PHARMACODYNAMICS AND DRUG ACTION

Sleep-inducing effects of low doses of melatonin ingested in the evening

We previously observed that low oral doses of melatonin given at noon increase blood melatonin concentrations to those normally occurring nocturnally and facilitate sleep onset, as assessed using an involuntary muscle relaxation test. In this study we examined the induction of polysomnographically recorded sleep by similar doses given later in the evening, close to the times of endogenous melatonin release and habitual sleep onset. Volunteers received the hormone (oral doses of 0.3 or 1.0 mg) or placebo at 6, 8, or 9 PM. Latencies to sleep onset, to stage 2 sleep, and to rapid eye movement (REM) sleep were measured polysomnographically. Either dose given at any of the three time points decreased sleep onset latency and latency to stage 2 sleep. Melatonin did not suppress REM sleep or delay its onset. Most volunteers could clearly distinguish between the effects of melatonin and those of placebo when the hormone was tested at 6 or 8 PM. Neither melatonin dose induced "hangover" effects, as assessed with mood and performance tests administered on the morning after treatment. These data provide new evidence that nocturnal melatonin secretion may be involved in physiologic sleep onset and that exogenous melatonin may be useful in treating insomnia. (CLIN PHARMACOL THER 1995;57:552-8.)

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In the initial description of melatonin (5-methoxy-N-acetyltryptamine) as the melanophore lightening agent present in bovine pineal glands, the hormone's

sedative effect on humans was noted.[1] Although melatonin was subsequently shown to be secreted primarily at night, when people sleep,[2] its role in physiologic sleep remained uncertain.[3] Pharmacologic doses of the hormone, which increase serum melatonin levels far beyond their normal nocturnal range, could be shown to produce hypnotic effects in humans[4-6]; however, smaller doses were rarely tested and yielded inconsistent results when examined.[7-9] We started by testing the midday administration of a pharmacologic dose (240 mg, administered orally) of melatonin and, on the basis of performance tests and self-reports, observed hypnotic-like effects.[10] We next tested four much lower (but still pharmacologic) doses of the hormone (10, 20, 40, or 80 mg) or placebo, given at noon. Although the resulting mean serum melatonin concentrations were roughly proportional to the doses administered, all doses were equally effective, relative to placebo, in inducing subjective sleepiness. These doses were also equally effective in decreasing oral

temperature, the number of correct responses in auditory vigilance, and self-reported vigor.[11] Because the effects were not related to dose, we concluded that the biochemical mechanisms that mediate the behavioral (and temperature) effects of melatonin had been saturated by all of the doses tested. In further pursuit of this line of investigation, we examined the effects of much lower oral doses (i.e., 1.0, 0.3, and 0.1 mg of melatonin or placebo, compared with a 10.0 mg reference dose) administered at noon. Sleep onset and duration were studied beginning 1¾ hours after the administration of melatonin or placebo by means of a positive pressure switch technique.[12] Serum melatonin concentrations were, again, roughly proportional to dose, but the peak amplitudes observed after the 0.1 and 0.3 mg doses (mean ± SEM, 48.4 ± 37.1 and 121.45 ± 24.21, respectively) were within the *normal* (nocturnal) range for human serum melatonin levels. Sleep onset latencies after ingestion of any of the doses tested were significantly shorter than those after ingestion of placebo: peak effects were observed with the 0.3 mg and 1.0 mg doses. This study was designed to determine whether such doses also facilitate sleep onset when given in the evening, close to the hour that most people elect to sleep, and whether the hypnotic effect of the hormone can be detected polysomnographically.

METHODS

Six healthy male volunteers (mean age ± SEM, 26.5 ± 1.3 years) were paid to participated in the study after each gave informed consent. The experimental protocol and the Subject's Consent Form was approved by the Massachusetts Institute of Technology (MIT) Committee on the Use of Humans as Experimental Subjects. All volunteers reported that they were free of habitual sleep disturbances and that they were drug-free. Self-report questionnaires completed by volunteers on each test day regarding their previous nights' sleep indicated that their mean bedtime occurred at 12:40 AM ± 22 minutes, mean sleep duration was 7.2 ± 0.32 hours, and mean estimated sleep onset latency at the habitual sleep time was 21.6 ± 4.26 minutes. Subjects were nonsmokers and were asked to not consume alcohol or caffeine for 24 hours before each test session. Meals (provided by the MIT Clinical Research Center) reflected a typical American diet: 15% protein, 35% fat, 50% carbohydrates; 3000 to 4000 calories per day.

The study consisted of three double-blind and placebo-controlled experiments. Treatment order for each experiment was determined with use of a 3 × 3 Latin-square design to counterbalance for order effects. Each volunteer participated in a total of nine test sessions, with at least 5 days elapsing between successive sessions. In the first experiment, subjects underwent three test sessions in which they received placebo or melatonin (0.3 or 1.0 mg; Nestle Co., Vevey, Switzerland) in gelatin capsules, orally at 6 PM. In the second and third experiments they received the same doses of melatonin or placebo at 8 or 9 PM. In the first two experiments, the sleep test consisted of 2 hours of polysomnographically recorded sleep that began when the lights were turned off 2 hours after administration of melatonin or placebo (i.e., at 8 or 10 PM). In the third experiment, subjects spent the night before the test session in the Clinical Research Center, and their sleep was recorded polysomnographically from 11 PM to 7 AM. On the following night they retired in darkness at 10 PM, 1 hour after ingestion of melatonin or placebo at 9 PM, and their sleep was recorded polysomnographically from 10 PM until 7 AM the following morning.

For polysomnographic recordings, electroencephalographic electrodes, placed according to the International 10/20 System (with recordings from C4-A1, C3-A2, C3-01, and C4-02), and electro-oculographic and submental electromyographic electrodes were used for sleep staging. Signals from the electrodes and transducers were amplified by 18-channel preamplifier/multiplexor units; they were then digitized and recorded (DigiTrace Care Services Inc., Boston, Mass.). Thirty-second epochs were staged according to the criteria of Rechtschaffen and Kales[13]: wake; stages 1, 2, 3, or 4; and rapid eye movement (REM) sleep. Sleep onset latency was defined as the time elapsing between the time that lights were turned off and the appearance of three consecutive epochs of stage 1 sleep, or of one epoch of any other sleep stage. Latency to REM sleep was defined as the time elapsing between sleep onset and the appearance of one epoch of REM sleep. Complete sets of polysomnographic recordings were obtained from all six of the subjects, except as noted below, after treatment at 8 and 9 PM and from two of the subjects after treatment at 6 PM. (No polysomnographic recording was obtained for subject 6 after he received 1.0 mg melatonin at 9 PM.) To test the subjective responses of the volunteers to treatment, subjects given melatonin or placebo at 6 or 8 PM were asked at the end of each sleep test whether they thought that the day's treatment had been a placebo or a hypnotic, and their answers were recorded.

On the morning after treatment, subjects completed a battery of computerized performance tasks and
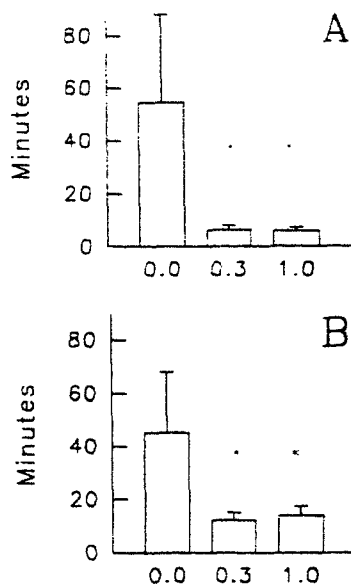
Fig. 1. Effects of melatonin (ingested at 8 PM) on average (SEM) latency to sleep onset (A) and sleep stage 2 (B). $*p < 0.001$.

Table I. Effects of melatonin, ingested at 9 PM, on sleep patterns*

Subject No.	Dose		
	Placebo	0.3 mg	1 mg
Sleep onset latency (min)			
1	187	12	8
2	23	4	7
3	23	6	6
4	34	8	7
5	5	2	1
6	56	10	†
Latency to stage 2 (min)			
1	214	17	28
2	25	6	14
3	28	13	7
4	35	10	9
5	6	2	1
6	59	12	†
Latency to REM sleep (min)			
1	163	95	173
2	80	89	78
3	103	67	144
4	63	70	78
5	41	48	65
6	141	106	†

*Six subjects received melatonin (oral doses of 0.3 or 1.0 mg) or placebo at 9 PM. and were allowed to sleep after 10 PM.
†Missing value.

mood inventories that previous studies have shown to be adequate for evaluating of the level of sleepiness: Four Choice Reaction Time, Simple Auditory Reaction Time, Profile of Mood States, and Stanford Sleepiness Scale (for details see Dollins et al.[11]).

Because of the high variability of characteristic sleep patterns among subjects, the nonparametric Friedman's test[14] was used to evaluate differences associated with the three treatment conditions. Group mean values were substituted for the missing data points from subject 6 (1.0 mg at 9 PM). Regression analysis was used to evaluate the relationship between the sleep onset latency after treatment and habitual sleep onset latency.

RESULTS

Both doses of melatonin given at 8 PM (Fig. 1) or at 9 PM (Table I) significantly decreased sleep onset latency and latency to stage 2 sleep ($p < 0.001$) relative to placebo, as assessed polysomnographically. The hormone was equally effective in the two subjects studied at 6 PM. Thus, latency to sleep onset after treatment at 6 PM decreased from 57.3 ± 17.88 (SEM) minutes (placebo) to 10.8 ± 0.13 minutes (0.3 mg dose) or 8.5 ± 1.50 minutes (1.0 mg dose); at 8 PM, latency decreased from 29.4 ± 10.77 minutes (placebo) to 6.4 ± 1.88 minutes (0.3 mg dose) or 7.2 ± 1.13 minutes (1.0 mg dose); and at 9 PM, latency

decreased from 54.8 ± 27.39 minutes (placebo) to 7.1 ± 1.61 minutes (0.3 mg dose) or 6.0 ± 1.07 minutes (1.0 mg dose). Latency to sleep stage 2 provided a more sensitive index of the hypnotic effect of melatonin. At 6 PM, latency decreased from 65.3 ± 14.38 minutes (placebo) to 19.0 ± 1.25 minutes (0.3 mg dose) or 14.0 ± 3.75 minutes (1.0 mg dose); at 8 PM, it decreased from 33.8 ± 10.06 minutes (placebo) to 7.2 ± 1.96 minutes (0.3 mg dose) or 11.4 ± 3.23 minutes (1.0 mg dose); at 9 PM, it decreased from 61.25 ± 31.34 minutes (placebo) to 10.1 ± 2.17 minutes (0.3 mg dose) or 11.3 ± 3.7 minutes (1.0 mg dose). The effects of the physiologic (0.3 mg) and low pharmacologic (1.0 mg) doses of melatonin did not differ significantly with respect to any of the parameters measured; therefore the higher dose did not enhance the hypnotic effect. We did not find statistically significant differences in latency to REM sleep after melatonin or placebo administration at 9 PM, although in three of the six subjects this interval decreased somewhat after administration of the lower dose. (Table I).

The ability of melatonin to decrease the sleep onset latency of any subject varied in proportion to his sleep
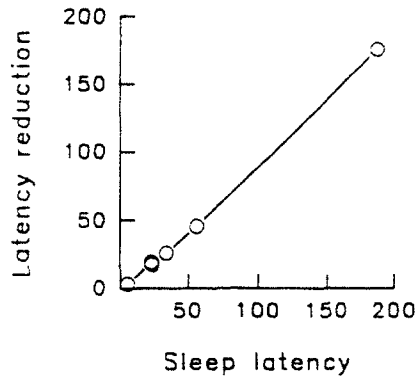
Fig. 2. The relation between the reduction in sleep onset latency after the ingestion of 0.3 mg dose of melatonin at 9 PM and the sleep latency in the untreated condition.

latency after placebo administration ($r = 0.99$ for the 0.3 mg dose and $r = 0.94$ for the 1.0 mg dose; Fig. 2), and in proportion to his self-reported sleep onset latency at his usual bedtime ($r = 0.86$). Thus, subjects who required more than 20 minutes to fall asleep after placebo treatment exhibited the more robust responses to the exogenous hormone (Table I). Analysis of subjective sleepiness and of reaction times in response to visual or auditory stimuli on the morning after treatment (at 7:30 or 9:30 AM) revealed no hangover effects of melatonin treatment (Fig. 3).

None of the volunteers mistook the placebo for melatonin in their self-reports. At 8 PM, four of the subjects receiving the 0.3 mg dose and five of the subjects receiving the 1.0 mg dose recognized that they had been treated with a hypnotic. Most subjects perceived the hypnotic effect within an hour of melatonin ingestion.

DISCUSSION

These data show that low oral doses of melatonin, which were previously shown to increase serum melatonin concentrations to levels normally occurring at night,[12] produce acute hypnotic effects when given in the evening (Fig. 1, Table I). Moreover, these effects are discernible both subjectively and by standard polysomnographic methods. These observations complement our earlier finding that similar small melatonin doses given at noon decrease sleep latency, as measured electromechanically,[12] and support the idea that the physiologic increase in blood melatonin levels that occurs late in the evening, 1 to 2 hours before habitual bedtime, is involved in the mechanisms that normally trigger sleep onset.

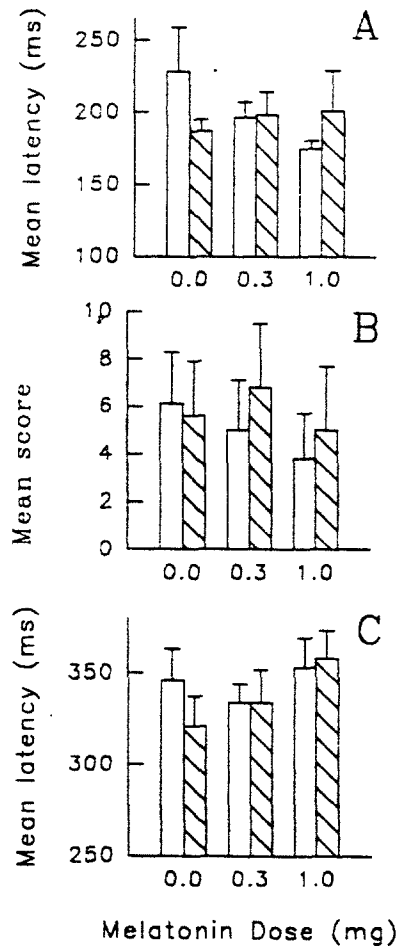Normally, the daily alternation of environmental

Fig. 3. Mood and performance on the morning after melatonin treatment: Simple Reaction Time (A); Profile of Mood States (fatigue/inertia scale) (B); and Four Choice Reaction Time, measured at 7:30 AM (open bars) or 9:30 AM (striped bars).

light and darkness, mediated through the eyes,[15] adaptively harmonizes the rhythmic secretion of melatonin from the pineal gland with other circadian rhythms. The nocturnal release of melatonin from the pineal gland is coincident with the habitual hours of sleep in people with entrained 24-hour sleep-wake rhythms.[2] In blind persons, deprived of this environmental zeitgeber, endogenously driven circadian rhythms tend to free-run and become, to varying degrees, dissociated; sleep becomes disrupted, and daytime alertness is diminished.[16] However, in a blind patient who exhibited free-running circadian rhythms, the daily pattern of sleep propensity and the onset of melatonin secretion were found to exhibit a normal relationship.[17] This as-

sociation supports the hypothesis that melatonin is involved in normal nocturnal sleep onset.

Because repeated daily doses of melatonin can shift the phasing of the daily rhythm in human melatonin levels,[18] it has been suggested that this treatment can also, as a consequence, shift the sleep rhythm[19-21] (i.e., that the effect of exogenous melatonin on sleep is regulated by induced changes in the endogenous rhythms). This does not seem to be a likely explanation for the changes in sleep latency observed here (Fig. 1; Table I) or in our previous study[12] in which melatonin was administered at noon and sleep onset measured at 2 PM. The extent to which a single dose of melatonin can shift the daily melatonin rhythm has been measured with use of repeated daily administration of the hormone at various time points,[21] or melatonin infusions over 3-hour intervals,[22] and found to be less than 1 hour per day of treatment, regardless of the time of day that melatonin was administered. Our present and previous findings[12] show that a single physiologic (0.3 mg) dose of melatonin can provoke sleep onset 5 to 11 hours earlier than sleep would otherwise occur. Similarly, Tzischinsky and Lavie,[23] using a pharmacologic dose of melatonin (5 mg), found that the hormone facilitated sleep onset and sleep propensity 2 hours after it was administered at noon or at 5 PM. Such effects are clearly unrelated to a general shift in circadian rhythms. Our findings and those of the Israeli group[23] suggest that the induced elevation of circulating melatonin concentrations triggers the onset of sleep, regardless of the prevailing phase of endogenous circadian rhythms. This acute effect of melatonin on sleep, considered in view of the reported increase in plasma melatonin levels[24] and the increase in urinary melatonin during sleep deprivation,[25] supports the idea that endogenous melatonin may serve as a link between circadian rhythmicity and the homeostatic mechanisms of sleep. This may account for the observed reduction of sleep latency as a function of sleep deficiency reported by Carskadon and Dement.[26] Thus a critical serum melatonin level may be basic to normal nocturnal sleep induction in humans.

In our studies, the extent to which melatonin accelerated sleep onset in any subject varied with his characteristic sleep latency in the untreated state. The sources of intersubject variability in sleep onset latency are unknown but could be related in part to differences in subjects' characteristic 24-hour serum melatonin patterns, which were not documented in this study. A similar relationship could also underlie the well-known increase of sleep pathology among older people,[27] inasmuch as nocturnal melatonin production

also declines with advancing age.[28,29] Because only young volunteers were involved in our studies, we do not have data on possible age-related differences in the sleep-inducing effect of melatonin. However, Haimov et al.[30] found that older people with insomnia have lower nocturnal melatonin levels than those of young people and of older subjects without insomnia.

Available data suggest that the sleep-inducing properties of melatonin may differ from those of the benzodiazepines. Benzodiazepines decrease the duration of REM sleep after the single administration of a high dose[31] or of longer-term administration of low doses[32,33]; they also reduce slow wave sleep,[34] thus negatively influencing sleep quality. In our study, using a single low melatonin dose, we observed no suppression of REM sleep, and some of the subjects underwent REM sleep sooner after melatonin treatment than after placebo (Table I). It is interesting to note that the benzodiazepines reportedly also suppress the nocturnal increase in plasma melatonin and may increase daytime plasma melatonin levels.[35,36] Such effects could account for the residual daytime sedation that frequently occurs after benzodiazepine treatment, as well as for the rebound insomnia that can follow the termination of such treatment. Infusion of L-tryptophan[37-39] or of Delta-Sleep-Inducing Peptide,[40] which can provoke sleep onset, also reportedly increase circulating melatonin levels.[41-43] In contrast, β-adrenergic blocking agents[44,45] or nonsteroidal antiinflammatory drugs (e.g., aspirin),[46,47] which disturb sleep, decrease blood melatonin levels.

In the design of our experiments, the objective "sleep tests" were initiated only 1 or 2 hours after ingestion of melatonin; thus we lack data on the minimum latency of the hormone's effect. However, self-reports of the volunteers indicated that their usual latency to its hypnotic effect was 25 to 60 minutes. In previous polysomnographic studies of the effect of melatonin on sleep latency positive results were obtained when melatonin was given 1 to 2 hours before the effect was observed (80 mg[6] and 5 mg[23]), and negative results were obtained when it was administered 15 minutes before bedtime (1 or 5 mg[8,9]). Further studies are needed to determine the time course of the effect of physiologic melatonin doses on sleep latency.

One of the major problems with existing hypnotic agents (e.g., the benzodiazepines) is a hangover effect; that is, on the morning after treatment, patients experience inappropriate sleepiness, changes in mood, and diminished performance. In this study, we used a battery of computerized tests previously shown to be

sensitive to the mood and performance changes induced by hypnotic agents. They revealed no hangover effects the morning after evening administration of melatonin.

Our findings[11] (Table I; Fig. 1) strongly suggest that the sleep onset, which is provoked by a single dose of melatonin, results not from its effect on biological timing mechanisms, but from a direct action of the elevated circulating melatonin per se.

We thank Joseph McCarthy for technical assistance. the nursing staff of the MIT Clinical Research Center for assistance in experiments. and DigiTrace Care Services. Inc.. Boston. Mass.. for loan of the recording equipment.

References

1. Lerner AB. Case JD. Melatonin. Fed Proc 1960;19: 590-2.
2. Lynch HJ. Wurtman RJ. Moskowitz MA. Archer MC. Ho MH. Daily rhythm in human urinary melatonin. Science 1975;187:169-71.
3. Wurtman RJ. Lieberman HR. Melatonin secretion as a mediator of circadian variations in sleep and sleepiness. J Pineal Res 1985;2:301-3.
4. Anton-Tay F. Diaz JL. Fernandez-Guardiola A. On the effect of melatonin upon human brain: its possible therapeutic implications. Life Sci 1971;10:841-50.
5. Cramer H. Rudolph J. Consbruch U. Kendel K. On the effects of melatonin on sleep and behavior in man. Adv Biochem Psychopharmacol 1974;11:187-91.
6. Waldhauser F. Saletu B. Trinchard-Lugan I. Sleep laboratory investigations on hypnotic properties of melatonin. Psychopharmacology (Berl) 1990;100:222-6.
7. Vollrath L. Semm P. Gammel G. Sleep induction by intranasal application of melatonin. Adv Biosci 1981; 29:327-9.
8. James SP. Mendelson WB. Sack DA. Rosenthal NE. Wehr TA. The effect of melatonin on normal sleep. Neuropsychopharmacology 1987;1:41-4.
9. James SP. Sack DA. Rosenthal NE. Mendelson WB. Melatonin administration in insomnia. Neuropsychopharmacology 1990;1:19-23.
10. Lieberman HR. Waldhauser F. Garfield G. Lynch HJ. Wurtman RJ. Effects of melatonin on human mood and performance. Brain Res 1984;323:201-7.
11. Dollins AB. Lynch HJ. Wurtman RJ. et al. Effect of pharmacological daytime doses of melatonin on human mood and performance. Psychopharmacology (Berl) 1993;112:490-6.
12. Dollins AB. Zhdanova IV. Wurtman RJ. Lynch HJ. Deng MH. Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep. mood. body temperature. and performance. Proc Natl Acad Sci USA 1994;91:1824-8. ·
13. Rechtschaffen A. Kales A. A manual of standardized terminology. techniques and scoring systems for sleep stages of human subjects. Los Angeles: Brain Information Service/Brain Research Institute. 1968:1-60.
14. Mendenhall W. Beaver RJ. Introduction to probability and statistics. Boston: PWS-Kent 1991:614-8.
15. Wurtman RJ. Axelrod J. Fischer JE. Melatonin synthesis in the pineal gland: effect of light mediated by the sympathetic nervous system. Science 1964;143: 1328-30.
16. Sack RL. Lewy AJ. Blood ML. Keith LD. Nakagawa H. Circadian rhythm abnormalities in totally blind people: incidence and clinical significance. J Clin Endocrinol Metab 1992;75:127-34.
17. Nakagawa H. Sack RL. Lewy AJ. Sleep propensity free-runs with the temperature. melatonin and cortisol rhythms in a totally blind person. Sleep 1992;4:330-6.
18. Sack RL. Lewy AJ. Melatonin administration phase advanced endogenous rhythms in humans. Sleep Res 1988;17:396-402.
19. Folkard S. Arendt J. Aldhous M. Kennett H. Melatonin stabilises sleep onset time in a blind man without entrainment of cortisol or temperature rhythms. Neurosci Lett 1990;113:193-8.
20. Dahlitz M. Alvarez B. Vignau J. English J. Arendt J. Parkes JD. Delayed sleep phase syndrome response to melatonin. Lancet 1991;337:1121-4.
21. Lewy AJ. Sack RL. Use of melatonin to assess and treat of circadian phase disorders. In: Touitou Y. Arendt J. Pevet P. eds. Melatonin and the pineal gland: from basic science to clinical application. Amsterdam: Excerpta Med. 1993:205-9.
22. Zaidan R. Geoffriau M. Brun J. et al. Melatonin is able to influence its secretion in humans: description of a phase-response curve. Neuroendocrinology 1994; 60:105-12.
23. Tzischinski O. Lavie P. Melatonin possesses time-dependent hypnotic effects. Sleep 1994;17:638-45.
24. Salin-Pascual RJ. Ortega-Soto H. Huerto-Deladillo. Camacho-Arroyo I. Roldan-Roldan G. Tamarkin L. The effect of total sleep deprivation on plasma melatonin and cortisol in healthy human volunteers. Sleep 1988;11:362-9.
25. Akerstedt T. Froberg JA. Friberg Y. Wetterberg L. Melatonin excretion. body temperature and subjective arousal during 64 hours of sleep deprivation. Psychoneuroendocrinology 1979;4:219-25.
26. Carskadon MA. Dement WC. Nocturnal determinants of daytime sleepiness. Sleep 1982;5:S73-81.
27. Zepelin H. Normal age related changes in sleep. In: Chase M. Weitzman ED. eds. Sleep disorders: basic and clinical research. New York: SP & Scientific Books 1983:431-44.
28. Iguchi H. Kato KI. Ibayashi H. Age-dependent reduction in serum melatonin concentrations in healthy human subjects. J Clin Endocrinol Metab 1982;1:27-9.

29. Grad BR. Rozencwaig R. The role of melatonin and serotonin in aging: update. Psychoneuroendocrinology 1993;18:283-95.

30. Haimov I. Laudon M. Zisapel N. et al. Sleep disorders and melatonin rhythms in elderly people [Letter]. Br Med J 1994;309:167.

31. Kales A. Kales JD. Scharf MB. Tan TL. Hypnotics and altered sleep-dream patterns: all night EEG studies of chloral hydrate. flurazepam and methqualone. Arch Gen Psychiatry 1970;23:219-25.

32. Dement WC. Zarcone VP. Haddes E. Smythe H. Carskadon M. Sleep laboratory and clinical studies with flurazepam. In: Garrattini S. Mussinin E. Randall LO. eds. The benzodiazepines. New York: Raven Press. 1973:599-611.

33. Oswald I. Adam K. Borrow S, Idzikowski C. The effects of two hypnotics on sleep. subjective feelings and skilled performance. In: Passonant P. Oswald I. eds. Pharmacology of the states of alertness. New York: Pergamon Press. 1979:51-9.

34. Frederickson PA. Krueger BR. Insomnia associated with specific polysomnographic findings. In: Kryger MH. Roth T. Dement WC. eds. Principles and practice of sleep medicine. Philadelphia: WB Saunders. 1989:484-5.

35. Kabuto M. Namura I. Saitoh Y. Nocturnal enhancement of plasma melatonin could be suppressed by benzodiazepines in humans. Endocrinol Jpn 1986;33: 405-14.

36. McIntyre I. Burrows GD. Norman TR. Suppression of plasma melatonin by a single dose of the benzodiazepine alprazolam in humans. Biol Psychiatry 1988; 24:105-8.

37. Thorliefsdottir B. Bjornsson JK. Kjeld M. Kristbjarnarson H. Effects of L-tryptophan on daytime arousal. Neuropsychobiology 1989;21:170-6.

38. George CF. Millar TW. Hanly PJ. Kryger MH. The effects of L-tryptophan on daytime sleep latency in normals: correlation with blood levels. Sleep 1989;12:345-53.

39. Scneider-Helmert D. Spinweber CL. Evaluation of L-tryptophan for treatment of insomnia: a review. Psychopharmacology (Berl) 1986;89:1-7.

40. Graf MV. Kastin AJ. Delta sleep inducing pepetide (DSIP): an update. Peptides 1986;7:1165-87.

41. Hajak G. Huether G. Blanke J. et al. The influence of intravenous L-tryptophan on plasma melatonin and sleep in men. Pharmacopsychiatry 1991;24:17-20.

42. Ouichou A. Pevet P. Implication of tryptophan in the stimulatory effect of delta-sleep-inducing peptide on indole secretion from perifused rat pineal glands. Biol Signals 1992;1:78-87.

43. Ouichou A. Zitouni M. Raynaud F. Simonneaux V, Gharib A, Pevet P. Delta-sleep-inducing peptide stimulates melatonin, 5-methoxytryptophol and serotonin secretion from perifused rat pineal glands. Biol Signals 1992;1:65-77.

44. Cowen PJ, Bevan JS. Gosden B, Elliott SA. Treatment with β-adrenoceptor blockers reduces plasma melatonin concentration. Br J Clin Pharmacol 1985;19:258-60.

45. Brismar K. Hylander B. Eliasson K, Rossner S, Wetterberg L. Melatonin secretion related to side-effects of β-blockers from the central nervous system. Acta Med Scand 1988;223:525-30.

46. Murphy P, Badia P. Myers BL, Boecker MR, Wright Jr EP. Nonsteroidal anti-inflammatory drugs affect normal sleep patterns in humans. Physiol Behav 1991;55: 1063-6.

47. Murphy P, Myers B, Wright K. Boecker M, Badia P. Nonsteroidal anti-inflammatory drugs and melatonin levels in humans [Abstract]. Sleep Res 1993;22:413.