia to resist minor perturbations in the CTS (17). At nighttime, melatonin may both transduce and amplify the circadian drive of the SCN. However, the synchronizing effects of melatonin in patients suffering from long-term internal desynchronization with reduced amplitude have not been tested.

In a number of case series studies, we observed that prolonged melatonin intake apparently led to a trend toward normalization of rapid-eye-movement (REM) sleep percentage, REM sleep quality, motor activity during sleep, temperature variation during sleep (unpublished), and blood

Abbreviations: aMT6s, 6-Sulfatoxymelatonin; CGI, clinical global impression; CTS, circadian timing system; EEG, electroencephalogram; NREM, non-REM sleep; PSG, polysomnography; PSQI, Pittsburgh sleep quality index; REM, rapid eye movement; SCN, suprachiasmatic nucleus.

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pressure accompanied by improvements in clinical symptoms (21, 22). Because no phase shift was observed, data from these studies suggest that the improvements seen in these patients may have been due to a restoration of the circadian pacemaker's output strength.

Two observations made in these pilot studies have lead to constraints in study procedure that are crucial to the design of the clinical trial described in this paper. First, responders and nonresponders in the pilot studies were best distinguished by evaluating each patient's sleep hygiene (*i.e.* stable or changing bedtimes and time of melatonin administration). As a result, it would seem crucial to keep the time of melatonin administration constant. Second, those patients who experienced a reoccurrence of symptoms did not do so until weeks or months after exogenous melatonin had been discontinued. The fact that the effects of melatonin clearly outlast the actual period of melatonin administration limits the usefulness of crossover study designs.

To investigate whether exogenous melatonin restores the amplitude of CTS output, three hypotheses were tested in patients diagnosed with reduced REM sleep duration; when timed appropriately, the administration of exogenous melatonin: 1) normalizes both REM sleep duration and continuity, which is accompanied by an improvement in subjective measures of daytime dysfunction; 2) promotes temperature decline during sleep; and 3) has effects that outlast the actual time of drug intake.

Subjects and Methods

Subjects

The study protocol was approved by the ethics committee of the Freie Universität Berlin. All participants gave written informed consent.

All patients included in the study had contacted the interdisciplinary sleep clinic voluntarily and were seeking treatment for neuropsychiatric sleep-related disturbances (International Classification of Sleep Disorders-criteria) (23). Polysomnography (PSG) was performed in cases in which the American Sleep Disorders Association indications for diagnostic PSG were met (24). Patients were included in the study if they were found to have a quantitative reduction in REM sleep duration 25% or more below their age norm (25). Exclusion criteria were: age less than 18 or more than 80, pregnancy, current or recent shift work (during the last year), poor sleep hygiene (sleep log or actigraphic proof of a more than 2-h variation in bedtime during a 14-d recruitment period), definite morning or evening types (26) (regular bedtime outside 10–12pm), transmeridian travel (during or within 1 month of the study), psychiatric disorders (except mood disorders in remission, Diagnostic and Statistical Manual of Mental Disorders-IV), pathological findings in brain imaging, recent changes in medication (within 1 month of the study), and the intake of any medication that might interfere with melatonin production/secretion or REM sleep (21, 27, 28).

Sixteen consecutive outpatients were initially included (six women, 10 men; mean age, 48 yr). Two of them were excluded from evaluation: a 29-yr-old man with narcolepsy, and a 32-yr-old woman suffering from idiopathic insomnia. Both individuals met the exclusion criteria for bad sleep hygiene and noncompliance with study medication. Noncompliance was primarily determined using sleep-log and actigraphy data.

In total, 24 sleep disturbances (23) were diagnosed in the 14 patients evaluated (five women, nine men, between 22 and 68 yr of age; mean age, 50 yr). These were: idiopathic insomnia (5), restless legs syndrome (4), periodic limb movement disorder (7), REM sleep behavior disorder (6), and narcolepsy (2). All patients had suffered from their respective disorders for a number of years, the majority of them for more than 10 yr. Three patients received medication for the following concomitant disorders: hypertension (two patients: one received captopril, the other nifedipine/furosemide), and bipolar II disorder (lithium). Medication remained unchanged in the 4 (or more) wk preceding diagnostic PSG. The 13 remaining patients did not receive any concomitant medication.

Study design

The melatonin used in the study was obtained from Helsinn Chemicals SA (Biasca, Switzerland) and analyzed for purity by the Department of Pharmacy at Freie Universität Berlin. The melatonin and placebo (mannitol filler) were administered orally to patients as hard-gelatin capsules, which were indistinguishable from one another by appearance, taste, and smell. The capsules provided for bioavailability within 30 min of ingestion. All capsules were dispensed using identical light-resistant bottles labeled "Phase I" for treatment in study I and "Phase II" for treatment in study II. The Department of Pharmacy supplied a computer-generated randomization list and sealed data regarding patient allocation, in envelopes, separately for each subject. The study code was broken only after all study procedures were terminated.

Two to 6 wk after diagnostic PSG (naturalistic setting), patients were randomly assigned to be treated with 3 mg melatonin daily over a 4-wk period in a double-blind, placebo-controlled, parallel design (study I). The supraphysiological dose of 3 mg melatonin was used because the hypothesized mode of action to be evaluated was an increase of output amplitude of the circadian pacemaker. After a 3- to 5-d washout period, patients treated with melatonin in study I received placebo in study II, and patients treated with placebo in study I received melatonin in study II (4-wk period). Patients stayed in their natural environment and were asked not to change their daily routines. Patients were allowed to administer melatonin: first, as closely as possible to their normal bedtime (2200–2400 h); and second, in the 30 min before their bedtime, but only in the time interval of 2200–2300 h and never later than 2330 h. However, this is not always feasible in a naturalistic setting. Thus, if unable to meet these intake requirements, patients were instructed to skip their dose of study medication on that particular evening. Bad sleep hygiene (an exclusion criterion) was defined as not going to bed at the instructed time for more than three nights within one treatment period, or once within 5 d of PSG. Sleep hygiene was monitored by actigraphy (ZAK, Germany) and by keeping a sleep log during both study periods.

An adaptation night and a PSG recording night were performed three times in all patients (at baseline and during the last two nights of each treatment period). Between adaptation and recording nights, participants left the clinical research center to attend to their normal activities. Patients were asked to refrain from napping (controlled by actigraphy), exercising, and alcohol consumption. That same day, they returned to the clinical research center at 2000 h and remained there until 0830 h.

Study procedures

Study procedures were identical for adaptation and recording nights, with the exception that no EEG (electroencephalogram), electrooculogram, or electromyogram recordings were made during adaptation nights. Patients slept in windowless, completely dark, sound attenuated, air-conditioned single bedrooms. PSG included a standard 19-channel montage for scoring sleep stages: horizontal and vertical electrooculogram, five central and occipital EEG leads, four electromyogram leads (mental, submental, tibiales left and right), ECG, snore microphone, bed actometry, nasal/oral airflow as well as thoracic respiratory effort. Signals were digitized and recorded using Walter Graphtek paperless PL-EEG (Walter Graphtek GmbH, Luebeck, Germany). Rectal temperature was continuously measured using a disposable thermistor (YSI, Inc., Yellow Springs, OH) and recorded at 30-sec intervals. Before adaptation nights, subjective sleep perception (Pittsburgh sleep quality index, PSQI) (29) and clinical global impression (CGI) (30) were assessed. Starting before adaptation nights and ending after PSG nights, urine was collected in five fractions (for protocol, see Ref. 31). The urinary concentrations of 6-sulfatoxymelatonin (aMT6s) were measured in duplicate using a highly sensitive, competitive ELISA kit (IBL, Hamburg, Germany; sensitivity, 1.7 ng/ml; intraassay variation, 4–9%; interassay variation, 9–12%).

All PSGs were scored visually (30-sec epochs) (32) by a highly experienced scorer (F.B.). The scorer was blind with respect to all patient data (*e.g.* age, sex, disease, and study-period).

Outcome measures

Primary outcome measures with respect to efficacy were: 1) change in REM sleep percentage at the end of study I, compared with baseline; 2) clinical relevance measured by CGI and subjective daytime dysfunction (item 7, PSQI); and 3) intrastudy confirmation assessed by change in REM sleep percentage and clinical relevance parameters in patients receiving melatonin in study II.

To test the second hypothesis that the effects of melatonin on REM sleep are mediated by an increase in CTS output amplitude, the following primary outcome measures were also established: 1) the absence of phase shift, as confirmed by a lack of changes in REM latency and time of core body temperature minimum; 2) in addition to REM sleep percentage, core body temperature decline during sleep [defined as the range between the temperature levels at 30 min after sleep onset (unmasked of motor activity resulting from the waking state) (33) and temperature minimum during sleep] as well as REM sleep continuity (defined as the percentage of stage shifts per minute REM, modified from Ref. 34); and 3) effects of melatonin outlast the actual period of melatonin administration in study I and continue until the end of study II (4-wk placebo treatment).

Statistical analysis

Based on pilot study data, the sample size required to prove the significance of increased REM sleep duration with melatonin was calculated to be seven for each group in a parallel design (α-error, 0.05; β-error, 0.2). Because of the low toxicity of melatonin, the drop-out rate was expected to be low, and total sample size was determined to be 16, because, in study II, the enduring effect was tested as a second hypothesis multiple adjustment was performed using the sequentially rejective test procedure according to Bonferroni-Holm.

PSG and temperature data are expressed as means ± SD, and nonparametric PSQI and CGI data are described using median and interquartile range. Data were analyzed for statistical significance using Student's *t* test or the paired *t* test for parametric variables, and the Mann-Whitney *U* test or Wilcoxon test for nonparametric variables. Direct comparisons were based on raw data, effect comparisons on pair differences. Primary outcome measures (REM percentage, stage shifts in REM, temperature amplitude, daytime dysfunction, and CGI) were tested one-tailed, to confirm hypotheses. All other tests were explorative and therefore performed two-tailed.

Results

None of the 14 patients missed medication more than once in one treatment period. Patients kept good sleep hygiene, as indicated by the low SD among bedtimes recorded in individual sleep logs and by actigraph. The 24-h excretion of aMT6s in urine at baseline was 26 μg, on average (range, 2–59 μg), which is similar to earlier published groups of patients and healthy subjects (21, 22, 31). After visual inspection, the temperature data for one patient were excluded from evaluation because of sensor malfunction.

Patients characteristics of groups M and P at baseline showed no statistical differences with respect to sex (two women, five men *vs.* three women, four men), age (mean/SD, 49 ± 18 yr *vs.* 52 ± 7 yr), sleep disorder (narcolepsy, 2 *vs.* 0; restless legs syndrome, 2 *vs.* 2; periodic limb movement disorder; 3 *vs.* 4; idiopathic insomnia, 1 *vs.* 4; REM sleep behavior disorder, 4 *vs.* 2), 24-h excretion of aMT6s in urine [22 μg (range, 5–48) *vs.* 26 μg (range, 20–32)], PSG data, temperature, PSQI, or CGI.

Except for a reduction in sleep onset latency, placebo did not significantly affect sleep (Table 1, placebo effect). Compared with placebo (Table 1, efficacy), melatonin significantly increased: 1) the percentage of REM sleep; 2) REM sleep continuity; and 3) temperature decline during sleep (see also Fig. 1). It improved the subjective measure of daytime dysfunction on the PSQI (item 7), as well as the CGI score. Temperature decline during sleep did not correlate with REM sleep improvement. Melatonin did not consistently change REM latency or time of temperature minimum (Fig. 1), indicating that the circadian phase remained unchanged. All results for the primary outcome measures in the group receiving melatonin in study I were confirmed in study II (Table 1, confirmation efficacy). In the active treatment group in study I, further sleep parameters [such as total sleep time, sleep efficiency, wake after sleep onset, and non-REM sleep (NREM2)] were significantly improved as well. When baseline and placebo in study II are compared (Table 1, enduring effect), the effects on primary outcome measures had slightly diminished but still remained, at least in part, significantly different. This would seem to indicate that melatonin has an enduring effect that outlasts the actual period of melatonin administration. Increases in REM sleep were primarily due to an increase of REM sleep episode duration (Fig. 2), which was most pronounced in the third and fourth REM sleep episodes. This indicates an increase in polarity (short REM sleep episodes at the beginning and long REM sleep episodes at the end of sleep period) (9).

A total of 11 of 14 patients reported clear improvements in symptoms during melatonin treatment. No side effects were reported. Placebo and melatonin were not distinguishable by frequency, intensity, quality, or content of dreams. CGI and self-rated daytime dysfunction were improved in patients on melatonin (Table 1). The most frequently cited subjective changes during melatonin treatment were: reduction of daytime fatigue (nine patients), a stronger sense of feeling refreshed after awakening, and increased sleepiness in the evening (eight patients).

Discussion

After much controversial debate, it has become increasingly clear that REM sleep is involved in learning processes, memory consolidation, the regulation of neural plasticity, and perhaps also in the coordination of metabolic processes and the proper maintenance of immune system function in humans (35–39). REM sleep is qualitatively disturbed and quantitatively reduced by many disorders that affect the brain, by the application of psychotropic drugs, and also by age (21, 28, 40). The data presented in this paper demonstrate that, regardless of the underlying pathology, properly administered melatonin can normalize nighttime REM sleep, with respect to REM sleep percentage, continuity, and polarity, and thereby improve daytime well-being. Considering the functions that have been attributed to REM sleep, this finding is of great importance.

In addition, our study demonstrates that melatonin can increase the amplitude of temperature decline during nighttime sleep, and that the effects of melatonin outlast the actual duration of melatonin intake. The amount of REM sleep obtained, REM sleep continuity, REM sleep polarity, and body temperature are under strong circadian control by the SCN (6, 11, 41, 42). The fact that the increase in temperature drop and the increase in REM sleep percentage were not directly associated with each other points toward two dif-

TABLE 1. Polysomnographic, temperature, and psychometric data of patients with reduced REM sleep duration

Polysomnography	Group M (n = 7)			Group P (n = 7)				*P* values		
	Baseline (bM)	Melatonin (mM)	Placebo (pM)	Baseline (bP)	Placebo (pP)	Melatonin (mP)	Placebo-effect pP *vs.* bP[a]	Efficacy mM-bM[b] *vs.* pP-bP[b]	Confirm. efficacy mP *vs.* pP[a]	Enduring effect pM-bM *vs.* pP-bP[b]
SOL (min)	44.3 (35.4)	19.9 (12.6)	20.6 (8.7)	29.1 (16.2)	19.0 (10.6)	15.5 (12.5)	0.014[c]	0.322	0.354	0.317
REM-Lat (min)	81.4 (53.4)	99.0 (53.8)	78.4 (54.6)	87.6 (53.6)	126.4 (94.3)	92.1 (58.2)	0.210	0.509	0.419	0.211
SPT (min)	473.9 (70.3)	471.5 (14.3)	479.1 (51.2)	473.4 (47.5)	443.2 (52.4)	467.4 (21.7)	0.190	0.405	0.277	0.227
TST (min)	358.4 (88.7)	390.4 (63.0)	361.6 (76.5)	383.3 (38.2)	344.6 (74.8)	403.3 (21.7)	0.142	0.105	0.013[c]	0.256
SE (%)	75.6 (14.6)	82.6 (12.2)	75.3 (11.7)	81.1 (4.1)	77.4 (13.9)	86.2 (10.2)	0.511	0.171	0.019[c]	0.609
WASO (%)	24.4 (14.6)	17.4 (12.2)	24.7 (11.7)	18.8 (4.11)	22.6 (13.9)	13.8 (10.2)	0.511	0.171	0.019[c]	0.609
NREM1 (%)	15.2 (5.5)	16.7 (6.5)	15.1 (6.6)	18.5 (10.4)	16.5 (3.7)	16.7 (6.0)	0.571	0.388	0.923	0.605
NREM2 (%)	31.1 (14.7)	32.8 (13.0)	31.0 (12.2)	39.3 (10.2)	38.2 (12.2)	43.7 (15.8)	0.731	0.655	0.043[c]	0.822
SWS (%)	13.1 (5.4)	10.4 (5.3)	11.8 (5.4)	7.8 (6.6)	8.5 (5.3)	6.0 (5.5)	0.741	0.258	0.250	0.532
REM (%)	14.7 (3.7)	17.8 (5.1)	16.2 (3.3)	14.3 (2.7)	12.0 (2.9)	17.9 (4.9)	0.094	**0.010**[c]	**0.005**[c]	**0.025**[c]
SiR (per min)	0.31 (0.13)	0.22 (0.11)	0.20 (0.09)	0.25 (0.11)	0.30 (0.17)	0.19 (0.07)	0.393	**0.024**[c]	**0.029**[c]	**0.023**[c]
REM-density (%)	50.1 (12.0)	55.7 (9.8)	49.9 (16.4)	52.9 (3.9)	46.2 (10.9)	47.5 (11.0)	0.213	0.061	0.781	0.331
REM-efficiency (%)	75.0 (12.3)	75.3 (12.3)	85.1 (9.3))	75.0 (28.7)	82.9 (17.9)	72.4 (30.3)	0.138	0.520	0.162	0.702
REM-period 1–4 (min)[d]	72.5 (19.8)	105.8 (22.0)	93 (21.4)	87.7 (10.7)	87.7 (25.4)	103.6 (29.2)				
Temperature-Amplitude (C)[e]	−0.13 (0.33)	−0.32 (0.12)	−0.23 (0.26)[f]	−0.11 (0.17)	−0.09 (0.14)	−0.39 (0.34)	0.321	**0.029**[c]	**0.019**[c]	**0.071**
PSQI-Global Score	7 (4–13)	4 (3–8)	5 (5–10)	12 (6–14)	11 (5–3)	4 (4–8)	0.393	0.456	0.017[c]	0.896
Subj. Sleep Perc.	2 (1–3)	1 (1–2)	2 (1–3)	2 (1–3)	2 (2–3)	1 (1–2)	0.180	0.620	0.046[c]	0.735
Daytime Dysf.	2 (1–3)	1 (0–2)	2 (1–3)	2 (1–2)	2 (1–3)	1 (1–3)	0.414	**0.037**[c]	**0.079**	**0.437**
CGI-Severity	7 (6–7)	5 (5–6)	5 (5–7)	6 (5–6)	6 (4–6)	4 (3–6)	0.317	**0.027**[c]	**0.017**[c]	**0.042**
Change		3 (2–4)	5 (4–5)		5 (4–5)	3 (2–5)	0.180	**0.019**[c]	**0.019**[c]	**0.500**

Group M, first treated with melatonin; group P, first treated with placebo; BM, baseline condition in group M; mM, melatonin in group M; pM, placebo in group M; bP, baseline in group P; pP, placebo in group P; mP, melatonin in group P; numbers refer to means ± SD; PSQI and CGI numbers: median and interquartile range (value of 25–75% range of raw data numbers on a continuous scale); "%" are expressed as percentage of SPT; SOL - sleep onset latency, interval between lights off and first epoch sleep other than stage NREM 1; REM-Lat - REM onset latency, interval between SOL and first epoch stage REM; SPT - sleep period time, interval between first and last epoch stage NREM 2, 3, 4 or REM; TST - total sleep time, sum of all epochs NREM 1, 2, 3, 4, REM; SE - sleep efficiency, percentage of TST on SPT; WASO - wake after sleep onset; SWS - slow wave sleep (NREM 3 + 4); SiR - stage shifts in REM - number of shifts between sleep stages as per minute REM sleep; REM-density - percentage of 3-sec mini-epochs REM with at least one REM; REM-efficiency - percentage of minutes REM over REM period. *Solid numbers* represent primary outcome measures (one-tailed testing).

[a] Paired t-test or Wilcoxon test (for PSQI and CGI).

[b] Student's *t* test or Mann-Whitney *U* test (for PSQI and CGI).

[c] Indicates significant differences (α-power: 0.05 for efficacy and 0.025 for enduring effect).

[d] Incomplete data (some patients only had three REM episodes - see legend figure 3).

[e] Variation between 30 min after sleep onset and temperature minimum - degree Celsius.

[f] n = 6 (one patient sensor malfunction).

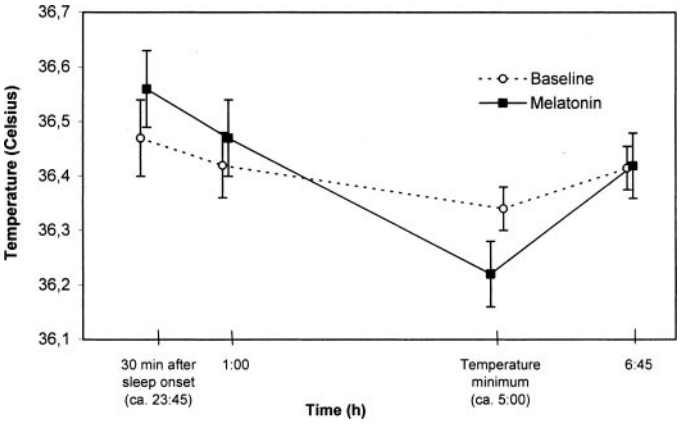

FIG. 1. Effects on temperature variation during sleep. Mean values are presented for: 30 min after sleep onset (unmasked of wake motor activity); 0100 h; temperature minimum; and 0645 h. One patient was excluded because of sensor malfunction. *Dotted line*, Baseline (n = 13); *solid line*, melatonin (n = 13). Note: 1) the phasemarker temperature minimum was nearly unchanged ($P = 0.554$); 2) temperature within 2.5 h after melatonin administration was increased; changes were not statistically significant.

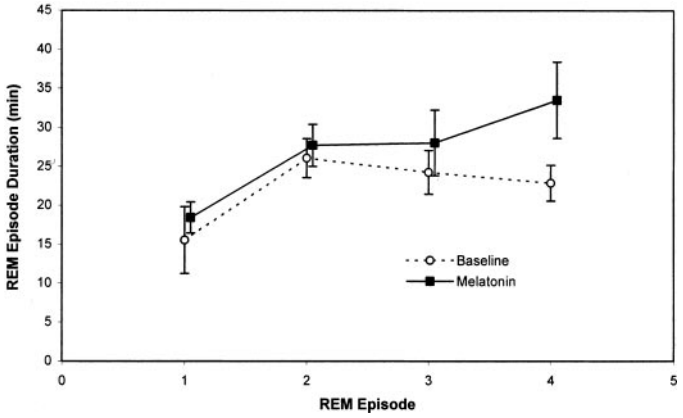

FIG. 2. Effects on REM episode duration in the course of the night. Mean values shown: *dotted line*, baseline; *solid line*, melatonin. At least three REM episodes (n = 14); four REM episodes (n = 7/14 at baseline, n = 9/14 with melatonin). Note highest increase of REM sleep episode duration in REM episode 3 and 4, which indicates increased polarity (9); changes were not statistically significant.

ferent pathways that lead to these effects. Thus, because no phase shift occurred, our results support the hypothesis that the effects of melatonin in our study are mediated by an increase of the output strength of the circadian pacemaker. Of course, this statement will need to be substantiated using protocols that control for the influence of a naturalistic setting on study results. Nevertheless, the possible impact of the reorganization of the CTS is a slowing or reversal of some age-related health problems that may result from a lifestyle that is continually in conflict with the rhythm of the internal clock.

Do any other of melatonin's known effects correspond with these results? First, some of melatonin's sleep-promoting effects have been attributed to the acute temperature suppression that occurs after daytime melatonin administration (43, 44). Because we did not assess daytime temperature before melatonin intake, this effect cannot be ruled out, but it seems unlikely to explain our results. In line with an earlier report (45), temperature at the beginning of the night, after chronic melatonin ingestion, was increased, when compared with baseline, in both of our treatment groups; and this effect continued for 4 wk after melatonin had been discontinued. The acute temperature-suppressing effect of melatonin is dose-dependent, with saturation being close to physiological nocturnal levels (46, 47). Thus, melatonin administration during a bright day (*i.e.* when the levels of endogenous melatonin are low) may affect temperature differently than nighttime administration, when melatonin levels due to endogenous secretion are high. Although the mechanisms underlying the acute effects of exogenous melatonin on temperature are still unknown, the authors of this study attribute the chronic effect to an increased output amplitude of the SCN. This would explain the increased amplitude of temperature decline during sleep, including increased absolute temperature in the first hours after melatonin ingestion (45) and reduced temperature minimum. Nevertheless, an additional mechanism of acute temperature suppression may have been present.

Another possible cause of the effects seen in our study could be melatonin replacement. However, in another study, low endogenous melatonin did not predict response to melatonin treatment in elderly insomniacs (45), and the absolute amount of melatonin excretion in our population did not differ from that in patients and healthy subjects (21, 22, 31). Alternatively, the increase of REM sleep in our patients could be explained by a change in the timing of sleep with respect to circadian phase (11, 41). This also seems unlikely, however, because no phase shift was observed, as was determined by unchanged temperature minimum and REM sleep latency. Finally, melatonin could have increased REM sleep by directly activating cholinergic REM-on or deactivating monoaminergic REM-off cells (27, 48). There is no evidence for such a mode of action, but it would be expected to occur in healthy subjects too, and not to be highly time-dependent.

Why did earlier melatonin drug trials yield conflicting results (49, 50)? Although some studies have shown melatonin to have a beneficial effect on subjective sleep perception and daytime behavior, this improvement was not substantiated by objective measures in most studies. In light of the chronobiotic effects of melatonin, several points need to be stressed.

First, melatonin exerts only minimal hypnotic effects on general sleep parameters. In contrast, earlier sleep studies did not specifically address the influence of melatonin on circadian components in the sleep-wake cycle.

Second, the effects of melatonin in humans are time-dependent (51); thus, the time of melatonin administration needs to be fixed. The greatest increase in endogenously secreted melatonin by the pineal gland in entrained humans (synchronized to the environmental light-dark cycle) occurs during the early night period. Only when exogenous melatonin is administered at that specific point in time can it support the functions of endogenous melatonin. In contrast, improper timing of exogenous melatonin may result in phase shifts. One consequence of phase shifts in the sleep-wake cycle is a reduced amplitude of circadian rhythms in humans (52, 53). Thus, a change in the time of administration with

changing bedtimes (*i.e.* "take one tablet, 30 min before bedtime") is likely to desynchronize the CTS and counteract any possible benefits. More clearly, our results indicate that the nonprescription use of melatonin is countertherapeutic when bedtimes and/or the time of administration are erratic.

Third, the effects mediated by increased CTS strength outlast the actual time of drug intake. In other words, once the clock ticks properly, it will keep ticking. Thus, a crossover design with intraindividual comparison of melatonin and placebo is inappropriate when evaluating the synchronizing effects of melatonin.

The practical consequences to be derived from the data presented here are still limited. None of the patients in the study showed melatonin excretion abnormalities, and the only viable concept for melatonin replacement therapy is still awaiting clinical validation (31). A reduction in REM sleep percentage can only be substantiated by PSG, which limits the indication for melatonin treatment at this time. The clinical improvements in our patients, which accompanied REM sleep normalization, suggest that compensation mechanisms for REM sleep reduction are limited. Nevertheless, the clinical features of a REM sleep deficit have yet to be defined.

Furthermore, the optimal dose of melatonin is still unknown (20, 54). Melatonin is a potent drug and will undoubtedly have side effects. Animal data suggest that melatonin may accelerate the course of Parkinson's disease (55). Melatonin is involved in sexual maturation, and hypermelatonism is being discussed as a cause of infertility (56). Thus, the unmonitored, over-the-counter intake of exogenous melatonin cannot be considered safe. Its application as a dietary supplement—in anyone, in any dose, at any time—is very likely to produce negative results in the long run.

The spectrum of diseases included in the present study calls the external validity of our results into question. No conclusion can be drawn with respect to the pathophysiology of these sleep disorders. Melatonin improved symptoms but did not cure the underlying disorder. However, the circadian variation of REM sleep expression, as well as the influence of melatonin on general physiology via the CTS, are fundamental and very active mechanisms in humans. Moreover, results were not obtained in the artificial environment of temporal isolation but while patients maintained their habitual daily activities with small changes in melatonin administration time allowed. It represents one strength of this study that exogenous melatonin was able to normalize parameters independent of pathology and in a natural setting.

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