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Human Melatonin During Continuous Magnetic Field Exposure

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This report describes the third in a series of double-blind, laboratory-based studies that were aimed at determining the effects of nocturnal exposure to power frequency magnetic fields on blood levels of melatonin in human volunteers. Our two earlier studies evaluated effects on melatonin of intermittent exposure to 60 Hz circularly polarized magnetic fields at 10 and 200 mG. No overall effects on melatonin levels were found. In the present study, men were exposed continuously rather than intermittently through the night to the same 200 mG magnetic field condition that was used previously; again, no overall effects on melatonin levels were found. We conclude that the intermittent and continuous exposure conditions used in our laboratory to date are not effective in altering nocturnal blood levels of melatonin in human volunteers. *Bioelectromagnetics 18:166–171, 1997.* © 1997 Wiley-Liss, Inc.

Key words: extremely low frequency; electromagnetic field; human; neuroendocrine; cancer

INTRODUCTION

Melatonin is a hormone that is secreted primarily at night by the pineal gland in the brain. Various reports suggest that it may be part of the body's natural defenses against cancer [Maestroni, 1993; Reiter, 1988; Wilson et al., 1990]. Animal studies, although they are not always consistent, suggest that nocturnal melatonin levels may be suppressed by electric or magnetic field (EMF) exposure [see, e.g., Kato et al., 1993; Lerchl et al., 1990], and this relationship has been proposed as a possible biological mechanism to account for epidemiological reports linking chronic EMF exposure and increased cancer risk [Stevens, 1987]. Research was needed to determine whether a similar suppression of melatonin occurs when humans are exposed to magnetic fields at night.

We recently performed two double-blind studies to determine whether blood levels of melatonin are reduced when people are intermittently exposed to magnetic fields at night [Graham et al., 1994a, 1995, 1996]. In study 1, 33 men were exposed either to sham or to 10 or 200 mG magnetic fields from 2300 to 0700 h under controlled environmental and exposure test conditions. Exposure to the circularly polarized magnetic field during the night was intermittent (1 h off/1 h on, with the field switched on and off every 15 s throughout each of the 1 h on periods). Overall, exposure had no effect on melatonin levels. However, men with preexisting low levels of melatonin did show significantly greater suppression of melatonin when they were exposed to light and also when they were exposed to the 200 mG magnetic field condition.

Study 2 was designed to examine the reproducibility of these results and to test directly the hypothesis that men with preexisting low levels of melatonin show greater suppression when exposed to light and to 200 mG magnetic fields. This study used a more powerful experimental design and included a larger sample of volunteers. Forty men were screened for peak melatonin level and light sensitivity prior to exposure. Each man then slept in the exposure facility on two nights. On one night, the men were sham exposed. On the other night, they were exposed to the same 200 mG field condition used in the previous study. Sham- and field-exposure sessions were counterbalanced. Blood samples, as before, were obtained each hour for melatonin analysis. Once again, exposure had no overall effect on melatonin levels. The original finding of enhanced sensitivity in low-melatonin subjects, however, failed to replicate in this study. Melatonin levels in field- and sham-exposure conditions were not different.

Intermittent field exposure was selected for use in the above two studies because of its effectiveness in altering human physiology in earlier work performed

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in our laboratory [Graham et al., 1990]. Given the paucity of studies on melatonin, however, arguments could have been made just as easily for the selection of other exposure conditions. Determination of the possible effects on melatonin of exposure to continuous magnetic fields is a test condition of obvious relevance and interest, because this type of exposure is more characteristic of the transmission line environment, with its greater stability and slower fluctuating fields [Heroux, 1987; Vinh et al., 1991].

In this report, we describe a study to evaluate the effects of continuous magnetic field exposure on nocturnal melatonin levels in human volunteers. The study followed the design and procedures of study 2 above. The direct parallels between the two studies enabled us to determine whether any of the effects found differed as a function of the type of exposure experienced by the subject.

MATERIALS AND METHODS

Experimental Design

When suppression of melatonin has been observed in animal exposure studies, it is typically in the order of a 25–30% reduction. Power analysis indicated that a sample of 40 volunteers would provide statistical power greater than .80 of detecting a similar degree of suppression in melatonin in humans at the .05 level of significance. The 40 men who participated in the study were initially prescreened for basal peak melatonin level (blood level at 0200 h) and sensitivity to bright light. Subjects were then randomly assigned to the two counterbalanced orders of sham and field exposure, and all test sessions were conducted double-blind. During one test session, each man was sham exposed from 2300 to 0700 h; in the other test session, each man was exposed to the continuous magnetic field. Hourly blood samples were collected for melatonin assay.

Subjects

Volunteers participated in the study after review and approval by the Human Subjects Committee at the Midwest Research Institute (MRI). Volunteers were screened to assure that they met specific criteria for participation (male, ages 18–35 years, no chronic disease or disability, no recent serious acute illness, not on medication, regular sleep habits, no night work). The test sample consisted of 40 young (mean = 24 years; range, 18–35 years), white (100%), nonsmoking (90%) men of average height (71.2 inches; range, 66–77 inches) and weight (176 pounds; range, 133–240 pounds) who typically slept 7-8 h per night.

Exposure Facility

The study was performed in the MRI Human Exposure Test Facility. The characteristics and control systems of this facility have been documented and are described in Cohen et al. [1992] and in Graham et al. [1994a]. A systematic protocol using test instruments with National Institute of Standards and Technology (NIST) traceability was followed to verify the exposure characteristics of the facility and to calibrate the recording equipment.

Subjects were exposed to a uniform (4–7%) circularly polarized 60 Hz magnetic field that was generated in the facility by six Helmholtz coils surrounding the exposure room in both the vertical and horizontal axes. The horizontal field axis was oriented from the doorway to the rear of the exposure room, and the vertical axis was oriented from floor to ceiling. Each field axis was independently energized from an adjustable autotransformer. The horizontal field current was shifted from the vertical field current by a phase angle of 90°. Subjects slept on a cot in the facility with their bodies oriented in line with the horizontal field component (north to south). The geomagnetic field density in the facility was 38 μT (380 mG) at an inclination of 78.7° north. Illumination in the facility was provided by incandescent lamps located above the translucent ceiling panels and was maintained at less than 10 lux during test sessions. Illumination levels were measured by using a Digital Photometer (model J16; Tektronix, Beaverton, OR).

Measures

Hourly melatonin concentrations measured in plasma provided the primary outcome measure of the study. Blood was drawn via intravenous catheter into EDTA tubes and immediately centrifuged. The aliquots were frozen at −20 °C for later blind assay using the melatonin radioimmunoassay kit distributed by American Laboratory Products Company, Ltd. (ALPCO; Windham, NH). This assay, which uses a double-antibody RIA based on the Kennaway G280 anti-melatonin antibody, is capable of measuring melatonin in concentrations as low as 0.5 pg/mL. Assay variability in our hands was slightly less than that reported by the manufacturer. Intra-assay coefficient of variation (CV%) ranged from 1.9 to 8.6%. Inter-assay variability was 5.4% for high standard and 11.3% for low standard.

Adequacy of the double-blind control procedures was assessed by using the Field Status Questionnaire (FSQ). The FSQ was independently completed by the subject and the experimenter in the morning after each test session. Each responded to same three questions: In your judgment, were the fields on or off? How con-

fident are you of this judgment (a 1–5 scale)? What are you basing this judgment on?

PROCEDURES

Preexposure screening procedures followed those described previously [Graham et al., 1996]. The purpose of the screening sessions was to measure each individual's basal peak melatonin level as well as the decrease in melatonin induced by controlled exposure to bright light (sensitivity). Groups of three or four subjects were scheduled for each night session. Each subject slept in the dark from 2230 to 0200 h, at which time a blood sample was obtained via venipuncture. The men then sat before a bank of fluorescent lighting (24, 4 foot, 40 W Sylvania Cool White tubes) for 60 min at a measured level of 5500 lux. A second blood sample was collected at 0300 h. The 0200 h blood samples provided a measure of basal peak melatonin level prior to exposure, and the 0300 h samples obtained after light exposure provided information about each individual's sensitivity to the known suppressor of melatonin.

Each subject then participated in two exposure test sessions. The identical protocol was followed in each session. On arrival at the laboratory at 2200 h, the subject changed into a surgical scrub suit, his vital signs were recorded, and the indwelling catheter for the collection of multiple blood samples was inserted in an arm vein. The subject got into bed in the exposure room, and the first blood sample was obtained at 2255 h. The double-blind/field control system was activated at 2300 h. The subject remained in bed in the facility until 0700 h. On sham test nights, the control system did not energize the field-generating equipment. On exposure test nights, the field-generating equipment was energized to present the 200 mG magnetic field continuously until 0700 h. The nurse entered the exposure facility each hour to collect blood samples. These collections occurred between 5 min before the hour and on the hour through the night. In the morning, the subject and the experimenter completed the FSQ.

STATISTICAL ANALYSIS

Statistical analysis tested the hypothesis that exposure to continuous magnetic fields has no effect on melatonin levels. Hourly melatonin values were entered into a repeated measures analysis of variance with order of exposure (field-sham, sham-field) and basal peak melatonin level as between-subject variables and with field condition as a within-subjects variable. The dependent variable was the melatonin level at each hourly blood sample. Alpha was set at $P < .05$. Multiple regression analysis was used to predict melatonin levels at 0700 h from age, field condition, basal peak melatonin level, and percent suppression by light. The multiple regressions were calculated separately for sham-control and exposure conditions. Data from the screening session were used to test the hypothesis that exposure to bright light results in suppression of melatonin that is proportionately greater for men with low melatonin levels than for those with high levels. Multiple regression and correlational techniques were used for this purpose. Where analysis of variance for repeated measures was used, probability values were corrected by using the Huynh-Feldt epsilon technique. The probability levels reported here are the corrected ones; the degrees of freedom (df) are not corrected to indicate the actual number of data points included in the statistical tests.

RESULTS

Double-Blind Control Measures

Analysis of the FSQ rating data indicated that the subjects were unable to judge whether the fields were on at better than chance levels ($\chi^2 = 1.27$; df = 1; $P > .20$). This was true even when subjects had strong confidence in the accuracy of their judgments. Experimenters also were unable to judge field status at better than chance levels ($\chi^2 = 0.80$; df = 1; $P > .30$). These results confirm the effectiveness of the double-blind control procedures.

Temperature and Humidity

Mean temperature was 74.1° F (S.E. .12), and mean humidity was 35.5% (S.E. .66). No overall significant differences in temperature or humidity were found as a function of exposure condition or time. Temperature changes over the night, however, were slightly different for the two exposure conditions; temperature decreased from 74.1 to 73.7 °F under sham conditions and increased from 74.1 to 74.4 °F under real field conditions.

Melatonin Concentration

Preexposure screening Light exposure had the expected suppressant effect. At 0200 h, mean melatonin level was 60.5 pg/mL (S.E. 5.4); after 1 h of light exposure, melatonin levels were significantly reduced to 26.2 pg/mL (S.E. 2.5; F = 215.03; df = 1,36; $P < .0001$). Melatonin levels at 0200 h ranged from 11.7 to 147.9 pg/mL. Figure 1 shows the broad range of individual peak melatonin levels observed during preexposure screening. Figure 2 shows the range of suppression effects on melatonin found when the men were

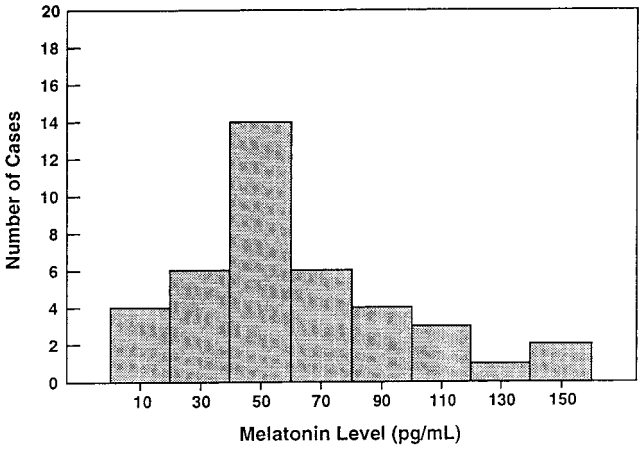

Fig. 1. Preexposure peak melatonin levels. Study volunteers exhibited the typical wide range of variation observed in peak blood concentrations of melatonin.

exposed to light at night. Percent suppression (0200 h value − 0300 h value ÷ 0200 h value) ranged from 29 to 79%, with the majority of subjects showing reductions of 50–60% in melatonin levels. No differences in suppression were found between subjects with high melatonin at 0200 h and those with low melatonin at 0200 h (median split.)

Experimental testing One of the 40 subjects was dropped from analysis, because it was not possible to obtain a blood sample at 0700 h. Figure 3 shows the mean levels of melatonin every hour from midnight to 0700 h for both sham-control and field-exposure sessions. The error bars indicate the standard error of the mean. The distribution of melatonin values over the night was as expected. Values began to increase rapidly in the early night, peaked about 0300 to 0500 h, and then declined. The distributions seen under sham and field exposure were very similar, and there was a high degree of overlap in the variances. Sham- and field-exposure nights were not significantly different from one another ($F = 0.09$; $df = 1,37$; $P > .75$).

To determine whether men with preexisting low levels of melatonin were significantly affected by field exposure, subjects were divided into two groups at the median peak melatonin value obtained at 0200 h in the preexposure screening session. The ''low'' group had values less than or equal to 55 pg/mL, and the ''high'' group had values greater than 55 pg/mL. These groups, as expected, differed significantly in melatonin levels ($F = 20.73$; $df = 1,37$; $P = .0001$). Melatonin values also showed the typical change pattern over the night as expected, peaking at 0400 h ($F = 25.05$; $df = 7,259$; $P < .0001$). There was a trend for an interaction between melatonin group and time of night ($F = 2.61$; $df = 7,259$; $P < .08$). This interaction indicated that the high group had a sharper increase in blood levels of melatonin in the early hours and a sharper decrease in late hours of the night than did the low group. Both groups, however, peaked at the same time of the night. Low- and high-melatonin groups did not show different responses to field exposure (melatonin Group × Field × Hour interaction: $F = 0.47$; $df = 7,259$; $P < .75$).

Prediction of morning melatonin level In our earlier work [Graham et al., 1994a], age, basal peak melatonin level, and percent suppression by light exposure significantly predicted an individual's morning melatonin level. Of these, age appeared to be the most potent predictor. In the present study, these factors were used again in a multiple regression analysis to predict melatonin levels at 0700 h. Separate regressions were performed for sham- and field-exposure test sessions. After field-exposure nights, basal peak melatonin level and age explained 55% of the variance in 0700 h melatonin level. After sham-exposure nights, the same variables predicted 46% of the variance in 0700 h melatonin. Percent suppression did not contribute significantly to prediction of melatonin levels at 0700 h under either sham- or field-exposure conditions.

DISCUSSION

Public concern has increased about the possible health risks associated with exposure to power-frequency EMFs. Laboratory-based studies aimed at elucidating possible mechanisms by which cancer risk

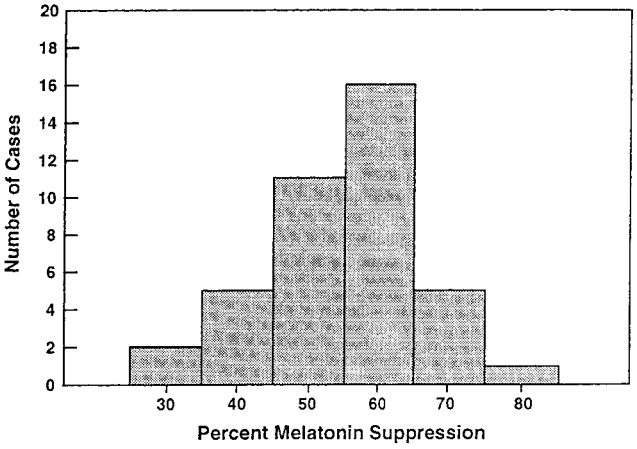

Fig. 2. Light-suppression effects on melatonin. Light exposure (0200–0300 h, 5,500 lux) had the expected suppressant effect on nocturnal blood concentrations of melatonin. Percent suppression ranged from 29 to 79%, with most volunteers showing reductions of from 50 to 60% in basal melatonin levels.

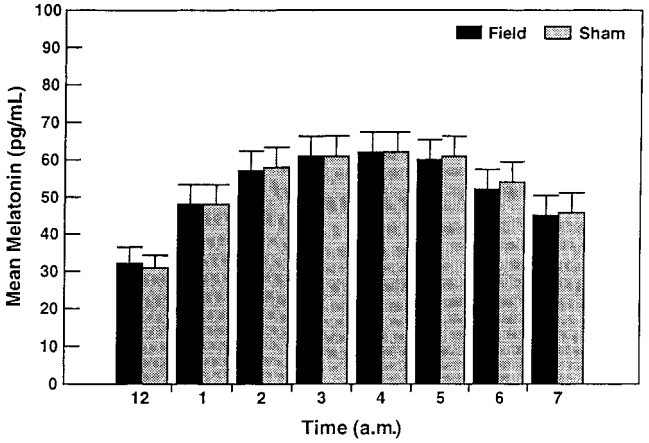

Fig. 3. Melatonin levels during sham and field exposure. Mean blood concentration of melatonin and standard error are plotted for sham control and magnetic field exposure conditions at each hour from midnight to 0700 h. Melatonin levels in the sham and exposed condition did not differ.

could be increased by such exposure are of the highest priority. Field-related suppression of nocturnal levels of the hormone melatonin is one of the mechanisms now receiving increasing research interest. The work reported here addressed public concern by testing research questions derived from the melatonin hypothesis and, by doing so in humans, the species of greatest interest. The present study evaluated the effects of continuous magnetic field exposure, and it followed the design and procedures of our previous study of intermittent exposure. The direct parallels between the two studies enabled us to determine whether the type of exposure a person experiences has differential effects on nocturnal melatonin levels. Like the previous intermittent exposure study, continuous exposure had no overall effect on melatonin levels, and no evidence was found for enhanced sensitivity in low-melatonin subjects. We conclude that the intermittent and continuous exposure conditions used in the above studies are not effective in altering nocturnal melatonin-release patterns in human volunteers.

In an industrialized society, magnetic field exposures from power distribution systems consist of relatively few components: the 50 or 60 Hz fundamental power frequency that is present at all times; harmonics; and transient events that result from normal utility operations, such as switching events, opening or closing of capacitor banks, etc. Transients can also result from the switching on and off of loads, whether residential or industrial. Recently, transients with a high-frequency content (ranging from several kilohertz to 10 megahertz) have been measured in homes [Guttman et al., 1993]. Biophysical calculations using realistic models of cells have shown that some of these transient events can induce transmembrane voltage changes in model cells that exceed thermal noise [Sastre et al., 1994; Vaughan and Weaver, 1994]. Therefore, these events, in principal, can affect biological systems. The effects of these fields, which are relevant both to power distribution systems and to the use of common electrical appliances, need to be examined in humans.

Similarly, brief daytime exposure to magnetic fields has been reported to produce alterations in nocturnal melatonin levels in rodents [Yellon and Hilliker, 1994]. In a recent field study of utility workers in Colorado, Reif et al. [1995] reported that daytime exposure to magnetic fields is associated with decreased nocturnal melatonin production. Such reports indicate the importance of further research to identify possible biologically relevant characteristics of the real world exposure environment as well as the need to determine whether such exposure alters nocturnal levels of melatonin in humans under controlled laboratory conditions.

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