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Evidence for an Effect of ELF Electromagnetic Fields on Human Pineal Gland Function

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A study was carried out to determine possible effects of 60-Hz electromagnetic-field exposure on pineal gland function in humans. Overnight excretion of urinary 6-hydroxymelatonin sulfate (6-OHMS), a stable urinary metabolite of the pineal hormone melatonin, was used to assess pineal gland function in 42 volunteers who used standard (conventional) or modified continuous polymer wire (CPW) electric blankets for approximately 8 weeks. Volunteers using conventional electric blankets showed no variations in 6-OHMS excretion as either a group or individuals during the study period. Serving as their own controls, 7 of 28 volunteers using the CPW blankets showed statistically significant changes in their mean nighttime 6-OHMS excretion. The CPW blankets switched on and off approximately twice as often when in service and produced magnetic fields that were 50% stronger than those from the conventional electric blankets. On the basis of these findings, we hypothesize that periodic exposure to pulsed DC or extremely low frequency electric or magnetic fields of sufficient intensity and duration can affect pineal gland function in certain individuals.

Key words: melatonin, electric blankets, electric field, magnetic field

INTRODUCTION

During the past two decades, interest has increased in the possibility that exposure to static or extremely low frequency (ELF: 10–100 Hz), including 50- or 60-Hz powerline-frequency electric and magnetic fields, may cause biological effects in human populations [Savitz and Calle, 1987]. Much of our work has been directed toward understanding the association between ELF electric- and

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magnetic-field exposure and alterations in pineal gland circadian rhythms [Wilson et al., 1989].

Melatonin (N-acetyl-5-methoxytryptamine), the principal hormone of the pineal gland, is produced by the action of N-acetyltransferase (NAT) and hydroxyindole-O-methyl transferase (HIOMT) on serotonin [Deguchi and Axelrod, 1972]. Melatonin concentrations normally increase during the hours of darkness in both the pineal gland and circulating blood. Maximum melatonin concentrations occur between approximately 0200 and 0400 h in humans. In all mammals, the internal clock that helps generate this pineal circadian rhythm resides in the suprachiasmatic nuclei. The pineal is richly innervated by fibers of the superior cervical ganglia (SCG) [Moore et al., 1968] as well as by fibers originating in the hypothalamus and optic regions of the brain [Zisapel et al., 1988]. Neuronal input from the eyes acts via the SCG as the principal regulator of the melatonin circadian rhythm in the pineal.

Light of sufficient intensity is effective in suppressing melatonin synthesis in many animals [Wurtman et al., 1963]. Lewy et al. [1982] reported that the light level required for suppression in humans is approximately 2,500 lux. It appears that the pineal gland of certain sensitive individuals, however, may respond to light levels as low as 200 lux [McIntyre et al., 1990]. Ingested alcohol [Wetterberg, 1978], β-adrenergic receptor-blocking drugs such as propranolol [Wetterberg, 1979], and certain kinds of stress [Troiani et al., 1987] have also been reported to reduce melatonin concentrations in the pineal and circulation of rats. Further, altering melatonin circadian rhythms by use of bright light has been effective in the treatment of seasonal affective disorder syndrome (SADS) [Lewy et al., 1987].

In the circulation, melatonin acts to suppress the function of several other endocrine glands, including the gonads. Melatonin also suppresses the growth of certain cancers in both in vitro and in vivo models [Blask, 1990]. Reduction in melatonin secretion has been associated with estrogen receptor-positive breast cancers [Sanchez Barcelo et al., 1988] and prostate adenocarcinoma [Buzzell et al., 1988]. Stevens [1987] proposed that, should there be increased cancer risk from ELF electromagnetic-field exposure, such risk may be a consequence of altered pineal gland function.

Chronic exposure to 60-Hz electric fields can reduce the normal nocturnal rise in both pineal NAT activity and melatonin concentration in laboratory rats [Wilson et al., 1981, 1983]. In 23-day-old rats maintained in a 60-Hz electric field for 20 h/day from conception, there was no difference among the pineal melatonin levels of animals exposed to field strengths of 10, 60, and 130 kV/m. Compared to controls, however, these exposed rats showed an approximate 40% reduction in maximal nighttime pineal melatonin levels and an approximate 1.4-h delay in the occurrence of the nighttime melatonin peak [Reiter et al., 1988]. Rats first exposed at 55 days of age to a 39-kV/m electric field showed no statistically significant difference between daytime and nighttime levels of pineal melatonin [i.e., no circadian rhythm in melatonin secretion) after 21 days of exposure. Within 3 days after cessation of ELF electric-field exposure, however, strong pineal melatonin rhythms were reestablished. This effect appeared to be an "all-or-none" response to electric fields between approximately 2 and 130 kV/m [Wilson et al., 1986].

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MATERIALS AND METHODS

Exposure Systems

Both conventional electric b
electric blankets were used. The
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Indeed, an accumulating body of data suggests that ELF electric- and
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marked changes in pineal serotonin metabolism in rats and mice expo
intermittent magnetic fields at night, but no such changes were observe
consequence of daytime exposure. Wever [1968] reported that expos
50-Hz electric or magnetic fields can act as a "zeitgeber," arresting the l
ening of the circadian cycle that normally occurs when humans are depri
temporal cues. However, we know of no direct experimental evidence th
electromagnetic-field exposure can affect human pineal gland function.

We have completed a study to determine if domestic ELF electri
magnetic-field exposure from using electric blankets could affect pineal
tonin secretion in humans. Use of electric blankets represents a periodic
sure to ELF fields that normally occurs at night when the pineal is most
Exposure to electric blankets, as used in this study, did not require altera
the normal lifestyle or daily routine of the subjects. To assess possible c
in pineal melatonin secretion, we determined overnight urinary 6-hy
melatonin sulfate (6-OHMS) excretion in healthy adult human voluntee

MATERIALS AND METHODS

Exposure Systems

Both conventional electric blankets and continuous polymer wire
electric blankets were used. The heating element of CPW blankets co
two parallel conductors separated by a resistive polymer material. Curre
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to temperature at any point along the element. This feature eliminates t
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vides some degree of auto temperature control. CPW blankets were
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blankets should have little or no effect on pineal gland function. (Af
studies were completed, however, Lerchl et al. [1990] showed that int
DC magnetic fields can indeed affect pineal gland function in rats.)
safety switches in the conventional electric blankets tested tended to a
DC power at temperatures greater than about 140°F. This arcing const
unacceptable fire hazard, and hence these blankets were deemed unsu
use with DC power.

Modifications to the CPW blankets consisted of power supplies
constructed in grounded metal boxes that could fit near, or under th
the bed. AC and DC power supply boxes could not be distinguishec
appearance or weight, and both types allowed use of the bedside ter
controllers that the manufacturer supplied with the blankets. Blanket
ture control units were dimly lit by an internal bulb that was the sam

Table 1. Measured Steady-State Magnetic Field Values[a] Generated at 10-cm Distance by Continuous Polymer Wire (CPW) Blanket in AC and DC Power Modes and by Conventional Electric Blanket in AC Power Mode

	Head	Chest	Knees
Background	0.78	0.89	0.84
Conventional	2.4	4.4	5.6
CPW (AC)[b]	4.2	6.6	5.6
CPW (DC)[b]	0.56	0.56	0.57

[a]Values are in milligauss (measured approximately 10 cm from blanket surface).
[b]Values were four to five times greater during warmup.

CPW and conventional electric blankets. When both husband and wife were participating in the study, a larger power supply was used to accommodate the individual temperature controllers for both sides of the bed. Subjects were not informed as to whether their blankets were powered by AC or DC at any given time. Nonfunctional (sham) power supply boxes were provided for use with the conventionally wired blankets.

Subjects

Volunteer subjects in the study consisted of 32 healthy, nonpregnant, pre-menopausal women and 10 healthy men. Male and female participants were randomly divided into three groups. Each of the groups provided early evening and morning urine samples for 2 weeks (period 1—preexposure) before beginning exposure. When exposure began, group 1 (n = 12 women, 2 men) slept nightly for 4 to 5 weeks (period 2) under AC-powered CPW blankets. Group 2 (n = 10 women, 4 men) used DC-powered blankets in the same manner. After 4 to 5 weeks of exposure, power modes on the blankets for groups 1 and 2 were switched, and exposure continued for an additional 4 to 5 weeks (period 3). Because of differences in the fields produced by AC-powered CPW and conventional electric blankets (Table 1), one group of 14 volunteers (group 3: n = 10 women, 4 men) used AC-powered, conventionally wired blankets for a total of 7 weeks of exposure. Urine samples were also collected from all three groups for 2 weeks (period 4) after cessation of exposure.

Because of the anticipated large variation in melatonin excretion among individuals, the study was designed so that volunteers would act as their own control. The study population was selected from residents of southeastern Washington State, a region centered around 46°15′ N latitude. At this latitude, winter solstice sunrise was at 0739 h and sunset at 1613 h. To control for possible changes in melatonin secretion arising from differences in the hours of daylight [Bojkowski and Arendt, 1988], study periods 1 and 2 were contiguous and ended just before the winter solstice. Periods 3 and 4 were contiguous and began just after the winter solstice. Because of the time required to change blanket power modes, there was essentially no break in exposure between periods 2 and 3.

The measure for assessing possible effects from ELF electromagnetic-field exposure was pineal gland function, as determined by radioimmunoassay (RIA) of urinary 6-OHMS. 6-OHMS is a stable metabolite of melatonin, and its levels in

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expressed as nanograms of 6-OHMS
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for further statistical analyses.

Assay for Urinary 6-Hydroxymelat

Urinary 6-OHMS excretion wa
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Statistical Analysis

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urine reflect pineal melatonin secretion over time [Arendt, 1986]. The sample collection method did not allow gathering of information on possible temporal shifts in the melatonin peak that might occur in the time span between the last urine voiding before retiring and the first morning urination.

Volunteers provided a set of two samples, a late afternoon/early evening urine (generally around 1700 h) and the first morning void urine (generally between 0600 and 0700 h), three times each week during the study. Samples taken in the late afternoon/early evening were used as controls for the morning void urine, which was used to assess overnight melatonin excretion. Volunteers recorded the clock time of last urination before retiring (urine not retained), as well as that for the evening and morning urine samples. Samples were refrigerated by the volunteers immediately after collection, picked up three times per week, and processed in the lab within a few hours of pickup. Total urine volumes were measured and recorded; three sets of aliquots (5 ml each) were then taken, one for analysis by RIA, one for creatinine determination, and one to be held for archival purposes. In total, more than 2,400 primary urine samples were collected and analyzed by RIA. Levels of 6-OHMS were normalized to creatinine content and to urinary volume and time. Excreted melatonin levels were thus expressed as nanograms of 6-OHMS per milliliters urine/hour, or as nanograms of 6-OHMS per milligram of creatinine; the measures were essentially equivalent. Cretainine normalization yielded lower variance and was therefore used for further statistical analyses.

Assay for Urinary 6-Hydroxymelatonin Sulfate

Urinary 6-OHMS excretion was determined using an RIA kit supplied by CIDtech Research Inc. [Mississauga, Ontario, Canada]. The assay is a modification of that described by Arendt [1986] in which 6-OHMS is iodinated with ^{125}I using a method adapted from Vakkuri et al. [1984]. The iodinated material (suspended in methanol) was separated on cellulose F thin-layer chromatography plates using a butanol, water, and acetic acid solvent (4:1.5:1). Measurements in unknown samples were based on a standard curve using known amounts of 6-OHMS antigen (0–200 pg/ml) diluted in stripped urine. The effective working range for the assay (linear portion of the curve) was between 0.5 and 100 pg/ml. Within-assay variance among triplicate samples averaged 9.5%; between-assay variance was 14%. Samples were run in triplicate at two or three different dilutions. Daytime urines were diluted between 50:1 and 250:1 and nighttime urines between 2000:1 and 8000:1.

Statistical Analysis

Results of daytime and nighttime 6-OHMS measurements were compiled for each subject and for the three groups of subjects during the study. All statistical analyses were performed on overnight 6-OHMS measurements. Data for each group were analyzed separately because of the significant difference in the measured preexposure urinary 6-OHMS excretion of groups 1 and 2, and the delay in the start of exposure of group 3.

Nested analysis of variance was used to test the hypothesis that the 6-OHMS means of preexposure, AC exposure, DC exposure, and postexposure

periods are equal for each group [Winer, 1971]. A subject within-period error term was used to test this hypothesis. A natural logarithmic transformation of the data was made before the analyses to achieve homogeneity of variances. Data for each subject were analyzed independently by one-way analysis of variance to test the hypothesis that the 6-OHMS means of the four periods were equal for that subject. The measurement within-period error term was used to test the hypothesis. Differences among means were delineated using the least-significant-difference test [Fisher, 1949]. Again, a natural logarithmic transformation of the data was made before the analysis to achieve homogeneity of variances. Also, the nonparametric procedure known as the sign test [Siegel, 1956] was used to evaluate the direction of the differences between pairs of period means for each subject and for each group of subjects. All statistical hypotheses were tested at the 0.05 level of significance. The general linear model (GLM) procedure from Statistical Analysis System (SAS, 1985) was employed for analysis of variance.

Electric Blanket Magnetic and Electric Fields

Magnetic fields associated with the CPW and conventional electric blankets were measured on three orthogonal axes using a Deno[1] meter magnetic-field measuring device. The blankets were suspended from the ceiling for these measurements. Instrument probe design obviated making actual measurements closer than 10 cm from the blankets. Table 1 shows the steady-state magnetic fields measured for both types of blankets at the human head, torso, and knee regions. AC magnetic fields produced in the DC power mode were approximately an order of magnitude less than those measured in the AC mode and were not distinguishable from background.

Both the average and maximum magnetic fields associated with the CPW blankets in the AC mode are approximately 50% higher than those for comparably sized conventional electric blankets. Florig and Holburg [1990] have carried out detailed computer simulations of both the electric and magnetic fields associated with conventional and CPW blankets of several sizes. Data from their work are in general agreement with our measurements. At initial switch-on, the CPW blanket may draw as much as five times its steady-state current, and during this period produces a proportionally higher magnetic field. During steady-state operation the modified CPW blankets had a slightly higher current just after switch-on than just before switch-off. Blanket duty cycles were characterized at a room temperature of 23.5°C while the blankets were maintained at approximately 26.5°C. A current shunt and a data-logging device were used to record current draw. Current levels and the on-off cycle for a queen-size CPW blanket with one side operating are shown in Figure 1A. Comparable data from a conventional queen-size electric blanket are shown in Figure 1B.

RESULTS

Table 2 shows the group means and corresponding log-transformed data, pressed as nanograms of 6-OHMS/mg creatinine, for each exposure period.

[1]Deno is a registered trademark of Electric Field Measurements Co., West Stockbridge, MA.

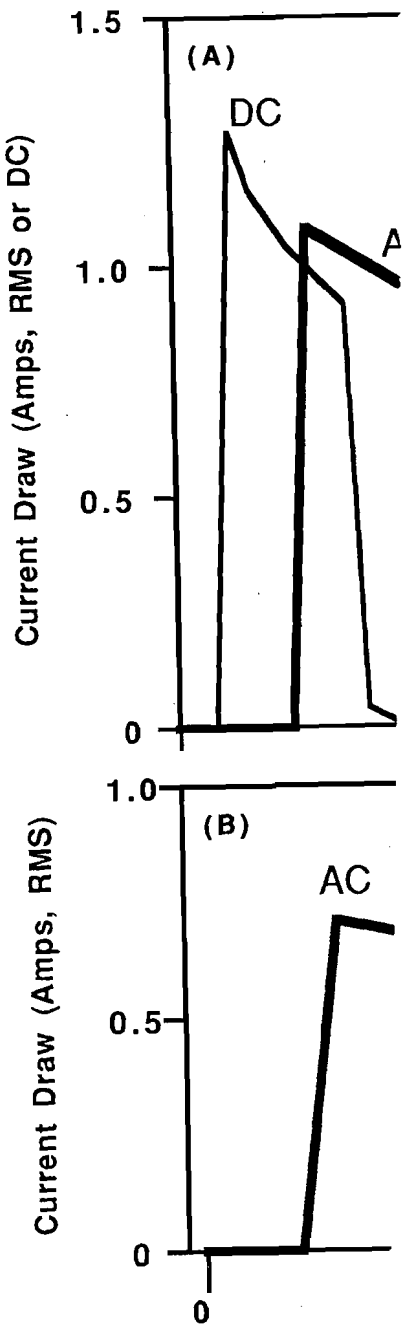

Fig. 1. (A) Plot of current draw du (CPW) electric blankets using AC pow draw during 150-sec interval for con

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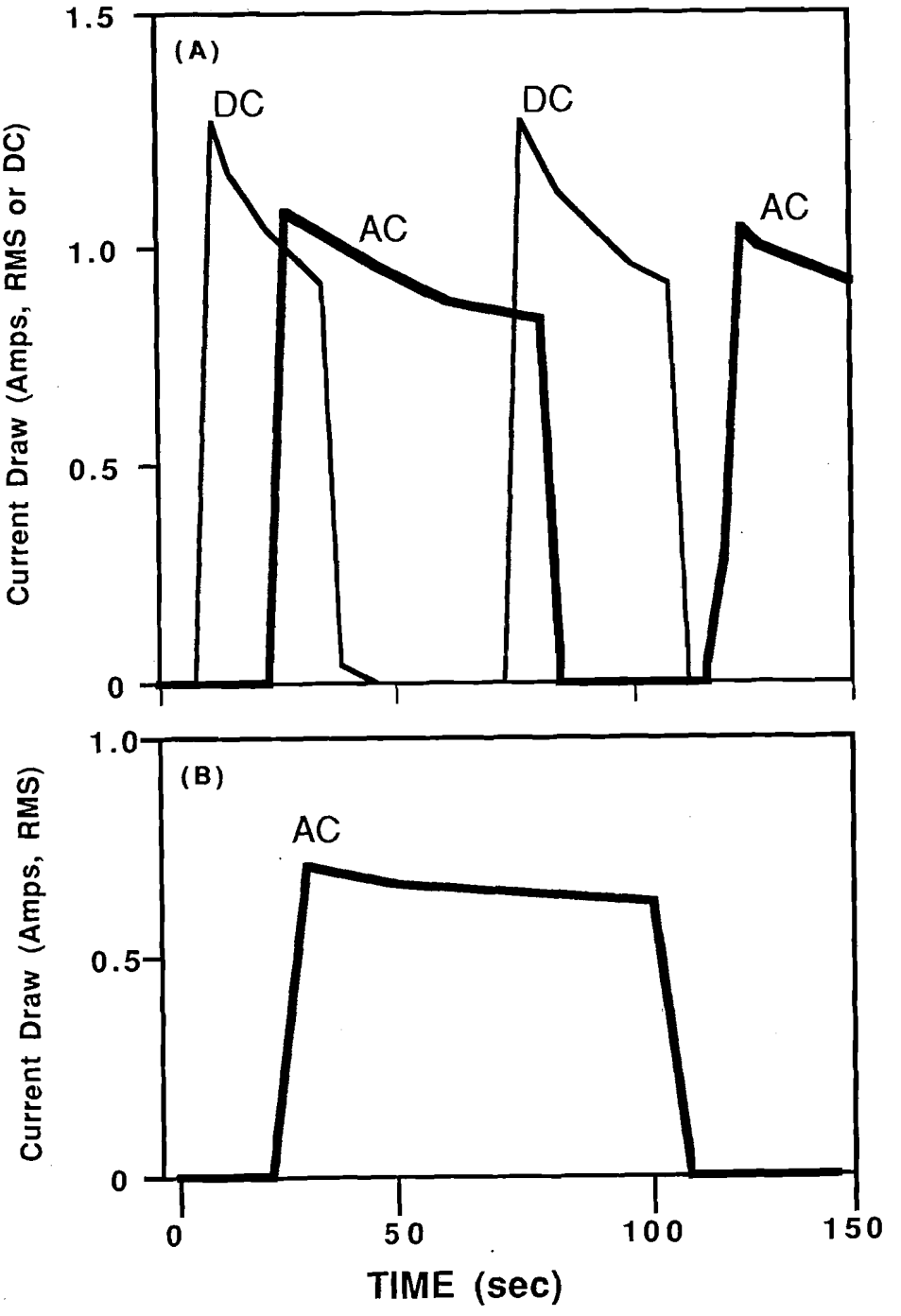

Fig. 1. (A) Plot of current draw during typical 150-sec interval for continuous polymer wire (CPW) electric blankets using AC power (thick line) and DC power (thin line). (B) Plot of current draw during 150-sec interval for conventional electric blanket using AC power.

Table 2. Group Means[a] for 6-Hydroxymelatonin Sulfate (6-OHMS) Excretion During Four Exposure Periods

	Exposure Period			
	1 (preexposure)	2	3	4 (postexposure)
		AC	DC	
Group 1 (CPW)	21.84 ± 3.74	23.46 ± 3.22	20.73 ± 3.41[b]	24.53 ± 3.26[b]
(n = 14)	2.88 ± 0.17[c]	2.92 ± 0.18	2.77 ± 0.18	3.01 ± 0.15
		DC	AC	
Group 2 (CPW)	14.13 ± 1.83	17.86 ± 2.10	13.97 ± 1.55	18.27 ± 2.89[b]
(n = 14)	2.49 ± 0.14	2.71 ± 0.13	2.48 ± 0.12	2.69 ± 0.16
		AC		
Group 3 (conventional)	18.89 ± 2.89	18.46 ± 2.95	——→	19.58 ± 3.49
(n = 14)	2.68 ± 0.21	2.60 ± 0.19	——→	2.68 ± 0.19

[a]± Values are standard error of the mean.
[b]Significantly different from previous exposure period by the sign test.
[c]Log-transformed (log e) values are listed beneath their respective means.

ere was no statistically significant difference in 6-OHMS excretion between AC and DC exposure periods as determined by analysis of variance of the group means. However, as determined by the nonparametric sign test, there was a significant difference in 6-OHMS excretion between periods 2 and 3, and between periods 3 and 4 in group 1, as well as between periods 3 and 4 in group 2.

Comparison of mean 6-OHMS excretion for individual subjects among the four test periods showed that seven CPW users (6 women and 1 man) had significant differences in the mean levels of 6-OHMS excretion as determined by analysis of variance. That is, there was a statistically significant difference between the levels of 6-OHMS excretion among at least two of the latter three test periods. Probabilities from analysis of variance on data for those individuals showing changes among exposure periods ranged between $P < 0.04$ and $P < 0.0001$.

Figure 2 is a plot of nightly 6-OHMS excretion from a CPW blanket user. Mean values for each exposure period are denoted by the height of the shaded area. There was a significant decrease ($P < 0.05$) during exposure period 3 as compared to exposure period 2 and a rebound to higher values after the cessation of exposure ($P < 0.05$). Six of the seven individuals exhibiting differences in 6-OHMS excretion showed this same pattern of melatonin excretion among the four exposure periods, as did the group 1 and group 2 populations in general (see Table 2).

Similar analysis of the conventional electric blanket data sets showed no such changes. Indeed, data from the conventional electric blanket users (group 3) showed no statistically significant changes among any of the exposure periods. As an additional check, we compared mean values before and after either 3 weeks of conventional electric blanket exposure. We found no significant individual or population changes by any of the foregoing criteria in group 3.

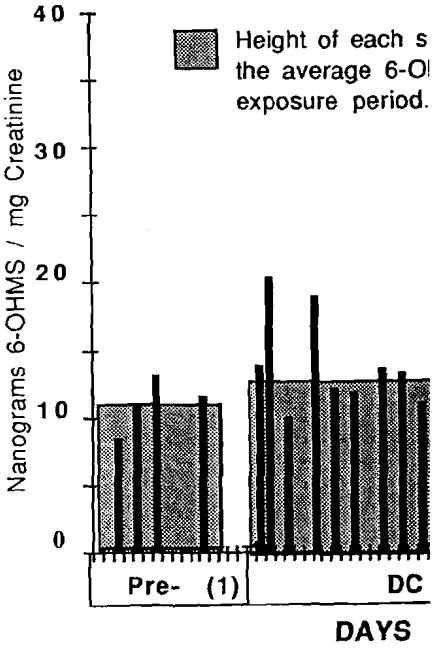

Fig. 2. Nightly 6-hydroxymelatonin sulfa blanket user. Height of shaded area repre immediately after onset and cessation of e

DISCUSSION

Data on individual subjects dence to suggest that exposure to electric or magnetic fields of suffi changes in melatonin excretion i OHMS excretion observed for tho fields, it appeared that there was response to onset of exposure an cessation of exposure.

During AC operation, the CP imately 50% higher than did the c duty cycle, CPW blankets switche did the conventional blankets. Oth outcome of the study include th differences in the switching tran presence of operating shielded tr unteers. It is also possible that melatonin peak for the conventi tected in the urinary 6-OHMS ass

It should be noted that ther heating was present without eithe however, we could find no eviden has a physiological effect differen

te (6-OHMS) Excretion During

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	3	4 (postexposure)
	DC	
2	20.73 ± 3.41[b]	24.53 ± 3.26[b]
8	2.77 ± 0.18	3.01 ± 0.15
	AC	
0	13.97 ± 1.55	18.27 ± 2.89[b]
3	2.48 ± 0.12	2.69 ± 0.16
5	——→	19.58 ± 3.49
9	——→	2.68 ± 0.19

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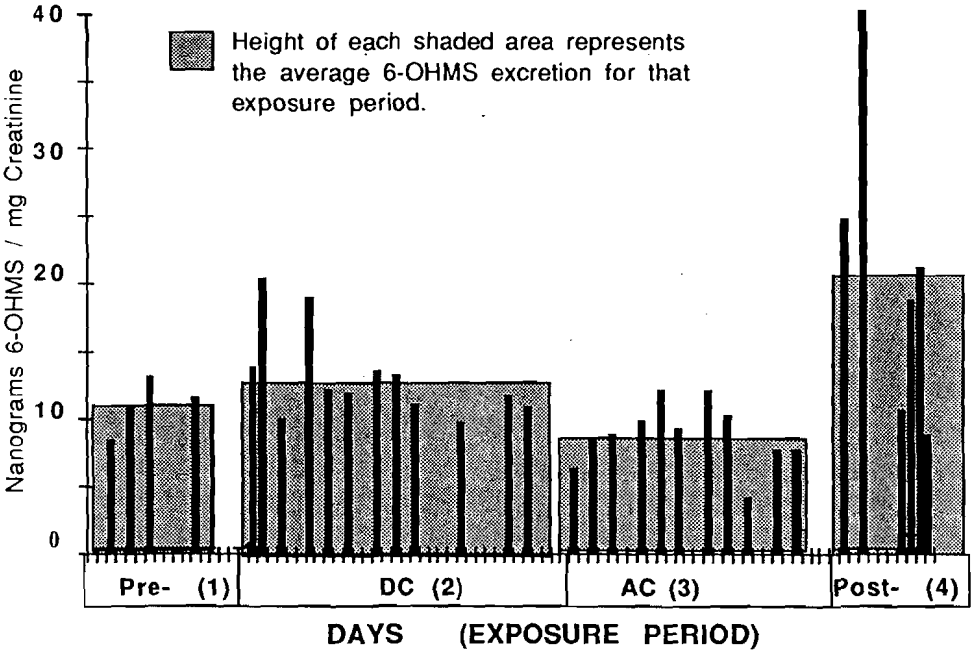

Fig. 2. Nightly 6-hydroxymelatonin sulfate (6-OHMS) excretion for continuous polymer wire blanket user. Height of shaded area represents period mean. Note increased 6-OHMS excretion immediately after onset and cessation of exposure.

DISCUSSION

Data on individual subjects serving as their own controls provided evidence to suggest that exposure to either or both intermittent DC, and 60-Hz AC, electric or magnetic fields of sufficient magnitude or duration may give rise to changes in melatonin excretion in some individuals. From the pattern of 6-OHMS excretion observed for those volunteers who showed a response to the fields, it appeared that there was a transient increase in 6-OHMS excretion in response to onset of exposure and a similar increase, of greater magnitude, at cessation of exposure.

During AC operation, the CPW blankets produced a magnetic field approximately 50% higher than did the conventional electric blankets. Owing to their duty cycle, CPW blankets switched on and off approximately twice as often as did the conventional blankets. Other possible factors that may have affected the outcome of the study include the combined effects of AC and DC exposure, differences in the switching transients of the two types of blankets, and the presence of operating shielded transformers in the bedrooms of the CPW volunteers. It is also possible that there were temporal shifts in the nighttime melatonin peak for the conventional electric blanket users that were not detected in the urinary 6-OHMS assay.

It should be noted that there was no group in the study wherein blanket heating was present without either an AC or a DC electric field. In the literature, however, we could find no evidence that warmth generated by a heated blanket has a physiological effect different from that achieved by using more or heavier

blankets. In addition, the conventional electric blanket users showed no changes in 6-OHMS levels, lending strength to the hypothesis that the electromagnetic fields associated with the CPW blankets, and not the heat that they generate, can affect human pineal function.

In further studies, it would be of interest to determine what, if any, physiological or genetic factors may be common to those individuals who exhibited change in 6-OHMS excretion as a consequence of electromagnetic field exposure. The report of McIntyre et al. [1990] cited earlier illustrated large variations in pineal gland sensitivity among individuals. Further work will be required to determine more precisely those electromagnetic-field characteristics that may be responsible for the observed changes in 6-OHMS excretion for certain individuals in the study.

ACKNOWLEDGMENTS

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A review of the evidence supporting melatonin's role as an antioxidant

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Reiter RJ, Melchiorri D, Sewerynek E, Poeggeler B, Barlow-Walden L, Chuang J, Ortiz GG, Acuña-Castroviejo D. A review of the evidence supporting melatonin's role as an antioxidant. J. Pineal Res. 1995;18:1–11.

Abstract: This survey summarizes the findings, accumulated within the last 2 years, concerning melatonin's role in defending against toxic free radicals. Free radicals are chemical constituents that have an unpaired electron in their outer orbital and, because of this feature, are highly reactive. Inspired oxygen, which sustains life, also is harmful because up to 5% of the oxygen (O_2) taken in is converted to oxygen-free radicals. The addition of a single electron to O_2 produces the superoxide anion radical (O_2^-); O_2^- is catalytic-reduced by superoxide dismutase, to hydrogen peroxide (H_2O_2). Although H_2O_2 is not itself a free radical, it can be toxic at high concentrations and, more importantly, it can be reduced to the hydroxyl radical (·OH). The ·OH is the most toxic of the oxygen-based radicals and it wreaks havoc within cells, particularly with macromolecules. In recent in vitro studies, melatonin was shown to be a very efficient neutralizer of the ·OH; indeed, in the system used to test its free radical scavenging ability it was found to be significantly more effective than the well known antioxidant, glutathione (GSH), in doing so. Likewise, melatonin has been shown to stimulate glutathione peroxidase (GSH-Px) activity in neural tissue; GSH-PX metabolizes reduced glutathione to its oxidized form and in doing so it converts H_2O_2 to H_2O, thereby reducing generation of the ·OH by eliminating its precursor. More recent studies have shown that melatonin is also a more efficient scavenger of the peroxyl radical than is vitamin E. The peroxyl radical is generated during lipid peroxidation and propagates the chain reaction that leads to massive lipid destruction in cell membranes. In vivo studies have demonstrated that melatonin is remarkably potent in protecting against free radical damage induced by a variety of means. Thus, DNA damage resulting from either the exposure of animals to the chemical carcinogen safrole or to ionizing radiation is markedly reduced when melatonin is co-administered. Likewise, the induction of cataracts, generally accepted as being a consequence of free radical attack on lenticular macromolecules, in newborn rats injected with a GSH-depleting drug are prevented when the animals are given daily melatonin injections. Also, paraquat-induced lipid peroxidation in the lungs of rats is overcome when they also receive melatonin during the exposure period. Paraquat is a highly toxic herbicide that inflicts at least part of its damage by generating free radicals. Finally, bacterial endotoxin (lipopolysaccharide or LPS)-induced free radical damage to a variety of organs is highly significantly reduced when melatonin is also administered; LPS, like paraquat, produces at least part of its damage to cells by inducing the formation of free radicals. Physiological melatonin concentrations have also been shown to inhibit the nitric oxide (NO·)-generating enzyme, nitric oxide synthase. The reduction of NO· production would contribute to melatonin's antioxidant action since NO· can generate the peroxynitrite anion, which can degrade into the ·OH. Thus, melatonin seems to have multiple ways either to reduce free radical generation or, once produced, to neutralize them. Melatonin accomplishes these actions without membrane receptors, indicating that the indole has important metabolic functions in every cell in the organism, not only those that obviously contain membrane receptors for this molecule.

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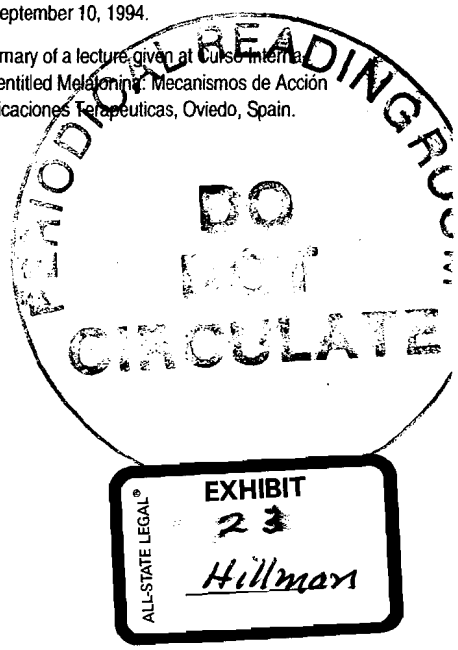
Dedicated to the memory of Dr. Armando Menendez-Pelaez, a dear friend, an outstanding colleague and an imaginative scientist; Armando died September 10, 1994.

A summary of a lecture given at Curso Internacional entitled Melatonina: Mecanismos de Acción e Implicaciones Terapéuticas, Oviedo, Spain.



Key words: melatonin – oxygen-based radicals – hydroxyl radical – peroxyl radical – antioxidative defense system – nitric oxide – lipid peroxidation – oxidative stress

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Recently, melatonin was shown to be a highly efficient scavenger of both the hydroxyl (·OH) (Tan et al., 1993) and peroxyl radical (ROO·) (Pieri et al., 1994). The initial in vitro findings suggest that melatonin is remarkably effective in these roles as indicated by the fact that when compared with the intracellular scavenger, glutathione

(GSH), melatonin proved five times better in neutralizing the ·OH and, when compared to vitamin E, melatonin was twice as effective in inactivating the ROO·. GSH (Meister, 1992) and vitamin E (Packer, 1994) are considered to be premier antioxidants within the cell. Besides these direct antioxidative actions of melatonin, there are indirect effects as well. Thus, melatonin stimulates glutathione peroxidase (GSH-Px) activity (Barlow-Walden et al., 1995) and inhibits nitric oxide synthase (NOS) (Pozo et al., 1994). GSH-PX is an important antioxidative enzyme because it metabolizes hydroperoxides including hydrogen peroxide (H_2O_2), thereby reducing the formation of the highly toxic ·OH (Liochev and Fridovich, 1994). By inhibiting NOS, melatonin reduces the formation of the free radical nitric oxide (NO·) (Palmer et al., 1988). Although NO· performs a variety of important functions in organisms (Moncada and Higgs, 1993), it also interacts with other radicals to produce the toxic peroxynitrite anion ($ONOO^-$), which can generate reactive oxygen-based radicals by way of its interaction with the superoxide anion radical ($O_2^{\cdot-}$) (Beckman, 1991; Radi et al., 1991).

The purpose of this brief review is to summarize the newly discovered intracellular functions of melatonin that relate to free radical generation. Other reviews discuss the potential implications of these new findings for aging (Reiter, 1994a; Reiter et al., 1994a) and age-related diseases (Poeggeler et al., 1993; Reiter et al., 1993, 1994b, 1994c).

Free radical generation and antioxidative defense mechanisms

A free radical is an atom or a molecule that contains an unpaired electron. Usually, electrons associated with atoms or molecules are paired; pairing of electrons makes molecules relatively stable and unreactive. Conversely, the loss of or addition of an electron leaves the atom or molecule unstable and relatively more highly reactive than its non-radical counterpart. The chemical reactivity of free radicals varies widely. The simplest free radical is the hydrogen radical (which is identical to the hydrogen atom); it contains a single proton and one unpaired electron. Removal of a hydrogen radical (or atom) from a polyunsaturated fatty acid (PUFA) in a cell membrane by a strong reducing agent can initiate radical chain reactions (such as in lipid peroxidation) (Kanner et al., 1987), which are highly destructive to cellular morphology and function.

Although there are a variety of free radicals produced in organisms, those that are byproducts of molecular oxygen (dioxygen or O_2) have received a great deal of investigative interest and they exert extensive damage, particularly over time (Harman, 1994). Although estimates vary somewhat, it is believed that up to 5% of the O_2 taken in by organisms may eventually end up as damaging

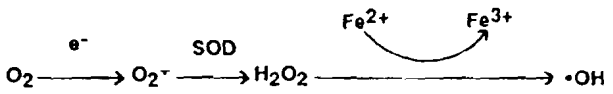

Fig. 1. The three-electron reduction of molecular oxygen (O_2) to the hydroxyl radical (·OH). The addition of a single e^- to O_2 produces the superoxide anion radical ($O_2^{\cdot-}$), which is catalytically converted by superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2). H_2O_2 can be metabolized to nontoxic products (see Fig. 3 below) or, in the presence of a transition metal, usually Fe^{2+}, it is reduced to the highly toxic ·OH.

oxygen-based radicals. In a human, this means that there could be the equivalent of 2 kg of $O_2^{\cdot-}$ produced each year (Halliwell and Gutteridge, 1989). $O_2^{\cdot-}$ is generated by the addition of a single electron to O_2 (Fig. 1); the $O_2^{\cdot-}$ is rather unreactive (Liochev and Fridovich, 1994). $O_2^{\cdot-}$ is usually classified as being generated accidentally, as in following the leakage of electrons from the mitochondrial electron transport chain and by the direct interaction of certain molecules, e.g., catecholamines, with O_2. On the other hand, $O_2^{\cdot-}$ is also deliberately formed by a variety of activated phagocytes, e.g., eosinophils, macrophages, monocytes, and neutrophils, for the purpose of killing bacteria and other foreign organisms (Babior and Woodman, 1990). In chronic inflammatory disease, the normal production of $O_2^{\cdot-}$ may induce damage to normal tissue. Other findings suggest that under certain conditions, low levels of free radical production are important because they may act as intracellular second messengers. For example, the response of cytosolic NF-κB to tumor necrosis factor, which acts via membrane receptors, relies on intracellularly produced oxygen radicals as second messengers (Schreck and Baeuerle, 1991; Schreck et al., 1991) (Fig. 2).

$O_2^{\cdot-}$ is enzymatically reduced to H_2O_2 in the presence of a ubiquitous enzyme, superoxide dismutase (SOD) (McCord and Fridovich, 1969). SOD, usually classified as an antioxidative enzyme that affords protection against free radical damage, in some cases can be associated with increased oxidative stress. Thus, the over-expression of SOD, such as occurs in trisomy 21 (Down syndrome), may be responsible for many of the neurodegenerative changes and cataracts these individuals experience at an early age (Kedziora and Bartosz, 1988).

H_2O_2 does not possess an unpaired electron and, therefore, is not a radical per se. Thus, it is usually classified as a reactive oxygen intermediate or species. H_2O_2 can diffuse through membranes and it has a half-life much longer than that of $O_2^{\cdot-}$. H_2O_2 has several fates intracellularly. It can be metabolized by one of two antioxidative enzymes, i.e., GSH-PX or catalase, and, in the worst case scenario, in the presence of the transition metals Fe^{2+} or Cu^{1+}, it is reduced to the ·OH via the Fenton reaction (Fig. 1) (Me-

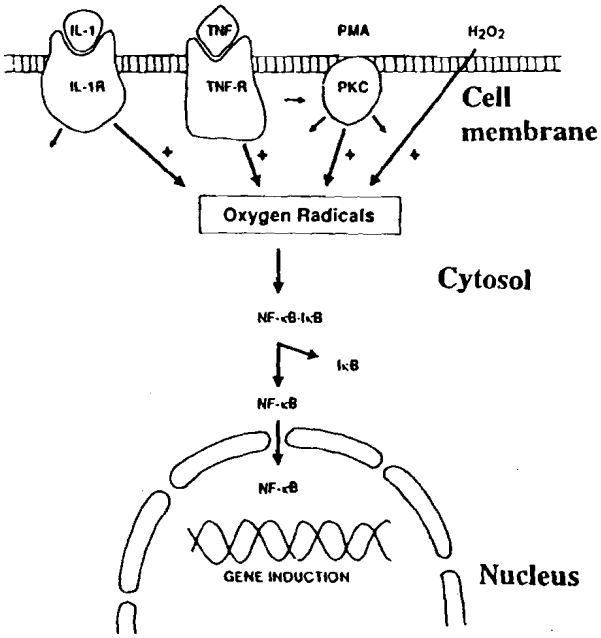

Fig. 2. Oxygen-based radicals may act as physiological second messengers, as illustrated in this figure. Thus, interleukin 1 (IL-1) and tumor necrosis factor (TNF) via their respective receptors generate oxygen radicals intracellularly; this is also the case for protein kinase C and hydrogen peroxide (H_2O_2). Oxygen radicals cause the dissociation of NF-κB, allowing NF-κB to translocate to the nucleus and to bind DNA. Phorbol ester PMA (phorbol 12-myristate 13-acetate). Modified from Schreck and Baeuerle (1991).

neghini and Martins, 1993).

The ·OH is fearsomely reactive and highly toxic. It indiscriminately reacts with any molecule it encounters. Among radicals, it could be classified as the radical's radical. Because of their large size and electroreactivity, it is not uncommon for ·OH to interact and damage macromolecules such as DNA, proteins, carbohydrates, and lipids (Kehrer, 1993). Oxidative damage to macromolecules is especially noticeable because, compared to the smaller molecules in cells, they are present in limited numbers. In the case of DNA, damage inflicted by the ·OH can lead to cancer (Dizdaroglu, 1993). ·OH are also generated within cells when they are exposed to ionizing radiation; in this case the electromagnetic radiation splits water molecules to produce the highly toxic ·OH (Littlefield et al., 1988).

The reactions of radicals with non-radicals, which most molecules in an organism are, result, by necessity, in the formation of a new radical; thus, radicals beget radicals. In some cases, these newly formed radicals may also be rather toxic and, in fact, they may initiate other damaging free radical reactions. An example of this type of chain reaction is lipid peroxidation, where the ROO·, once produced, abstracts a hydrogen atom from another PUFA (Girotti, 1985).

Radicals, however, can also interact with another radical to form a stable molecule. In this case, the unpaired electrons in each radical form a covalent bond. This is what happens when a $O_2^{\cdot-}$ encounters NO· with the resultant formation of peroxynitrite anion.

$O_2^{\cdot-} + NO\cdot \rightarrow ONOO^-$

$ONOO^-$ by itself can damage proteins and can also decompose into toxic products including nitrogen dioxide gas ($NO_2\cdot$), ·OH, and the nitronium ion (NO^{2+}). Thus, both $ONOO^-$, as well as the products it generates, are toxic to cellular elements.

The phrase given to describe the damage done by free radicals in oxidative stress (Sies, 1991). The degree of oxidative stress a cell endures may determine whether it remains healthy or becomes diseased. Under conditions of severe oxidative damage, many cells undergo either necrosis or apoptosis. There are a variety of conditions that increase oxidative stress, including ingestion of toxins, excessive exercise, ionizing radiation, infection, ischemia/reperfusion, and thermal damage (Farrington et al., 1973; Freeman et al., 1987; Keizer et al., 1990; Aust et al., 1993; Zimmerman and Granger, 1994). The accumulated subcellular damage caused by a lifetime of oxidative stress may also be related to the degenerative diseases of aging and to aging itself (Subborao et al., 1990; Taylor et al., 1993; Harman, 1994; Reiter, 1994b, 1994c).

Fortunately, cells have means to resist free radical abuse. Collectively, this is referred to as the antioxidative defense system (Sies, 1991). Enzymatic antioxidants, which have already been mentioned, include SOD (McCord and Fridovich, 1969), GSH-PX (Maiorino et al., 1991), and catalase (Chance et al., 1979). These enzymatic antioxidants catalytically metabolize either a free radical ($O_2^{\cdot-}$ in the case of SOD) or a reactive oxygen intermediate (H_2O_2 in the case of GSH-PX and catalase) to generally less toxic or non-toxic products (Fig. 3). Since SOD reduces $O_2^{\cdot-}$ to H_2O_2, which can be converted to the highly

$$2O_2^{\cdot-} + 2H^+ \xrightarrow[\text{dismutase}]{\text{superoxide}} H_2O_2 + O_2$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

$$H_2O_2 + 2GSH \xrightarrow[\text{peroxidase}]{\text{glutathione}} 2H_2O + GSSG$$

$$GSSG + NADPH + H^+ \xrightarrow[\text{reductase}]{\text{glutathione}} NADP^+ + 2GSH$$

Fig. 3. Hydrogen peroxide can be metabolized to nontoxic products by the enzymes catalase and glutathione peroxidase. In the process glutathione peroxidase also oxidizes glutathione (GSH) to its disulfide form (GSSG). GSSG is recycled back to GSH in the presence of the enzyme glutathione reductase.

toxic ·OH, it is important that the antioxidative enzymes GSH-PX and catalase, both of which metabolize H_2O_2, work in concert with SOD (Chance et al., 1979).

In the process of the conversion of H_2O_2 to water by GSH-PX, the tripeptide GSH is converted to its disulfide oxidized form, GSSG (Fig. 3). GSH is an important antioxidant itself. It is found in millimolar concentrations within cells and it has important roles in xenobiotic metabolism and leukotriene synthesis (Chance et al., 1979). GSH-PX, which removes H_2O_2, is a selenium containing molecule; a related enzyme removes lipid hydroperoxides, which are formed during lipid peroxidation, from cellular membranes (Maiorino et al., 1991).

As shown in Figure 1, the reduction of H_2O_2 to ·OH requires a transition metal, usually Fe^{2+} but occasionally Cu^{1+}. Because of this, it is important that these metals are not in the free state in cells and any molecule that binds them and renders them incapable of interacting with H_2O_2 is classified as part of the antioxidative defense system. A common storage-form of iron in serum is transferrin (Winterboum and Sutton, 1984), whereas copper is often sequestered by ceruloplasmin (Goldstein et al., 1979). In these forms, the transition metals cannot promote free radical reactions. Besides those mentioned here, there are a wide variety of other antioxidative enzymes, free radial scavengers, and transition metal binders that contribute to the total antioxidant capacity of the organism.

The role of melatonin in the antioxidative defense system

For the last decade, some reports related to the actions of melatonin on metabolic processes have been considered inconsistent with the rather limited distribution of membrane receptors in cells (Reiter, 1991). It seemed likely that certain actions of melatonin, e.g., those related to the regulation of reproduction (Reiter, 1980) and those concerned with circadian regulation (Armstrong, 1989), will prove to be mediated by membrane receptors on specific cells related to these functions (Vanecek et al., 1987; Morgan and Williams, 1989). However, the existence of melatonin in unicellular organisms (Poeggeler et al., 1991), as well as its widespread actions, described elsewhere (Reiter, 1991), in multicellular organisms led us to speculate that melatonin performed functions within cells that did not require an interaction with a receptor, particularly not a receptor located on the limiting membrane of the cell. Furthermore, the high lipid solubility of the indole allows it ready access to the interior of all cells, also indicating that the melatonin's actions may not be limited to actions at the cell membrane level. Interestingly, the recent demonstration that melatonin is also quite soluble in aqueous media is consistent with the intracellular actions of melatonin (Shida et al., 1994). Finally, the recent finding that melatonin intracellularly may be in rather high concentrations in the nuclei (Mennenga et al., 1991; Menendez-Pelaez and Reiter, 1993; Menendez-Pelaez et al., 1993) and that there may be specific binding sites for melatonin associated with nucleoproteins (Acuña Castroviejo et al., 1993, 1994), suggest the possibility that melatonin may function like some other hormones, e.g., steroid and thyroid hormones, on molecular events in the nuclei of cells.

The initial studies from which we deduced that melatonin may alter the redox state of the cell were those of Chen et al. (1993). In this investigation Ca^{2+}-stimulated + Mg^{2+}-dependent ATPase (Ca^{2+}-pump) activity in the heart was found to be influenced by the pineal gland and melatonin. Initially, a day/night difference in Ca^{2+}-pump activity was noted with highest levels at night. When animals were pinealectomized, the nighttime rise in the activity of the pump did not occur, so it was assumed that the rise was probably mediated by melatonin. When cardiomyocyte membranes were in fact incubated with melatonin, Ca^{2+}-ATPase activity increased in a dose-dependent manner (Chen et al., 1993). Since the activity of this enzyme is normally depressed in a high free radical atmosphere (Kaneko et al., 1989), we speculated that melatonin altered the redox state of the cell by neutralizing toxic free radicals, which then allowed Ca^{2+}-pump activity to rise passively. This idea is also supported by more recent studies wherein rats were treated with alloxan, which is known to generate free radials. This treatment significantly reduced Ca^{2+}-pump activity, which was again reversed by concurrent melatonin treatment (Chen et al., 1994). Although the evidence is indirect, both studies indicated a potential involvement of melatonin with the oxidative status of cardiac cells.

These initial studies were followed by a series of investigations that were designed to specifically examine the ability of melatonin to function as a free radical scavenger and antioxidant. Of specific interest was the interaction of melatonin with the highly toxic ·OH. To check this, we developed a simple in vitro system in which H_2O_2 was exposed to 254 nm ultraviolet light to generate the ·OH (Tan et al., 1993a). However, because of their extremely short half-life (1×10^{-9} sec at 37°C), ·OH are difficult to measure directly. To overcome this, a spin trapping agent, 5,5-dimethylpyrroline N-oxide, or DMPO, was added to the mixture. DMPO forms an adduct with the ·OH and, since the adducts have a much longer half-life, they can be quantitated as an index of ·OH generation. The adducts (DMPO--OH) were qualitatively and quantitatively evaluated using both high pressure liquid chromatography with electrochemical detection and electron spin resonance spectroscopy (Tan et al., 1993a). By also adding melatonin (or other known scavengers) to the mixture, it was possible to estimate the ·OH scavenging capacity of the compounds of interest. In this system, melatonin proved to be very significantly more efficient than either GSH or mannitol

TABLE 1. Concentration of various constituents required to scavenge 50% (IC_{50}) of the ·OH generated in vitro following the exposure of H_2O_2 to ultraviolet light

Scavenger	IC_{50}
Melatonin (N-acetyl-5-methoxytryptamine)	21μM
Reduced glutathione	123μM
Mannitol	183μM

TABLE 2. Peroxyl radical (ROO·) scavenging capacity, as measured in oxygen radical absorbing capacity (ORAC) units, of the four compounds indicated[a]

Scavenger	$ORAC_{peroxyl}$
Melatonin (N-acetyl-5-methoxytryptamine)	2.04
Vitamin C (ascorbate)	1.12
Trolox (water soluble vitamin E analogue)	1.00
Reduced glutathione	0.68

[a] The findings suggest that, of the four ROO· scavengers checked, melatonin is the most efficient.

in scavenging the ·OH (Table 1). This finding generated considerable interest because both GSH and mannitol are very effective intracellular free radical scavengers, suggesting that melatonin may well have a physiologically significant role as an antioxidant. More importantly, of all the radicals produced in the organism, the ·OH is considered the most toxic; thus, any compound that neutralizes this radical could play an important role in the antioxidative defense system.

The free radical scavenging capacity of melatonin may extend to other radicals as well. A year following our reported demonstration of melatonin as a neutralizer of the ·OH (Tan et al., 1993a), Pieri and colleagues (1994) claimed that the indole exhibits a similar action in reference to the peroxyl radical (ROO·). Using a well established in vitro system for evaluating the radical scavenging capacity of a compound (Cao et al., 1993), Pieri et al. (1994) claimed that melatonin was better than vitamin E in scavenging the ROO·, which is a consequence of lipid peroxidation (Table 2). Clearly, in this system melatonin was twice as effective as vitamin E, a well known and important chain-breaking antioxidant (Packer, 1994), in halting lipid peroxidation. Thus, melatonin would be expected to be highly effective against lipid peroxidation in vivo for several reasons: 1) melatonin is highly lipophilic and should, therefore, normally be found in rather high concentrations in cellular membranes; 2) melatonin, like vitamin E, is an effective chain breaking antioxidant and, thus, it would reduce oxidation of lipids; and 3) melatonin, by virtue of its ability to scavenge the ·OH, would also reduce the initiation of lipid peroxidation. The ·OH is one of the radicals that is sufficiently toxic to abstract a hydrogen atom, i.e., initiate lipid peroxidation, from a PUFA (Niki et al., 1993).

The demonstration that melatonin affords protection against oxidative stress in vivo followed soon after the in vitro studies indicating that melatonin is a potent scavenger of both the ·OH (Tan et al., 1993a) and ROO· (Pieri et al., 1994). In reference to oxidative damage to nuclear DNA, Tan and co-workers (1993b, 1994) in a series of two reports found that hepatic DNA damage inflicted by safrole, a chemical carcinogen, was highly significantly reduced when the rats also received melatonin. Safrole damages DNA at least in part because it generates the production of toxic-free radicals (Boberg et al., 1983). Perhaps the most remarkable feature of melatonin's protection against safrole-induced DNA damage was that it was effective at very low concentrations relative to the very large dose of safrole administered. Thus, even when the amount of melatonin administered was 1,000-fold lower than the dose of safrole, most of the DNA damage was prevented. Furthermore, when safrole was given either during the day or at night, in the latter case DNA damage was less. The implication of this observation is that even the nighttime rise in endogenous melatonin is sufficient to provide protection against oxygen toxicity resulting from xenobiotic administration (Tan et al., 1994).

The protective effect of melatonin against oxygen radical damage to DNA was also observed in another model system (Vijayalaxmi et al., 1995). In this case, we incubated human lymphocytes and subjected them to 150 cGy ionizing radiation with and without concurrent treatment of the cells with melatonin. Damage to DNA was then cytogenetically evaluated by an investigator who was unaware of the experimental design of the study. Melatonin, in a dose-response manner, significantly reduced the number of micronuclei, the number of cells with exchange aberrations (both of which are indices of genomic damage), and the total number of cell with any type of chromosomal damage (Fig. 4). At a concentration of 2 mM melatonin reduced ionizing radiation-induced damage by about 70%. For dimethylsulfoxide (DMSO), a known radioprotective agent (Littlefield et al., 1988), to provide a similar level of DNA protection a dose of 1 M was required (Fig. 4) (Vijayalaxmi et al., 1995). Thus, in this system melatonin seemed to be on the order of 500 times more effective than DMSO as a radioprotector. Free radicals induced by ionizing radiation are the causative factor in damage to the genomic material (Okada et al., 1983).

Melatonin as a general protector against ionizing radiation is certainly also suggested by the observations of Blinkenstaff and co-workers (1994). This group found that almost 50% of mice treated with melatonin prior to exposure to 950 cGy ionizing radiation survived at least 30 days, whereas within the same time frame all irradiated

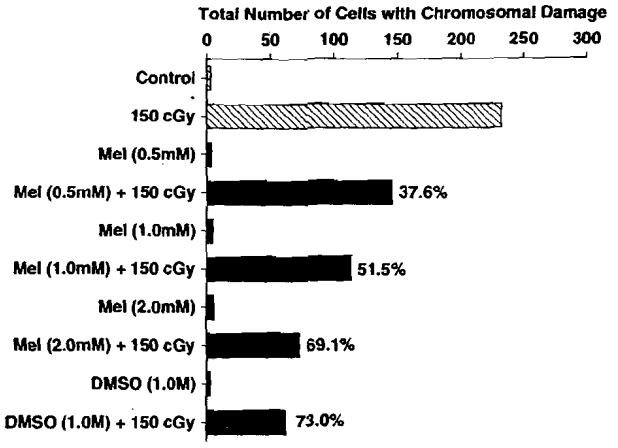

Fig. 4. Percentage reduction of the number of human lymphocytes exhibiting chromosomal damage after their exposure to 150 cGy ionizing radiation. At a concentration of 2.0 mM in the incubation medium, melatonin reduced the percentage of damaged cells by 69.1%. For the known radioprotector dimethylsulfoxide (DMSO) to reduce chromosomal damage by roughly the same percentage (73%), its concentration had to be 1 M. Modified from Vijayalaxmi et al. (1994).

mice that did not receive melatonin died.

The protection of macromolecules from oxidative stress by melatonin is not restricted to nuclear DNA. In a study where oxidative damage to the lens of the eye was assessed, we found that melatonin also provided significant protection against lenticular degeneration (Abe et al., 1994). Cataractogenesis is known to be a free radical-mediated condition where the lens becomes cloudy following oxidative attack on lenticular protein and other macromolecules (Spector, 1991). One of the major antioxidative defense constituents in the lens is GSH (Pau et al., 1990). One model in which to investigate the importance of GSH in protecting the lens from oxygen radical-based cataracts is to inject newborn rats with a drug (buthionine sulfoximine or BSO) that depletes the organisms of this key antioxidant; BSO acts by inhibiting γ-glutamylcysteine synthase, which regulates GSH formation (Martensson et al., 1989; Meister, 1992). When BSO is given shortly after birth, rats typically have cataracts at the time their eyes open (around 2 weeks of age). Interestingly, the pineal gland of newborn rats also produces only small amounts of melatonin during the first 2 weeks of life (Reiter, 1991). Thus in reality, following BSO administration, the newborn animals are really deficient in two important antioxidants, i.e., GSH and melatonin.

Considering this, we treated BSO-injected (to deplete their GSH levels) newborn rats with melatonin for the first 2 weeks of life to determine if the indole would alter cataractogenesis (Abe et al., 1994). The animals receiving BSO only exhibited the usual high incidence of cataracts, whereas those treated with BSO and melatonin had a very low incidence of cataracts (Table 3). In these animals, BSO had indeed highly significantly reduced lenticular GSH levels whether or not they had been given melatonin. The clear implication is that melatonin was the active agent in reducing oxidative damage and suppressing cataract formation. Furthermore, although the evidence is obviously indirect it seems likely melatonin was effective in this model system because it reduced oxidative damage to protein (Spector, 1991).

There is, of course, a great deal of interest in lipid peroxidation because it is devastating to cell membranes and it either disrupts the functions of these critical cellular organelles or, in the worst case scenario, it leads to the death of the cell (Ursini et al., 1991). As mentioned previously, the best known lipid antioxidant is vitamin E, usually represented by α-tocopherol (Packer, 1994). However, Pieri and colleagues' demonstration (1994) showing that, at least in an in vitro situation, melatonin is a more efficient scavenger of the ROO· than is vitamin E itself, led us to examine melatonin's ability to reduce peroxidation of lipid in the lungs of rats treated with the highly toxic herbicide paraquat. Although the mechanisms by which paraquat inflicts its damage to lipid membranes is complex, the damage is believed in part to be a consequence of the induction of oxygen-free radicals (Ogata and Manobe, 1990). Thus, we administered paraquat to rats with and without concurrent melatonin treatment and biochemically evaluated the degree of oxidative damage in the lungs using three indices, i.e., the concentration of malondialdehyde (MDA) and 4-hydroxyalkenals, total glutathione levels, and the ratio of oxidized glutathione (GSSG) to total glutathione (Melchiorri et al., 1994). MDA and 4-hydroxyalkenals are degraded lipid products in cell membranes that are taken as an index of oxidative damage (Ursini et al., 1990). In this experimental system, as in the others where it has been tested, melatonin provided remarkably potent protection against lipid peroxidation (Fig. 5). All indices of oxidative stress were returned to normal levels when paraquat-treated rats were also given melatonin. Furthermore, in yet-unpublished findings we have found that the lethal dose of paraquat required to kill 50% of the animals (LD_{50}) increases several-fold in melatonin pretreated rats (D. Melchiorri and R.J. Reiter, unpublished observations).

TABLE 3. Incidence of cataracts in newborn rats after various treatments

Treatment	Incidence of cataracts	Percent of rats with cataracts
None (controls)	0/17	0
Buthionine sulfoximine	18/18	100
Buthionine sulfoximine + Melatonin	1/15	7

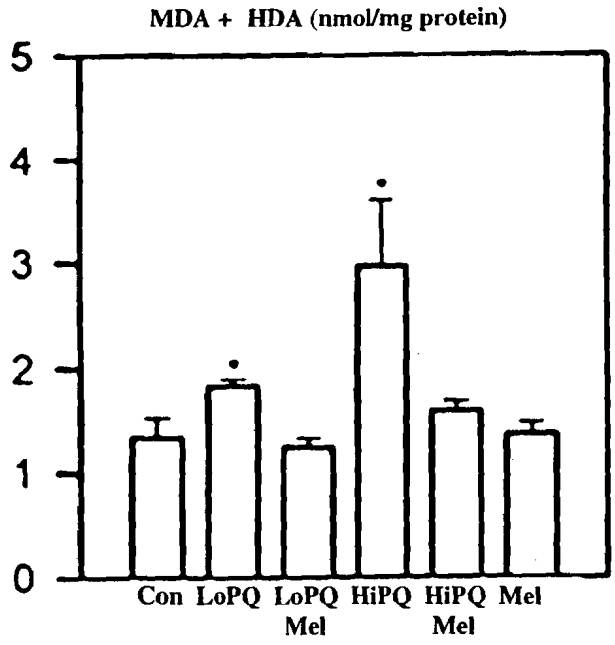

Fig. 5. Lipid peroxidation products (MDA + HDA) in lungs of paraquat (PQ)-treated rats. One of two doses of paraquat (LoPQ = 20 mg/kg and HiPQ = 70 mg/kg) was given to rats with or without concurrent melatonin (Mel = 10 mg/kg) treatment. Melatonin cotreatment overcame the effects of paraquat. Modified from Melchiorri et al. (1994).

This remarkably potent protection against paraquat toxicity by melatonin certainly exceeded the most optimistic expectations. Seemingly, the results cannot be explained by the mere ability of melatonin to interrupt propagation of lipid peroxidation by scavenging the ROO· (Pieri et al., 1994). Protection is also likely afforded by melatonin's ability to scavenge the ·OH (Tan et al., 1993a), which is certainly a sufficiently toxic radical to initiate lipid peroxidation. Even these two mechanisms alone may not account for the remarkable ability of melatonin to curtail the peroxidative processes in the lungs of paraquat-treated rats. Several other potential mechanisms are currently being investigated. Pierrefiche and colleagues (1993), using an in vitro system and brain homogenates, also report that melatonin may prevent lipid peroxidation in the brain but the protection in this organ was reportedly not as great as that provided by its metabolite, 6-hydroxymelatonin. This leaves open the possibility that some of melatonin's antioxidative protection in vivo may follow its hepatic metabolism to its hydroxylated metabolite.

More recently, we have used another model system to examine melatonin's protective actions against peroxidative damage. Bacterial lipopolysaccharide (LPS) is a highly toxic endotoxin that induces extensive cellular damage in many organs (Ghezzi et al., 1986; Peavy and Fairchild, 1986) because of its ability to generate free radicals (Gram et al., 1986). We have recently found that melatonin highly resists the peroxidative effects of LPS (Sewerynek et al., 1995) with the degree of efficiency being equal to that when paraquat is used as the free radical-generating molecule. The significance of the findings relates to the fact that LPS causes widespread oxidative damage in a number of organs, all of which are negated by melatonin treatment (Sewerynek et al., 1995); thus, the protection against free radical attack by melatonin is obviously not confined to a single organ but probably extends to every organ and cell in an organism.

There are also several important enzymes that are part of the antioxidative defense system of animals that are influenced by melatonin. In the brain, GSH-PX is a premier enzyme in warding off oxidative attack since this enzyme metabolizes H_2O_2 to water, thereby reducing the formation of the toxic ·OH (Halliwell and Gutteridge, 1989). Indeed, GSH-PX activity is considered possibly the most important means by which neural tissue protects itself from the devastating actions of free radicals. In a series of studies, we have shown that melatonin greatly promotes GSH-PX activity in the brain (Fig. 6) (Barlow-Walden et al., 1995). This correlates with the rapid uptake of melatonin by the brain when it is administered to animals (Menendez-Pelaez et al., 1993). The clear implication of the findings of Barlow-Walden and co-workers (1995) is that besides its direct scavenging ability, melatonin stimulates the most important antioxidative enzyme in the brain, GSH-PX, and thus provides indirect as well as direct protection against free radical attack.

We have also found that the activity of NOS, which controls the quantity of NO· produced (Mayer et al., 1990), is suppressed in the cerebellum by physiological concentrations of melatonin (Pozo et al., 1994). This finding has numerous implications in terms of melatonin regulation of neural as well as cardiovascular physiology, but also could be another mechanism by which the indole quells free radical generation. NO·, itself a free radical, can, in the presence of O_2^- induce the formation of ONOO–, which, although not a free radical itself, is rather toxic within cells and can also degrade to the ·OH via peroxynitrous acid (Beckman et al., 1990). Thus, by virtue of melatonin's ability to reduce NO· formation by limiting NOS activity, free radical production from this source would be limited (Pozo et al., 1994) thereby reducing the likelihood of oxidative destruction.

Finally, another enzyme closely related to the antioxidative defense system of any organism is cytochrome P450. This microsomal complex enzyme often is involved in the metabolism of xenobiotics with the resultant production of free radicals (Gram et al., 1986; Coon et al., 1992). Kothari and Subramanian (1992) have recently found that the activity of cytochrome P450 is reduced in the presence of melatonin; we have confirmed this finding by showing

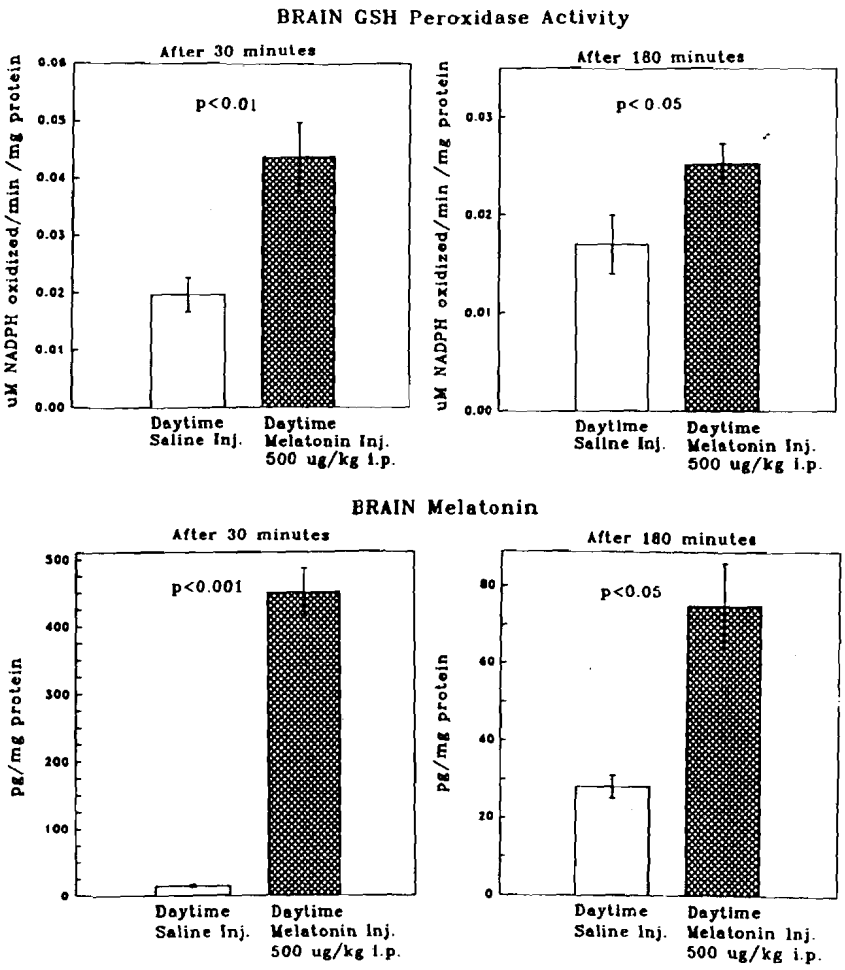

Fig. 6. Brain glutathione peroxidase activity and melatonin levels, measured by radioimmunoassay, after the treatment of rats with 500 μg/kg at either 30 or 180 min before the measurements were made. The levels of melatonin in the brain were correlated with stimulation of antioxidative enzyme glutathione peroxidase. Modified from Barlow-Walden et al. (1995).

in vivo that melatonin lowers the activity of this enzyme by about 30% (E. Sewerynek, R.J. Reiter, unpublished). This reduction would also lower the generation of free radicals and thus reduce oxidative damage.

Final comments

Free radical research has flourished in the last decade and it has become increasingly apparent that they may be in part or wholly responsible for a number of debilitating diseases as well as for aging itself. Free radicals attack any molecule they encounter with the resultant destruction of macromolecules such as DNA, proteins, and lipids being most noticeable. Although there are a variety of antioxidative defense mechanisms with which organisms are endowed, they do not totally protect against the ravages of the most toxic free radicals. One newly discovered component of the antioxidant defense repertoire appears to be melatonin. The small indole has already been shown in vitro to be an efficient scavenger of both the ·OH (Tan et al., 1993a) and ROO· (Pieri et al., 1994). Additionally, it stimulates the antioxidant enzyme, GSH-PX (Barlow-Walden et al., 1995) while inhibiting an enzyme, NOS (Pozo et al., 1994) that promotes the generation of free radicals. Considering its multiple actions, melatonin is certainly one of the most versatile antioxidants thus far discovered. This is certainly compatible with its distribution within cells. Most other intracellular antioxidants are compartmentalized within cells, e.g., vitamin E in the lipid-rich cell membranes, vitamin C in the cytosol, etc.; on the contrary, melatonin seems to have actions in the membrane, in the cytosol and in the nucleus suggesting its presence at all these locations. While additional experiments are required to definitively define the extent of melatonin's role as an antioxidant, data accumulated to date suggest it may play a very significant role in protecting organisms from free radical damage.

A major question that remains is whether melatonin's ability as an antioxidant is purely a pharmacological observation or whether melatonin produced by the pineal gland and other organs is physiologically relevant in terms of an antioxidant action, as is suggested by the observations of Tan and coworkers (1994). The melatonin molecule detoxifies at least two different radical species, i.e.,

the reactive initiating and propagating ·OH and ROO·, by electron donation and the relatively inert $O_2^{\cdot-}$ by adduct formation in a two-step process (Hardeland et al., 1993). In the first step, the indolyl cation radical is formed when melatonin donates an electron; thereafter, the indolyl cation radical is quickly oxidized by the omnipresent $O_2^{\cdot-}$ to form 5-methoxy-N-acetyl-N-formyl-kynuramine. Thus, melatonin is irreversibly oxidized and cannot be regenerated as is the case with some other antioxidants.

Considering the large number of radicals produced in an organism it seems that there may be an insufficient number of melatonin molecules produced endogenously to provide a significant radical-scavenging action. However, the multiplicity of melatonin's action as both a free radical scavenger (Hardeland et al., 1993; Reiter et al., 1993; Tan et al., 1993); Pieri et al., 1994) and as an antioxidant (Poeggeler et al., 1993, 1994; Reiter et al., 1993; 1994a; 1994c; Pozo et al., 1994; Barlow-Walden et al., 1995) greatly increases the likelihood that the quantity of endogenously produced melatonin provides a significant defense against oxidative attack (Tan et al., 1994); this possibility is supported by the findings that melatonin may be produced in organs in addition to the pineal gland. However, even if melatonin is only pharmacologically relevant as an antioxidant its therapeutic value and potential, considering its virtual lack of toxicity, would be seemingly almost limitless.

Acknowledgments

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Mini Review

Melatonin in relation to physiology in adult humans

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Abstract: The role exerted by melatonin in human physiology has not been completely ascertained. Melatonin levels have been measured in different physiopathological conditions, but the effects induced by melatonin administration or withdrawal have been tested only recently. Some effects have been clearly documented. Melatonin has hypothermic properties, and its nocturnal secretion generates about 40% of the amplitude of the circadian body temperature rhythm. Melatonin has sleep inducing properties, and exerts important activities in the regulation of circadian rhythms. Melatonin is capable of phase shifting human circadian rhythms, of entraining free-running circadian rhythms, and of antagonizing phase shifts induced by nighttime exposure to light. Its effect on human reproduction is not completely clear, but stimulatory effects on gonadotropin secretion have been reported in the follicular phase of the menstrual cycle. Direct actions on ovarian cells and spermatozoa have been also documented. Beside these, new important actions for melatonin may be proved. Melatonin may exert protective effects on the cardiovascular system, by reducing the risk of atherosclerosis and hypertension, and may influence immune responses. Finally, by acting as an antioxidant, melatonin could be important in slowing the processes of ageing.

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Key words: Melatonin – humans – reproduction – temperature – sleep – ageing – circadian rhythms

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Introduction

Physiology of melatonin has been extensively studied in animals. For years data obtained in animals have been extrapolated to humans without critical evaluation. Indeed, only more recent studies have tried to investigate the mechanisms of synthesis, regulation, and action of melatonin in humans. The present review will focus almost entirely on data obtained in humans that have defined mechanisms of melatonin production by the pineal gland, and the effects of melatonin on biological and endocrine functions.

Melatonin synthesis

Studies performed in vitro and in animals have clarified the mechanisms involved in the regulation of melatonin synthesis by the pineal gland [Cardinali and Vacas, 1987; Krause and Dubocovich, 1990; Reiter, 1991]. Tryptophan is taken up by the pinealocyte, is transformed to serotonin, and serotonin is finally converted into melatonin by a two-step process that involves the sequential activities of two enzymes, N-acetyltransferase (NAT), which is believed to be the limiting enzyme for the synthesis of melatonin, and hydroxyindole-O-methyltransferase (HIOMT). The synthesis of melatonin is initiated by the binding of norepinephrine to adrenergic β1 receptors, subsequent activation of pineal adenylate cyclase, increase in cyclic AMP (cAMP), binding, and de novo synthesis of NAT or of its activator. The potent cAMP-induced gene transcription repressor (ICER) is activated in conjunction with NAT and represents a mechanism that limits the nocturnal production of melatonin [Stehle et al., 1993]. The β1 adrenergic receptor stimulus is enhanced by α1- adrenoceptors, via calcium (Ca^{2+})-phospholipid-dependent protein kinase C (PKC) and by prostaglandins, whose synthesis is activated by

the influx of Ca^{2+} into the pinealocyte that follows $\alpha 1$ adrenergic action [Cardinali and Vacas, 1987; Krause and Dobocovich, 1990].

Additional stimuli to melatonin synthesis derive from VIPergic neurons that reach the pineal gland through the pineal stalk [Cardinali and Vacas, 1987] by opioids that bind to σ receptors [Jansen et al., 1990] and by pituitary adenylate cyclase-activating polypeptide [Chik and Ho, 1995; Yuwiler et al, 1995]. By contrast, GABA (and benzodiazepines), dopamine, glutamate, and delta-sleep-inducing peptide seem to inhibit melatonin production [Krause and Dubocovich, 1990].

Whether all the above mechanisms are relevant to melatonin secretion in humans is not completely known. As in animals, in humans melatonin synthesis also depends upon tryptophan availability and is reduced by acute tryptophan depletion [Zimmermann et al., 1993]. Evidence indicated that also in humans the adrenergic stimulus is important for melatonin secretion. Beta 1-adrenergic blockers suppress the nocturnal secretion of melatonin [Cowen et al., 1983; Arendt at al., 1985; Brismar et al., 1987; Demitrack et al., 1990, Cagnacci et al., 1994], with an effect that seems to be inversely related to nocturnal levels of the hormone [Cagnacci et al., 1994]. Similarly, a reduction of nocturnal melatonin secretion can be obtained with the administration of either clonidine, which reduces the endogenous adrenergic tonus [Lewy et al., 1986], or alpha-methyl-para-tyrosine, which reduces presynaptic catecholamine synthesis [Zimmermann et al., 1994]. Conversely, melatonin secretion is increased by the administration of drugs capable of augmenting catecholamine availability, such as MAO inhibitors or tricyclic antidepressants [Murphy et al., 1986; Skene et al., 1994]. The importance of intracellular calcium is supported, although not conclusively, by the capability of dihydropyridine calcium antagonists to markedly reduce nocturnal melatonin levels in subhuman primates [Meyer et al., 1986], whereas the stimulatory effect of prostaglandins is apparent from the decrease in melatonin production that follows the administration of prostaglandin inhibitors [Murphy et al., 1996]. Opiate administration enhances melatonin production [Chazot et al., 1985; Lissoni et al., 1986], but opioid receptor blocking agents, such as naloxone or naltrexone, do not reduce melatonin levels [Strassman et al., 1989; Laughlin et al., 1991]. Activation of GABA receptors by benzodiazepines reduces melatonin at night [Monteleone et al., 1989; McIntyre et al., 1993], whereas manipulation of dopaminergic receptors, with either agonists [Lal et al., 1987; Murphy et al., 1986] or antagonists [Murphy et al., 1986; Laughlin et al., 1991], is not capable of markedly modifying melatonin levels.

Environmental control of the pineal melatonin synthesis

The pineal gland is the major site of melatonin production [Neuwelt and Lewy, 1983]. Melatonin is secreted by the pineal gland in a marked circadian fashion. Its circulating levels begin to rise in the evening, progressively increase to reach maximal values in the middle of the night and then progressively decrease to reach minimal values in the morning [Cagnacci et al., 1992, 1994]. The circadian rhythm of melatonin secretion originates in the suprachiasmatic nuclei (SCN) of the hypothalamus [Kruase and Dubocovich, 1990; Hofman and Swaab, 1993]. SCN outputs modulate in a circadian fashion the activity of noradrenergic neurons originating in superior cervical ganglia and impinging upon pinealocytes [Bruce et al., 1991]. In addition, a circadian rhythm of $\beta 1$ adrenergic receptors has been found on human pinealocytes [Oxenkrug et al., 1990]. Peak values of adrenergic receptors are reached between 16.00 hr and 20.00 hr. At this time, the pineal content of serotonin and N-acetylserotonin begin to increase to reach maximal values between 20.00 hr and 24.00 hr. The serotonin and N-acetylserotonin peaks coincide with the increase of melatonin, that reaches maximal values between 24.00 hr and 04.00 hr.

Light perceived by the retina, reaches the SCN through a non-visual pathway, the retinohypothalamic tract [Sadun et al., 1984; Czeisler et al., 1995]. Light, by influencing SCN output, suppresses melatonin secretion in a dose dependent fashion [Lewy et al., 1980; McIntyre et al., 1989; Brainard et al., 1988; Petterborg et al., 1991; Dollins et al., 1993a; Cagnacci et al., 1993]. Minimal suppressive effects are observed with full spectrum light intensities of 200–300 lux [McIntyre et al., 1989; Dollins et al., 1993a], whereas complete melatonin suppression is obtained with light intensities above 2,000–2,500 lux [Lewy et al, 1989; Cagnacci et al., 1993].

The response to light is rapid, and only 15 min of bright light exposure (1,500 lux] are sufficient to shut down melatonin production [Petterborg et al., 1991]. However, as a consequence of melatonin half-life in blood, a prolonged exposure is necessary to reduce circulating melatonin to daytime levels. Removal of the light stimulus is associated with an immediate resynthesis of melatonin and restoration of normal night-time levels [Petterborg et al., 1991; Dollins et al., 1993a; Cagnacci et al., 1993]. The prompt increase in melatonin that follows the termination of the light stimulus is probably a mechanism of defense aimed to limit the impact of occasional night-time bright light exposure on endogenous circadian rhythms [Cagnacci et al., 1993].

In the absence of light inputs, as in some artificial experimental conditions [Weaver, 1989] or in

some blind people [Czeisler et al., 1995; Lewy and Newsome, 1983; Sack et al., 1992], the circadian secretion of melatonin, as the circadian rhythms of other biological functions, free runs with a period of about 25 hr; under these conditions the rhythm is not entrained to day-night changes. Thus, as for other biological rhythms, light is necessary to synchronize and entrain the circadian melatonin rhythm to a 24 hr period [Weaver, 1989]. Experimental evidence indicates that, beside light, weak electromagnetic fields [Reiter, 1993] and temperature may influence the endogenous production of melatonin in animals [Underwood and Calaban, 1987; Firth and Kenneway, 1989; Stokkan et al., 1991; Ulrich et al., 1973, 1974], but no data are available for humans.

Distribution of melatonin

Following its synthesis, melatonin is not stored, because of its small size and its lipophilic and hydrophilic properties, passively diffuses out of pinealocytes [Reiter, 1991]. The published evidence do not allow to where melatonin is primarily secreted [Cagnacci, 1996]. Evidence in animals indicates that during its endogenous secretion, the levels of melatonin in cerebrospinal fluid (CSF) of the lateral ventricles is much higher than in blood [Shaw et al., 1989; Kanematsu et al., 1989], and that during peripheral administration, this concentration can be probably achieved only by inducing pharmacological levels of the hormone in blood [Reppert et al., 1979; Kanematsu et al., 1989; Vitte et al., 1988]. The presence of a concentration gradient between CSF and blood may indicate a simultaneous secretion of melatonin into both compartments.

In CSF, melatonin is not bound to proteins, whereas in blood 70% of it is bound to albumin [Cardinali et al., 1972]. From the CSF melatonin disappears with an half-life of 40 min, at least in primates [Reppert et al., 1979]. In humans, the half-life of melatonin in blood is of about 28.4 min [Mallo et al., 1990] and is dependent on both its diffusion into body fluids [Reiter, 1991], including CSF [Partridge and Mietus, 1980], and its massive metabolism by the liver, where 90% of it is hydroxylated within a single passage. Hydroxylated metabolites are then excreted in urine as sulphate and, to a lesser extent, glucuronide conjugates [Cardinali et al., 1972; Reiter, 1991].

Circulating melatonin can reaches all body tissues [Reiter, 1991], including the brain [Anton-Tay and Wurtman, 1969; Vitte et al., 1988], where, at least in animals, it is reported concentrated in several regions of the cortex, bulb-pons, cerebellum, thalamus, and paraventricular nuclei of the hypothalamus [Anton-Tay and Wurtman, 1969; Vitte et al., 1988; Menendez-Pelaez and Reiter, 1993]. However, it may be the CSF that is the preferential route for melatonin to enter the brain. In rats, concentrations of melatonin in the brain are 100 times higher following CSF than blood administration, and the hypothalamus is the structure where melatonin is most highly concentrated [Anton-Tay and Wurtman, 1969; Cardinali et al., 1973]. Besides CSF and blood, elevated concentrations of melatonin have been detected in other biological fluids. Quite high levels of melatonin have been detected in the fluid of the anterior chamber of the eye [Martin et al., 1992; Viggiano et al., 1994], where concentrations are parallel to those of plasma. However, local melatonin synthesis by the ciliary body has been also observed [Martin et al., 1992]. Melatonin has been detected in urine [Vakkuri et al., 1985] and in saliva [Miles et al., 1985; Laasko et al., 1990], where it seems to derive from plasma. Relevant melatonin concentrations have been also found in biological fluids strictly linked to reproduction such as fluid of preovulatory follicles [Brzezinski et al., 1987; Ronnberg et al., 1990; Yie et al., 1995a], male semen [Oosthuizen et al., 1986; Bomman et al., 1989], amniotic fluid [Mitchell et al., 1978; Kivela et al., 1989], and breast milk [Illnerova et al., 1993]. Evidence indicates that melatonin is not synthesized at the site but diffuses from the plasma. In some cases plasma levels are not strictly correlated with those of follicular or semen fluids, but these discrepancies are probably the result of the presence of proteins retaining melatonin in follicular or seminal fluid, when the hormone is rapidly cleared from the circulation.

Melatonin receptors

Recently, a high affinity melatonin receptor has been cloned, and its signal has been found in the hypophyseal pars tuberalis and in hypothalamic SCN of humans [Reppert et al., 1994]. Furthermore melatonin may bind and activate an orphan of the nuclear receptor superfamily [Becker-Andre et al., 1994; Wiesenberg et al., 1995]. In studies where melatonin binding is considered equivalent to receptors, the presence of melatonin receptors has been found in pituitary pars distalis [Weaver et al., 1993], hypothalamic SCN [Weaver et al., 1993; Reppert et al., 1988], wall of the arteries of both rats [Viswanathan et al., 1991] and primates [Stankov et al., 1993], retina [Nash and Osborne, 1995], platelets [Vacas et al., 1992], lymphocytes [Steinhilber et al., 1995], kidney [Song et al., 1995], prostate [Laudon et al., 1996], spermatozoa [Van Vuuren et al., 1992] and ovarian granulosa cells [Yie et al., 1995b], and liver [Acuna-Castroviejo et al., 1994].

The presence of specific receptors defines the targets for melatonin, but the absence of receptors does not imply a lack of effect of the hormone, because melatonin rapidly diffuses into all cellular compartments [Poeggler et al., 1993; Reiter et al., 1995] where it may exert more basic functions. Indeed, melatonin may bind to calmodulin and through this mechanism may modulate cytoskeletal and mitotic cellular function [Benitez-King and Anton-Tay, 1993]. Furthermore, melatonin is an antioxidant molecule and may exert hydroxyl scavenging actions in every cell compartment [Poeggler et al., 1993; Reiter et al., 1995].

Variations in the levels or in the effects of melatonin

Modifications in melatonin levels have been found in several conditions from physiology to pathology. A reduction in circulating levels of melatonin have been observed in aged individuals [Nair et al., 1986; Sack et al., 1986], in those with a low intake of tryptophan [Zimmermann et al., 1993], in individuals suffering from insomnia [Haimow et al., 1995], fatal familial insomnia [Portaluppi et al., 1994], cephalgia [Waldenlind et al., 1994; Brun et al., 1995], depression [Wetterberg et al., 1979; Mendlewicz et al., 1979], coronary artery disease [Brugger et al., 1995], diabetic neuropathy [O'Brein et al., 1986], rheumatoid arthritis [West and Oosthuinen, 1992], porphyria [Puy et al., 1993], and liver cirrhosis [Steindl et al., 1995]. Also, melatonin levels are reduced in individuals using β-blockers [Cowen et al., 1983; Arendt et al., 1985; Brismar et al., 1987; Demitrack et al., 1990, Cagnacci et al., 1994], clonidine [Lewy et al., 1986], prostaglandin inhibitors [Murphy et al., 1996], benzodiazepines [Monteleone et al., 1989; McIntyre et al., 1993], probably alcohol [Ekman et al., 1993], and calcium antagonists [Meyer et al., 1986]. Furthermore, intense physical training seems to reduce melatonin levels [Skrinar et al., 1989]. By contrast, an increase in melatonin levels have been found in amenorrheic women [Brzezinski et al., 1988; Berga et al., 1988; Lauglin et al., 1991; Okatani and Sagara, 1995], in individuals taking tricyclic antidepressants or MAO inhibitors [Murphy et al., 1986; Skene et al., 1994], and by some authors [Ferrari et al., 1989; Arendt et al., 1992], but not by others [Kennedy et al., 1993; Mortola et al., 1993], in women suffering from anorexia nervosa.

The effect of melatonin is also different in different physiopathological states. In animals, gonadal steroids modulate the expression of melatonin receptors [for review, see Cagnacci and Volpe, 1996], and evidence in humans indicates that they modulate the effects of melatonin. Indeed, the melatonin effects on body temperature regulation [Cagnacci et al., 1992], gonadotrophin [Cagnacci et al., 1991; Cagnacci et al.,1995a,b] and TSH secretion [Melis et al., 1995], evident in women during the follicular phase of the menstrual cycle, disappears during the luteal phase [Cagnacci et al., 1995a–c; Melis et al., 1995; Cagnacci et al., 1996a]. Administration of melatonin enhances cortisol levels in postmenopausal women [Cagnacci et al., 1995c] with this effect being reversed by estrogen supplementation [Cagnacci and Soldani, 1992].

Beside gonadal steroids, ageing also seems to influence biological responses to melatonin. Melatonin receptors decline in aged animals [Laitinen et al., 1992], and in aged women the response of body temperature to melatonin administration seems to be reduced and inconsistent [Cagnacci et al., 1995d].

Melatonin and reproduction

The effect of melatonin as a transducer of photoperiodic information to animal reproduction has been known for a long time. Modifications, particularly in the duration, of nocturnal melatonin production, represents the signal through which melatonin influences the reproductive axis of seasonal breeder animals [Reiter, 1991; Cagnacci and Volpe, 1996]. In some species, such as hamsters, a prolongation of the nocturnal melatonin production induces reproductive quiescence, whereas in other species, such as the ovine, the same signal induces gonadal recrudescence. Although humans are not strictly considered seasonal breeders, seasonality of conceptions is evident in the human [Roennenberg and Aschoff, 1990a,b; Cagnacci and Volpe, 1996]. The seasonal rhythm of conception seems to be influenced by environmental factors among which photoperiod and temperature are the most obvious [Roenneberg and Aschoff, 1990b]. A lengthening of the nighttime period, and a 24 hr minimal temperature of about 12°C represent the conditions favoring conception [Roenneberg and Aschoff, 1990b]. It is possible that in humans, as in other species, melatonin interferes with the reproductive axis of both men and women and plays a critical role in determining a seasonal rhythm of conception. The effect of melatonin on the male reproductive system has not been extensively investigated. Although, melatonin does not seem to influence gonadotropin secretion [Strassman et al., 1991a], receptors for melatonin have been detected in human spermatozoa [Van Vuuren et al., 1992], and melatonin seems to reduce sperm motility [Irez et al., 1992]. More studies have been performed to evaluate the effect exerted by melatonin on the reproductive axis of women. In the follicular phase of the normal menstrual cycle, gonadotropin secretion, particularly the

amplitude of secretory LH pulse, increases at night [Filicori et al., 1986; Rossmanith and Yen, 1987] when melatonin is normally secreted. In this menstrual phase the administration of melatonin during the day enhances the amplitude of spontaneous LH pulses, and the responses of both LH and FSH to simil-physiological GnRH stimuli [Cagnacci et al., 1991, 1995a,b]. The relevance of these findings to human reproduction is unclear. Indeed, in nocturnal animals, such as rats, it has been demonstrated that the preovulatory LH surge begins in the afternoon, due to a daily circadian stimulus, and is evident also in diestrus II or diestrus I; this is translated into the preovulatory LH surge by sufficient levels of gonadal steroids [for review, Cagnacci and Volpe, 1996]. In humans, which are diurnal, the preovulatory LH surge seems to begin at night, around 03.00 hr [Testart et al., 1982], and the nocturnal amplification of LH pulses may represent the circadian input that is transformed into a preovulatory LH surge by critical estradiol levels. Accordingly, the circadian secretion of melatonin may help to synchronize the preovulatory LH surge to the nighttime period.

The impact that a prolonged amplification of LH pulses, associated with a prolonged night, may have on women's reproductive functions is not clear. LH hypersecretion is associated with alterations in ovulatory processes [Stanger and Yovich, 1985; Regan et al., 1990], and it is possible that an amplification of LH pulses beyond a critical time in the 24 hr period may have deleterious effects on ovulation. This view is in line with a highly credible theory that the effect of melatonin on reproductive function is dependent on the length of its nocturnal secretion [Reiter, 1991]. Recent evidence showing that in Arctic regions the dark season is associated with a prolonged melatonin secretion [Stokkan and Reiter, 1994], enhanced LH levels and defective ovulation seem to further support this hypothesis [Martikainen et al., 1996]. Furthermore, in all cases in which melatonin has been considered to induce gonadal quiescence, as in hypothalamic [Brzezinsky et al., 1988; Berga et al., 1988] or exercise induced-amenorrhea [Laughlin et al., 1991], one of the features of its circadian rhythm is a prolongation of its nocturnal secretion. However, the causative effect between prolonged melatonin secretion and anovulation is not firmly proven, and evidence that a reduction in melatonin causes a reactivation of the reproductive axis is lacking. By contrast, data indicating that the altered secretion of melatonin, observed in hypogonadal males and females, may be reduced towards normality by steroid administration [Okatani and Sagara, 1995; Luboshitzky et al., 1996] seems to indicate that the increased levels of melatonin are not the cause but the consequence of hypogonadism. This possibility is further supported by the finding showing that the administration of melatonin, even in large doses (300 mg/day for 4 months), may induce a defective luteal phase but it is not capable of blocking ovulation [Voordouw et al., 1992].

The effect of melatonin on other hormones that may influence reproductive processes, such as prolactin, growth-hormone or thyroid hormones, is not well known. Melatonin may amplify the nocturnal rise in prolactin [Waldhauser et al., 1987; Okatani and Sagara, 1993; Cagnacci et al., 1995e], and probably that of TSH, without modifying circulating levels of thyroid hormones [Melis et al., 1995]. Melatonin's effect on growth hormone has not been investigated in women, whereas in men melatonin has been reported to stimulate [Valcavi et al., 1993] or to exert no effect [Waldhauser et al., 1987] on growth hormone secretion. Whether these reported modifications play any role in ovulatory processes and testicular function is unknown.

In the luteal phase of the menstrual cycle, the effect of melatonin on both gonadotropins [Cagnacci et al., 1995a,b] and TSH disappear [Melis et al., 1995], but influences on prolactin [Okatani and Sagana, 1993] and on ovarian function still occur. Receptors for melatonin have been detected in human granulosa cells [Yie et al., 1995b]. Melatonin stimulates androstenedione synthesis from ovarian stroma [MacPhee et al., 1975] and enhances basal and hCG-stimulated progesterone production from preovulatory granulosa cells [Webley and Luck, 1986] and from cells of day 18–27-day-old corpora lutea [MacPhee et al., 1975]. These data are consistent with a direct effect of melatonin on progesterone production from the human ovary, but, at the present time, the implications of this effect on human reproduction are unclear.

Role of melatonin in the regulation of the circadian body temperature rhythm

Following the first evidence that melatonin may exert hypothermic effects [Carman et al., 1976], it has been ascertained that its nocturnal secretion plays an important role in generating the amplitude of the circadian rhythm of body temperature [Strassman et al., 1991b; Cagnacci et al., 1992, 1993, 1994]. Following its administration during the day, and its suppression at night, it has been shown that melatonin, above threshold levels, induces about a 40–50% reduction of the circadian body temperature rhythm amplitude [Cagnacci et al., 1992, 1994]. This effect is evident in men [Strassman et al., 1991b] and in women during the follicular phase of the menstrual cycle [Cagnacci et al., 1992], and is reduced or abolished in at least two physiological situations, i.e., in

the luteal phase of the menstrual cycle and in aged women. In the luteal phase of the menstrual cycle, the administration of melatonin does not induce a decrease in body temperature, and this lack of response is associated with a 40% reduction in the nocturnal decrease of body temperature [Cagnacci et al., 1996a]. Similarly, in aged women the amplitude of the circadian rhythm of body temperature is blunted, and the decline of body temperature during melatonin administration is reduced and inconsistent [Cagnacci et al., 1995d]. Thus, in aged subjects, a reduction in its action along with lower levels of melatonin could be related to circadian rhythm abnormalities [Weitzman et al., 1982, Van Coeverden et al., 1991].

The site(s) where melatonin acts to regulate the circadian rhythm of body temperature is unclear. Thermoregulatory centers are localized in the preoptic area of the anterior hypothalamus, and melatonin receptors have been detected in preoptic area neurons [Krause and Dubocovich, 1990]. Central serotoninergic activation induces a decrease in core body temperature, and at least in animals brain serotonin levels are believed to be enhanced by melatonin administration [Anton-Tay et al., 1968; Cassone et al., 1983].

Modifications of circulatory functions may also have an impact on body temperature regulation. In rats melatonin influences arterial tonus of both cerebral and peripheral arteries [Viswanathan et al., 1990; Krause et al., 1995]. Modifications in the vascular tone of cerebral arteries, by modifying the flow of cool or warm blood [Capsoni et al., 1995], may regulate the frequency of discharge of thermosensitive neurons in the preoptica area [Boulant, 1981], while influences on peripheral blood flow may modulate heat loss [Viswanathan et al., 1990; Krause et al., 1995]. Similar effects seem to be exerted by melatonin in humans, where its administration influences blood flow in cerebral arteries and enhances peripheral heat loss [Cagnacci et al., 1995f, 1996b].

An influence of melatonin on heat production is also possible. Non-shivering heat production is mainly a consequence of catecholamine and thyroid hormone secretion [Swanson, 1956; Leduc, 1976]. Administration of melatonin reduces stimulated norepinephrine levels [Cagnacci et al., 1996b,c] and probably decreases the activity of thyroid hormones. Indeed, during melatonin administration, the increases of TSH associated with unmodified thyroid hormone levels seem to support a reduced capability of the latter hormones to exert a central negative feedback [Melis et al., 1995].

The body temperature modification induced by melatonin may also be a consequence of an alteration in SCN activity. The circadian rhythm of body temperature, like that of many other biological rhythms, is dependent upon hypothalamic SCN activity. Receptors for melatonin have been detected in the SCN [Reppert et al., 1988; Krause and Dubocovich, 1990; Reppert et al., 1994]. Melatonin modifies the metabolic [Cassone et al., 1988] and electrical [McArthur et al., 1991; Margraf and Lynch, 1993; Jiang et al., 1995] activity of SCN neurons, and when administered to humans it is capable of phase shifting circadian rhythms [Lewy et al., 1992; Zaidan et al., 1994]. However, the possibility that the decline of body temperature, induced by melatonin, represents the expression of a circadian phase shift is not supported by the findings that both the magnitude and the direction of the circadian phase shifts are dependent upon the circadian time of melatonin administration [Lewy et al., 1992; Zaidan et al., 1994], whereas the response of body temperature is similar throughout the 24 hr period [Cagnacci et al., 1992]. The same consideration argues against the inverse possibility, suggested by Deacon et al. [1994], that the decline of body temperature is responsible for the circadian phase shifts induced by melatonin administration.

Melatonin and sleep

The effect of melatonin on sleep has been clearly documented. The daytime administration of melatonin in doses ranging from 10 to 80 mg induces sleepiness both after a single [Dollins et al., 1993b] or a 1 week administration [Nickelsen et al., 1989]. Studies in which melatonin was administered in the evening around 17.00 hr was without any relevant effect on sleep [Arendt et al., 1984]. By contrast, when melatonin was given 2 hr before sleep onset [Haimov et al., 1995] or at bed-time [Jan et al., 1994], it significantly improved sleep. In aged individuals with insomnia 2 mg of melatonin 2 hr before sleep for 7 days significantly reduced sleep latency [Haimov et al., 1995], and similar effects were described in normal adult individuals with doses ranging from 0.3 mg to 1 mg [Zhadanova et al., 1995]. The short half-life of melatonin [Mallo et al., 1990] results in a rapid disappearance of the hormone from blood and probably a lack of effect of its administration on late night sleep. By contrast, when melatonin was administered in high doses (80 mg) to normal individuals placed in a high-noise environment, it improved the efficiency of sleep throughout the entire night [Waldhauser et al., 1990]. The same results were obtained with slow release formulations of melatonin. Two milligrams of melatonin in a slow-release formulation administered at bedtime were sufficient to reduce sleep

latency and to improve the quality and the efficiency of sleep in elderly insomniacs [Garfinkel et al., 1995; Haimov et al., 1995]. Furthermore, this effect was enhanced by the prolonged administration of the hormone [Garfinkel et al., 1995]. More recently, doses of only 0.3 mg have been reported to improve sleep efficiency of insomniac individuals [Wurtman and Zhadanova, 1995]. The mechanisms mediating the sleep inducing properties of melatonin are not clear. The decrease of body temperature induced by melatonin may be involved [Dawson et al., 1995], but GABAergic properties are also very likely to play a major role [Tenn and Niles, 1995].

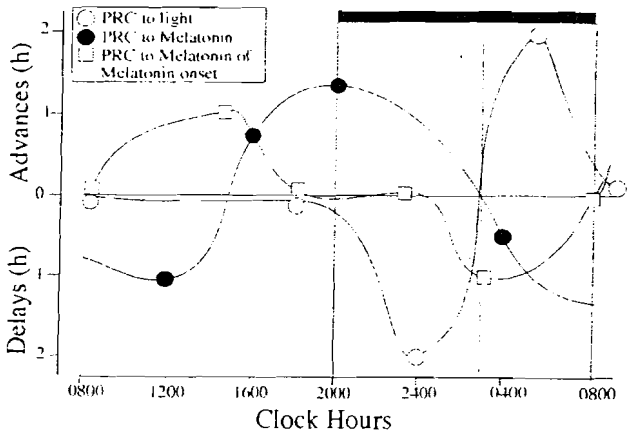

Fig. 1. Phase response curveS (PRC) of human circadian rhythms to light (open circles) or melatonin. The PRC to melatonin has been graphed in terms of the melatonin acrophase (closed circles) and melatonin onset (open squares). The graph was obtained by combining the PRC to light [Minors et al., 1991] with those of the PRCs to melatonin of the melatonin acrophase [Zaidan et al., 1994] and of the melatonin onset [Lewy et al., 1992]. In order to have comparable curves, the PRCs have been normalized to the initiation of the light stimulus, or to the time of melatonin infusion or administration. The black bar indicates the usual time of the nocturnal melatonin secretion, and the vertical line the time of the core body temperature nadir at 03.00 hr.

Melatonin and circadian rhythms

The effect of melatonin on circadian rhythms seems to be opposite to that of light. Exposure to bright light has no effect on body temperature when given during the day, a period in which melatonin is not secreted, but when given during the night it induces an increase in body temperature [Badia et al., 1991; Cagnacci et al., 1993]; this latter observation is coincident with the suppression of melatonin and is abolished by the simultaneous administration of the hormone [Strassman et al., 1991, Cagnacci et al., 1993]. Since only bright light is capable of suppressing melatonin secretion and in eliciting a "hyperthermic" response, it is likely that the nocturnal production of melatonin represents a mechanism aimed to consolidate circadian rhythmicity, and to oppose circadian alterations induced by weak signals such as low intensity light stimuli. The phase response curve (PRC) to light of human circadian rhythms is similar to that of animals [Honma et al., 1988; Beersma and Daan, 1989; Minors et al., 1991]. Light stimuli of sufficient strength given in the first part of the night phase delay and in the second part of the night phase advance circadian rhythms. Minimal changes are obtained when light stimuli are given during the daytime.

Receptors for melatonin have been detected in SCN [Reppert et al., 1988, 1994], and melatonin administration is capable of entraining free-running circadian rhythms [Arendt et al., 1986; Sack et al., 1992; Tzischinsky et al., 1992; Petrie et al., 1993] and of inducing phase shifts of human circadian rhythms [Lewy et al., 1992; Zaidan et al., 1994]. The PRC of human circadian rhythms to melatonin seems to be opposite to that of light (Fig. 1). The opposite PRC to melatonin and to light may indicate that melatonin antagonizes the circadian effects of light and that its suppression is probably necessary for light to induce circadian phase shifts. This possibility has been recently tested [Cagnacci et al., 1995f, 1996d]. The administration of a 4 hr bright light stimulus initiated at the time of the body temperature nadir and repeated for 3 nights, induced a 2 hr phase advance of the circadian rhythms of body temperature, cortisol and melatonin secretion. This effect did not occur in those subjects that, in conjunction with the bright light stimulus, received melatonin (1 mg just before the start of exposure and 1 mg after 2 hr of exposure) [Cagnacci et al., 1995f, 1997]. These data confirm that the effect of melatonin on human circadian rhythms is opposite to that of light and that bright light must inhibit melatonin production to exert its phase shifting properties.

Influence of melatonin on the seasonal modifications of its own secretion

Seasonal modifications in the duration of nocturnal melatonin secretion have been clearly documented in animals [Reiter, 1991; Cagnacci and Volpe, 1996]. In humans, by inducing an artificial prolongation of the night it has been possible to show a prolongation of the nocturnal secretion of melatonin [Wehr et al., 1993, 1995a,b], although the same results have not been obtained in men studied in normal conditions throughout the year [Wehr et al., 1995b]. Preliminary data seem to support a seasonal modulation of melatonin in women [Wehr et al., 1995b], and indeed seasonal variations in the length of the nocturnal melatonin production have been

detected in some studies [Illrenova et al., 1985; Kauppila et al., 1987; Bojkowski and Arendt, 1988; Martikanen et al., 1996; Levine et al., 1994; Stokken and Reiter, 1994]. Thus, it is very likely that in humans, particularly in women, as in other animals, photoperiodic modifications are associated with variations in the length of the nocturnal melatonin production. A variation in the length of the signal may only occur when two different mechanisms regulate the onset and the offset of nocturnal melatonin secretion. Indeed, the presence of one circadian clock governing the melatonin onset and another governing melatonin offset of the nocturnal melatonin rise has been hypothesized in animals [Illrenova and Vanecek, 1982, Illrenova et al., 1989; Elliot and Tamarkin, 1994], and more recently in humans [Wehr et al., 1993, 1995a, Cagnacci et al., 1995f, 1997]. Exposure to light in the morning is believed to compress nocturnal secretion of melatonin, by simultaneously phase advance the offset and phase delay the onset. Indeed, exposure of women for 3 nights to a 4 hr bright light stimulus initiated at the time of the body temperature nadir phase advanced the offset and slightly less the onset, so that there was a tendency to compress of the nocturnal melatonin rise [Cagnacci et al., 1995f, 1997]. Interestingly, when melatonin was administered in conjunction with bright light, the phase advance of the offset was completely abolished, whereas that of the onset was enhanced and almost doubled [Cagnacci et al.,1995f, 1997]. Thus, melatonin itself seems to exert a differential effect in the regulation of the onset and offset of its own secretion. Evidence of a different regulation by melatonin of the two indices derive also from the two studies in which the PRC to melatonin was investigated. In one study the PRC of melatonin onset to melatonin administration was investigated [Lewy et al., 1992], whereas in the other the onset, the acrophase, and the offset of the nocturnal melatonin rise were considered [Zaidan et al., 1994]. After normalization of the data, it is evident that the PRCs for the onset and for the offset reported in the two studies are different [Lewy et al., 1992; Zaidan et al., 1994] (Fig. 1). In accordance with the reported PRCs, exposure to light in the morning may induce phase advances that are favored by the elimination of the delaying effect of melatonin on the circadian indices of its own production. However, since in the morning the phase delaying properties of melatonin are more pronounced for the offset than for the onset (Fig. 1), removal of melatonin results in a greater advance of the offset than of the onset, and ultimately in a compression of the nocturnal melatonin rise. On the contrary, a prolongation of melatonin in the late morning, following hormone administration or delayed exposure to light, may phase advance the onset and phase delay the offset. Thereby rapidly prolong the nocturnal melatonin rise (Fig. 1). This theoretical model may easily explain the rapid adaptation of the nocturnal melatonin signal to photoperiodic modifications, but focused studies are needed to confirm or to deny its validity.

The sites where melatonin may act to induce a differential control of the onset and offset of its own secretion are unknown, but SCN cannot be excluded, since populations of neurons responding to melatonin in an opposite fashion have been detected in this nuclear group [Margraf and Lynch, 1993].

Future perspectives

The role exerted by melatonin in humans is being delineated. Actions clearly ascertained are those exerted on body temperature and sleep regulation, while less clear are the effects of melatonin on reproductive processes. Emerging evidence indicates that melatonin also exerts important effects on the regulation of human circadian rhythms. In this regard, melatonin not only transmits photoperiodic information to all body compartments but actively influences the mechanisms that generate and regulate circadian rhythms. Therapeutic implications from these actions can be envisioned.

In addition to these effects, there is an increasing amount of data supporting other important functions for melatonin. Melatonin is a scavenger of free radicals [Reiter et al., 1993], and its decline with age may render the body more sensible to oxidative damage. Accordingly, slowing of ageing processes are a reported consequence of prolonged melatonin supplementation, as supported by recent data in mice [Pierpaoli et al., 1995].

Reduced levels of melatonin have been found in humans with coronary artery disease [Brugger et al., 1995], and the possibility that such a reduction may favor the development of cardiovascular diseases should not be disregarded. Indeed, melatonin reduces platelet aggregations [Del Zar et al., 1990] and lipid oxidation [Melchiorri et al., 1995; Reiter, 1995; Reiter et al., 1995], both of which are involved in atherogenesis [Fuster et al., 1992; Regnstrom et al., 1992; Reiter et al., 1995] reduces stimulated norepinephrine levels [Cagnacci et al., 1996b,c], lowers blood pressure both in normotensive [Cagnacci et al., 1996b,c] and hypertensive [Birau et al., 1981] individuals, and reduces resistance to blood flow in great vessels [Cagnacci et al., 1996b,c]. These actions may indicate a protective effect of melatonin on cardiovascular diseases, but clinical trials are needed to prove this possibility.

Finally, future clinical implications may derive from the immunomodulatory functions, which have been demonstrated for melatonin in experimental models [Conti and Maestroni,1995].

In conclusion, the role of melatonin in humans has been delineated only in part. Additional important implications for melatonin on human physiology, pathology, and therapy seem to be warranted in light of available preliminary clinical data.

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Mechanisms of Disease

FRANKLIN H. EPSTEIN, M.D., *Editor*

MELATONIN IN HUMANS

AMNON BRZEZINSKI, M.D.

THREE centuries ago, the French philosopher René Descartes described the pineal gland as "the seat of the soul," but it was not until the late 1950s that melatonin, the principal substance secreted by the pineal gland, was identified.[1] There is now evidence that melatonin may have a role in the biologic regulation of circadian rhythms, sleep, mood, and perhaps reproduction, tumor growth, and aging (Table 1). However, uncertainties and doubts still surround the role of melatonin in human physiology and pathophysiology. This review summarizes current knowledge about melatonin in humans and its clinical implications.

PHYSIOLOGY AND PHARMACOLOGY

In humans, the pineal gland lies in the center of the brain, behind the third ventricle (Fig. 1). The gland consists of two types of cells: pinealocytes, which predominate and produce both indolamines (mostly melatonin) and peptides (such as arginine vasotocin), and neuroglial cells. The gland is highly vascular.

Melatonin, or *N*-acetyl-5-methoxytryptamine, was first identified in bovine pineal extracts on the basis of its ability to aggregate melanin granules and thereby lighten the color of frog skin.[1] In the biosynthesis of melatonin, tryptophan is first converted by tryptophan hydroxylase to 5-hydroxytryptophan, which is decarboxylated to serotonin. The synthesis of melatonin from serotonin is catalyzed by two enzymes (arylalkylamine *N*-acetyltransferase and hydroxyindole-*O*-methyltransferase) that are largely confined to the pineal gland.[2,3]

The mammalian pineal gland is a neuroendocrine transducer. Photic information from the retina is transmitted to the pineal gland through the suprachiasmatic nucleus of the hypothalamus and the sympathetic nervous system (Fig. 1). The neural input to the gland is norepinephrine, and the output is melatonin. The synthesis and release of melatonin are stimulated by darkness and inhibited by light. During daylight hours, the retinal photoreceptor cells are hyperpolarized, which inhibits the release of norepinephrine.[4] The retinohypothalamic–pineal system is quiescent, and little melatonin is secreted. With the onset of darkness, the photoreceptors release norepinephrine, thereby activating the system, and the number of α_1- and β_1-adrenergic receptors in the gland increases.[5] The activity of arylalkylamine *N*-acetyltransferase, the enzyme that regulates the rate of melatonin synthesis, is increased, initiating the synthesis and release of melatonin.

As the synthesis of melatonin increases, the hormone enters the bloodstream through passive diffusion. In humans, melatonin secretion increases soon after the onset of darkness, peaks in the middle of the night (between 2 and 4 a.m.), and gradually falls during the second half of the night. Serum melatonin concentrations vary considerably according to age. Infants younger than three months of age secrete very little melatonin. Melatonin secretion increases and becomes circadian in older infants, and the peak nocturnal concentrations are highest (average, 325 pg per milliliter [1400 pmol per liter]) at the age of one to three years, after which they decline gradually.[6] In normal young adults, the average daytime and peak nighttime values are 10 and 60 pg per milliliter (40 and 260 pmol per liter), respectively. The daytime rhythm in serum melatonin concentrations parallels the day–night cycle.[7,8] However, a rhythm of about 24 hours' duration also persists in normal subjects kept in continuous darkness.

The circadian rhythm of melatonin secretion is of endogenous origin, reflecting signals originating in the suprachiasmatic nucleus.[9] Environmental lighting does not cause the rhythm but entrains it (alters its timing). Light has two effects on melatonin: day–night light cycles modify the rhythm of its secretion (Fig. 2), and brief pulses of light of sufficient intensity and duration abruptly suppress its production.[10] In normal subjects, exposure to light inhibits melatonin secretion in a dose-dependent manner.[11] The threshold is 200 to 400 lux (equivalent to ordinary fluorescent light), and maximal inhibition occurs after exposure to intense light (600 lux or higher) for one hour. A longer exposure to light has no further suppressive effect on serum melatonin concentra-

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TABLE 1. BIOLOGIC FUNCTIONS AND PROCESSES THAT MAY BE AFFECTED BY MELATONIN AND SUGGESTED MECHANISMS OF ACTION IN HUMANS.

FUNCTION OR PROCESS	EFFECT	SUGGESTED MECHANISM	TYPE OF EVIDENCE
Sleep	Hypnotic effect and increased propensity for sleep	Hypothermic effect (at pharmacologic doses) Receptor-mediated action on limbic system	Placebo-controlled clinical trials
Circadian rhythm	Control of circadian rhythms and entrainment to light–dark cycle	Secretion of melatonin in response to neural input from the eyes and suprachiasmatic nucleus Receptor-mediated effects on neural and peripheral tissues Thermoregulation	Studies in animals and in humans on the effects of light and the light–dark cycle on the pattern of melatonin secretion
Mood	Possible role in cyclic mood disorders (seasonal affective disorder, depression)	Unknown	Comparative clinical studies of the pattern of melatonin secretion and studies of phototherapy for mood disorders
Sexual maturation and reproduction	Inhibition of reproductive process	Inhibition of hypothalamic–pituitary–gonadal axis Effect on ovarian steroidogenesis	Studies in animals and comparative clinical studies of the pattern of melatonin secretion (during puberty and in women with amenorrhea)
Cancer	Antiproliferative effects	Direct antiproliferative effect Enhanced immune response Scavenging of free radicals	In vitro and in vivo studies in animals, in vitro studies of human neoplastic cells and cell lines, and a few small clinical studies
Immune response	Enhanced immune response	Increased interleukin production by T-helper lymphocytes	Studies in animals and a few uncontrolled studies in humans
Aging	Possible protective effects and decreased cell damage	Scavenging of free radicals	In vitro and in vivo studies in animals

tions. Some blind persons with no pupillary light reflexes and no conscious visual perception have light-induced suppression of melatonin secretion,[12] suggesting the existence of two photoreceptive systems: one mediating melatonin secretion and the other mediating the conscious perception of light.

Melatonin is rapidly metabolized, chiefly in the liver, by hydroxylation (to 6-hydroxymelatonin) and, after conjugation with sulfuric or glucuronic acid, is excreted in the urine. The urinary excretion of 6-sulfatoxymelatonin (the chief metabolite of melatonin) closely parallels serum melatonin concentrations.[7] Intravenously administered melatonin is rapidly distributed (serum half-life, 0.5 to 5.6 minutes) and eliminated.[13] The bioavailability of orally administered melatonin varies widely. For example, in normal subjects given 80 mg of melatonin in a gelatin capsule, serum melatonin concentrations were 350 to 10,000 times higher than the usual nighttime peak 60 to 150 minutes later, and these values remained stable for 90 minutes.[14] Much lower oral doses (1 to 5 mg), which are now widely available in drugstores and food stores, result in serum melatonin concentrations that are 10 to 100 times higher than the usual nighttime peak within one hour after ingestion, followed by a decline to base-line values in four to eight hours. Very low oral doses (0.1 to 0.3 mg) given in the daytime result in peak serum concentrations that are within the normal nighttime range.[15]

No serious side effects or risks have been reported in association with the ingestion of melatonin. The dose-dependent physiologic effects of the hormone, however (e.g., hypothermia, increased sleepiness, decreased alertness, and possibly reproductive effects), have not yet been properly evaluated in people who take large doses for prolonged periods of time. Despite the general absence of a marked endocrine action, decreased serum luteinizing-hormone concentrations and increased serum prolactin concentrations have been reported after the administration of pharmacologic doses of melatonin in normal subjects.[16,17]

Numerous synthetic melatonin preparations are currently available at health-food stores and drugstores. The purity of some of these preparations is questionable. The consumer's only guarantee of purity is to purchase a preparation made by a company that follows good manufacturing practices (i.e., is able to pass an inspection by the Food and Drug Administration).

MECHANISMS OF ACTION

Receptors

Two membrane-bound melatonin-binding sites belonging to pharmacologically and kinetically distinct groups have been identified: ML1 (high-affinity [picomolar]) sites and ML2 (low-affinity [nanomolar]) sites.[18,19] Activation of ML1 melatonin receptors, which belong to the family of guanosine triphosphate–binding proteins (G protein–coupled receptors),[20] results in the inhibition of adenylate cyclase activity in target cells. These receptors are probably involved in the regulation of retinal function, circa-

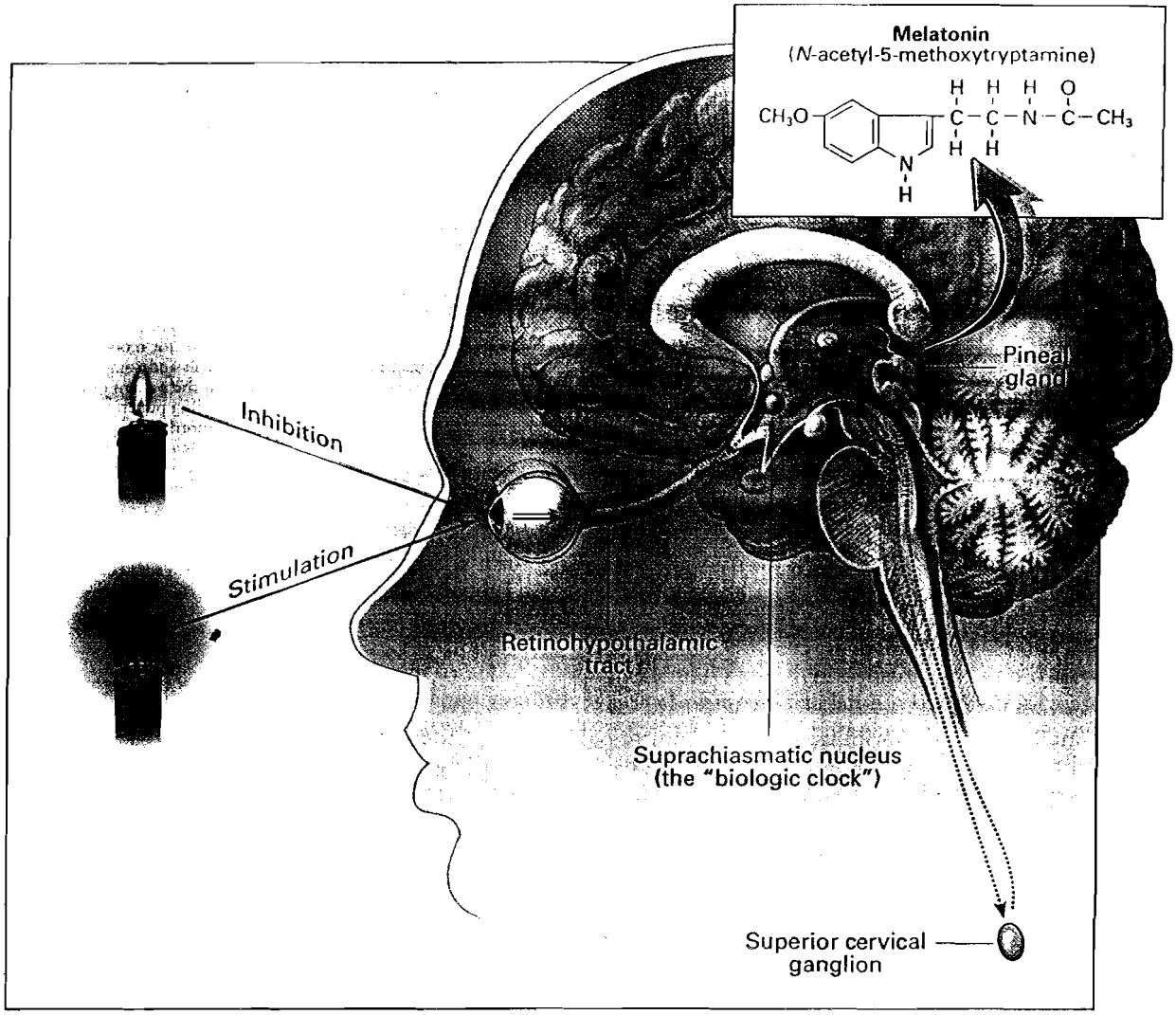

Figure 1. Physiology of Melatonin Secretion.

Melatonin (inset) is produced in the pineal gland. The production and secretion of melatonin are mediated largely by postganglionic retinal nerve fibers that pass through the retinohypothalamic tract to the suprachiasmatic nucleus, then to the superior cervical ganglion, and finally to the pineal gland. This neuronal system is activated by darkness and suppressed by light. The activation of α_1- and β_1-adrenergic receptors in the pineal gland raises cyclic AMP and calcium concentrations and activates arylalkylamine *N*-acetyltransferase, initiating the synthesis and release of melatonin. The daily rhythm of melatonin secretion is also controlled by an endogenous, free-running pacemaker located in the suprachiasmatic nucleus.

dian rhythms, and reproduction. The ML2 receptors are coupled to the stimulation of phosphoinositide hydrolysis, but their distribution has not been determined (Fig. 3). With the use of the polymerase chain reaction (PCR), two forms of a high-affinity melatonin receptor, which have been designated Mel1a and Mel1b, were cloned from several mammals, including humans.[21,22] The Mel1a receptor is expressed in the hypophysial pars tuberalis and the suprachiasmatic nucleus (the presumed sites of the reproductive and circadian actions of melatonin, respectively). The Mel1b melatonin receptor is expressed mainly in the retina and, to a lesser extent, in the brain.

Melatonin may also act at intracellular sites. Through binding to cytosolic calmodulin, the hormone may directly affect calcium signaling by interacting with target enzymes such as adenylate cyclase and phosphodiesterase, as well as with structural proteins.[23] Melatonin has recently been identified as a ligand for two orphan receptors (α and β) in the family of nuclear retinoid Z receptors.[24] The binding

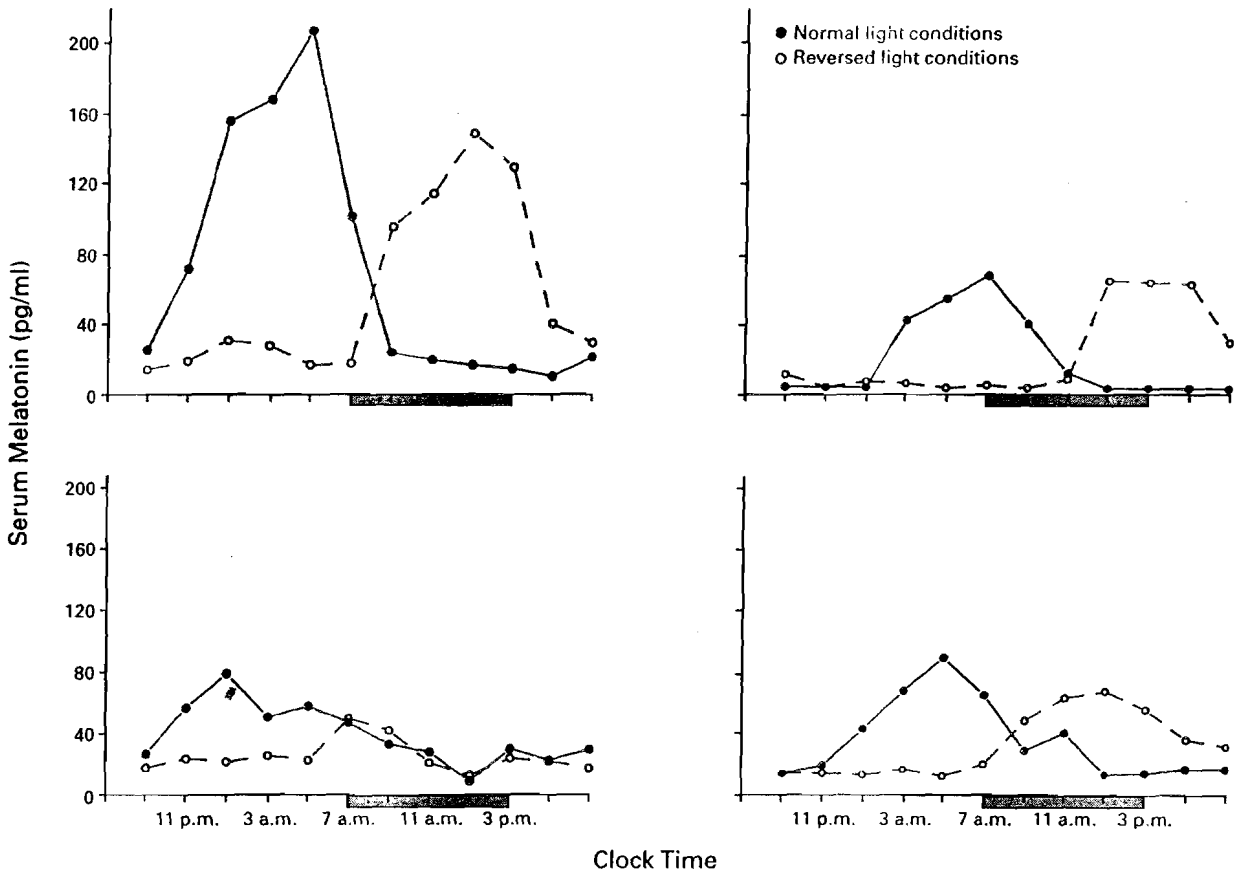

Figure 2. Serum Melatonin Concentrations in Four Normal Men (22 to 35 Years Old) Living under Normal Light Conditions (Solid Circles) and after Living under Reversed Light Conditions for Seven Days and Six Nights (Open Circles).

Under reversed light conditions, lights were out between 7 a.m. and 3 p.m. (shaded bars). The peak serum melatonin concentrations shifted from the nighttime, under normal conditions, to the daytime, under reversed light conditions. To convert values for serum melatonin to picomoles per liter, multiply by 4.31.

was in the low nanomolar range, suggesting that these receptors may be involved in nuclear signaling by the hormone.

Autoradiography and radioreceptor assays have demonstrated the presence of melatonin receptors in various regions of the human brain[25] and in the gut,[26] ovaries,[27] and blood vessels.[28] Neural receptors (e.g., those in the suprachiasmatic nucleus of the hypothalamus) are likely to regulate circadian rhythms. Non-neural melatonin receptors (such as those located in the pars tuberalis of the pituitary) probably regulate reproductive function, especially in seasonally breeding species, and receptors located in peripheral tissues (e.g., arteries) may be involved in the regulation of cardiovascular function and body temperature.

Free-Radical Scavenging

Both in vitro studies[29] and in vivo studies[30] have shown that melatonin is a potent scavenger of the highly toxic hydroxyl radical and other oxygen-centered radicals, suggesting that it has actions not mediated by receptors.[31] In one study, melatonin seemed to be more effective than other known antioxidants (e.g., mannitol, glutathione, and vitamin E) in protecting against oxidative damage.[31] Therefore, melatonin may provide protection against diseases that cause degenerative or proliferative changes by shielding macromolecules, particularly DNA, from such injuries. However, these antioxidant effects require concentrations of melatonin that are much higher than peak nighttime serum concentrations. Thus, the antioxidant effects of melatonin in humans probably occur only at pharmacologic concentrations.

Enhancement of Immune Function

Melatonin may exert certain biologic effects (such as the inhibition of tumor growth and counteraction of stress-induced immunodepression) by augmenting

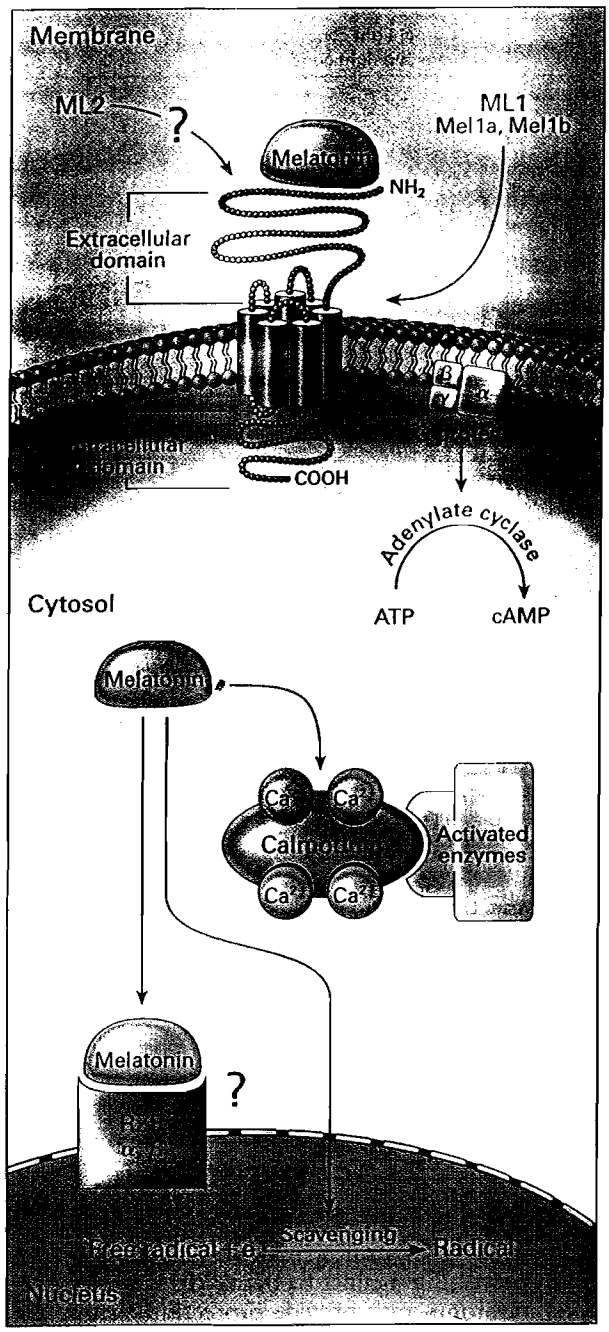

Figure 3. Suggested Sites and Mechanisms of Action of Melatonin at the Cellular Level.

Two membrane-bound melatonin receptors have been identified: ML1 (a high-affinity receptor) and ML2 (a low-affinity receptor). ML1 has two subtypes, designated Mel1a and Mel1b. By binding to its membrane-bound receptors, melatonin changes the conformation of the α subunit of specific intracellular G proteins, which then bind to adenylate cyclase and activate it. Cytosolic and nuclear binding sites have also been described. On binding to cytosolic calmodulin, melatonin may directly affect calcium signaling by interacting with target enzymes, such as adenylate cyclase and phosphodiesterase, and structural proteins. The nuclear binding sites are retinoid Z receptors (RZR) α and β. Melatonin scavenges oxygen-centered free radicals, especially the highly toxic hydroxyl radical, and neutralizes them by a single electron transfer (e), which results in detoxified radicals. The hormone may therefore protect macromolecules, particularly DNA, from oxidative damage. The question marks indicate mechanisms of action that have not been proved. cAMP denotes cyclic AMP.

the immune response.[32] Studies in mice have shown that melatonin stimulates the production of interleukin-4 in bone marrow T-helper cells and of granulocyte–macrophage colony-stimulating factor in stromal cells,[33] as well as protecting bone marrow cells from apoptosis induced by cytotoxic compounds.[34] The purported effect of melatonin on the immune system is supported by the finding of high-affinity (K_d, 0.27 nM) melatonin receptors in human T lymphocytes (CD4 cells) but not in B lymphocytes.[35]

SLEEP AND CIRCADIAN RHYTHMS

Sleep

In humans, the circadian rhythm for the release of melatonin from the pineal gland is closely synchronized with the habitual hours of sleep. Alterations in synchronization due to phase shifts (resulting from transmeridian airline flights across time zones or unusual working hours) or blindness are correlated with sleep disturbances. In the initial description of melatonin as a melanophore-lightening agent, its sedative effect in humans was noted.[36] More recently, serum melatonin concentrations were found to be significantly lower, with later peak nighttime concentrations, in elderly subjects with insomnia than in age-matched controls without insomnia.[37] Electrophysiologic recordings demonstrated that the timing of the steepest increase in nocturnal sleepiness (the "sleep gate") was significantly correlated with the rise in urinary 6-sulfatoxymelatonin excretion.[38]

Ingestion of melatonin affects sleep propensity (the speed of falling asleep), as well as the duration and quality of sleep (Table 2), and has hypnotic effects.[40,41] In young adults, oral administration of 5 mg of melatonin caused a significant increase in sleep propensity and the duration of rapid-eye-movement (REM) sleep.[48] In other studies, sleep propensity was increased in normal subjects given much lower doses of melatonin (0.1, 0.3, or 1 mg), either in the daytime[15] or in the evening,[46] and sleepiness in the morning was not increased. The time to the maximal hypnotic effect varies linearly from about three hours at noon to one hour at 9 p.m.[48] The administration of melatonin for three weeks in the form of sustained-release tablets (1 mg or 2 mg per day) may improve the quality and duration of sleep in elderly persons with insomnia.[44]

These results indicate that increasing serum mela-

TABLE 2. SUMMARY OF STUDIES OF THE EFFECTS OF EXOGENOUS MELATONIN ON SLEEP VARIABLES AND SLEEP DISTURBANCES.*

STUDY	YEAR	SUBJECTS	ADMINISTRATION OF MELATONIN		EFFECTS
			DOSE AND ROUTE	TIMING AND DURATION	
Cramer et al.[39]	1974	15 normal subjects	Single dose of 50 mg intravenously	At 9:30 p.m.	Decreased sleep-onset latency
Vollrath et al.[40]	1981	10 normal subjects	Single dose of 1.7 mg intranasally	During daytime	Induction of sleep
Lieberman et al.[41]	1984	14 normal subjects	Total dose of 240 mg intravenously (80 mg given three times over a 2-hr period)	During daytime	Reduced alertness, increased fatigue and sleepiness
Dahlitz et al.[42]	1991	8 patients with delayed-sleep-phase syndrome	Single dose of 5 mg orally	At 10 p.m., for 4 wk	Earlier onset of sleep and wake-up time
Haimov et al.[43]	1995	26 elderly subjects with insomnia	Single dose of 2 mg orally (sustained release in one group and fast release in another)	2 Hr before bedtime for 1 wk	Increased efficiency and duration of sleep in sustained-release group, improved initiation of sleep in fast-release group
Garfinkel et al.[44]	1995	12 elderly subjects with insomnia	Single dose of 2 mg orally, controlled release	At night for 3 wk	Increased efficiency of sleep, no effect on total sleep time
Oldani et al.[45]	1994	6 patients with delayed-sleep-phase syndrome	Single dose of 5 mg orally	For 1 mo	Advanced onset of sleep
Dollins et al.[15]	1994	20 young subjects	Single dose of 0.1 or 0.3 mg orally	At midday	Increased duration of sleep, decreased sleep-onset latency
Zhdanova et al.[46]	1995	6 young subjects	Single dose of 0.3 or 1.0 mg orally	At 6, 8, or 9 p.m.	Decreased sleep-onset latency, no effect on REM sleep
Wurtman and Zhdanova[47]	1995	9 elderly subjects with insomnia	Single dose of 0.3 mg orally	30 min before bedtime	Increased efficiency of sleep, decreased sleep-onset latency

*All studies except that by Oldani et al. were placebo-controlled. REM denotes rapid eye movement.

tonin concentrations (to normal nighttime values or pharmacologic values) can trigger the onset of sleep, regardless of the prevailing endogenous circadian rhythm. The hypnotic effect of melatonin may thus be independent of its synchronizing influence on the circadian rhythm and may be mediated by a lowering of the core body temperature.[49] This possibility is supported by the observations that the circadian cycle of body temperature is linked to the 24-hour cycle of subjective sleepiness and inversely related to serum melatonin concentrations and that pharmacologic doses of melatonin can induce a decrease in body temperature.[50,51] However, physiologic, sleep-promoting doses of melatonin do not have any effect on body temperature.[47] Alternatively, melatonin may modify brain levels of monoamine neurotransmitters, thereby initiating a cascade of events culminating in the activation of sleep mechanisms.

Circadian Rhythms

A phase shift in endogenous melatonin secretion occurs in airplane passengers after flights across time zones,[52] in night-shift workers,[53] and in patients with the delayed-sleep-phase syndrome (delayed onset of sleep and late waking up).[42] Subjects kept under constant illumination and some blind subjects have a 25-hour cycle of melatonin secretion.[54]

Bright light and ingestion of melatonin may alter the normal circadian rhythm of melatonin secretion,[55] but the reports on this effect are inconsistent, probably because of variations in the timing of the exposure to bright light or the administration of melatonin in relation to the light–dark cycle. The onset of nocturnal melatonin secretion begins earlier when subjects are exposed to bright light in the morning and later when they are exposed to bright light in the evening. The administration of melatonin in the early evening results in an earlier increase in endogenous nighttime secretion.[55] In a study of subjects traveling eastward across eight time zones,[52] 5 mg of melatonin given at 6 p.m. before their departure and at bedtime after their arrival apparently hastened their adaptation to sleep and alleviated self-reported symptoms of jet lag. In a study of flight-crew members on round-trip overseas flights,[56] those who took 5 mg of melatonin orally at bedtime on the day of the return to the point of origin and for the next five days reported fewer symptoms of jet lag and sleep disturbances, as well as lower levels of tiredness during the day, than those taking placebo. However, crew members who started to take melatonin three days before the day of arrival reported a poorer overall recovery from jet lag than the placebo group.

Exogenous melatonin thus appears to have some beneficial effects on the symptoms of jet lag, although the optimal dose and timing of ingestion have yet to be determined. It is also unclear whether the benefit of melatonin is derived primarily from a hypnotic effect or whether it actually promotes a resynchronization of the circadian rhythm.

Abnormal circadian rhythms have also been implicated in affective disorders, particularly in those char-

acterized by diurnal or seasonal patterns, such as endogenous depression and seasonal affective disorder (winter depression). Low nighttime serum melatonin concentrations have been reported in patients with depression,[57] and patients with seasonal affective disorder have phase-delayed melatonin secretion.[58] Although bright-light therapy reduced the depression scores of such patients in one study, a direct association with the phase-shifting effect of light on melatonin secretion was not substantiated.[59]

SEXUAL MATURATION AND REPRODUCTION

There is abundant evidence that the pineal gland, acting through the release of melatonin, affects reproductive performance in a wide variety of species. The efficacy of exogenous melatonin in modifying particular reproductive functions varies markedly among species, according to age and the timing of its administration in relation to the prevailing light–dark cycle or the estrus cycle. In some species melatonin has antigonadotropic actions, and the responses to it are greater in those species with greater seasonal shifts in gonadal function. Changes in the number of hours of darkness each day, and therefore the number of hours that melatonin is secreted, mediate the link between reproductive activity and the seasons. For example, in hamsters (a seasonal-breeding species) the reproductive system is inhibited by long periods of darkness, when more melatonin is secreted, leading to testicular regression in males and anestrus in females.[60] Although humans are not seasonal breeders, epidemiologic studies in several geographic areas point to a seasonal distribution in conception and birth rates.[61] Among people living in the Arctic, pituitary–gonadal function and conception rates are lower in the dark winter months than in the summer.[61,62]

The idea that the pineal gland may affect puberty dates back to 1898, when Heubner[63] described a 4.5-year-old boy with precocious puberty and a nonparenchymal tumor that had destroyed the pineal gland. Many similar cases were subsequently described, most of which involved boys. These cases support the idea that a melatonin deficiency can activate pituitary–gonadal function. As noted earlier, peak nighttime serum melatonin concentrations decline progressively throughout childhood and adolescence. Whether this reduction is related to changes in the secretion rate[64] or to increasing body size, without changes in secretion, is not known. If melatonin inhibits the activity of the hypothalamic gonadotropin-releasing–hormone pulse generator (as in ewes) or attenuates the response of the pituitary gland to stimulation by a gonadotropin-releasing hormone (as in neonatal rats), the onset of puberty in humans may be related to the decline in melatonin secretion that occurs as children grow.

No data are available from studies in humans to support either of these mechanisms. However, some children with precocious puberty have low levels of melatonin secretion for their age.[65] There is also a report of a man with hypogonadotropic hypogonadism, delayed puberty, and high serum melatonin concentrations in whom gonadotropin secretion increased and pubertal development occurred after a spontaneous decrease in the secretion of melatonin.[66] These findings provide some support for the hypothesis that melatonin has a role in the timing of puberty. Longitudinal studies are needed to determine whether there is a causal relation between the decline in serum melatonin concentrations and the time at which puberty occurs, as well as its rate of progression.

Melatonin secretion does not change during the menstrual cycle in normal women.[67] Similarly, substantial increases in serum estradiol concentrations do not alter melatonin secretion in infertile women with normal cycles.[68] On the other hand, serum melatonin concentrations are increased in women with hypothalamic amenorrhea[67,69,70] (Fig. 4). Men with hypogonadotropic hypogonadism also have increased serum melatonin concentrations, which decline in response to treatment with testosterone.[71] These findings suggest that changes in melatonin secretion may affect the production of sex steroids, and the converse may also be true.

In both animals that breed seasonally and those that do not, melatonin inhibits pituitary responses to gonadotropin-releasing hormone or its pulsatile secretion.[60] Although there are no similar data in humans, the increase in serum melatonin concentrations in women with hypothalamic amenorrhea raises the possibility of a causal relation between high melatonin concentrations and hypothalamic–pituitary–gonadal hypofunction. Serum melatonin concentrations also increase in response to fasting and sustained exercise, both of which, if prolonged, may cause amenorrhea. However, the hypersecretion of melatonin may merely be coincidental. In a study of normal young women, a very large daily dose of melatonin (300 mg) given orally for four months suppressed the midcycle surge in luteinizing-hormone secretion and partially inhibited ovulation, and the effects were enhanced by concomitant administration of a progestin.[72]

Melatonin may also modulate ovarian function directly. Ovarian follicular fluid contains substantial amounts of melatonin (average daytime concentration, 36 pg per milliliter [160 pmol per liter]),[73] and granulosa-cell membranes have melatonin receptors.[27] In addition, melatonin stimulates progesterone synthesis by granulosa–lutein cells in vitro.[74] Collectively, these findings suggest that melatonin plays a part in the intraovarian regulation of steroidogenesis.

AGING

The decrease in nighttime serum melatonin concentrations that occurs with aging, together with its multiple biologic effects, has led several investigators to suggest that melatonin has a role in aging and age-related diseases.[75,76] Studies in rats[77] and mice[78] suggest that diminished melatonin secretion may be associated with an acceleration of the aging process. Melatonin may provide protection against aging through attenuation of the effects of cell damage induced by free radicals or through immunoenhancement. However, the age-related reduction in nighttime melatonin secretion could well be a consequence of the aging process rather than its cause, and there are no data supporting an antiaging effect of melatonin in humans.

CANCER

There is evidence from experimental studies that melatonin influences the growth of spontaneous and induced tumors in animals. Pinealectomy enhances tumor growth, and the administration of melatonin reverses this effect or inhibits tumorigenesis caused by carcinogens.[79]

Data on the relation between melatonin and oncogenesis in humans are conflicting, but the majority of the reports point toward protective action. Low serum melatonin concentrations and low urinary excretion of melatonin metabolites have been reported in women with estrogen-receptor–positive breast cancer and men with prostatic cancer.[80-82]

The mechanism by which melatonin may inhibit tumor growth is not known. One possibility is that the hormone has antimitotic activity. Physiologic and pharmacologic concentrations of melatonin inhibit the proliferation of cultured epithelial breast-cancer cell lines (particularly MCF-7)[83] and malignant-melanoma cell lines (M-6) in a dose-dependent manner.[84] This effect may be the result of intranuclear down-regulation of gene expression or inhibition of the release and activity of stimulatory growth factors. Melatonin may also modulate the activity of various receptors in tumor cells. For example, it significantly decreased both estrogen-binding activity and the expression of estrogen receptors in a dose-specific and time-dependent manner in MCF-7 breast-cancer cells.[85] Another possibility is that melatonin has immunomodulatory activity. In studies in animals, melatonin enhanced the immune response by increasing the production of cytokines derived from T-helper cells (interleukin-2 and interleukin-4),[32] and as noted earlier, in mice melatonin protects bone marrow cells from apoptosis by enhancing the production of colony-stimulating factor by granulocytes and macrophages.[34] Lastly, as a potent free-radical scavenger, melatonin may provide protection against tumor growth by shielding molecules, especially DNA, from oxidative damage.[31] However, the antioxidant effects of melatonin occur only at very high concentrations.

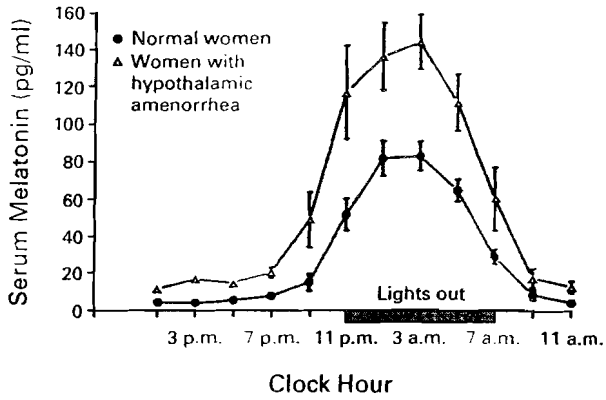

Figure 4. Mean (±SE) Serum Melatonin Concentrations Measured at 2-Hour Intervals for 24 Hours in 14 Normal Women (Circles) and 7 Women with Hypothalamic Amenorrhea (Triangles).

To convert values for serum melatonin to picomoles per liter, multiply by 4.31. Adapted from Brzezinski et al.[67] with the permission of the publisher.

The effects of melatonin have been studied in some patients with cancer, most of whom had advanced disease. In these studies, melatonin was generally given in large doses (20 to 40 mg per day orally) in combination with radiotherapy or chemotherapy. In a study of 30 patients with glioblastomas, the 16 patients treated with melatonin and radiotherapy lived longer than the 14 patients treated with radiation alone.[86] In another study by the same investigators, the addition of melatonin to tamoxifen in the treatment of 14 women with metastatic breast cancer appeared to slow the progression of the disease.[87] In a study of 40 patients with advanced malignant melanoma treated with high doses of melatonin (up to 700 mg per day), 6 had transient decreases in the size of some tumor masses.[88] It has been claimed that the addition of melatonin to chemotherapy or radiotherapy attenuates the damage to blood cells and thus makes the treatment more tolerable.[89] All these preliminary results must be confirmed in much larger groups followed for longer periods of time.

CONCLUSIONS

There is now evidence to support the contention that melatonin has a hypnotic effect in humans. Its peak serum concentrations coincide with sleep. Its administration in doses that raise the serum concentrations to levels that normally occur nocturnally can promote and sustain sleep. Higher doses also promote sleep, possibly by causing relative hypothermia. Exogenous melatonin can also influence circadian rhythms, thereby altering the timing of fatigue and sleep.

Abnormally high (or pharmacologic) concentrations of melatonin in women are associated with altered ovarian function and anovulation. It is tempting to speculate that the hormone also has antigonadal or antiovulatory effects in humans, as it does in some seasonal and nonseasonal mammalian breeders, but this possibility has not been substantiated. The antiproliferative and antiaging effects of melatonin are even more problematic. Uncontrolled use of melatonin to obtain any of these effects is not justified.

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Melatonin Metabolite Levels in Workers Exposed to 60-Hz Magnetic Fields: Work in Substations and with 3-Phase Conductors

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Melatonin suppression by 50/60-Hz magnetic fields represents a plausible biological mechanism for explaining increased health risks in workers. Personal exposure to magnetic fields and ambient light, and excretion of the melatonin metabolite 6-hydroxymelatonin sulfate (6-OHMS), were measured over 3 consecutive workdays in electric utility workers. There was a magnetic field–dependent reduction in adjusted an nocturnal and post-work 6-OHMS levels among men working more than 2 hours per day in substation and 3-phase environments and no effect among those working 2 hours or less. No changes were observed among men working in 1-phase environments. The results suggest that circular or elliptical magnetic field polarization, or another factor linked to substations and 3-phase electricity, is associated with magnetic field induced melatonin suppression in humans.

The issue of whether exposure to power frequency (50/60-Hz) electric and magnetic fields (EMFs) is associated with health effects in humans remains uncertain in part because human biological responses to EMF exposure have not been reproducibly characterized. The hormone, melatonin, has oncostatic,[1–2] immunological,[3–4] and antioxidant properties[5–6]; thus its suppression by EMFs represents a biologically plausible mechanism for increased cancer risks that have been observed in electric utility workers.[7–8]

Melatonin synthesis and secretion follow a diurnal pattern synchronized by ambient light, thereby exerting significant effects on circadian physiology.[9–10] Peak melatonin concentrations occur in the dark phase (0200 to 0400 hours), and lowest concentrations occur during the light phase (1200 to 1800 hours) of the 24-hour light–dark cycle.[9–10] Circulating melatonin levels are age dependent, although only small differences have been reported in subjects between the ages of 20 and 60 years.[11–12] Urinary concentrations of the major metabolite, 6-hydroxymelatonin sulfate (6-OHMS), are well correlated with circulating melatonin, and overnight 6-OHMS excretion represents an integrated measure of nocturnal melatonin production.[13–14]

In experimental animals, exposure to 50/60-Hz magnetic fields has been associated with reduced circulating and pineal melatonin concentrations, although these effects have not been

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b' :d consistently.[15–16] Differ- nc.. in genetic composition; the ming, duration, or intensity of exosure; field polarization; lighting onditions; or other factors may exlain divergent findings among labratory species. Epidemiological tudies of human melatonin levels in esponse to EMF exposure have been erformed in male utility work- :rs,[17–18] healthy women,[19] male railway workers,[20] electric blanket us- :rs,[21] and workers using video lisplay terminals.[22] There was wide variation in the exposure conditions; the duration, precision, and type of measures obtained; the presence of possible confounders (light at night, shift work), and the general characteristics of participants among these studies. Although the response to individual exposure metrics was not always consistent, each study showed some decrement in urinary 6-OHMS excretion.[23]

asons for the inconsistency a ag the various human and animal studies remain to be elucidated. One potential explanation is that EMFs have no effect on melatonin production and that some unidentified factor produced a number of false positives.[16] Alternatively, one or more critical factors that can modify the effects of EMFs on melatonin may not have been carefully considered in all studies.[16] Kato and coworkers[24–27] reported that circularly polarized fields or elliptical fields with a small axial ratio were most effective at suppressing nocturnal melatonin production in rats, whereas linearly polarized fields or elliptical fields with a large axial ratio had little or no effect. Although numerous investigations of melatonin levels in response to 50/60-Hz EMF exposure have been performed subsequently in rodents, no other studies used circularly or elliptically larized magnetic fields. Magnetic ds in close proximity to energized 3-phase conductors (eg, 3-phase distribution lines and substations) have circular or elliptical polarization,[28] whereas those associated with single phase conductors are linearly polarized. Exposure monitoring in substations as well as in residential settings has confirmed the presence of elliptically polarized fields.[29] The purpose of this analysis was to test the hypothesis that the effect of 60-Hz magnetic field exposure on 6-OHMS excretion was greatest among utility employees working in substations or in the vicinity of energized 3-phase conductors, and that work around 1-phase conductors had little or no effect on 6-OHMS excretion.

Methods

The study population was comprised of male workers from six utilities who were engaged in electric power generation (power plant operators, mechanics, electricians), distribution (linemen, meter readers, substation operators), and comparison (utility administrative and maintenance) activities. Data collection was performed between January and September 1997, using procedures similar to those reported previously.[17–18] Serial biological monitoring of urinary 6-OHMS excretion was combined with concomitant measurement of personal exposure to 60-Hz magnetic fields and ambient light. Magnetic field and light exposures were recorded at 15-second intervals over the first 3 days of the subjects' workweek using EMDEX II meters (Enertech Consultants, Campbell, CA) worn at the waist. The light sensor was adapted to the EMDEX via the external sensor jack. A custom computer program was developed to calculate magnetic field and light exposure metrics. Work-related activities (work in substations, in the vicinity of 3-phase or 1-phase conductors, office, and travel) were recorded in 30-minute increments in a log kept by each participant. Subjects were instructed to log their activities if they had been within approximately 1 meter (arm's length) of an energized conductor (3-phase, 1-phase, or within a substation) for at least 30 minutes.

Melatonin production was assessed by radioimmunoassay of urinary 6-OHMS concentrations (CIDtech, Mississagua, Ontario, Canada).[30–31] Participants provided overnight urine samples, combining any voids after bedtime with the first morning void on each day of participation. Daily post-work urine samples were also collected. Total overnight 6-OHMS excretion was estimated as the product of the overnight urine volume and the 6-OHMS concentration in each sample. Nocturnal and post-work 6-OHMS concentrations normalized to creatinine (6-OHMS/cr) were also analyzed. The interassay coefficient of variation for 6-OHMS was 8% at 10.5 ng/mL; within-assay variability ranged from 4% to 10% (mean, 6%); and the limit of detection was 0.1 ng/mL.

Data analyses were performed by using the Proc Mixed procedure for repeated measures in version 6.12 of the Statistical Analysis Software computer package (SAS Institute Inc, Cary, NC). Workplace exposure metrics based on either field intensity (time-weighted geometric mean) or temporal stability (standardized rate of change metric [RCMS]) were calculated for each workday of participation.[17–18] The RCMS estimates first-lag serial autocorrelation of personal magnetic field exposures; low values of RCMS represent temporally stable exposures.[32] Ambient light exposure was summarized using the workshift arithmetic time-weighted average. Analyses were performed using log-transformed values of overnight 6-OHMS, 6-OHMS/cr, ambient light, and geometric mean magnetic field exposures (RCMS was untransformed). Mean values were back-transformed for presentation in the tables.

Subjects were first grouped into tertiles of workplace magnetic field exposure and then into groups who spent more than 2 hours, or 2 hours or less, per day in substations or 3-phase environments. Because substation and 3-phase environments

LE 1
Magnetic Field Exposures for Work Activities

Time Spent Performing Activity	Workplace Exposure Tertiles: Substation and 3-Phase Activities			Workplace Exposure Tertiles: 1-Phase Activities		
	1	2	3	1	2	3
Geometric mean (μT)						
≤2 hours	0.04 ± 0.10 (142)*	0.08 ± 0.10 (133)	0.20 ± 0.10 (96)	0.04 ± 0.10 (137)	0.08 ± 0.10 (146)	0.22 ± 0.10 (133)
>2 hours	0.03 ± 0.12 (6)	0.09 ± 0.11 (18)	0.27 ± 0.11 (52)†	0.04 ± 0.11 (11)	0.10 ± 0.12 (5)	0.20 ± 0.11 (15)
RCMS[a] exposures (per 15 sec)						
≤2 hours	1.04 ± 0.01 (140)	0.74 ± 0.01 (125)	0.46 ± 0.01 (106)	1.03 ± 0.01 (142)	0.73 ± 0.01 (135)	0.42 ± 0.01 (139)
>2 hours	0.95 ± 0.04 (9)‡	0.68 ± 0.02 (22)‡	0.36 ± 0.02 (45)†	1.05 ± 0.04 (7)	0.70 ± 0.03 (12)	0.48 ± 0.03 (12)

[a] RCMS, standardized rate of change metric.
* Mean ± standard error of the mean (worker-days of exposure in parentheses).
† $P < 0.01$ vs ≤2 hour group.
‡ $P < 0.05$ vs ≤2 hour group.

were both expected to have circularly or elliptically polarized magnetic fields, these activities were combined. Mean magnetic field exposures among subjects with more than 2 hours, or 2 hours or less, of work in substation or 3-phase environments were compared statistically

hin each tertile by using the least ificant differences method in SAS. Least-squares means of 6-OHMS excretion (adjusted for the effects of age, ambient light exposure, and month of participation) were then calculated by exposure tertile in groups with more than 2 hours, or 2 hours or less, of work in substations and in 3-phase environments. Adjusted mean 6-OHMS levels in the high and low exposure tertiles were compared statistically for each group. The study population was then reclassified on the basis of work in the vicinity of 1-phase conductors, and analyses of mean 6-OHMS excretion in groups with more than 2 hours, or 2 hours or less, per day of 1-phase work were performed in the same manner. Additional analyses were performed using 0.5-, 1.0-, and 1.5-hour periods to assess cut point bias. There were insufficient worker-days of exposure t- ssess outcomes using cut points e 2 hours. Results of separate analyses incorporating potential confounding variables obtained from questionnaires, including personal, occupational, medical, and lifestyle factors, were consistent with those presented below.

Results

Complete data were available for 149 of 161 subjects; the mean age was 44 ± 9 years; and approximately 91% were Caucasian and non-Hispanic. There were 60 (40%) electric power distribution, 50 (33%) generation, and 39 (26%) comparison workers. Geometric mean magnetic field exposures for subjects working in substations and in the vicinity of 3-phase conductors were similar among subjects in the first and second exposure tertiles (Table 1). For subjects in the highest exposure tertile, geometric mean magnetic field exposures were greater for those with more than 2 hours of work in substations and in 3-phase environments (Table 1). Magnetic field exposures among men working more than 2 hours in substation/3-phase environments were more temporally stable than those with 2 hours or less (Table 1). For those working in 1-phase environments, there were no statistically significant differences in geometric mean or RCMS magnetic field exposures among those with more than 2 hours, or 2 hours or less, of work (Table 1).

A diurnal variation in mean urinary 6-OHMS excretion was observed among all subjects; mean concentrations were 3.0 ng/mg creatinine in the post-work and 18.2 ng/mg creatinine in the overnight samples. Results summarizing 6-OHMS excretion in response to occupational magnetic field exposure and substation/3-phase work activities are presented in Table 2. In workers with more than 2 hours of substation or 3-phase work, there was a clear trend of decreasing nocturnal 6-OHMS/cr excretion with increasing magnetic field exposure using either the geometric mean ($P = 0.03$) or the temporal stability metric ($P = 0.01$). Adjusted mean overnight 6-OHMS levels and post-work 6-OHMS/cr concentrations also exhibited a decreasing trend across tertiles of magnetic field exposure for those participating in more than 2 hours of substation and 3-phase activities, although statistically significant differences between the upper and lower tertiles were observed only for the temporal stability metric (Table 2). In contrast, no decrease in 6-OHMS excretion was observed among those with 2 hours or less of substation/3-phase work (Table 2). An increase in overnight 6-OHMS excretion was observed with increasing exposure to temporally stable magnetic fields among those with 2 hours or less of substation/3-phase work. However, statistically significant increases were not observed in this group for any of the other 6-OHMS variables

TABLE 2
Melatonin Metabolite Excretion* in Electric Utility Workers with Substation and 3-Phase Activities

Substation and 3-Phase	1	2	3	Difference: Tertile 1 vs 3	*P*-value: Tertile 1 vs 3
Workplace geometric mean exposure tertiles					
Nocturnal 6-OHMS/cr concentration (ng/mg cr)					
≤2 hours	15.0	14.9	14.7	−2%	0.84
>2 hours	23.5	18.0	13.5	−43%	0.03
Overnight 6-OHMS excretion (μg)					
≤2 hours	7.9	7.9	8.2	+4%	0.81
>2 hours	13.1	8.8	8.0	−39%	0.11
Post-work 6-OHMS/cr concentration (ng/mg cr)					
≤2 hours	2.1	2.4	2.5	+19%	0.19
>2 hours	3.5	1.8	2.3	−34%	0.21
Workplace temporal stability exposure tertiles					
Nocturnal 6-OHMS/cr concentration (ng/mg cr)					
≤2 hours	13.7	15.2	15.7	+13%	0.11
>2 hours	23.6	16.1	13.8	−42%	0.01
Overnight 6-OHMS excretion (μg)					
≤2 hours	7.2	8.1	8.8	+22%	0.05
>2 hours	13.5	8.9	7.8	−42%	0.03
Post-work 6-OHMS/cr concentration (ng/mg cr)					
≤2 hours	2.2	2.3	2.3	+5%	0.87
>2 hours	3.5	2.7	1.8	−49%	0.02

* Least squares means adjusted for the effects of age, average workplace light exposure, and month of participation.

TABLE 3
Melatonin Metabolite Excretion* in Electric Utility Workers with 1-Phase Activities

1-Phase Activities	1	2	3	Difference: Tertile 1 vs 3	*P*-value: Tertile 1 vs 3
Workplace geometric mean exposure tertiles					
Nocturnal 6-OHMS/cr concentration (ng/mg cr)					
≤2 hours	15.4	15.2	14.1	−8%	0.37
>2 hours	13.5	16.9	15.1	+12%	0.66
Overnight 6-OHMS excretion (μg)					
≤2 hours	8.1	8.0	8.1	0%	0.99
>2 hours	8.4	7.8	8.4	0%	0.98
Post-work 6-OHMS/cr concentration (ng/mg cr)					
≤2 hours	2.1	2.3	2.4	+14%	0.30
>2 hours	2.3	2.7	2.3	0%	0.96
Workplace temporal stability exposure tertiles					
Nocturnal 6-OHMS/cr concentration (ng/mg cr)					
≤2 hours	14.3	14.9	15.4	+8%	0.35
>2 hours	13.4	20.0	12.7	−5%	0.84
Overnight 6-OHMS excretion (μg)					
≤2 hours	7.5	8.0	8.5	+13%	0.20
>2 hours	7.4	9.5	7.9	+7%	0.82
Post-work 6-OHMS/cr concentration (ng/mg cr)					
≤2 hours	2.3	2.4	2.1	−9%	0.38
>2 hours	2.3	2.2	2.4	+4%	0.78

* Least squares means adjusted for the effects of age, average workplace light exposure, and month of participation.

or for magnetic field intensity. When the same analysis was performed for work in 1-phase environments, there were no statistically significant differences in mean 6-OHMS excretion for those with or without 2 hours of 1-phase work when using either the geometric mean or the temporal stability metric (Table 3).

Results obtained among workers with more than 1.0 or 1.5 hours of substation/3-phase work (Table 4) were very similar to those obtained using the 2-hour cut point (Table 3). Differences between the upper and lower tertiles were progressively greater as the duration of time spent in substation/3-phase environments increased. There were no statistically significant differences in mean 6-OHMS excretion among subjects below the chosen cut points for substation/3-phase activities or among those with 1-phase work activities above or below the cut points (results not shown).

Discussion

Decreased nocturnal or post-work urinary 6-OHMS excretion have been associated with magnetic field exposures in studies of electric railway workers[20] and in our earlier studies of electric utility workers.[17–18] In the present study, another population of male electric utility workers had decreased overnight 6-OHMS levels as well as lower nocturnal and post-work 6-OHMS/cr concentrations with increasing exposure to 60-Hz magnetic fields in substations or near energized 3-phase conductors. Differences in mean 6-OHMS excretion between the upper and lower exposure tertiles became progressively greater as the cut point for the amount of time spent in substations and in 3-phase environments increased from 0.5 to 2 hours. These findings are consistent with the hypothesis that magnetic fields with circular or elliptical polarization are more effective at suppressing melatonin production than linearly polarized fields.[24–27] The lack of effects observed in those with 2 hours or less of substation/3-phase work or among those with 1-phase exposures further supports the hypothesis. Alternatively, this classification scheme may have simply selected those with more intense and temporally stable exposures. How-

.E 4
Melatonin Metabolite Excretion*: Cut Point Analysis

Melatonin Metabolite	Above Cut Point for Substation and 3-Phase Activities		
	0.5 hours	1.0 hours	1.5 hours
Workplace geometric mean			
Nocturnal 6-OHMS/cr	−14% (P = 0.42)	−40% (P = 0.02)	−42% (P = 0.02)
Overnight 6-OHMS	−5% (P = 0.82)	−34% (P = 0.12)	−37% (P = 0.09)
Post-work 6-OHMS/cr	−12% (P = 0.55)	−33% (P = 0.21)	−32% (P = 0.23)
Workplace temporal stability			
Nocturnal 6-OHMS/cr	−26% (P = 0.11)	−37% (P = 0.02)	−39% (P = 0.02)
Overnight 6-OHMS	−22% (P = 0.23)	−36% (P = 0.06)	−38% (P = 0.04)
Post-work 6-OHMS/cr	−37% (P = 0.04)	−44% (P = 0.02)	−44% (P = 0.02)

* Difference in adjusted mean melatonin metabolite levels between the upper and lower magnetic field exposure tertiles.

ever, if intensity or temporal stability was the critical parameter, then one might also expect to observe a trend of decreasing mean 6-OHMS excretion among those with 2 hours or less of substation/3-phase work or among those with 1-phase exposures. A trend of decreasing mean 6-OHMS excretion was observed only among those with more than 2 hours of tation/3-phase work, even gh a gradient of exposure across tertiles and similar magnitudes of magnetic field intensity or temporal stability were observed among subjects in each group of substation/3-phase and 1-phase activity. Clearly, further investigation of magnetic field exposures in substations and in the vicinity of 3-phase and 1-phase conductors is needed. The intensity, temporal stability, and degree of magnetic field polarization in each environment should be quantitatively assessed along with other potentially relevant magnetic field parameters, such as high frequency transients and harmonic content.

Temporally stable magnetic field exposures that occurred in substation/3-phase environments were more strongly associated with decreased mean 6-OHMS excretion than magnetic field intensity, as measured by the geometric mean. These f ngs are consistent with previous s es in electric utility workers that indicated decreased 6-OHMS excretion in response to temporally stable magnetic field exposures.[17–18] The importance of temporally stable magnetic field exposures in eliciting biological effects was originally described by Litovitz and coworkers.[33] The basis for the biological activity of temporally stable exposures remains unexplained but may provide a clue as to the fundamental mechanism of interaction between 60-Hz magnetic fields and melatonin production. Kruglikov and Dertinger[34] indicate that a highly correlated exposure is required for stochastic resonance at a cellular level. However, further work is required to determine whether such a mechanism might mediate the effects of temporally stable magnetic field exposures on 6-OHMS excretion in humans.

Studies performed in rats by Kato and coworkers indicated that circularly polarized magnetic fields were more effective at inducing melatonin suppression than linearly polarized fields.[24–27] They observed decreased circulating melatonin concentrations in rats when using 1.4 μT circularly polarized magnetic fields.[24,25,27] The same group reported that chronic exposure to a horizontally polarized magnetic field was effective at a higher intensity of 5 μT but not at 1 μT.[26–27] Linearly polarized 50/60-Hz magnetic fields have been effective at reducing circulating melatonin levels in other rodent studies,[35–38] although results have been inconsistent.[39–42] Sheep penned under a 3-phase transmission line had no noticeable changes in circulating melatonin levels after 6 to 10 months of exposure.[42a] Field polarization at ground level under the power lines was not reported, although a large axial ratio (ie, close to linear polarization) would have been expected.[27–28] Inasmuch as no other laboratory has attempted to evaluate the effects of field polarization on magnetic field induced melatonin suppression in experimental animals, the role of this parameter remains undefined.

Human laboratory-based studies, performed using either circularly[43–44] or linearly polarized[45] magnetic fields, have generally yielded negative results. However, it is difficult to draw conclusions regarding the effectiveness of circular polarization from these studies owing to questions concerning the timing of exposure. Magnetic field induced delays in human melatonin secretion were observed by using circularly polarized fields when 20-μT exposures of 1.5 to 4.0 hours duration commenced before the nocturnal melatonin onset.[46] Similarly, decreased nocturnal 6-OHMS excretion in utility workers occurred in response to magnetic field exposures occurring at home, or for work and home exposures combined, but not during sleep.[17] Repeated short-term exposure (20 minutes per day for 3 weeks) to a high-intensity, 2900-μT magnetic field delivered before the nocturnal melatonin onset (1000 or 1800 hours) was also associated with reduced nocturnal melatonin production in humans.[47]

nd Shigemitsu[27] presented
al calculations to explain
y circularly or elliptically polar-
d fields would be more effective
suppressing melatonin than lin-
ly polarized fields. These authors
icate that magnetic fields with
cular or elliptical polarization are
pected to more effectively induce
ctrical currents in the rat pineal
nd. Recent estimates suggest that
cupationally relevant electric field
posures (10 kV/m) in humans may
sult in average induced current
nsities of 1451 $\mu A/m^2$ in the pi-
al gland compared with average
rrent densities of 6 $\mu A/m^2$ attained
wing to endogenous electrical ac-
vity.[48] However, differences due to
eld polarization were not ad-
ressed.

The characterization of human bi-
logical responses to 60-Hz mag-
netic fields is critical for determining
whether concern over potential
ıf effects is warranted. Melato-
uppression is a plausible link to
increased cancer risks that have been
associated with such exposures. Re-
sults from the present analysis sug-
gest that magnetic field induced mel-
atonin suppression seems to be
enhanced by work in substations and
with energized 3-phase conductors.
Failure to characterize magnetic field
polarization or other potentially im-
portant modifying factors[18,49] may
partially explain the inconsistent
findings reported to date. Recently
developed personal exposure devices
are now available to evaluate the role
of field polarization and other bio-
logically based exposure parameters
on human 6-OHMS excretion.[50] Re-
duced melatonin secretion may serve
as an important model for under-
standing human biological responses
to magnetic field exposures.

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Reduced Excretion of a Melatonin Metabolite in Workers Exposed to 60 Hz Magnetic Fields

ALL-STATE LEGAL®
EXHIBIT
27
Hillman

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The effects of occupational 60 Hz magnetic field and ambient light exposures on the pineal hormone, melatonin, were studied in 142 male electric utility workers in Colorado, 1995–1996. Melatonin was assessed by radioimmunoassay of its metabolite, 6-hydroxymelatonin sulfate (6-OHMS), in post-work shift urine samples. Personal magnetic field and light exposures were measured over 3 consecutive days using EMDEX C meters adapted with light sensors. Two independent components of magnetic field exposure, intensity (geometric time weighted average) and temporal stability (standardized rate of change metric or RCMS), were analyzed for their effects on creatinine-adjusted 6-OHMS concentrations (6-OHMS/cr) after adjustment for age, month, and light exposure. Geometric mean magnetic field exposures were not associated with 6-OHMS/cr excretion. Men in the highest quartile of temporally stable magnetic field exposure had lower 6-OHMS/cr concentrations on the second and third days compared with those in the lowest quartile. Light exposure modified the magnetic field effect. A progressive decrease in mean 6-OHMS/cr concentrations in response to temporally stable magnetic fields was observed in subjects with low workplace light exposures (predominantly office workers), whereas those with high ambient light exposure showed negligible magnetic field effects. Melatonin suppression may be useful for understanding human biologic responses to magnetic field exposures. *Am J Epidemiol* 1999;150:27–36

electricity; electromagnetic fields; 6-hydroxymelatonin sulfate; pineal body

Research on the biologic effects associated with occupational exposure to power frequency (50/60 Hz) electric and magnetic fields (EMFs) has intensified in recent years due to reported associations with leukemia and brain cancer (1, 2). Some biologic effects of EMF exposure may be mediated by the hormone, melatonin (3, 4). Melatonin is produced primarily by the pineal gland and its synthesis is directly inhibited by ambient light exposure, resulting in a diurnal secretory pattern (high at night, low during the day) (5). Melatonin suppression in response to magnetic field exposure has been reported both in experimental animals and humans (3, 4, 6, 7) and light exposure may be required to elicit a magnetic field effect (8–10). In addition to its well-characterized relation with endogenous circadian rhythms (11, 12), melatonin exerts physiologic effects that are relevant to carcinogenesis, including suppression of tumor growth in humans and experimental animals (13–15), enhancement of the immune response (15, 16), and scavenging of free radicals (17–19). Disrupted melatonin secretion following magnetic field exposure could therefore influence carcinogenesis via alteration of these processes. Melatonin also inhibits the secretion of estrogen and other tumor-promoting hormones (11, 12, 20, 21). Therefore, suppression of melatonin, induced either by EMFs alone or in combination with light-at-night, could enhance estrogen secretion, leading to increased breast cancer risk (4, 22). In support of this hypothesis, elevated breast cancer risks have been reported in male (23–26) and female (27–29) EMF-exposed workers although such effects have not been observed consistently (30–33).

Electric utility workers have occupational magnetic field exposures that are elevated relative to other occupations and they work in a complex electromagnetic environment with respect to the intensity and temporal characteristics of their exposure (34–38). Although magnetic field intensity (summarized by the time-weighted average [TWA]) is a commonly evaluated exposure metric, temporal characteristics of magnetic field exposure may be important for eliciting biologic effects, such as the increased enzymatic activity of ornithine decarboxylase (39–41). The temporal autocorrelation between successive EMF measurements

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Abbreviations: EMF, electric and magnetic field; 6-OHMS/cr, creatinine-adjusted 6-hydroxymelatonin sulfate; RCMS, standardized rate of change metric; TWA, time-weighted average.

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has been identified as a component of personal EMF exposure in electric utility workers that is independent of magnetic field intensity (38). Further, the temporal autocorrelation of residential magnetic field exposures may be important for predicting childhood leukemia risk when combined with other EMF exposure metrics (42).

Reduced excretion of the major urinary melatonin metabolite, 6-hydroxymelatonin sulfate (6-OHMS), has been shown in two studies of occupational EMF exposure (6, 7). Swiss railway workers were found to have reduced evening 6-OHMS excretion after 5 days of exposure to 16.7 Hz fields (7). Recently, we demonstrated decreased nocturnal 6-OHMS excretion associated with exposure to temporally stable 60 Hz magnetic fields in male electric utility workers (6). Temporal stability was assessed using an estimate of autocorrelation, and the effect was most pronounced when both residential and occupational exposures were combined (6). The current study reports the effects of occupational exposures to 60 Hz magnetic fields on post-work shift 6-OHMS excretion in the same population of electric utility workers using measures of field intensity and temporal autocorrelation.

MATERIALS AND METHODS

The study population was derived from three municipal electric utilities in Colorado. All employees, aged 20–60 years, with at least one month of electric utility work experience were contacted via orientation meetings or by telephone. The goal, based on statistical power calculations, was to obtain 200 participants; 195 subjects were eventually recruited. Of those 195 workers, data were available for 173, of which 142 were men. Workers with electric power generation, distribution, or administrative job descriptions were studied over a one-year period during daytime work hours (approximately 7:00 a.m. to 6:00 p.m.). Data collection was scheduled for the first 3 days of the work week to permit evaluation of changes in melatonin after time away from work (6, 7). Non-shift workers participated during the daytime after 2 days of non-occupational magnetic field exposure. In order to generate comparable data for shift workers, they participated while they were working during the day; however, their schedule provided 3 days off prior to commencing their day shift.

A questionnaire was used to collect additional information concerning factors that might influence magnetic field or light exposure and melatonin production. Potential confounders or modifiers included personal (age, race, body mass index), occupational (job title, years work experience, physical activity, work with specific chemicals, e.g., creosote, solvents, pesticides), life-style (tobacco and alcohol consumption, light-at-night, electrical appliance use, exercise), and medical factors (medications, disease history). No subjects were taking exogenous melatonin during participation.

Subjects collected one urine sample immediately following their work shift on each of 3 consecutive days (usually Monday, Tuesday, and Wednesday) for determination of 6-OHMS. Subjects also collected four consecutive overnight urine samples; the Monday morning sample was used to evaluate baseline nocturnal melatonin prior to resuming work. However, the logistics of having subjects collect a baseline "post-work shift" urine sample while off duty were considered not practical due to concerns about subject compliance and quality assurance. The melatonin metabolite 6-OHMS was measured in urine by radioimmunoassay (43-45) using materials supplied by CIDtech (Mississauga, Ontario, Canada). The interassay coefficient of variation for the slope of the standard curves obtained during this study was 4 percent and the limit of detection for 6-OHMS was 0.1 ng/ml. Concentrations of 6-OHMS were normalized to urinary creatinine concentrations (6-OHMS/cr) and are presented as nanograms 6-OHMS per milligram creatinine (ng/mg cr).

Work shift personal magnetic field and ambient light exposures were logged daily for all subjects at a rate of once every 15 seconds using EMDEX C meters (Electric Field Measurements, Stockbridge, Massachusetts) worn at the waist. Light exposure was measured with a light sensor (model LX101, Grasby Optronics, Orlando, Florida) adapted to the meter's external jack. This photoelectric detector produces a linear output current in proportion to light intensity, from less than 1 lux to approximately 100,000 lux. Exposure assessment was performed for 3 work days due to battery life and the capacity of digital memory in the meter. Subjects logged their work activities and hours on duty, permitting the calculation of daily workplace exposure metrics. Light exposure was summarized by calculating the work shift arithmetic TWA. The geometric TWA was used to assess the intensity of magnetic field exposure, and the standardized rate of change metric (RCMS), which estimates first-lag autocorrelation, was used to assess the temporal stability of exposure (6). Low values of RCMS represent relatively small differences between successive magnetic field measurements and are indicative of temporally stable exposures.

Analyses were performed with the Statistical Analysis Software (SAS) computer program (SAS Institute Inc., Cary, North Carolina) using log-transformed values for 6-OHMS/cr, geometric mean magnetic field exposures (untransformed values for RCMS), and light data. A

univariate procedure (*t*-test or analysis of variance (ANOVA) for categorical data and linear correlation for continuous data) was used to screen 98 questionnaire items for a potential association with 6-OHMS/cr using a cutpoint of $p \leq 0.10$. Multivariate statistical evaluations of the effects of magnetic field exposure on 6-OHMS/cr excretion were conducted using Proc Mixed for repeated measurements. Analyses were performed with adjustment for age, month of participation, and TWA light exposure, which were considered potential confounders a priori. The results were unchanged when other potential confounders selected using the univariate screening process were also included in the analysis (height, tobacco consumption, self-reported stress, exercise, shift work, use of electric ovens, use of cellular telephones, use of acetaminophen). Workplace magnetic field exposures were divided into quartiles and daily least-squares mean 6-OHMS/cr concentrations were estimated for each quartile. Mean 6-OHMS/cr levels in the lowest and highest quartiles were then compared using the least significant difference procedure in SAS. Data were also analyzed with Proc Mixed using magnetic field exposure metrics as continuous variables with age, month, and light exposure included as covariates. Potential interactions between magnetic field intensity and temporal stability were analyzed by including these metrics and their cross-product in the statistical model. Interaction terms for magnetic field metrics with light exposure were also analyzed.

RESULTS

The study population comprised 142 males: 56 (39 percent) distribution, 29 (20 percent) generation, and 57 (40 percent) administrative and maintenance (comparison) workers. The mean age (±standard error) of the population was 41 (±0.6) years; approximately 75 percent of the study population was between 30 and 50 years old. Hispanics and other non-Anglo or nonwhite racial/ethnic groups accounted for 10.5 percent of the population.

As expected, a diurnal variation in mean 6-OHMS/cr concentrations was observed; unadjusted mean 6-OHMS/cr concentrations were 38.3 (±1.5) ng/mg cr in the nocturnal (first void) samples and 9.0 (±0.4) ng/mg cr in the post-work shift samples for all subjects combined. Mean 6-OHMS/cr concentrations for selected personal and occupational factors are presented in table 1. A seasonal pattern in post-work 6-OHMS/cr concentrations was present with a peak during the winter and a trough during the summer months. In contrast, there were no statistically significant differences in mean 6-OHMS/cr levels across quartiles of workplace light exposure (table 1). When analyzed as a continuous variable, workplace light exposure was negatively associated with 6-OHMS/cr excretion ($p = 0.06$). The crude mean 6-OHMS/cr concentrations were elevated for electric power generation and shift workers. These differences were reduced after adjustment for month and light exposure. Generation and shift workers participated mainly during the winter and fall (97 percent and 82 percent, respectively), which is likely to explain the differences between crude and adjusted mean 6-OHMS/cr levels. Subjects who smoked more than one pack of cigarettes per day had higher 6-OHMS/cr excretion than those smoking less than one pack or nonsmokers. A slight reduction in 6-OHMS/cr concentrations was noted among workers who consumed alcohol. Among the other variables listed in table 1, statistically significant ($p < 0.05$) differences between crude means for recreational exercise and use of acetaminophen disappeared after adjustment for a priori confounders.

Crude and adjusted means for post-work shift 6-OHMS/cr levels are presented by quartile of workplace geometric mean magnetic field exposure in table 2. There were no statistically significant differences in 6-OHMS/cr excretion among subjects in the highest and lowest exposure quartiles although a tendency toward decreasing adjusted mean 6-OHMS/cr excretion was apparent on Day 3. Table 3 presents mean 6-OHMS/cr concentrations by quartile of temporally stable (RCMS) magnetic field exposure at work. A statistically significant difference in unadjusted mean 6-OHMS/cr excretion was observed on each day. After adjustment for age, month, and light exposure, there were no differences in 6-OHMS/cr concentration on Day 1 (table 3). However, men with temporally stable magnetic field exposures (quartile 4) had lower adjusted 6-OHMS/cr concentrations on Day 2 and Day 3, respectively, compared with those with temporally unstable exposures (quartile 1, table 3).

When analyzed as a continuous variable, geometric mean magnetic field exposure was not associated with 6-OHMS/cr excretion. A negative association was observed between 6-OHMS/cr excretion and temporally stable (RCMS) magnetic field exposure ($p = 0.06$). More stable magnetic field exposures were associated with lower concentrations of the melatonin metabolite. Neither the interaction term for geometric mean with RCMS magnetic field exposure nor the interaction term for the geometric mean magnetic field with ambient light exposure was associated with 6-OHMS/cr. However, there was a statistically significant interaction between temporally stable magnetic fields and ambient light exposures ($p = 0.02$). In subjects with workplace light exposures below the median, temporally stable magnetic field exposures were associated with decreased 6-OHMS/cr excretion ($p < 0.01$), whereas no

TABLE 1. Mean* creatinine-adjusted 6-hydroxymelatonin sulfate (6-OHMS/cr) concentrations for selected variables in male electric utility workers, Colorado, 1995–1996†

Variable	Crude mean‡ (ng/mg cr)		Adjusted mean‡ (ng/mg cr)	
Age group (years)				
20–30 (n = 17)	4.1	(2.6–6.6)	4.8	(3.5–6.5)
31–40 (n = 47)	6.1	(4.7–7.9)	4.1	(3.4–5.1)
41–50 (n = 59)	7.0	(5.5–8.9)	5.4	(4.6–6.3)
51–60 (n = 19)	7.4	(4.7–11.6)	4.6	(3.5–6.1)
Race				
Nonwhite or Hispanic (n = 15)	4.7	(2.7–8.3)	3.7	(2.7–5.3)
White (n = 125)	6.4	(5.5–7.5)	4.9	(4.4–5.5)
Occupational group				
Administrative/maintenance (n = 57)	6.5	(5.1–8.4)	4.6	(3.8–5.4)
Distribution (n = 56)	4.6	(3.6–5.8)	4.5	(3.8–5.4)
Generation (n = 29)	10.9	(8.8–13.5)	6.5	(4.8–8.7)
Season				
Winter (n = 45)	11.2	(9.4–13.4)	10.3	(8.5–12.6)
Spring (n = 21)	3.9	(3.1–4.8)	3.6	(2.7–4.8)
Summer (n = 32)	2.6	(1.8–3.6)	1.9	(1.5–2.4)
Fall (n = 44)	8.7	(7.0–10.8)	8.0	(6.5–9.8)
Mean light exposure				
≤262 lux (n = 30)	8.7	(6.4–11.9)	5.6	(4.8–6.5)
263–572 lux (n = 30)	5.4	(4.0–7.3)	4.3	(3.6–5.2)
573–1,791 lux (n = 31)	8.0	(5.8–10.9)	5.2	(4.3–6.2)
>1,791 lux (n = 30)	4.6	(3.4–6.1)	4.8	(3.9–5.8)
Cigarette smoking				
Nonsmokers (n = 113)	6.1	(5.1–7.3)	4.6	(4.1–5.3)
≤1 pack/day (n = 22)	6.5	(4.4–9.7)	5.5	(4.2–7.2)
>1 pack/day (n = 5)	11.8	(7.8–17.8)	8.0	(4.5–14.3)
Alcohol consumption				
Nondrinker (n = 39)	8.0	(6.0–10.7)	5.9	(4.8–7.2)
≤12 drinks/month (n = 51)	5.7	(4.4–7.3)	4.0	(3.3–4.7)
>12 drinks/month (n = 48)	5.6	(4.2–7.3)	5.1	(4.2–6.2)
Recreational Exercise				
>Once per week (n = 92)	5.6	(4.6–6.9)	4.8	(4.2–5.5)
Seldom or never (n = 50)	7.8	(6.3–9.6)	4.8	(4.0–5.8)
Use of acetaminophen				
Yes (n = 36)	8.3	(6.3–10.8)	5.3	(4.2–6.8)
No (n = 105)	5.7	(4.8–6.9)	4.7	(4.2–5.4)
Body mass index (kg/m^2)				
≤26 (n = 71)	6.6	(5.3–8.2)	5.0	(4.2–5.8)
>26 (n = 71)	6.0	(4.9–7.5)	4.7	(4.1–5.5)
Shift work				
Yes (n = 17)	11.0	(7.9–15.5)	6.0	(4.2–8.6)
No (n = 124)	5.8	(4.9–6.7)	4.6	(4.1–5.2)
Use of cell phone at work				
Never (n = 33)	6.5	(4.7–8.9)	5.0	(3.9–6.4)
Seldom <1x/week (n = 38)	7.7	(6.0–9.9)	4.7	(3.9–5.7)
Occasional 1x/day (n = 44)	6.0	(4.6–8.0)	5.1	(4.1–6.3)
Often >1x/day (n = 26)	4.8	(3.1–7.3)	4.5	(3.6–5.7)

* 95% confidence interval in parentheses.

† Variations in subject number are due to missing data for selected variables.

‡ Individual results were averaged across 3 days of observation and crude means were then compared by t-test or analysis of variance. Proc Mixed for repeated measurements was used to calculate least-squares means adjusted for the effects of age, month of participation, and workplace light exposure.

TABLE 2. Mean* creatinine-adjusted 6-hydroxymelatonin sulfate (6-OHMS/cr) concentrations by quartile of geometric mean magnetic field exposure at work in male electric utility workers, Colorado, 1995–1996†

	Workplace geometric mean magnetic field exposure quartile (μT)‡									
	I (≤ 0.078)		II (0.079–0.10)		III (0.10–0.135)		IV (> 0.135)		Relative change (%) in 6-OHMS/cr concentration, quartile I vs. IV	
	Mean (ng/mg cr)		Mean (ng/mg cr)		Mean (ng/mg cr)		Mean (ng/mg cr)		Mean (95% CI)	*p* value
Day 1										
Crude	4.8	(3.8–6.0)	7.1	(5.6–9.1)	5.9	(4.5–7.6)	5.0	(3.8–6.6)	4 (–35 to 33)	0.79
Adjusted	4.2	(3.3–5.4)	5.5	(4.4–7.1)	5.0	(3.9–6.4)	4.5	(3.5–6.0)	7 (–34 to 36)	0.68
Day 2										
Crude	5.2	(4.1–6.6)	4.6	(3.4–6.2)	6.1	(4.7–7.8)	5.7	(4.2–7.6)	10 (–33 to 39)	0.63
Adjusted	4.9	(3.8–6.4)	4.1	(3.0–5.5)	5.1	(4.1–6.5)	4.8	(3.6–6.5)	–2 (–48 to 30)	0.91
Day 3										
Crude	5.8	(4.6–7.5)	5.8	(4.4–7.6)	5.5	(4.1–7.4)	5.9	(4.4–7.9)	2 (–44 to 31)	0.99
Adjusted	5.8	(4.5–7.6)	4.8	(3.8–6.2)	4.5	(3.4–5.8)	4.4	(3.4–5.9)	–24 (–70 to 7)	0.14

* 95% confidence interval (CI) in parentheses.
† Least-squares means based on adjustment for age, season, and mean workplace light exposure.
‡ Data arranged from lowest (I) to highest (IV) quartile of workplace geometric mean magnetic field exposure. μT, microtesla.

TABLE 3. Mean* creatinine-adjusted 6-hydroxymelatonin sulfate (6-OHMS/cr) concentrations by quartile of temporally stable magnetic field exposure at work in male electric utility workers, Colorado, 1995–1996†

	Workplace RCMS magnetic field exposure quartile (per 15 seconds)‡									
	I (> 0.90)		II (0.89–0.75)		III (0.74–0.58)		IV (≤ 0.58)		Relative change (%) in 6-OHMS/cr concentration, quartile I vs. IV	
	Mean (ng/mg cr)		Mean (ng/mg cr)		Mean (ng/mg cr)		Mean (ng/mg cr)		Mean (95% CI)	*p* value
Day 1										
Crude	6.7	(5.2–8.7)	5.2	(4.0–6.7)	6.0	(4.6–7.9)	4.8	(3.8–6.1)	–28 (–65 to –1)	0.04
Adjusted	4.9	(3.8–6.3)	4.2	(3.3–5.3)	5.3	(4.1–7.0)	5.0	(3.9–6.4)	2 (–42 to 28)	0.95
Day 2										
Crude	7.5	(5.8–9.7)	5.8	(4.4–7.5)	5.0	(3.8–6.5)	4.4	(3.5–5.5)	–41 (–78 to –14)	<0.01
Adjusted	6.3	(4.8–8.3)	4.8	(3.8–6.2)	4.5	(3.4–5.9)	4.1	(3.2–5.2)	–35 (–79 to –5)	0.02
Day 3										
Crude	7.3	(5.4–9.8)	5.8	(4.2–8.0)	6.0	(4.7–7.8)	5.0	(4.0–6.2)	–31 (–72 to –2)	0.03
Adjusted	6.1	(4.6–8.0)	4.7	(3.5–6.4)	5.0	(4.0–6.3)	4.2	(3.3–5.2)	–31 (–73 to –2)	0.03

* 95% confidence interval (CI) in parentheses.
† Least-squares means based on adjustment for age, season, and mean workplace light exposure.
‡ Data arranged from highest (I) to lowest (IV) quartile of workplace RCMS magnetic field exposure. Low values of RCMS indicate temporally stable exposures. RCMS, standardized rate of change metric.

association was noted in workers with workplace light exposures above the median ($p = 0.40$). This interaction is illustrated in figure 1. Individuals in the lowest quartile of workplace light exposure showed a clear trend of decreasing mean 6-OHMS/cr concentrations with increasing exposure to temporally stable magnetic fields, whereas subjects in the highest (or intermediate [results not shown]) quartile of ambient light exposure had no differences in 6-OHMS/cr excretion across quartiles of temporally stable magnetic fields. The proportion of subjects who reported office work on their activity logs was greater for subjects in the lowest quartile of light exposure (71 percent) compared with those in the highest quartile (49 percent) ($p < 0.01$ by the chi-square test). When subjects were stratified according to the season in which they participated, subjects with low light exposures tended to have reduced mean 6-OHMS/cr levels in response to temporally stable magnetic field exposures regardless of their season of participation.

DISCUSSION

Exposure to temporally stable magnetic fields may elicit biologic effects in cellular systems (39–41). In our earlier analysis of electric utility workers (6), temporally stable 60 Hz magnetic field exposures at home or at home and work combined were associated with reductions in total overnight 6-OHMS excretion and nocturnal urinary 6-OHMS/cr concentration. In the current study, we provide evidence that occupational exposure to temporally stable magnetic fields is also associated with a reduction in post-work shift 6-OHMS/cr excretion. Adjusted mean post-work shift 6-OHMS/cr concentrations were unchanged on the first day (typically Monday) but were reduced on the second and third days of occupational exposure to temporally stable magnetic fields. This suggests that suppression of post-work shift 6-OHMS/cr excretion by RCMS magnetic fields is dependent on exposure duration and that several days may be required to elicit an effect.

These findings are reasonably consistent with those in Swiss railway workers (7), where statistically significant decreases in mean evening (samples collected at 6:00 p.m.) 6-OHMS concentrations were found in workers 1 and 5 days after occupational exposure to 16.7 Hz magnetic fields. In contrast to the Swiss study (7), we did not observe a reduction in mean 6-OHMS/cr on Day 1, which may have been due to differences in the intensity of magnetic field exposures, the duration of time off prior to resuming work (2–3 days vs. 7–21 days), or differences in the frequency of the magnetic field exposures (60 Hz vs. 16.7 Hz).

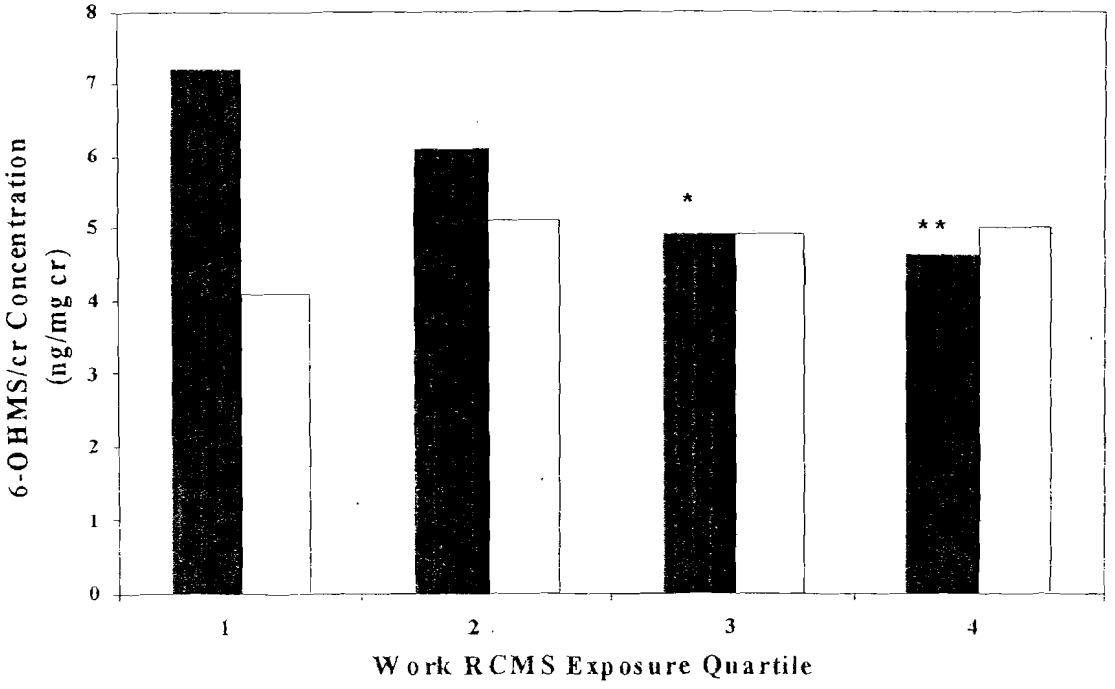

FIGURE 1. Least-squares means (adjusted for age and season) of daytime urinary creatinine-adjusted 6-hydroxymelatonin sulfate (6-OHMS/cr) concentrations for male electric utility workers in the lowest (black bars) and highest (white bars) quartiles of time-weighted average light exposure at work. Data are arranged by increasing quartile of temporally stable magnetic field exposure at work (i.e., 1 = highest quartile of standardized rate of change metric (RCMS), 4 = lowest quartile, etc.). *$p < 0.05$ vs. quartile 1; **$p < 0.01$ vs. quartile 1.

Some investigators have reported apparent compensatory increases in nocturnal (46) or evening (7) 6-OHMS excretion following termination of exposure. We did not measure 6-OHMS/cr levels over the weekend and thus were unable to determine whether increases occurred at those times. Any compensatory increases that may have occurred on Day 1 (Monday) due to cessation of occupational exposure over the weekend may have been negated by magnetic field-induced suppression of 6-OHMS/cr that occurred on Day 1 due to the resumption of workplace exposures.

The possibility that confounding could be introduced in this study was considered carefully. The effects of ambient light, perhaps the most important factor that influences melatonin synthesis, were carefully monitored by assessing personal light exposures concurrently with magnetic field exposures and by incorporating month of participation into the analysis. Other factors that affect light exposure or circadian rhythmicity, such as shift work and travel across time zones, were also considered in the analysis.

An effort was made to account for other factors that influence melatonin production (11, 12). Melatonin synthesis from tryptophan is mediated primarily by the binding of norepinephrine to its beta-1 receptor on pineal cells (11, 12). This activation can be enhanced by alpha-adrenergic stimulation, increased intracellular calcium, and prostaglandin production (11). The use of medications that influence these processes, such as beta adrenergic and calcium channel blockers, tranquilizers, antidepressants, and non-steroidal anti-inflammatory agents (aspirin, acetaminophen), was included in the questionnaire (11). Similarly, information was collected on other factors known to influence melatonin production, including age, body mass index, cigarette smoking, alcohol consumption, and exercise (11, 12). Alcohol and tobacco consumption can induce metabolic enzymes and may therefore increase melatonin metabolism and excretion. Evidence for such an effect was observed with cigarette smoking in this analysis but not with alcohol consumption (table 1). Substantial inter-individual differences in melatonin secretion have led some to suggest that racially distributed genetic polymorphisms may also influence melatonin production (47), although the difference between whites and nonwhites/Hispanics in this study was negligible (table 1).

The well-known negative association between age and melatonin secretion was not apparent in this study, which may have been due to the relative homogeneity in age among subjects. Decreases in melatonin production that occur between ages 30 and 50 years are moderate (48, 49) and results from this study are consistent with other studies in which no differences in circulating melatonin levels were observed among subjects within a limited age range (48, 50, 51). Although the possibility of residual confounding by some unmeasured factor cannot be excluded, screening for all known potential confounders as included in the questionnaire, and statistical adjustment for factors associated with 6-OHMS/cr did not alter the interpretation of the results when analyzed either individually or collectively.

Light exposure that occurred during work was analyzed because it coincided directly with the magnetic field exposure that was being assessed and because it was considered the most relevant time frame for influencing post-work shift 6-OHMS/cr levels. Pineal melatonin is released directly to the bloodstream following synthesis (11). The half-life of melatonin in circulation has been estimated at 20 to 30 minutes (52, 53), and metabolic clearance occurs within 4–8 hours (12). Thus, post-work shift sample collection should provide the best opportunity to evaluate workplace magnetic field induced changes in melatonin production. Measured light exposure outside this time frame was not considered relevant for post-work shift 6-OHMS/cr levels.

The seasonal variation in mean 6-OHMS/cr excretion observed in this study was consistent with previous reports (54–58). Ambient light exposure was not strongly associated with 6-OHMS/cr excretion after statistical adjustment for month of participation, indicating that seasonal photoperiodic changes were more important than workplace light exposures in determining post-work 6-OHMS/cr levels.

Because of its relatively rapid metabolic clearance, the timing of exposure in relation to sample collection may explain why workplace RCMS exposures had more of an effect on post-work shift rather than nocturnal 6-OHMS/cr levels. Temporally stable magnetic field exposures at work were associated with 31 percent and 35 percent decreases in mean post-work 6-OHMS/cr concentrations, whereas nocturnal 6-OHMS/cr levels from this population were only 7 percent lower in response to workplace RCMS magnetic field exposures (6). For nocturnal 6-OHMS/cr determinations in our earlier study (6), urine samples were collected on the morning after workplace exposures occurred, whereas samples were obtained immediately following the work shift in the present analysis. This may also explain why reduced concentrations of 6-OHMS were observed in post-work shift urine samples but not in first morning voids of railway workers exposed to 16.7 Hz magnetic fields (7).

The physiologic significance of nocturnal melatonin secretion is well established. Less is understood about the effects of melatonin secretion during the afternoon

or evening, but there are several reasons why reductions in melatonin at these times may be important. Mean daytime melatonin levels in circulation are approximately 10 pg/ml (12). These levels coincide with those required for activation of the melatonin receptor (approximately 5 to 14 pg/ml) (59, 60). Thus, modest (~30 percent) decreases in evening melatonin levels may reduce melatonin receptor activation, thereby altering functional melatonin responses. In humans, ambient light or magnetic field exposures that influence afternoon/evening melatonin levels also suppress or delay the onset of nocturnal melatonin production (6, 61–64). The combined reduction of both daytime and nocturnal melatonin secretion would lead to reduced 24-hour melatonin secretion, which could alter immunologic (15, 16), oncostatic (13–15), or antioxidant (17–19) processes influenced by melatonin.

The effects of temporally stable magnetic fields on 6-OHMS/cr excretion were modified by workplace light exposure. Adjusted mean 6-OHMS/cr concentrations among subjects within the highest quartile of ambient light exposure were 14 percent lower than those in the lowest quartile, whereas those in the highest quartile of temporally stable magnetic field exposures had adjusted mean 6-OHMS/cr levels that were 31 to 35 percent lower compared with those in the lowest quartile. Among individuals in the lowest quartile of ambient light exposure, there was a 36 percent difference in adjusted mean 6-OHMS/cr levels between those in the upper and lower quartiles of temporally stable magnetic field exposures. A dose-response trend of progressively lower 6-OHMS/cr levels with increasing exposure to temporally stable magnetic fields was noted for those with low workplace light exposure. The basis for the effect modification is uncertain; one possibility is that elevated light exposure suppressed post-work 6-OHMS/cr levels to such an extent that further decreases associated with magnetic field exposure were not detectable in those groups.

Alternatively, light exposure may be linked to the biologic mechanism of magnetic field effects. Perception of the earth's magnetic field in animals has been associated with photoreceptors located in the retina and/or the pineal gland (10, 65). In experimental animals, artificial manipulation of the earth's magnetic field suppresses melatonin production (8–10); in some studies, this effect was dependent on an intact visual system (9) or exposure to long wavelength (red) light (8). In our study, low levels of light exposure were most strongly associated with a magnetic field effect and subjects with low TWA light exposures were primarily engaged in office work. Artificial lighting has a different spectral composition and in some cases a greater red component than natural light (66, 67). Thus, spectral or other properties of artificial lighting may enhance the effects of magnetic fields on melatonin production.

In conclusion, results presented here provide further evidence that occupational exposure to magnetic fields is associated with reduced post-work shift 6-OHMS/cr excretion. Low ambient light exposures appear to have an important modifying effect. Additional research that incorporates a wide range of ambient light and temporally stable magnetic field exposure is needed to confirm these results and to elucidate the differential response to magnetic fields in subjects with high and low light exposure.

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Nocturnal excretion of a urinary melatonin metabolite among electric utility workers

by James B Burch, PhD,[1] John S Reif, DVM,[1] Michael G Yost, PhD,[2] Thomas J Keefe, PhD,[1] Charles A Pitrat, MS[1]

Burch JB, Reif JS, Yost MG, Keefe TJ, Pitrat CA. Nocturnal excretion of a urinary melatonin metabolite among electric utility workers. *Scand J Work Environ Health* 1998;24(3):183—189.

Objectives The effects of 60-Hz magnetic field and ambient light exposures on the pineal hormone melatonin were studied among electric utility workers.

Methods Personal exposure was measured at 15-second intervals over 3 consecutive 24-hour periods. Exposure metrics based on magnetic field intensity, intermittence, or temporal stability were calculated for periods of work, home, and sleep. A rate-of-change metric (RCM) was used to estimate intermittence, and the standardized RCM (RCMS = RCM/standard deviation) was used to evaluate temporal stability. The effects of magnetic field exposure on total overnight 6-hydroxymelatonin sulfate (6-OHMS) excretion and creatinine-adjusted nocturnal 6-OHMS (6-OHMS/cr) concentration were analyzed with adjustment for age, month, and light exposure.

Results Magnetic field intensity, intermittence, or cumulative exposure had little influence on nocturnal 6-OHMS excretion. Residential RCMS magnetic field exposures were associated with lower nocturnal 6-OHMS/cr concentrations. In multivariate statistical analyses, the interaction term for geometric mean and RCMS magnetic field exposures at home was associated with lower nocturnal 6-OHMS/cr and overnight 6-OHMS levels. Modest reductions in the mean 6-OHMS levels occurred after RCMS exposures during work. The greatest reductions occurred when RCMS exposures both at work and at home were combined; therefore the effects of temporally stable magnetic fields may be integrated over a large portion of the day.

Conclusions Results from this study provide evidence that temporally stable magnetic field exposures are associated with reduced nocturnal 6-OHMS excretion in humans.

Key terms electromagnetic fields, human, 6-hydroxymelatonin sulfate, 60 Hz, magnetic fields, pineal.

The potential health effects associated with exposure to power frequency (50/60 Hz) magnetic fields have received considerable attention in recent years, due in part to the pervasiveness of such exposures in the home and workplace. The characterization of human biological responses to magnetic field exposures is critical in determining whether such exposures result in adverse health effects. Some magnetic field effects may be mediated through reduced secretion of the hormone melatonin. Melatonin secretion follows a diurnal rhythm (night high, day low) that is synchronized by ambient light exposure (1). Through this mechanism, melatonin influences sleep and other physiological processes with circadian rhythms (2, 3). Melatonin is also associated with suppressed tumor growth (3—6), enhanced immunity (6—9), antioxidant effects (10—12), and reduced secretion of tumor-promoting hormones (13, 14). Decreased melatonin production could therefore have important biological consequences.

Although a reduction in melatonin synthesis following exposure to magnetic fields has been reported for a variety of experimental animal models (15), only a few studies have attempted to determine whether such effects occur in humans. In a study of electric blanket users, Wilson et al (16) found a reduction in nocturnal urinary concentrations of the major melatonin metabolite 6-hydroxymelatonin sulfate (6-OHMS or 6-sulfatoxymelatonin) in some persons after 8 weeks of electric blanket use. Cessation of electric blanket use was accompanied by an increase in 6-OHMS excretion (16). A reduction in early evening, but not overnight, 6-OHMS excretion was reported recently in a study of railway workers with occupational exposure to 16.7 Hz magnetic fields (17).

Elevated magnetic field exposures have been reported for electric utility workers (18—21). Numerous epidemiologic studies have identified this occupational group as having an elevated risk for developing leukemia (22)

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or brain cancer (23). Therefore, this study was designed to test the hypothesis that electric utility workers exposed to magnetic fields exhibit a decrease in nocturnal melatonin biosynthesis.

Subjects and methods

The study population comprised 142 male electric power utility workers aged 20 to 60 years. Generation workers [N=29] (utility electricians and operators), distribution workers [N=56] (linemen and substation operators), and a comparison group of utility maintenance and administrative staff [N=57] were studied concurrently over a 1-year period. The mean age was 41 (SD 0.6) years; approximately 90% were Caucasian and non-Hispanic. All the subjects worked a normal daytime shift during their participation in the study. A questionnaire was administered to collect information concerning personal (age, race, body mass index), occupational (job title, employment duration, use of cell phones and other equipment, physical activity, work with chemicals), life-style (tobacco and alcohol use, sleep habits, electrical appliance use, exercise), and medical factors (medication, disease history) that might influence magnetic field exposure or melatonin production. None of the subjects were taking exogenous melatonin.

Exposure assessment

Personal exposure to magnetic fields and ambient light was measured over a period of 3 consecutive workdays, and during the night preceding the first day of work. Twenty-four hour magnetic field and light exposures were recorded at 15-second intervals with EMDEX C meters (19). Light exposure was measured by a Grasby Optronics photometric sensor adapted to the external jack of the EMDEX. The meter was worn in a belt pack with the subject at work and off duty; it was placed beside the bed adjacent to the waist during the worker's sleep. Calibration logs and recordings of magnetic fields, light, and motion were inspected, and data were excluded if the meter was out of calibration, malfunctioning, or not worn. The participants logged their times at work, at home, and in bed, and exposures were partitioned accordingly. Home exposures were comprised mainly of time spent at the residence in the evening with a small component due to time at home prior to work.

Exposure metrics

Magnetic field and light exposure metrics were calculated for each exposure period and day of study. The arithmetic time-weighted average (TWA) was used to summarize personal light exposure. Magnetic field exposure metrics were selected a priori and included the arithmetic TWA, the geometric TWA, cumulative exposure, and cumulative exposure above 0.2 μT (24, 25). Two other metrics were calculated according to proposed mechanisms of magnetic field action. Exposure to fields with many switching events may have important biological implications (26—28). Therefore, a "rate of change metric" (RCM) based on the root-mean-square variation in successive magnetic field measurements was used to measure the intermittence of exposure (29):

$$RCM\ (\mu T/15\ s) = \sqrt{[\Sigma(MF_1 - MF_2)^2/(n-1)]},$$

where MF_1 and MF_2 are successive 15-second magnetic field measurements and n is the number of measurements within a given exposure period. The RCM provides an estimate of both the variability and the first-lag autocorrelation in a series of measurements. Higher RCM values indicate greater variability or less autocorrelation between successive readings or both. Others have suggested that temporally stable magnetic fields induce biological effects (30—32). The standardized RCM (RCMS) was therefore derived as follows:

$$RCMS\ (\text{per } 15\ s) = RCM/SD,$$

where SD is the standard deviation of the magnetic field measurements in a given period. The RCMS estimates the first-lag autocorrelation. Low RCMS values correspond to relatively small differences between successive measurements and represent magnetic field exposures that are stable over time. Thus low RCMS values should be directly associated with low 6-OHMS levels.

The geometric mean and RCMS magnetic field exposures are summarized in table 1 by worker group and exposure period. In general, the measures of magnetic field intensity correlated well. The geometric mean magnetic field exposures at work were higher for the generation workers than for the comparison workers ($P<0.01$). For comparison with other studies, the arithmetic means for the workplace exposures were 0.23, 0.32, and 0.15 μT for the distribution, generation, and comparison workers, respectively.

Determination of 6-hydroxymelatonin sulfate

Morning urine samples were collected daily for 4 days to determine the 6-OHMS levels. The base-line sample was obtained prior to the beginning of the workweek. The participants then submitted a morning sample on each of 3 consecutive workdays. Night-time and first morning voids were pooled to provide a total overnight sample. Melatonin production was assessed by a radioimmunoassay of urinary 6-OHMS concentrations (33, 34) (CI-Dtech, Mississauga, Ontario, Canada), which follow a diurnal pattern that is highly correlated with circulating melatonin (35). Total overnight 6-OHMS excretion and the nocturnal 6-OHMS concentration adjusted for creatinine (6-OHMS/cr) were calculated for each day.

Data analyses

Statistical analyses were performed using log-transformed data (log of the reciprocal for RCMS). Magnetic field exposures were compared among the distribution, generation, and comparison groups with a repeated-measures analysis of variance. Analyses for magnetic field effects were adjusted for age, month of participation, and TWA light exposure for the same period using Proc Mixed for repeated measures (SAS Institute Inc. Cary, NC, USA). Additional analyses were performed to evaluate potential confounding by the questionnaire variables; the results were essentially unchanged from those presented in this text. The potential effects of magnetic fields on the 6-OHMS excretion were modeled in 2 ways. First, 6-OHMS excretion was analyzed using each magnetic field metric as a continuous variable with age, month, and light exposure included as covariates in the Proc Mixed analysis, along with "day" and "magnetic fields by day". Second, magnetic field exposures were divided into quartiles, and the least-squares means (adjusted for age, month, and light exposure) were estimated for the 6-OHMS for each quartile of exposure. The means in the lowest and highest exposure quartiles were compared by Fisher's least significant difference method.

Results

The overall mean of the overnight 6-OHMS excretion was 22.7 (SD 1.3) µg, a value consistent with previously published data (35, 36). There was a statistically significant association between month of participation and both measures of 6-OHMS excretion ($P<0.01$): mean levels were higher in winter and lower in summer. Light exposures (TWA or cumulative lux) at home and during commutes from work to home were associated with lower 6-OHMS levels.

When each magnetic field metric was analyzed separately as a continuous variable, no statistically significant associations between 6-OHMS excretion and magnetic field intensity (TWA and cumulative exposures) or intermittence (RCM) were found. When RCMS was analyzed as a continuous variable, low RCMS exposures at home were associated with lower nocturnal 6-OHMS/cr concentrations ($P<0.01$) and lower overnight 6-OHMS excretion ($P=0.06$). No statistically significant reductions in 6-OHMS were found for RCMS exposures during work or sleep.

Further analyses were performed to determine whether temporally stable magnetic field exposures that occurred at higher field strengths had more of an effect than stable exposures at lower intensities. The geometric mean, RCMS, and their interaction term were included in the analysis as continuous variables. The interaction term for the geometric mean and RCMS magnetic field exposure at home was associated with lower 6-OHMS/cr concentrations ($P<0.01$) and with reduced overnight 6-OHMS excretion ($P<0.01$). Subjects with exposure to high-intensity magnetic fields that were also temporally stable had the lowest 6-OHMS levels. Similar results were obtained using the interaction term for RCMS with other intensity metrics at home. Interaction terms for RCMS with other exposure metrics during work or sleep were not associated with lower 6-OHMS levels.

The mean nocturnal 6-OHMS/cr concentrations and total overnight 6-OHMS excretion are presented by the quartile of the geometric mean and RCMS magnetic field exposures in tables 2 and 3, respectively. Quartile 4 represents the highest level of magnetic field intensity or temporal stability. Thus the mean 6-OHMS levels are arranged by increasing quartile of the geometric mean and decreasing quartile of the RCMS magnetic field exposure.

There was little evidence for reduced 6-OHMS excretion with increasing intensity of magnetic field exposure as measured by the geometric mean (table 2). However, the mean nocturnal 6-OHMS/cr concentrations were consistently lowest in subjects that were in the lowest quartile of RCMS exposure during work, home, or sleep (table 3). The difference between the highest and lowest quartiles was statistically significant for the home RCMS exposures ($P<0.01$). The mean overnight 6-OHMS excretion was also the lowest for the subjects that were in the lowest quartile of RCMS exposure although the differences

Table 1. Summary statistics for the magnetic field exposures of the male electric utility workers by exposure period. (RCMS = standardized rate of change metric)

Worker group	Geometric mean (µT)						RCMS (per 5 s)					
	Work[a]		Home[a]		Sleep[a]		Work[a]		Home[a]		Sleep[a]	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Distribution (N=56)	0.10	0.03	0.11	0.04	0.08	0.05	0.64	0.04	0.55**	0.03	0.50	0.04
Generation (N=29)	0.22**	0.07	0.14	0.05	0.11*	0.07	0.69	0.05	0.59	0.05	0.45*	0.04
Comparison (N=57)	0.10	0.03	0.09	0.04	0.06	0.05	0.73	0.03	0.68	0.04	0.58	0.04

[a] Mean and standard error of each exposure metric for days 1, 2, and 3 combined.
* $P \leq 0.05$ versus comparison group. ** $P \leq 0.01$ versus comparison group.

Table 2. Nocturnal 6-hydroxymelatonin sulfate (6-OHMS) excretion by the quartile of the geometric mean magnetic field exposure. (cr = creatinine)

Quartile of magnetic field exposure	Nocturnal 6-OHMS / cr concentration[a] (ng/mg)				Total overnight 6-OHMS excretion[a] (µg)			
	1	2	3	4	1	2	3	4
Work	29.6	25.2	30.0	28.5	17.7	15.0	17.8	14.8
Home	27.4	27.3	31.3	25.9	16.0	16.9	16.6	15.6
Sleep	25.3	31.9	30.6	26.8	15.1	17.7	17.7	16.3

[a] Least-square means based on adjustment for age, month of participation, and mean light exposure during the same period.

Table 3. Nocturnal 6-hydroxymelatonin sulfate (6-OHMS) excretion by the quartile of temporally stable magnetic field exposure. (cr = creatinine)

Quartile of magnetic field exposure	Nocturnal 6-OHMS / cr concentration[a] (ng/mg)				Total overnight 6-OHMS excretion (µg)			
	1	2	3	4	1	2	3	4
Work	29.2	27.5	30.9	27.2	17.6	17.3	16.6	15.1
Home	32.8	28.4	30.1	24.5**	18.4	15.6	18.6	14.9
Sleep	29.1	28.3	29.2	26.3	17.5	16.8	16.2	15.7

[a] Least-square means based on adjustment for age, month of participation, and mean light exposure during the same period.
** $P \leq 0.01$ for 1st versus 4th quartile.

between the highest and lowest quartiles were not statistically significant.

Additional analyses were performed to evaluate whether temporally stable magnetic field exposures over a larger portion of the day influenced 6-OHMS excretion. The subjects who were in the lowest quartile of RCMS exposure both at home and at work had mean nocturnal 6-OHMS/cr concentrations that were 39% lower than those in the highest quartile at home and at work (23.3 ng/mg versus 38.2 ng/mg, P=0.02) (figure 1). Similar results were obtained when these analyses were performed using the mean overnight 6-OHMS excretion as the dependent variable (12.9 µg versus 20.5 µg, P=0.03). A similar trend was noted for the subjects with temporally stable magnetic field exposure both at home and during sleep (results not shown).

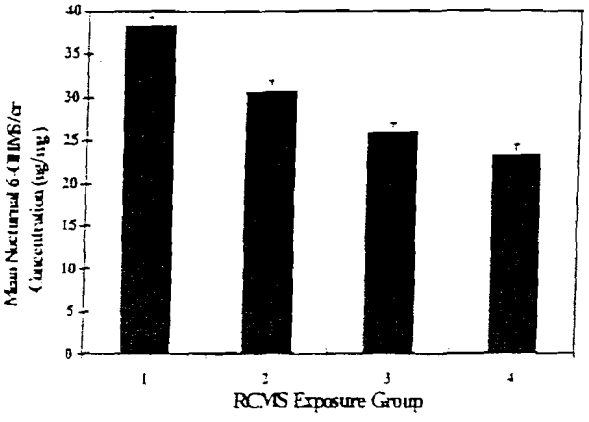

Figure 1. Least-square means of the nocturnal 6-hydroxymelatonin sulfate (6-OHMS) concentrations (ng 6-OHMS per mg creatinine) adjusted for the effects of age, month of participation, and ambient light exposure. The data have been summarized by group of magnetic field exposure using the standardized rate-of-change metric (RCMS): 4 = lowest quartile of RCMS exposure both at work and at home; 1 = highest quartile of RCMS exposure both at work and at home; 3 = lowest quartile of RCMS at work or at home, but not both; and 2 = all remaining subjects (P=0.02 for group 1 versus group 4).

Discussion

Our findings indicate that the temporal stability of magnetic fields may be important for eliciting biological effects in humans. This hypothesis was based on the findings of Litovitz et al, who measured ornithine decarboxylase (ODC) activity in vitro after exposure to magnetic fields in which the frequency was shifted at various time intervals (30). The ODC activity doubled when the frequency of a 10-µT magnetic field remained stable for intervals of at least 10 seconds (30); this finding suggests that 60-Hz magnetic fields must remain stable over time in order elicit effects (31, 32).

Based in part on these findings, the RCMS was developed as an estimate of the temporal stability of exposure. Consistent with this hypothesis, low RCMS values were associated with reduced 6-OHMS excretion. Time constants calculated from the lower quartile of the RCMS at work or at home indicated that exposures remaining highly correlated for intervals of at least 3 to 5 minutes on the average (assuming a first-order autoregressive model) were associated with reduced 6-OHMS levels. When analyzed separately, magnetic field intensity, intermittence, or cumulative exposure had little or no influence on

6-OHMS excretion although the intensities were relatively low. However, the interaction between residential magnetic field intensity and temporal stability was associated with a reduction in both 6-OHMS variables and therefore suggests that the effects of temporally stable magnetic fields are enhanced at higher field strengths.

Our results indicate that the timing of exposure to temporally stable fields may be important for suppressing 6-OHMS excretion. Light exposures that occur at times when people are expected to be at home (ie. near dawn and dusk) influence nocturnal melatonin production (37—39), and the magnetic field suppression of melatonin may be mediated by retinal photoreceptors (40—42). If so, magnetic field exposures may need to coincide with specific periods of photosensitivity for melatonin suppression to occur. In controlled human experiments, magnetic field exposures that occurred prior to the onset of nocturnal melatonin production resulted in a delay in onset and a suppression in peak nocturnal plasma melatonin concentrations (43). Nocturnal melatonin onset usually occurs between 1600 and 2000 hours (2), which corresponds to the time of day when most of the subjects were at home. Other investigators have failed to elicit a reproducible suppression of nocturnal melatonin production in humans using only overnight magnetic field exposures that started at 2300 hours, after the nocturnal melatonin onset (44—46). Similarly, we found no statistically significant reductions in 6-OHMS in association with exposures that occurred only during sleep.

Reductions in mean nocturnal 6-OHMS levels were modest after RCMS magnetic field exposures at work. The greatest reductions in the mean 6-OHMS levels were observed when RCMS exposures at work and at home were combined (figure 1). This finding suggests that the effects of temporally stable magnetic fields are integrated over a longer time span than the approximate 8-hour periods that were used in this study and that exposures occurring during the day influence melatonin production at night. Animal experiments indicate that several weeks of exposure to 50- to 60-Hz electric or magnetic fields over a large portion of the day appear to be the most effective means of suppressing melatonin (47—52) although there are some inconsistencies (53—56). Short-term exposures have been ineffective (57, 58) unless repeated daily for several weeks (59).

Melatonin suppression has been reported for experimental animals after exposure to rapidly switched magnetic fields (27, 28). Our results do not support a role for intermittent exposures in suppressing 6-OHMS excretion. However, intermittent changes in magnetic fields at intervals of less than 15 seconds could not be evaluated. The rate-of-change metric was designed to capture switching events but, like the EMDEX meter, it does not specifically quantify transient exposures. Thus the negative findings for RCM in this study and the positive findings of others (27, 28) suggest that future studies should more carefully characterize exposure to high-frequency transients.

One strength of our study was the ability to measure light exposure and adjust for its effects on melatonin production. However, the light sensor response was matched to that of the human eye and was not maximal at all wave lengths that produce the greatest melatonin inhibition (60). Thus the effects of the measured light on 6-OHMS excretion may have been somewhat attenuated due to the misclassification of exposures.

The reductions in mean nocturnal 6-OHMS excretion associated with RCMS magnetic field exposures in this study (approximately 20—40%) were consistent with those reported elsewhere (15—17), and the results were in general agreement whether nocturnal 6-OHMS/cr concentration or overnight 6-OHMS excretion was used as the outcome variable. Residential, rather than occupational, magnetic field exposures were most strongly associated with a reduction in nocturnal 6-OHMS excretion, which does not support the hypothesis that workplace exposures reduce 6-OHMS levels. However, the mean workplace exposures were lower than those reported by others (18—21), and they were only marginally higher than the mean residential exposures. Thus it was not possible to determine the effects of higher workplace magnetic field exposures on 6-OHMS excretion in our population. The finding that temporally stable magnetic field exposures, as measured by RCMS, are associated with reduced 6-OHMS excretion is unique and requires confirmation. Further work is also needed to determine whether 6-OHMS excretion is chronically suppressed in electric utility workers and to determine whether the effects are due to a reduction in the biosynthesis of melatonin, a phase shift in nocturnal melatonin production, or an increase in melatonin metabolism. Melatonin suppression may serve as a valuable tool for understanding human biological responses to magnetic fields.

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Melatonin metabolite excretion among cellular telephone users

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EXHIBIT
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Abstract.
Purpose: The relationship between cellular telephone use and excretion of the melatonin metabolite 6-hydroxymelatonin sulfate (6-OHMS) was evaluated in two populations of male electric utility workers (Study 1, $n=149$; Study 2, $n=77$).
Materials and methods: Participants collected urine samples and recorded cellular telephone use over 3 consecutive workdays. Personal 60-Hz magnetic field (MF) and ambient light exposures were characterized on the same days using EMDEX II meters. A repeated measures analysis was used to assess the effects of cellular telephone use, alone and combined with MF exposures, after adjustment for age, participation month and light exposure.
Results: No change in 6-OHMS excretion was observed among those with daily cellular telephone use >25 min in Study 1 (5 worker-days). Study 2 workers with >25 min cellular telephone use per day (13 worker-days) had lower creatinine-adjusted mean nocturnal 6-OHMS concentrations ($p=0.05$) and overnight 6-OHMS excretion ($p=0.03$) compared with those without cellular telephone use. There was also a linear trend of decreasing mean nocturnal 6-OHMS/creatinine concentrations ($p=0.02$) and overnight 6-OHMS excretion ($p=0.08$) across categories of increasing cellular telephone use. A combined effect of cellular telephone use and occupational 60-Hz MF exposure in reducing 6-OHMS excretion was also observed in Study 2.
Conclusions: Exposure-related reductions in 6-OHMS excretion were observed in Study 2, where daily cellular telephone use of >25 min was more prevalent. Prolonged use of cellular telephones may lead to reduced melatonin production, and elevated 60-Hz MF exposures may potentiate the effect.

1. Introduction

The use of cellular or mobile telephones has expanded rapidly in recent years. It is unclear whether exposure to the fields generated by these devices is linked with health effects. Some epidemiologic investigations indicate that cellular telephone exposures may be associated with elevated brain or ocular cancer risks (Hardell *et al.* 1999, 2000, Stang *et al.* 2001), particularly in brain regions closest to where the cellular telephone was held (Hardell *et al.* 1999, 2000). Others have reported no association between cellular telephone use and brain or other cancer risks (Dreyer *et al.* 1999, Morgan *et al.* 2000, Muscat *et al.* 2000, Inskip *et al.* 2001, Johansen *et al.* 2001). The interpretation of these initial studies is hindered by the relatively short follow-up periods in relation to tumour latency and with difficulties in accurately reconstructing the degree and type of exposure to cellular telephones (Rothman 2000, Frey 2001). Findings from two recent studies add to evidence suggesting that analogue cellular telephone use may be linked with increased brain cancer risks (Hardell *et al.* 2000, 2002, Auvinen *et al.* 2002). In Sweden, analogue cellular telephone use was associated with a brain tumour odds ratio (OR) of 1.3 (95% confidence interval [CI]: 1.02–1.6) (Hardell *et al.* 2002). The OR for acoustic neurinoma was 3.5 (1.8–6.8), whereas no clear association was observed for digital or cordless telephone use (Hardell *et al.* 2002).

Cellular telephone use has also been associated with cognitive and neurological symptoms as well as with altered EEG activity, sleep patterns and neuroendocrine function (Hyland 2000, Krewski *et al.* 2001). Reduced secretion of the hormone melatonin or the excretion of its major urinary metabolite, 6-OHMS, has been reported in some studies of humans exposed to magnetic fields (MFs) (Wilson *et al.* 1990, Pfluger and Minder 1996, Burch *et al.* 1998, 1999, 2000, Karasek *et al.* 1998, Wood *et al.* 1998, Juutilainen *et al.* 2000). Because melatonin has oncostatic (Conti and Maestroni 1995, Panzer and Viljoen 1997, Fraschini *et al.* 1998), immune-enhancing (Conti and Maestroni 1995, Fraschini *et al.* 1998) and antioxidant properties (Reiter 1998), reduced secretion of this hormone in response to MF exposure has been suggested as a plausible mechanism to explain increased cancer risks in human populations exposed to MFs (Stevens and Davis 1996). Studies of human melatonin production in response to cellular telephone exposures have been limited to small groups of healthy, young, white male subjects in laboratory-based settings using digital

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exposure) of overnight 6-OHMS excretion and nocturnal or post-work 6-OHMS/cr concentrations were calculated for each group. Least-squares means of the dependent variable are obtained in a multivariate model by holding other covariates in the model to their means (Searle *et al.* 1980). Adjusted mean 6-OHMS levels among workers without cellular telephone use were compared with those with >25 min of use via the least significant differences statistic in SAS. Separate analyses were performed for the Study 1 and 2 populations. Trend tests across categories of cellular telephone use were performed using linear contrasts in Proc Mixed based on coefficients that accounted for unequal cell sizes and uneven intervals between categories of cellular telephone use (Kirk 1982). To evaluate potential effect modification by 60-Hz fields, participants in different cellular telephone use categories were stratified into tertiles of mean workplace MF exposure and adjusted mean 6-OHMS levels in each stratum were compared as described above. In addition, questionnaire items were individually screened for potential associations with the 6-OHMS variables using a cut point of $p<0.10$. The interpretation of the results did not change when additional confounders selected for nocturnal 6-OHMS/cr (use of chemicals at work, exercise, use of electric bed heaters, electric appliance use, consumption of ibuprofen), overnight 6-OHMS (use of chemicals at work, height, ethnicity, eye colour, computer use, work outdoors, consumption of ibuprofen), and post-work 6-OHMS/cr (body mass index, employer, electric and microwave oven use, use of electric power tools) were included in the analysis.

3. Results

In Study 1, the mean (±SD) age of participants was 44±9 years; approximately 91% were Caucasian and non-Hispanic. There were 60 (40%) electric power distribution, 50 (34%) generation, and 39 (26%) administrative and maintenance (comparison) workers. The mean age of the Study 2 workers was 41±8 years and approximately 88% were Caucasian and non-Hispanic. There were 29 (38%) electric power distribution, 22 (29%) generation and 23 (30%) comparison workers (no response, $n=3$). As expected, there was a clear diurnal variation in 6-OHMS excretion among workers in both populations. Mean nocturnal 6-OHMS/cr levels (Study 1 = 18.2 ng mg^{-1} cr; Study 2 = 20.5 ng mg^{-1} cr) were approximately six times greater than post-work 6-OHMS cr levels (Study 1 = 3.1 ng mg^{-1} cr; Study 2 = 3.5 ng mg^{-1} cr). Inspection of covariance parameter estimates for 6-OHMS excretion indicated that within-subject variability was equal to or less than between-subject variability in both study populations (Littell *et al.* 1998).

The prevalence of cellular telephone use differed among workers in the two studies (figure 1). In Study 1, three subjects reported cellular telephone use of >25 min day^{-1} (5 worker-days total). Only one individual was in this category on all 3 days and there was no cellular telephone use >30 min day^{-1}. No statistically significant difference or trend in adjusted mean 6-OHMS excretion was observed among men with elevated cellular telephone use compared with those without cellular telephone use in Study 1 (table 1). In Study 2, five participants used cellular telephones for >25 min day^{-1} (13 worker-days total). Four of the five individuals used a cellular telephone daily for >25 min. In the Study 2 population, cellular telephone use >25 min day^{-1} was associated with lower adjusted mean nocturnal 6-OHMS/cr concentrations ($p=0.05$) and overnight 6-OHMS excretion ($p=0.03$) compared with those without cellular telephone use (table 1). The adjusted mean post-work 6-OHMS/cr concentrations were elevated among those with >25 min of cellular telephone use compared with those with none, although the difference was not statistically significant ($p=0.08$, table 1). There was a decreasing trend of adjusted mean nocturnal 6-OHMS/cr concentrations ($p=0.02$) and overnight 6-OHMS excretion ($p=0.08$) and an increasing trend of post-work 6-OHMS/cr levels ($p=0.09$) across categories of increasing cellular telephone use (table 1). Potential cut point bias was evaluated by re-analysing the data using 20 or 30 min of cellular telephone use per day to define the highest exposure group and the results were consistent with those described above (data not shown). Analyses

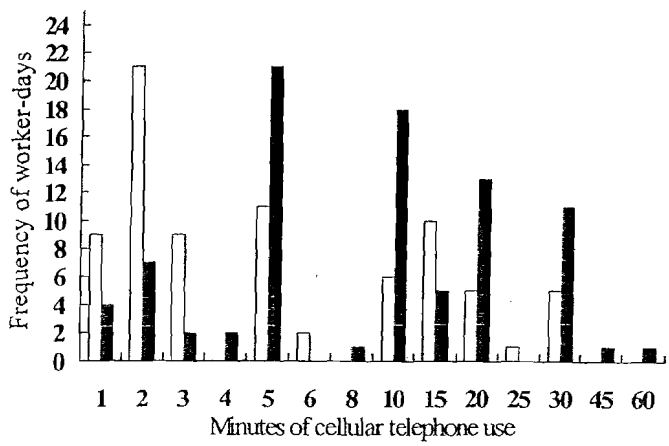

Figure 1. Number of worker-days of participation for different categories of cellular telephone use plotted among participants in Study 1 (1997, white bars) and Study 2 (1998, black bars).

Table 2. Melatonin metabolite excretion[a] in cellular phone users stratified by mean workplace magnetic field exposure, Colorado, 1998.

Categories of cellular phone use at work	Workplace arithmetic mean exposure tertiles[b] 1 (0.5 ± 0.2 mG)	2 (1.1 ± 0.2 mG)	3 (5.0 ± 8.3 mG)	Two-tailed *p*: 1 versus 3
Nocturnal 6-OHMS/cr (ng mg^{-1} cr)				
0 min	16.7 ± 1.1 (n = 38)	19.3 ± 1.1 (49)	20.6 ± 1.1 (50)	0.08
1–10 min	17.5 ± 1.1 (26)	15.1 ± 1.1 (14)	16.5 ± 1.2 (14)	0.75
> 10 min	17.3 ± 1.3 (5)	18.2 ± 1.2 (13)	7.8 ± 1.2 (10)	0.01
Two-tailed *p*: 0 versus > 10 min	0.90	0.74	< 0.01	
Overnight 6-OHMS (μg)				
0 min	10.3 ± 1.1 (n = 38)	10.5 ± 1.1 (49)	8.8 ± 1.1 (50)	0.18
1–10 min	10.0 ± 1.1 (26)	10.1 ± 1.1 (14)	10.5 ± 1.1 (14)	0.77
> 10 min	9.3 ± 1.2 (5)	11.0 ± 1.2 (13)	5.6 ± 1.2 (10)	0.04
Two-tailed *p*: 0 versus > 10 min	0.65	0.78	0.02	
Post-work 6-OHMS/cr (ng mg^{-1} cr)				
0 min	2.6 ± 1.1 (n = 38)	2.7 ± 1.1 (49)	2.6 ± 1.1 (50)	0.99
1–10 min	2.7 ± 1.1 (26)	2.8 ± 1.2 (14)	2.7 ± 1.2 (14)	0.96
> 10 min	2.6 ± 1.4 (5)	3.1 ± 1.2 (13)	3.8 ± 1.2 (10)	0.31
Two-tailed *p*: 0 versus > 10 min	0.99	0.46	0.10	

[a] Least-squares means ± SEM adjusted for age, light exposure at work, and month of participation (number of worker-days exposure in parentheses). The average number of workers in each category can be obtained by dividing worker-days by 3.
[b] Time-weighted arithmetic mean work shift 60-Hz magnetic field exposure (± SD) in parentheses.

reductions in adjusted mean 6-OHMS levels occurred on the third day of participation. The results suggest that a minimum daily and/or a multi-day threshold of cellular telephone use may be necessary to reduce 6-OHMS excretion.

Uncertainties about RF exposures and the small proportion of workers with extensive cellular telephone use limit the interpretation of our results. The dose of non-ionizing radiation received by a cellular telephone user depends on the duration of telephone use and on the type of telephone, the power output, the hand placement, the system traffic and the management software used, and the distance to a cellular telephone tower (ICNIRP 1996, Hyland 2000, Krewski *et al.* 2001). The power output of analogue cellular telephones is greater than that of digital telephones. However, it is possible that subjects with high daily cellular telephone use preferred digital telephones because of their longer battery life. Also, non-work cellular telephone use was not determined in our studies, although personal (off-duty) cellular telephone use is not expected to have been highly prevalent in 1997 and 1998 when these studies were performed.

An increase in adjusted mean post-work 6-OHMS/cr levels among workers with cellular telephone use > 25 min was observed in both studies, although neither finding was statistically significant. These results were not consistent with our previous findings (Burch *et al.* 1997, 1999), making it difficult to draw conclusions about post-work 6-OHMS/cr excretion and cellular telephone use. The finding that cellular telephone use during work was associated with a reduction in 6-OHMS excretion occurring later that night is consistent with previously observed decreases in nocturnal 6-OHMS excretion following residential and/or workplace exposure to power frequency MFs earlier in the day (Karasek *et al.* 1998, Burch *et al.* 1998, 2000, Juutilainen *et al.* 2000).

The population sizes in our studies were approximately three to 10 times greater than prior laboratory-based studies of human melatonin production in response to cellular telephone exposure. In addition

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