XETAPHARM™
Natural Preventive Healthcare

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XetaPharm, Inc.
100 Jersey Avenue
Building B, Suite 310
New Brunswick, NJ 08901-3279
(908) 249-0133
Fax (908) 247-4090

June 21, 1996

Dr. Elizabeth A. Yetley, Director
Office of Special Nutritionals HFS-450
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, N.W.
Washington, D.C. 20204

Dear Dr. Yetley:

Notice is hereby given pursuant to the requirements of Section 403(r)(6) (21 U.S.C. 343 (r)(6)) of the Federal Food, Drug and Cosmetic Act of Statements of nutritional support which have been made on the label and in the labeling in connection with the marketing of the dietary supplement Melatonin 1 mg with Vitamin B-6 1 mg tablet. Melatonin 1 mg with Vitamin B-6 1 mg tablet, packaged in blister pack will be first marketed with these statement of nutritional support on July 1, 1996. The statements of nutritional support are as follows:

Blister Pack Label:

DIRECTIONS: For restoring normal sleep in individuals whose natural melatonin has been altered, take 1 tablet at bedtime, or as directed by your health care professional.

WARNINGS: TAKE ONLY ONE TABLET PER DAY. NOT FOR CHILDREN UNDER 18. DO NOT USE IF PREGNANT OR NURSING, OR IF TRYING TO CONCEIVE (BOTH MEN AND WOMEN). FOR RELIEF OF OCCASIONAL SLEEPLESSNESS. IF SLEEPLESSNESS PERSISTS CONTINUOUSLY FOR MORE THAN 2 WEEKS, CONSULT YOUR DOCTOR. DO NOT TAKE THIS PRODUCT IF YOU ARE TAKING SEDATIVES OR TRANQUILIZERS OR UNDER MEDICATION WITHOUT CONSULTING YOUR DOCTOR (SEE ENCLOSED INSERT).*

975-0162

LET 35917
A 10

GOLD LEAF

Melatonin helps regulate your sleep cycle, resulting in quality sleep. This is especially useful for travelers and shift workers. This product is also an excellent source of Vitamin B-6 which is needed for your body's natural ability to produce melatonin.*

*THESE STATEMENTS HAVE NOT BEEN EVALUATED BY THE FOOD AND DRUG ADMINISTRATION. THIS PRODUCT IS NOT INTENDED TO DIAGNOSE, TREAT, CURE, OR PREVENT ANY DISEASE.

A Label copy is attached.

Labelings:

Number 1:

Introducing Melatonin with Vitamin B-6 Nutritional Supplement*

We are pleased to offer Melatonin - Low Dose, containing 1mg of Melatonin and 1mg of Vitamin B-6.

- **Melatonin:** Helps to regulate the onset and quality of sleep, especially in those individuals whose natural ability to produce melatonin has been altered.[1,2]

- **Low-Dosage:** Because consumer safety is our primary concern, our Melatonin comes in a 1mg effective dose.

- **Vitamin B-6:** Maintains the body's natural ability to produce Melatonin.

- **For Shift Workers and Travelers:** Melatonin is particularly useful for those who have their light patterns disrupted-such as shift workers, travelers across time zones, and individuals with difficulty adjusting to seasonal changes in day/night length.

- **Lower-Dose Option:** Tablets are scored for the option of 0.5mg.

*THESE STATEMENTS HAVE NOT BEEN EVALUATED BY THE FOOD AND DRUG ADMINISTRATION. THIS PRODUCT IS NOT INTENDED TO DIAGNOSE, TREAT, CURE OR PREVENT ANY DISEASE.

A Labeling copy is attached.

1. Dollins et al. Effect of inducing nocturnal serum melatonin concentration in daytime sleep, mood, body temperature, and performance. Proc Natl Acad Sci USA. 1994;91:1824-1828.

2. Zhdanova et al. Sleep-inducing effects of low doses of melatonin ingested in the evening. Clin Pharmacol Ther. 1995;57:5512-558.

Number 2 and 3:

There's melatonin, and now there's Melatonin from XetaPharm™, Inc.

Melatonin helps regulate your sleep cycle, resulting in quality sleep. This is especially useful for travelers and shift workers. Also, as you age, your body's production of melatonin slowly decreases, often leading to sleep disturbances. Scientific data indicates that supplementing with melatonin may help prevent these sleep disturbances.*

This product is also a source of Vitamin B-6, which is needed for your body's natural ability to produce melatonin.*

Our Melatonin

Low Dosage*

- Because consumer safety is our primary concern, our Melatonin comes in an effective 1mg dose[1,2].

- Tablets are scored for the lower-dosage option of 0.5mg.

Purest Grade*

- Product is prepared with the purest grade of Melatonin and Vitamin B-6 available.
- Finished products are tested for potency, purity, weight variation, disintegration, and dissolution as per Nutritional Supplements, *United States Pharmacopeia* (USP).
- Products are manufactured with the same care and quality control necessary for a pharmaceutical product.

*THESE STATEMENTS HAVE NOT BEEN EVALUATED BY THE FOOD AND DRUG ADMINISTRATION. THIS PRODUCT IS NOT INTENDED TO DIAGNOSE, TREAT, CURE OR PREVENT ANY DISEASE.

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Very truly yours,
XetaPharm, Inc.
A subsidiary of
Xechem, International Inc.

RCPandey

Ramesh Chandra Pandey, Ph.D.
President and CEO

PROD#: P102

Melatonin 1mg

with Vitamin B-6
Dietary Supplement

See back panel for supplement facts

10 Tablets

TRAVELER PACK

GOLD LEAF

XETAPHARM™

Distributed by: XetaPharm, Inc.
A Subsidiary of Xechem International, Inc.
New Brunswick, New Jersey 08901 USA

Directions: For restoring normal sleep in individuals whose natural melatonin has been altered, take 1 tablet at bedtime, or as directed by your healthcare professional.

Warnings: TAKE ONLY ONE TABLET PER DAY. NOT FOR CHILDREN UNDER 18. DO NOT USE IF PREGNANT OR NURSING, OR IF TRYING TO CONCEIVE (BOTH MEN AND WOMEN). FOR RELIEF OF OCCASIONAL SLEEPLESSNESS. IF SLEEPLESSNESS PERSISTS CONTINUOUSLY FOR MORE THAN 2 WEEKS, CONSULT YOUR DOCTOR. DO NOT TAKE THIS PRODUCT IF YOU ARE TAKING SEDATIVES OR TRANQUILIZERS OR UNDER MEDICATION WITHOUT CONSULTING YOUR DOCTOR (SEE ENCLOSED INSERT).*

Melatonin helps regulate your sleep cycle, resulting in quality sleep. This is especially useful for travelers and shift workers. This product is also an excellent source of Vitamin B-6 which is needed for your body's natural ability to produce melatonin.*

*These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

Supplement Facts: Serving Size: 1 Tablet

	Amount per tablet	% Daily Value
Vitamin B-6	1mg	50%
Melatonin	1mg	*

*Daily value not established

Other Ingredients: Cellulose, Calcium Phosphate, Magnesium Stearate and Sodium Starch Glycolate.

Free From: Artificial colors, preservatives or flavors, sugar, soy, yeast, dairy products, gluten, animal or fish derivatives.

Store in a dry room, 59-77°F (15-25°C).

Safety Seal: Each tablet is individually blister sealed for your protection. Do not use if blister seal is broken.

KEEP OUT OF REACH OF CHILDREN.

6 0920-4110-09 8

Lot #:

Exp.:

Gold Leaf Quality... Bridging the Gap Between Nutritional Supplements and Pharmaceuticals, Symbolizing Purity, Quality, and Reliability.

Gold Leaf Brand Challenge: Potency and purity of this brand is verifiable from any independent, outside testing laboratory in the world.

There's melatonin, and now there's Melatonin from XetaPharm™, Inc.

Melatonin helps regulate your sleep cycle, resulting in quality sleep. This is especially useful for travelers and shift workers. Also, as you age, your body's production of melatonin slowly decreases, often leading to sleep disturbances. Scientific data indicates that supplementing with melatonin may help prevent these sleep disturbances.*

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Our Melatonin

Low-Dosage*

- Because consumer safety is our primary concern, our Melatonin comes in an effective 1mg dose[1,2].
- Tablets are scored for the lower-dosage option of 0.5mg.

Purest Grade*

- Product is prepared with the purest grade of Melatonin and Vitamin B-6 available.
- Finished products are tested for potency, purity, weight variation, disintegration, and dissolution as per Nutritional Supplements, *United States Pharmacopeia* (USP).
- Products are manufactured with the same care and quality control necessary for a pharmaceutical product.

*These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

Melatonin is available in bottles of 90 tablets, and also in Traveler Packs of 10 tablets. To place your order call Customer Service at 1-800-858-5854

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Natural Preventive Healthcare

XetaPharm™, Inc.
A subsidiary of Xechem® International, Inc.
100 Jersey Avenue
Building B, Suite 310
New Brunswick, NJ 08901-3279
TEL: (908) 249-0133 FAX: (908) 247-4090

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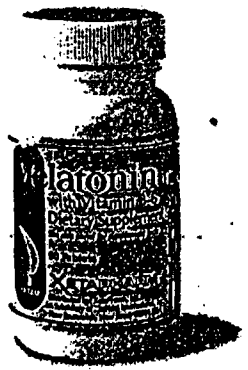
- **Lower-Dose Option:** Tablets are scored for the option of 0.5mg.



* These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

Nutritional Supplements, Pharmaceutical Standards.

XetaPharm demands that the same rigid quality standards must go into developing and manufacturing nutritional supplements as go into our pharmaceutical products. We measure quality with only one yardstick.

We follow a strict code of ethics in regards to label directions, declarations of identity, strength and purity, daily intake values, conditions of use, and warnings.

- **Purest Grade:** Product is prepared with the purest grade of Melatonin and Vitamin B-6 available.
- **Potency, Purity:** Finished products are tested for potency, purity, weight variation, disintegration, and dissolution as per Nutritional Supplements, *United States Pharmacopeia* (USP).
- **Lab Tested:** Laboratory control and test procedures are designed to assure that the components, in-process materials, labeling, and finished products conform to appropriate standards of identity, strength, quality, and purity.

From XetaPharm™, Inc.
Where Higher Standards are Standard Practice

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Introducing Melatonin with Vitamin B-6 Dietary Supplement

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Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 1824–1828, March 1994
Physiology

Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance

(vigilance/fatigue/hypnotic/circadian)

ANDREW B. DOLLINS*, IRINA V. ZHDANOVA, RICHARD J. WURTMAN†, HARRY J. LYNCH, AND MEI H. DENG

Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139

Communicated by Walle J. H. Nauta, November 23, 1993 (received for review July 20, 1993)

ABSTRACT We examined effects of very low doses of melatonin (0.1–10 mg, orally) or placebo, administered at 1145 h, on sleep latency and duration, mood, performance, oral temperature, and changes in serum melatonin levels in 20 healthy male volunteers. A repeated-measure double-blind Latin square design was used. Subjects completed a battery of tests designed to assess mood and performance between 0930 and 1730 h. The sedative-like effects of melatonin were assessed by a simple sleep test: at 1330 h subjects were asked to hold a positive pressure switch in each hand and to relax with eyes closed while reclining in a quiet darkened room. Latency and duration of switch release, indicators of sleep, were measured. Areas under the time–melatonin concentration curve varied in proportion to the different melatonin doses ingested, and the 0.1- and 0.3-mg doses generated peak serum melatonin levels that were within the normal range of nocturnal melatonin levels in untreated people. All melatonin doses tested significantly increased sleep duration, as well as self-reported sleepiness and fatigue, relative to placebo. Moreover, all of the doses significantly decreased sleep-onset latency, oral temperature, and the number of correct responses on the Wilkinson auditory vigilance task. These data indicate that orally administered melatonin can be a highly potent hypnotic agent; they also suggest that the physiological increase in serum melatonin levels, which occurs around 2100 h daily, may constitute a signal initiating normal sleep onset.

Serum melatonin levels in normal humans are very low during most of the day but increase significantly to a mean of 80 pg/ml (range, 0–200) between 0200 and 0400 h (1 pg/ml = 4.31 pmol/liter) and remain elevated during the normal hours of sleep, falling sharply to daytime values around 0900 h (1). The physiological significance of the nocturnal increase in serum melatonin could derive from acute effects of the hormone [e.g., its ability to reduce core body temperature (2), alter thermoregulation (3), modify brain levels of monoamine neurotransmitters (4), stimulate prolactin secretion (5), or induce sleepiness (6, 7)].

Alternatively, the nocturnal increase in serum melatonin could constitute a time signal, affecting the temporal characteristics of other circadian rhythms. Oscillations in the concentration of circulating melatonin could directly affect circadian rhythms (8, 9) or could provide humoral communication of information about environmental lighting (and thus about time of day), which entrains endogenous physiological and behavioral rhythms (e.g., those associated with photoperiodism or seasonality; ref. 10). Some studies also suggest a role for melatonin in human development. The decrease in amplitude of the melatonin rhythm, which occurs late in the 1st decade of life, has been proposed as a factor contributing to pubescence (11), while further decrease, which occurs after the 6th decade, may contribute to disruptions in circadian rhythmicity reported by the elderly (12).

The acute effects of exogenous melatonin on human behavior have been studied only sporadically and have used melatonin doses that raise serum melatonin levels well beyond their normal nocturnal range. Thus, Lieberman *et al.* (13) found that a dose (240 mg over a 2-h period) that raised serum melatonin levels several thousandfold impaired mood and performance. More recently, we found similar behavioral changes after a considerably lower dose of the hormone (10 mg), which still raised serum levels to 40–50 times the normal nocturnal level (i.e., 4072 pg/ml; ref. 14).

The present study was designed to determine whether much lower daytime doses, which elevate serum melatonin levels significantly but keep these levels within the normal nocturnal range, are also sufficient to produce short-term behavioral effects. If so, this would suggest a similar role for the normal nocturnal increase in serum melatonin levels. We gave the melatonin at midday (9 or more h before the nocturnal increase) and measured mood, performance, sleepiness, and (indirectly) sleep onset.

METHODS AND MATERIALS

Twenty healthy male volunteers [mean age, 23.05 ± 4.22 (SEM) years; range, 18–24 years] participated. Before admission to the study, each subject gave his informed consent, had a physical examination to ensure that he was in good health, and completed two 1.5-h training sessions to become familiar with testing procedures and the performance test battery. Subjects were also screened for depressive symptoms by using the Hamilton psychiatric rating scale for depression (15) with a special addendum for seasonal affective disorder (16); any with a history or findings of depression were excluded. All subjects were paid for their participation in the experiment.

The study was double blind and placebo controlled. A repeated-measures, within-subjects, 5 × 5 Latin square design was used. The subjects participated in five 8-h (0930–1730 h) testing sessions. At least 5 days elapsed between successive test sessions. Capsules containing 0.1, 0.3, 1.0, or 10 mg of melatonin or placebo were administered orally (p.o.) at 1145 h each test day. Treatment order was determined by the balanced Latin square design.

Oral temperatures were measured hourly and blood was sampled at regular intervals via an indwelling venous catheter for subsequent serum melatonin measurement. Serum samples were separated by centrifugation and stored at −20°C

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Abbreviations: p.o., orally; RT, reaction time; POMS, profile of mood states; SSS, Stanford sleepiness scale.

*Present address: DoD Polygraph Institute, Building 3195, Ft. McClellan, AL 36205.
†To whom reprint requests should be addressed at: Massachusetts Institute of Technology, E25-604, Cambridge, MA 02139.

until they could be assayed by radioimmunoassay for melatonin (17).

Throughout test sessions, subjects were required to sit at an assigned computer workstation with eyes open. The task order and time of testing were held constant across test days. All instructions, performance tasks, and mood questionnaires were automated to reduce the possibility of experimenter-induced bias. The performance tasks used were coded in-house and included measures of (*i*) auditory vigilance (18), (*ii*) four-choice reaction time (RT) (19), (*iii*) simple RT, and (*iv*) symbol digit (modalities) substitution (20). Mood questionnaires included the profile of mood states (POMS; ref. 21) and the Stanford sleepiness scale (SSS; ref. 22). Details of the tasks and their administration are published elsewhere (14). The mood questionnaires were completed at 1030 h and at hourly intervals beginning at 1200 h. The simple and four-choice RTs and symbol digit substitution tasks were completed at 1030, 1300, 1500, 1600, and 1700 h. The auditory vigilance task was administered at 1200 and 1400 h. Subjects were allowed to leave their workstations during lunch (a standard lunch was served between 1100 and 1130 h), toilet breaks (<5 min), and during the half-hour sleep test.

Subjects participated in a sleep test between 1330 and 1400 h. They were asked to recline (on either a bed or a reclining chair) and relax with their eyes closed in a quiet, darkened room. In each hand they held a 1-in (2.54 cm) plastic tube that bore a positive pressure switch. They were asked to rest their hands, palm up, alongside their body and to depress the switches with the last segment of their index fingers. Release of the switch on either tube was recorded as a pen deflection on an event recorder. An investigator remained in attendance with the subjects to ensure that they followed instructions. An event-timer solenoid was randomly activated to reduce the possibility that the soft click of the switch would be misconstrued by the subjects as a significant event. After 30 min, the subjects were asked to stop relaxing and/or were awakened. They were then asked (*i*) if they fell asleep, (*ii*) if so, how long it took to fall asleep, and (*iii*) to respond to the SSS. Latency to switch release was measured from the beginning of instruction presentation to the first full minute of switch release. Total switch release time was measured as the total length of time a recording pen was deflected (the smallest interval of pen deflection measured was 1 min; accuracy, 0.25 min). One subject failed to release a switch during a sleep test session but was identified as asleep by his prolonged snoring. Sleep onset for this subject was recorded as occurring after 2 min of continuous snoring. When questioned later, the subject reported that he had indeed fallen asleep.

The after-treatment-dependent measures were each assessed by using a repeated-measures, within-subjects, 5 × 5 Latin square analysis. Orthogonal planned comparisons (23) were used to evaluate differences among the melatonin/placebo treatment conditions when a significant ($P < 0.05$) treatment effect was found. The comparisons chosen were (*i*) placebo vs. all melatonin treatments, (*ii*) 0.1 vs. 0.3, 1.0, and 10 mg of melatonin, (*iii*) 0.3 vs. 1.0 and 10 mg of melatonin, and (*iv*) 1.0 vs. 10 mg of melatonin. Pairwise comparisons were subsequently calculated for these measures (repeated-measures *t* tests for the sleep test and melatonin data and ANOVAs for the other measures) because inspection of the data suggested that the planned comparisons provided an insufficient basis for interpreting results. Only main effects that resulted in significant contrasts and interaction effects are reported. There were some missing data due to difficulties with equipment. Group means were therefore substituted for the 1700-h (0.1 mg), 1300-h (1.0 mg), 1700-h (1.0 mg), and 1000-h (10 mg) measures for subjects 15, 03, 16, and 15, respectively, on most performance measures. Blood samples were not drawn during placebo testing of subject 01 due to difficulties with catheterization, and group means were substituted for the data. Group means of serum melatonin levels were also substituted for six other missing data points. The Greek symbol Δ is used to indicate "an average change of."

RESULTS

Serum Melatonin Levels. Mean serum melatonin levels are illustrated in Fig. 1. The mean (SEM) areas under the time–melatonin concentration curve (AUC) between 1000 and 1730 h for the placebo and 0.1-, 0.3-, 1.0-, and 10-mg treatment conditions were 87.7 (5.11), 213.2 (25.02), 459.9 (62.7), 1599.0 (141.7), and 21,000.4 (3752.3), respectively. Serum melatonin AUC differed significantly among the five treatment conditions ($F_{(4,60)} = 34.34$; $P < 0.001$) and all planned contrasts were significant ($P < 0.001$). All pairwise comparisons were also significant ($P < 0.001$). The order and treatment-by-order effects were not significant.

Melatonin Treatment Effects. Significant melatonin treatment effects were found for oral temperature ($F_{(4,60)} = 7.90$; $P < 0.001$); sleep test sleep-onset latency ($F_{(4,60)} = 6.65$; $P < 0.001$); sleep duration ($F_{(4,60)} = 7.80$; $P < 0.001$); self-reported sleep-onset latency ($F_{(4,60)} = 10.52$; $P < 0.001$); postsleep test SSS responses ($F_{(4,60)} = 3.11$; $P < 0.05$) (Fig. 2); POMS vigor–activity ($F_{(4,60)} = 4.16$; $P < 0.01$) and fatigue–inertia ($F_{(4,60)} = 3.05$; $P < 0.05$) (Fig. 3) responses; SSS responses ($F_{(4,60)} = 2.79$; $P < 0.05$); number of correct responses on the Wilkinson auditory vigilance task ($F_{(4,60)} = 3.42$; $P < 0.05$); and four-choice RT response latency ($F_{(4,60)} = 3.84$; $P < 0.01$). Table 1 summarizes the planned comparison results and Table 2 contains the mean (SEM) levels measured. The treatment-by-order interaction effects were nonsignificant for all measures except the SSS ($F_{(4,60)} = 4.68$; $P < 0.001$). There were no significant differences among the baseline (1000 h) oral temperature, SSS, or POMS measures. An order effect was found among the four-choice RT response latency baseline measures ($F_{(4,60)} = 15.13$; $P < 0.001$), but the treatment and treatment-by-order interaction effects were nonsignificant.

As Table 2 indicates, response levels for some measures did not consistently increase or decrease relative to serum melatonin levels. For example, self-reported SSS responses were greatest and POMS vigor–activity scale responses were smallest after ingesting 0.3 mg, rather than higher doses, of melatonin. Pairwise comparisons were calculated to aid in interpretation of these data. Mean oral temperature measures were significantly less, relative to placebo, after ingesting 1.0 and 10 mg of melatonin [Δ −0.24°F and −0.37°F, respec-

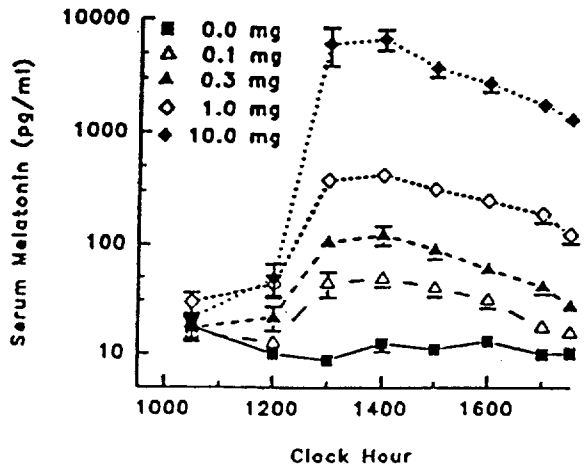

FIG. 1. Mean (SEM) serum melatonin profiles of 20 subjects sampled at intervals after ingesting 0.1, 0.3, 1.0, and 10 mg of melatonin or placebo at 1145 h.

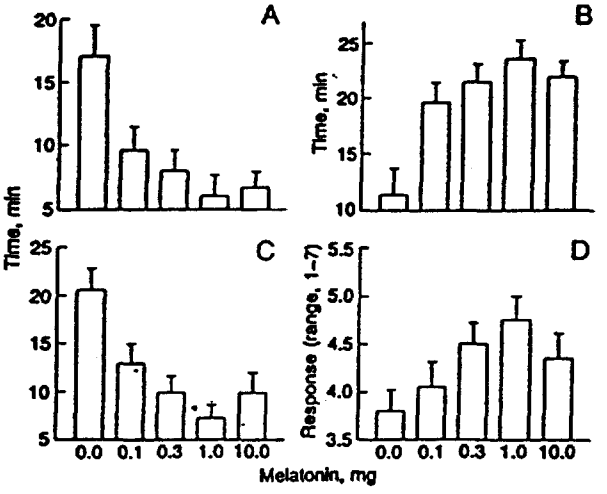

FIG. 2. Mean (SEM) sleep-onset latencies (A), sleep durations (B), self-reported sleep-onset latencies (C), and posttest SSS responses (D) after ingestion of melatonin or placebo at 1145 h ($n = 20$).

tively; 1°F = (°C × 9/5) + 32]. Oral temperatures measured after ingesting 1.0 (Δ −0.16°F) and 10 (Δ −0.29°F) mg of melatonin were also less than those measured after ingestion of 0.1 mg of melatonin. Ingestion of 10 mg of melatonin also decreased oral temperature relative to the 0.3- and 1.0-mg doses (Δ −0.21°F and −0.13°F, respectively). SSS responses indicated greater feelings of sleepiness, relative to placebo, after ingesting 0.3, 1.0, and 10 mg of melatonin (Δ +0.51, +0.47, and +0.46, respectively). POMS responses showed a decrease in self-reported feelings of vigor–activity, relative to placebo, after ingesting 0.3, 1.0, and 10 mg of melatonin (Δ −2.77, −1.90, and −1.95, respectively). Feelings of vigor–activity were also decreased after ingesting 0.3 mg relative to 1.0 mg of melatonin (Δ −1.33). POMS responses indicated an increase in self-reported feelings of fatigue–inertia, relative to placebo, after ingesting 0.3, 1.0, and 10 mg of melatonin (Δ +2.14, +1.56, and +2.28, respectively). Sleep test sleep-onset latencies were shorter, relative to placebo, after ingesting 0.1, 0.3, 1.0, and 10 mg of melatonin (Δ −7.45, −9.03, −11.02, and −10.32 min, respectively). The duration of sleep (i.e., switch release) experienced during the sleep test was greater, relative to placebo, for the 0.1-, 0.3-, 1.0-, and 10-mg melatonin doses (Δ +8.20, +10.04, +12.09, and +10.47 min, respectively). Sleep test self-reported sleep latencies were smaller, relative to placebo, for the 0.1-, 0.3-, 1.0-, and 10-mg melatonin doses (Δ −7.60, −10.60, −13.27, and −10.65 min). Subjects also indicated that they slept more quickly after ingesting 1.0 mg, relative to 0.1 mg, of melatonin (Δ −5.67). Responses to the postsleep test SSS indicate that subjects felt sleepier after ingesting 1.0 and 10 mg of melatonin than after ingesting placebo (Δ +0.70 and +0.95, respectively). Postsleep test SSS responses also indicate that 1.0 mg of melatonin caused greater feelings of sleepiness than 0.1 mg (Δ +0.70). Fewer Wilkinson auditory vigilance task correct responses were recorded, relative to placebo, after subjects ingested 1.0 and 10 mg of melatonin (Δ −4.75 and −5.67, respectively). Correct four-choice RT response latencies were greater (i.e., longer) after ingesting 10 mg, relative to placebo, and 0.1 mg of melatonin (Δ +17.21 and +14.46 ms, respectively). All of the pairwise comparison results reported above were significant at the $P < 0.05$ level.

Order and Time Effects. Significant order effects were found on the Wilkinson auditory vigilance, simple RT, four-choice RT, and symbol digit substitution tasks. These results indicate that subjects tended to respond more accurately (symbol digit substitution responses and RT response latencies) with practice and less frequently (Wilkinson auditory vigilance) on subsequent test days. These changes are of little interest because (i) significant treatment-by-order interactions were not found on these measures, (ii) the Latin square is balanced to compensate for order effects, and (iii) similar results have been observed previously (14).

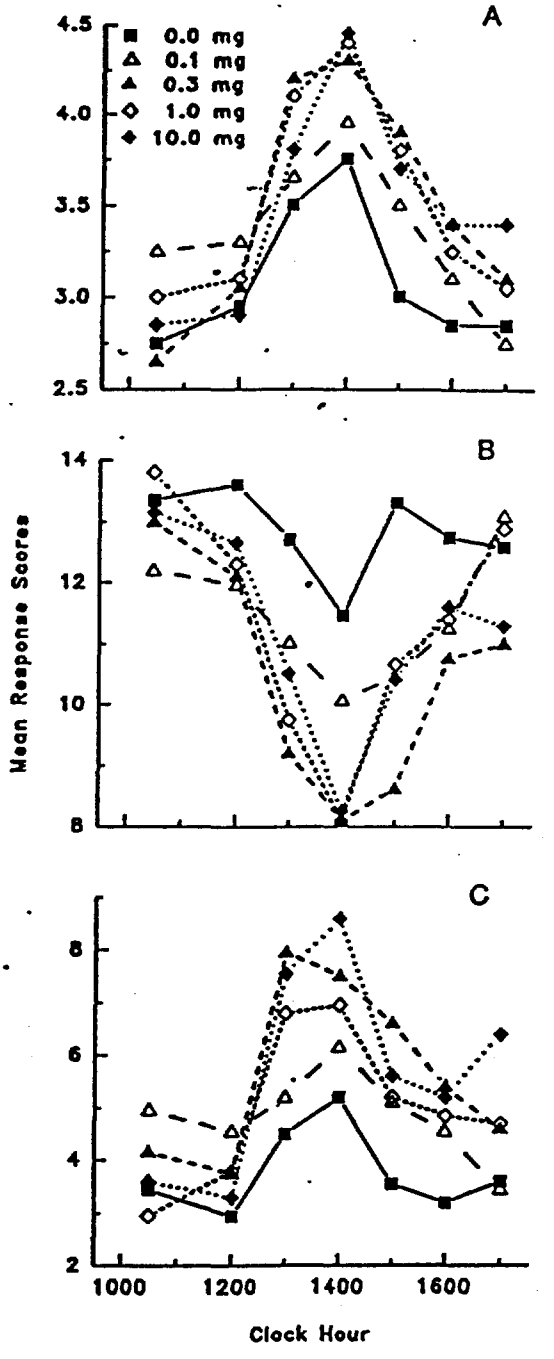

FIG. 3. Mean response scores on the SSS (A), POMS vigor–activity scale (B), and POMS fatigue–inertia scales (C) throughout testing. Melatonin or placebo was ingested at 1145 h ($n = 20$). Increased feelings of sleepiness, vigor, and fatigue are indicated by higher scores.

There were consistent patterns of variance over time among the mood scale measures. Subjects' SSS ($F_{(5,75)} = 17.50$; $P < 0.001$) and POMS fatigue–inertia ($F_{(5,75)} = 8.04$; $P < 0.001$) responses indicate that they felt sleepiest and most fatigued and that they felt the least vigorous [POMS vigor–activity scale ($F_{(5,75)} = 12.79$; $P < 0.001$)] at 1400 h, 2.25 h after melatonin ingestion (Fig. 3). Oral temperatures were consistently low at 1200 and 1400 h (means, 96.85°F and

Table 1. Mean differences of planned comparisons following significant overall *F* tests

	0.0 vs. 0.1, 0.3, 1.0, and 10	0.1 vs. 0.3; 1.0, and 10	0.3 vs. 1.0 and 10	1.0 vs. 10
Oral temperature, °F	−0.21**	−0.18**	−0.14**	−0.13
SSS	0.42**	0.25**	−0.05**	−0.01
Profile of mood states				
Vigor–activity scale	−2.02**	−0.77**	0.84**	−0.05
Fatigue–inertia scale	1.75**	0.97**	−0.22**	0.72
Sleep test				
Sleep-onset latency, min	−9.46**	−2.67**	−1.64**	0.70
Sleep duration, min	10.20**	2.67**	1.24**	−1.62
Self-reported sleep latency, min	−10.53**	−3.91**	−1.36**	2.62
SSS responses	0.61**	0.48**	0.05**	−0.40*
Wilkinson auditory vigilance, correct responses	−3.72*	−3.03	−2.19*	−0.92*
Four-choice RT correct response latency, ms	10.06**	13.25	7.57*	2.62*

Values in table are an average of the subsequent melatonin doses (mg) minus the first dose listed in the heading. All comparisons were *F* tests calculated with 5 and 15 degrees of freedom; $n = 20$ (see text for details of missing value substitutions). *, $P < 0.05$; **, $P < 0.001$.

97.09°F, respectively). Mean four-choice RT response latencies were the greatest at 1300 h (i.e., 377.77 ms) and decreased to 360.15 ms at 1700 h. Significant treatment-by-time interactions were found in oral temperature ($F_{(24,360)} = 1.60$; $P < 0.05$) and number of correct responses on the four-choice RT task ($F_{(12,180)} = 1.92$; $P < 0.05$).

DISCUSSION

Ingestion of melatonin (0.1–10 mg) at 1145 h resulted in correspondingly increased circulating melatonin levels. Serum melatonin concentrations observed after the 0.1- and 0.3-mg doses were within the normal dynamic range for nocturnal melatonin concentrations (1). Sleep test results indicate that acute administration of melatonin (0.1–10 mg, p.o.) at midday decreased objective and self-reported sleep-onset latencies by an average of 9.46 and 10.53 min, respectively, relative to placebo. Sleep duration was increased by an average of 10.2 min during the 30-min sleep test, relative to placebo, by ingestion of melatonin. Pairwise comparison of SSS scores, after the sleep test, indicates that the 0.3- and 1.0-mg doses of melatonin increased self-reported feelings of sleepiness, relative to placebo, and that feelings of sleepiness after the 1.0-mg dose were greater than those after the 0.1-mg dose. These results indicate that ingestion of an acute dose of melatonin, sufficient to increase circulating melatonin to levels within the normal nocturnal physiologic range, has hypnotic effects. The acute nature of the hypnotic effect of melatonin suggests that it may constitute a direct physiologic effect of the hormone independent of its action as a circadian zeitgeber time signal (24–26). This hypothesis is supported by the recent independent observation that melatonin (5 mg, p.o.) administered at 1200, 1700, or 1900 h exerted a direct hypnotic effect, increasing sleep propensity within 90–120 min of ingestion (27). The phase shifts in circadian rhythms in sleep (27) or endogenous melatonin secretion (28, 29) previously seen after a single acute dose of the hormone, as used here, were of insufficient magnitude to support the hypothesis that the hypnotic effects found in the current study could be caused by melatonin acting as a zeitgeber time signal. Such a hypothesis would suggest that a 9-h phase shift in sleep onset can occur after a single acute dose of melatonin at midday; this seems highly improbable.

The observed significant decreases in oral temperature after ingestion of 1.0 and 10 mg of melatonin are consistent with previous reports (2, 14). Alterations in mood and performance measures after melatonin ingestion, relative to placebo (e.g., increase in feelings of sleepiness and fatigue; increase in four-choice RT latency; decrease in feelings of vigor, decrease in number of correct responses on the Wilkinson auditory vigilance task), are also consistent with previous reports in both direction and magnitude of measure (13, 14). The direction and magnitude of response change, relative to placebo, among the melatonin doses administered

Table 2. Mean (SEM) measured responses

	Melatonin ingested, mg				
	0.0	0.1	0.3	1.0	10.0
Oral temperature, °F	97.55 (0.06)	97.47 (0.06)	97.39 (0.05)	97.31 (0.05)	97.18 (0.05)
SSS	3.15 (0.10)	3.38 (0.10)	3.66 (0.10)	3.62 (0.12)	3.61 (0.13)
Profile of mood states					
Vigor–activity scale	12.73 (0.59)	11.29 (0.64)	9.96 (0.57)	10.83 (0.65)	10.78 (0.63)
Fatigue–inertia scale	3.83 (0.40)	4.85 (0.42)	5.97 (0.44)	5.39 (0.49)	6.11 (0.53)
Sleep test					
Sleep-onset latency, min	17.06 (2.43)	9.61 (1.84)	8.03 (1.60)	6.04 (1.65)	6.74 (1.24)
Sleep duration, min	11.36 (2.34)	19.56 (1.79)	21.40 (1.63)	23.45 (1.67)	21.83 (1.41)
Self-reported sleep latency, min	20.55 (2.23)	12.95 (2.07)	9.95 (1.69)	7.28 (1.33)	9.90 (2.07)
SSS responses	3.80 (0.22)	4.05 (0.26)	4.50 (0.22)	4.75 (0.25)	4.35 (0.26)
Wilkinson auditory vigilance, correct responses	27.30 (1.48)	25.85 (1.32)	24.28 (1.53)	22.55 (1.66)	21.63 (1.56)
Four-choice RT correct response latency, ms	359.41 (8.25)	359.54 (7.36)	367.74 (7.65)	374.00 (9.08)	376.62 (9.86)

were consistent on both the mood and performance measures. It thus seems likely that failure to find significant differences in mood or performance between placebo and the lower melatonin doses (0.1 and 0.3 mg) is due to the limited sensitivity of the measures used rather than an absence of effect.

The results of this study are consistent with the observations of Vollrath *et al.* (6), who report a decrease in daytime latency of sleep onset in subjects given 1.7 mg of melatonin nasally and with those of Lavie and colleagues (27) described above. Nickelsen *et al.* (30) reported that 50 mg of melatonin, administered p.o. at 0900 or 1900 h, caused nonsignificant decreases in sleep latency but increased feelings of sleepiness only after the administration at 0900 h. Others (31, 32) reported that evening ingestion of melatonin (1 and 5 mg) did not influence sleep onset or duration but did cause an increase in rapid eye movement (REM) sleep-onset latency. These studies suggest that the magnitude of melatonin's sedative-like effects may be significantly influenced by the time of its administration. Alternatively, the experimental designs used in the negative studies might have precluded observing the hypnotic effect seen here (e.g., by not allowing subjects to modify their sleep times or by forbidding afternoon napping; refs. 28 and 29). It should be noted that the pattern of physiologic and performance responses observed here resembles that observed for drugs in the benzodiazepine family (33–35).

In summary, administration of a small melatonin dose (0.1–0.3 mg, p.o.) during the daytime, which raises serum melatonin concentrations to within the normal nocturnal range, or of slightly higher doses (1.0–10 mg, p.o.) was shown to cause hypnotic effects relative to placebo. These effects include a decrease in objective and self-estimated sleep-onset latency, an increase in sleep duration, and sleepiness upon waking. Self-reported feelings of sleepiness and fatigue were increased and feelings of vigor diminished. Oral temperature and the number of correct responses on the Wilkinson auditory vigilance task were found to decrease significantly after ingestion of 1.0 and 10 mg of melatonin. These results are similar to those reported after ingestion of benzodiazepines and suggest that melatonin may find use as a hypnotic drug. They also suggest that the normal physiologic secretion of melatonin may be an important and direct-acting factor in bringing about sleep onset.

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1. Lynch, H. J., Brzezinski, A., Deng, M. H. & Lieberman, H. (1987) in *Advances in Pineal Research*, eds. Fraschini, F. & Reiter, R. J. (Libbey, London), Vol. 2, pp. 181–189.
2. Cagnacci, A., Elliott, J. A. & Yen, S. S. C. (1992) *J. Clin. Endocrinol. Metab.* 75, 447–452.
3. Viswanathan, M., Laitinen, J. T. & Saavedra, J. M. (1990) *Proc. Natl. Acad. Sci. USA* 87, 6200–6203.
4. Anton-Tay, F., Chou, C., Anton, S. & Wurtman, R. J. (1968) *Science* 162, 277–278.
5. Waldhauser, F., Lieberman, H. R., Lynch, H. J., Waldhauser, M., Herkner, K., Frisch, H., Vierhapper, H., Waldhausl, W., Schemper, M., Wurtman, R. J. & Crowley, W. F. (1987) *Neuroendocrinology* 46, 125–130.
6. Vollrath, L., Semm, P. & Gammel, G. (1981) *Adv. Biosci.* 29, 327–329.
7. Wurtman, R. J. & Lieberman, H. (1987) *Integr. Psychiatry* 5, 13–14.
8. Lewy, A. J., Sack, R. L. & Latham, J. M. (1991) *Adv. Pineal Res.* 5, 285–293.
9. Lewy, A. J., Ahmed, S., Jackson, J. M. L. & Sack, R. L. (1992) *Chronobiol. Int.* 9, 380–392.
10. Reiter, R. J. (1988) in *Melatonin: Clinical Perspectives*, eds. Miles, A., Philbrick, D. R. S. & Thompson, C. (Oxford Univ. Press, New York), pp. 1–42.
11. Waldhauser, F., Weiszenhacher, G., Frisch, H., Zeitlhuber, U., Waldhäuser, M. & Wurtman, R. J. (1984) *Lancet* i, 362–365.
12. Czeisler, C. A., Dumont, M., Duffy, J. F., Steinberg, J. W., Richardson, G. S., Brown, E. N., Sanchez, R., Rios, C. D. & Ronda, J. M. (1992) *Lancet* 340, 933–936.
13. Lieberman, H. R., Waldhauser, F., Garfield, G., Lynch, H. J. & Wurtman, R. J. (1984) *Brain Res.* 323, 201–207.
14. Dollins, A. B., Lynch, H. J., Wurtman, R. J., Deng, M. H., Kischka, K. U., Gleason, R. E. & Lieberman, H. R. (1993) *Psychophysiology* 112, 490–496.
15. Hamilton, M. (1967) *Br. J. Soc. Clin. Psychol.* 6, 278–296.
16. Rosenthal, N. E., Genhart, M., Sack, D. A., Skewer, R. G. & Wehr, T. A. (1987) in *The Psychobiology of Bulimia*, eds. Hudson, J. I. & Pope, H. G., Jr. (Am. Psychiatr. Press, Washington, DC), pp. 205–228.
17. Brzezinski, A., Seibel, M. M., Lynch, H. J., Deng, M. H. & Wurtman, R. J. (1987) *J. Clin. Endocrinol. Metab.* 64, 865–867.
18. Wilkinson, R. T. (1969) *Psychol. Bull.* 72, 260–272.
19. Wilkinson, R. T. & Houghton, D. (1975) *Behav. Res. Methods Instrum.* 7, 441–446.
20. Smith, A. (1967) *Educ. Psychol. Meas.* 27, 1077–1083.
21. McNair, P. M., Lorr, M. & Droppleman, L. F. (1971) *Profile of Mood States Manual* (Educational and Industrial Testing Service, San Diego).
22. Hoddes, E., Dement, W. & Zarcone, V. (1973) *Psychophysiology* 10, 431–436.
23. Winer, B. J. (1971) *Statistical Principles in Experimental Design* (McGraw-Hill, New York), 2nd Ed. pp. 384–388.
24. Arendt, J. & Broadway, J. (1987) *Chronobiol. Int.* 4, 273–282.
25. Petrie, K., Conaglen, J. V., Thompson, L. & Chamberlain, K. (1989) *Br. Med. J.* 298, 705–707.
26. Claustrat, B., Brun, J., David, M., Sassolas, G. & Chazot, G. (1992) *Biol. Psychiatry* 32, 705–711.
27. Tzischinski, Q., Lavie, P. & Pal, I. (1992) *J. Sleep Res.* 1, Suppl. 1, 234.
28. Lewy, A. J. & Sack, R. L. (1993) in *Melatonin and the Pineal Gland, from Basic Science to Clinical Application*, eds. Tiouitou, Y., Arendt, J. & Pevet, Y. (Elsevier Science, Amsterdam), pp. 205–210.
29. Zaidan, R., Geoffriau, M., Claustrat, B., Brun, J., Taillard, J., Bureau, C. & Chazot, G. (1993) in *Melatonin and the Pineal Gland, from Basic Science to Clinical Application*, eds. Tiouitou, Y., Arendt, J. & Pevet, Y. (Elsevier Science Publishers B.V., Amsterdam), pp. 235–239.
30. Nickelsen, T., Demisch, L., Demisch, K., Radermacher, B. & Schoffling, K. (1989) *J. Pineal Res.* 6, 325–334.
31. James, S. P., Mendelson, W. B., Sack, D. A., Rosenthal, N. E. & Wehr, T. A. (1987) *Neuropsychopharmacology* 1, 41–44.
32. James, S. P., Sack, D. A., Rosenthal, N. E. & Mendelson, W. B. (1990) *Neuropsychopharmacology* 3, 19–23.
33. Koelega, H. S. (1989) *Psychopharmacology* 98, 145–156.
34. Greenblatt, D. J., Harmatz, J. S., Engelhardt, N. & Shader, R. I. (1989) *Arch. Gen. Psychiatry* 46, 326–332.
35. Walsh, J. K., Schweitzer, P. K. & Parwatiker, S. (1983) *Clin. Pharmacol. Ther.* 34, 496–500.

2. Zhdanova et al. Sleep-inducing effects of low doses of melatonin ingested in the evening. Clin. Pharmacol. Therap. 57, 552 - 558, 1995.

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.)

Irina V. Zhdanova, PhD, Richard J. Wurtman, MD, Harry J. Lynch, PhD, John R. Ives, BS, Andrew B. Dollins, PhD, Claudia Morabito, BS, Jean K. Matheson, MD, and Donald L. Schomer, MD *Cambridge and Boston, Mass.*

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

From the Clinical Research Center and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, and the Department of Neurology, Beth Israel Hospital, Harvard Medical School, Boston.

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Reprint requests: Richard J. Wurtman, MD, Cecil H. Green Distinguished Professor, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, E25-604, 45 Carleton St., Cambridge, MA 02142.

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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; Nestlé 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

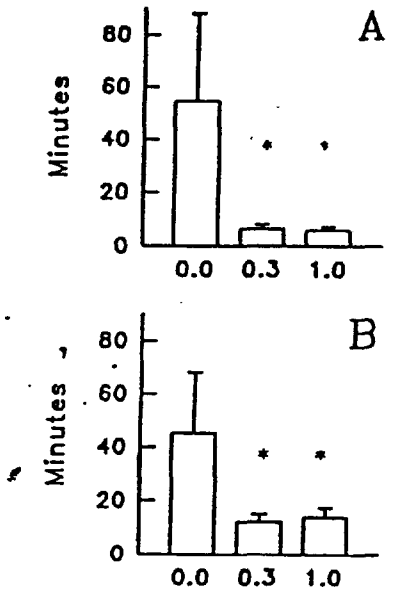

Fig. 1. Effects of melatonin (ingested at 8 PM) on average (SEM) latency to sleep onset (A) and sleep stage 2 (B). *$p < 0.001$.

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

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.

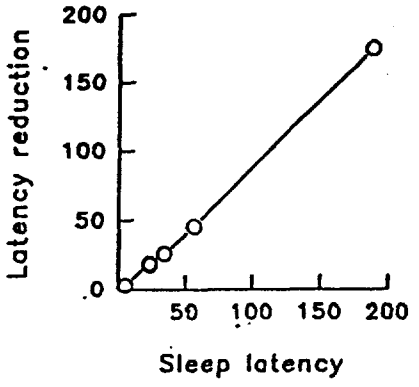

Fig. 2. The relation between the reduction in sleep onset latency after the ingestion of 0.3 mg dose of melatonin at 9 PM and the sleep latency in the untreated condition.

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.

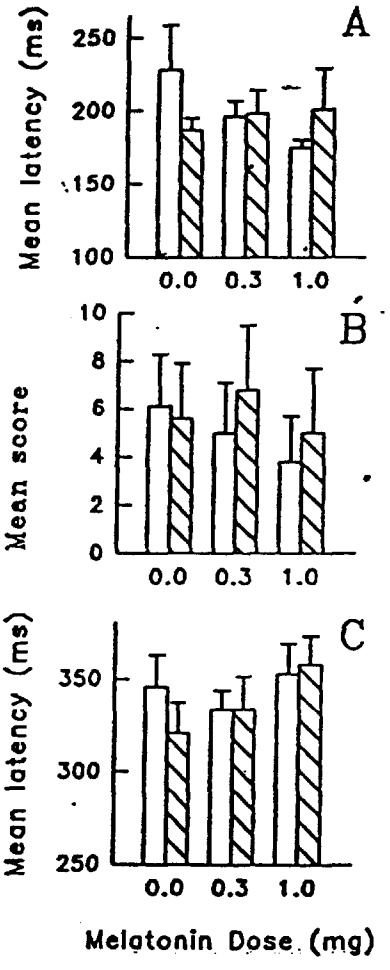

Fig. 3. Mood and performance on the morning after melatonin treatment: Simple Reaction Time (A); Profile of Mood States (fatigue/inertia scale) (B); and Four Choice Reaction Time, measured at 7:30 AM *(open bars)* or 9:30 AM *(striped bars)*.

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 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 anti-inflammatory 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.

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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.