Exposure to a 50-Hz Magnetic Field Induces a Circadian Rhythm in 6-hydroxymelatonin Sulfate Excretion in Mice

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Melatonin/6-OHMS/Mice/Magnetic field/Circadian rhythm.

The effect of magnetic field (MF) exposure on melatonin production was studied in female CD2F1 (BALB/c x DBA/2) mice. The mice were exposed to a 50 Hz MF at 100 µT for 52 days and nocturnal urine was collected 1, 3, 7, 14, 16 and 23 days after the beginning of MF exposure. The animal room was illuminated for 12 h daily at 200 lux. To study the circadian rhythm of melatonin production, night and day samples of urine were collected once, at about 40 days after the beginning of MF exposure. Urinary 6-hydroxy melatonin sulfate (6-OHMS) was determined to assess melatonin production. The pineal glands were analyzed for melatonin content at the middle of the dark period. No statistically significant peak of melatonin was observed in either group. The light-regulated natural melatonin rhythm was absent in sham-exposed mice. The MF exposure caused a significant day-night difference in the 6-OHMS levels, but did not affect the total excretion of 6-OHMS during the 24-hour period. A possible interpretation of the findings is that MF exposure increases the sensitivity of the pineal gland to light in this strain normally insensitive to the circadian light variations. Further studies on interaction of light and MF exposure might help in understanding the inconsistencies of earlier research on MFs and melatonin.

INTRODUCTION

Epidemiological studies indicate that extremely low frequency (ELF) magnetic fields (MFs) might increase the risk of cancer, especially childhood leukemia.1–3 The results of experimental studies on the cancer risk of ELF MFs are inconclusive.4,5 Several mechanisms have been offered to explain the proposed effects of ELF fields on carcinogenesis in vivo, including modification of melatonin secretion by the pineal gland.6,7 Melatonin is the main secretory product of the pineal gland. Its synthesis is driven by the biological clock in the suprachiasmatic nuclei and usually follows a circadian pattern, with maximal production occurring during the night. Light exposure at night reduces melatonin production.8 Melatonin is released rapidly into the blood from the pineal gland. Circulating melatonin is taken up and metabolized by the liver and eliminated mainly as 6-hydroxy melatonin sulfate (6-OHMS) in the urine. Urinary 6-OHMS excretion is thought to reflect the pineal production of melatonin,9,10 although this is not necessarily always the case.11 Melatonin has been reported to be an effective free radical scavenger and an antioxidant12 and it is also thought to have cancer-protective effects.13 Melatonin is understood to behave as an antitumoral agent through its antigonadotropic and antiestrogenic actions.14 Extremely low frequency magnetic fields have been reported to inhibit the nocturnal production of melatonin in rats5–17 and in hamsters.18 The decrease in melatonin levels has usually been moderate and in most of the experiments no effects have been shown in rats19,20 or in mice.21,22 Evidence of MF effects on nocturnal melatonin levels in humans23–27 is limited and inconsistent.

We have shown that long-term exposure of CD2F1 (BALB/c x DBA/2) mice to 50 Hz magnetic fields can facilitate the growth of skin tumors induced by simulated solar radiation.28 We have already investigated polyamine metabolism29 and apoptosis30 in skin as possible factors to explain this process. In the present study we tested the effects of a MF exposure on melatonin production in the same mouse strain.

MATERIALS AND METHODS

Study design

The study design, including MF exposures, urine collection periods and sampling of pineal glands is presented in Table 1.

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Animals and their maintenance
Sixty female hybrid CD2F1 (BALB/c x DBA/2) mice were used. The animals were produced in the barrier unit of the National Laboratory Animal Centre, University of Kuopio (Kuopio, Finland). They were 8 weeks old at the start of the MF exposures and were randomized to the exposure groups five days before the start. The animals were housed in polycarbonate cages (3 animals/cage) with lids made of steel wire. Granular aspen chips were used as bedding material (Tapvei Oy, Kaavi, Finland). Tap water and commercial rodent feed (Lactamin R36, Lactamin AB, Stockholm, Sweden) were available *ad libitum* during the exposures. During the urine collection periods, however, ground feed was given. The animal room was illuminated for 12 hours daily (7.00–19.00 h). The intensity of light in the animal room was around 200 lux during the daytime. During the first 4 nights there was uncontrolled dim light (max. 0.8 lux) coming from the corridor to the animal room. Thereafter, the windows were covered with black plastic sheet, so the room was completely dark during the nights. The room temperature was about 22°C during the experiment. The investigations were carried out in compliance with the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. The research plan was approved by the Research Animal Use Committee of the University of Kuopio.

Magnetic field exposure
The MF was produced by a system consisting of two paired exposure racks, with five coils in each to produce vertical MF. The dimensions of the coils were 0.4 × 1.2 m, and the vertical distances among the five coils were 0.25 m. The structures supporting the coils were made of wood. The 50 Hz current for the MF was taken from the 230 V power network through an adjustable transformer. The exposure system was described in detail by Kumlin *et al.*. Half of the mice were exposed to a vertical, sinusoidal 50 Hz MF at 100 µT and sham-exposed mice served as controls. The sham-exposed animals were in an identical but non-energized system in the same room. The 50 Hz MF exposure of the sham-exposed animals was less than 0.05 µT. The static (geomagnetic) flux density measured with a fluxgate magnetometer (JH-13, Fiskars Geoinstruments, Espoo, Finland) varied between 48 and 52 µT (inclination of 60–80°C) in the animal room. The animals were in the exposure system all the time for the 52 day-exposure period, except for the urine collection periods. They were removed from the exposure system also for the changing of water bottles, feed and cages.

Collection of urine
Nocturnal samples: To study the difference between nocturnal excretion of 6-OHMS, the animals were transferred into metabolic cages (3 animals/cage) at 18.30–19.00 for one night at 1, 3, 7, 14, 16 and 23 days after the beginning of MF exposure. The mice were removed from the cages the following morning at 8.30–9.00. The collection time was thus 14 hours during which the room was illuminated the last two hours.

Night and day samples: To study the difference between night and day 6-OHMS excretion, the animals were transferred into metabolic cages (3 animals/cage) for one night (19.00–7.00) and for one day (7.00–19.00). The timing of sampling varied from 36 to 43 days after the beginning of MF exposure.

The urine from 3 animals was collected as one sample. The volume and the weight of the excreted urine were measured. The urine samples were collected at room temperature and stored at –70°C.

Pineal gland samples
At the end of the study (after 52 days of exposure and about one week after the last collection of urine) the pineal glands were dissected for analysis of their melatonin content. The first pineal glands were taken six hours after darkness onset. The next pineal glands were taken at four time points every 15 min after the first sampling. Middle of the

Table 1. The study design

| Time frame          | Treatment                                                                 | Details                                                                 |
|---------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------|
| From day 1 to 52    | Magnetic field exposure 50 Hz, 100 µT sham-magnetic field                 | 24h/day, 7 days/week, except during the urine collection periods         |
| Days 2, 4, 8, 15, 17 and 24 | Urine collection nocturnal 6-OHMS level                              | 14-hour sample/cage (3 mice)                                           |
| Day 40 *            | Urine collection nocturnal 6-OHMS level day 6-OHMS level               | Two 12-hour samples/cage (night and day) to study the difference between night and day levels |
| Day 53              | Pineal gland sampling nocturnal melatonin level                         | In the middle of the dark period (6–7 hours after darkness onset)       |

* varied from day 37 to day 44
dark period (usually 6–7 hours after darkness onset) has been reported to be the time of the melatonin peak in mice.\textsuperscript{34)}

Three animals per group were killed at each of the five time points using a mixture of carbon dioxide and oxygen. The pineal glands were removed and frozen in dry ice under dim red light within 12 min after killing. One exposed and one sham-exposed mouse were operated simultaneously. The pineal glands were then stored at \(-70^\circ\text{C}.\)

**Determination of urinary 6-hydroxymelatonin sulfate**

Urine samples were assayed for 6-OHMS concentration using a direct radioimmunoassay\textsuperscript{31)} with rat urine 6-OHMS standards (Stockgrand Ltd, Surrey, UK), as previously described.\textsuperscript{11)} Validation experiments (not shown) indicated complete parallelism between mouse urine dilutions and rat urine 6-OHMS standards. The assay precision was evaluated as the within-assay (intra) variance and the between-assay (inter) variance from duplicate measurements of control sample containing 1.9 ng/ml 6-OHMS. For this sample, the intra- and inter-assay coefficients of variation were 5.8 and 16.6%, respectively (n = 6 assays). The normalized concentration of 6-OHMS was determined as 6-OHMS/creatinine (ng/mg). Creatinine is considered to be an appropriate reference for 6-OHMS because the excretion of creatinine stays constant in healthy animals.\textsuperscript{32)}

**Determination of pineal melatonin content**

Individual pineal glands were homogenized by sonication in 0.5 ml ice-cold phosphate-buffered saline, pH 7.0 (NaH\(_2\)PO\(_4\), 3.33 mmol/l; Na\(_2\)HPO\(_4\), 6.7 mmol/l; NaCl 140 mmol/l; Na\(_2\)S, 23 mmol/l) containing gelatine (1 g/l). The homogenate material was centrifuged at 14000 g for 10 min at 4\(^\circ\text{C}\) and the supernatant (0.1 ml in duplicates) was assayed for melatonin using a specific radioimmunoassay with the GS/707-8483 antiserum (Stockgrand Ltd, Surrey, UK) and tritiated melatonin tracer, essentially as previously described.\textsuperscript{11,33)} The assay precision was evaluated as the within-assay (intra) variance and the between-assay (inter) variance from duplicate measurements of control sample containing 21 pg/ml melatonin. For this sample, the intra- and inter-assay coefficients of variation were 2.7 and 2.0%, respectively (n = 2 assays).

**Data analysis**

The normality of the parameter distributions was tested by the Kolmogorov-Smirnov and Shapiro-Wilk- tests, using SPSS for Windows v. 8.0.1. (SPSS Inc. Chicago, IL, USA). No statistically significant deviations from normality were seen. The differences between MF-exposed and sham-exposed animals in the nocturnal 6-OHMS levels were evaluated using two-way ANOVA (mixed model, repeated measures analysis by time). The differences between day and night values were studied by paired and unpaired t-tests. ANOVA and t-tests were performed using GraphPad Prism v.4.02 for Windows (GraphPad Software, San Diego, CA, USA).

**RESULTS**

**Nocturnal excretion of 6-OHMS in MF-exposed and sham-exposed mice**

There was no statistically significant difference in the nocturnal 6-OHMS levels between the MF-exposed and sham-exposed mice (p = 0.174; ANOVA, two way, repeated measures) (Fig. 1), although the values were slightly higher in the MF-exposed animals.

**Day and night excretion of 6-OHMS**

No difference between day and night 6-OHMS excretions (1.3 ng/mg creatinine in both) was found in the sham-exposed mice (Fig. 2). In MF-exposed mice, however, the nocturnal levels of 6-OHMS (1.7 ng/mg creatinine) were significantly higher than the day levels (0.9 ng/mg creatinine).

![Fig. 1. Nocturnal excretion of 6-OHMS in magnetic field-exposed and sham-exposed mice. Each bar shows mean \(\pm\) S.E.M. of 10 urine samples, 3 mice/sample.](https://example.com/fig1)

![Fig. 2. Day and night excretion of 6-OHMS in sham-exposed mice, 3 animals/cage. Average values are given as mean \(\pm\) S.E.M.](https://example.com/fig2)
The pineal melatonin content in MF-exposed and sham-exposed mice

It is apparent that there were no statistically significant differences in the pineal melatonin content between the different time points in the middle of the dark period in the MF-exposed mice (Fig. 4a). In the sham-exposed animals the peak was even less clear (Fig. 4b).

DISCUSSION

The production of melatonin appeared to be weak in the presently used CD₂F₁ mice and the midnight levels (below 15 pg/pineal gland) are comparable to earlier findings in inbred mice.³⁴,³⁵ No statistically significant melatonin peak was seen. This may be selective factor in the development of laboratory strains of mice because melatonin can inhibit reproduction and modifies circadian rhythmicity.³⁶,³⁷ Roseboom et al.³⁷ found that C57BL/6J mice have a natural mutation in serotonin N-acetyltransferase gene causing a ‘knockdown’ in melatonin synthesis. Similar mutations may explain the decreased melatonin production also in other strains of mice. Some researchers have even stated that domesticated inbred strains of mice, such as BALB/c, AKR and C57/6J have no detectable melatonin production in their pineal gland.³⁸,³⁹ These findings are probably due to the long sampling intervals (3–4 hours) or insufficient assay sensitivity in these studies. In later reports melatonin production and its nocturnal peak have been detected but the duration of the peak has been very short, only 15–30 minutes.³⁴,³⁵ This means that very short sampling intervals are needed to detect the peak. We did not find statistically significantly different melatonin contents between the collection time points, although it seems visually (Fig. 4a) that there may be a small peak of melatonin production about 7 hours after the lights are turned off.

The light-regulated natural rhythm of melatonin was absent in sham-exposed CD₂F₁ mice when measured as an excreted 6-OHMS in day and night urine samples. Exposure to 50-Hz MF seemed to cause a rhythm in the 6-OHMS excretion, however, so that the excretion was depressed at daytime and increased at night. A possible explanation to this finding is that MF exposure enhances the sensitivity of the pineal gland to circadian light rhythm. It may be hypothesized that MF exposure stimulates pineal cells so that they are more receptive to signals from the biological clock. Another possibility is the stimulation of retinal photoreceptors so that stronger signals of light variations reach the pineal gland. According to studies reported by Olcese et al.⁴⁰,⁴¹ and Reuss and Olcese,⁴² retinal photoreceptors of rats might be capable of responding to a magnetic field. Reuss and Olcese⁴² reported that exposure to dim red light at night is a necessary predisposing factor for magnetic fields to inhibit the melatonin production in the mammalian pineal gland. These findings suggest that the MF signal goes to the pineal gland through the eyes. Recently, exposure to a 60 Hz MF has been reported to modulate the diurnal rhythm of the pain threshold in mice.⁴³ The authors concluded that MFs are acting on the system that is associated with the environmental light-dark cycle. Thoss et al.¹⁴ reported changes in oscillations of visual sensitivity in human volunteers after artificial changes in the direction of earth’s magnetic field.

Most of the animal studies investigating MF effects on melatonin have used rats and only few researchers²¹,²²,⁴³ have used mice. As far as we know, this is the first time when...
effects of ELF MFs on melatonin production in mice have been reported. Earlier studies using rodents have shown mainly inhibiting effects on melatonin production in rats, whereas we found an enhancing effect on the circadian rhythm of melatonin production in a strain of mice that has no or very weak natural melatonin rhythm.

Our findings do not support the reported reduced melatonin production in MF-exposed animals, but nevertheless suggest effects of MF on the pineal gland. The results are consistent with earlier studies reporting that light and MFs interact in producing changes in melatonin secretion. Uncontrolled and consequently varying light conditions could also possibly explain at least part of the inconsistencies in earlier studies on MFs and melatonin. We conclude that further studies of MF effects on melatonin production in animals and humans might be more productive, if they focus on possible interaction of light and MF exposure.

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