Bright Light Exposure of a Large Skin Area Does Not Affect Melatonin or Bilirubin Levels in Humans

Niki Lindblom, Taina Hätönen, Maija-Liisa Laakso, Aino Alila-Johansson, Marja-Leena Laipio, and Ursula Turpeinen

Background: Light treatment through the eyes is effective in alleviating the symptoms of some psychiatric disorders. A recent report suggested that skin light exposure can affect human circadian rhythms. Bilirubin can serve as a hypothetical blood-borne mediator of skin illumination into the brain. We studied whether bright light directed to a large body area could suppress the pineal melatonin secretion or decrease serum total bilirubin in conditions that could be used for therapeutic purposes.

Methods: Seven healthy volunteers participated in two consecutive overnight sessions that were identical except for a light exposure on the chest and abdomen in the second night from 12:00 AM to 6:00 AM (10,000-lux, 32 W/m² cool white for six subjects and 3000-lux, 15 W/m² blue light for one subject). Hourly blood samples were collected from 7:00 PM to 7:00 AM for melatonin radioimmunoassays. Bilirubin was measured by a modified diazo method in blood samples taken at 12:00 AM and 6:00 AM and in urine samples collected from 7:00 PM to 11:00 PM and from 11:00 PM to 7:00 AM.

Results: The skin light exposure did not cause any significant changes in serum melatonin or bilirubin levels. The excretion of bilirubin in urine was also the same in both sessions.

Conclusions: Significant melatonin suppression by extracocular light does not occur in humans. Robust concentration changes of serum total bilirubin do not have a role in mediating light information from the skin to the central nervous system. Biol Psychiatry 2000;48:1098–1104 © 2000 Society of Biological Psychiatry

Key Words: Extraocular phototransduction, skin light exposure, light treatment, pineal gland, melatonin suppression, bilirubin

Introduction

The mechanisms by which light affects the regulation of sleep, circadian rhythms, and mood are poorly understood. Nevertheless, since the 1980s numerous studies have shown that light therapy has beneficial effects when applied in certain types of sleep and mood disorders (e.g., Eastman et al 1998; Lewy et al 1982; Rosenthal et al 1984; Terman et al 1995, 1998). Today, research is in progress for defining the optimum quality and quantity of light, and the proper timing of exposure in various disorders (e.g., Chesson et al 1999; Lewy et al 1998).

To date, it was thought that in adult mammals light can affect brain functions only through the eyes (Nelson and Zucker 1981; Underwood and Groos 1982); however, this concept has to be re-evaluated, since it was reported that the human body temperature rhythm and melatonin onset time were shifted by bright light directed to the skin behind the knee (Campbell and Murphy 1998). The finding is theoretically interesting, and in addition, it might have practical applications.

The level of effective illuminance in light treatment directed to the eyes is highly dependent on the subject’s behavior. For example, the threshold illuminance needed to suppress melatonin varies greatly according to the experimental conditions. Illuminances as low as 6–17 photopic lux have been reported to suppress melatonin in strictly controlled conditions (monochromatic light of 509 nm, light beam directed uniformly on the retina, dilated pupils, and subject’s head kept motionless; Brainard et al 1988). In another study, when the subjects were sitting in front of a light source and gazed at the source for 10 sec every 2 min, a significant suppression was produced by 500-lux but not by 200-lux illuminance (Hashimoto et al 1996). This variability does not cause problems if the patient participating in the light therapy is well motivated and able to follow the instructions; however, sleep disorders are extremely common and harmful in, for example, mentally retarded people and in patients with Alzheimer’s disease, and some of them can benefit from light therapy (Guilleminault et al 1993; Van Someren et al 1997). In their case, it would be useful if the light treatment could be
delivered without worrying about the gaze direction or openness of the eyes.

In attempts to determine the effectiveness of light, the melatonin suppression test is often used. Although it is quite possible that light affects the brain functions without changing the secretion of the pineal hormone, the suppression of nocturnal melatonin synthesis can be a sign of the light stimulus reaching the hypothalamus, the site of the main body clock. This assumption is valid at least if the light stimulus is directed to the eyes and mediated through the retinohypothalamic pathways. Two independent studies have shown that bright light directed to the small skin area behind the knee for 3 hours does not suppress melatonin secretion in humans (Hébert et al. 1999; Lockley et al. 1998). It is possible, however, that the efficacy of skin light exposure depends on the size of the exposed area and the duration of the exposure. Therefore, we decided to study whether extraocular light exposure delivered to a larger body area and for a longer period would produce melatonin suppression.

The mechanisms involved in the possible humoral phototransduction are completely unknown; however, an attractive hypothesis has been presented. According to the original model, some light-sensitive molecules, such as bilirubin or hemoglobin, circulating in retinal blood vessels could mediate the effects of light to the circadian regulatory machinery (Oren 1996). Later this hypothesis was further developed to include the concept of extraocular phototransduction through the skin (Oren and Terman 1998).

Bilirubin is formed mainly in the cells of the reticuloendothelial system as a product of heme catabolism. Despite seemingly only a waste product, it has been shown to possess strong antioxidant activity (Stockker et al. 1987). This and its well-known sensitivity to light may be in favor of the assumption that it might function as a blood-borne mediator of extraocular phototransduction. In hyperbilirubinemic newborns the renal excretion of bilirubin can be substantially increased by skin phototherapy (McDonagh and Lightener 1985). The effect of phototherapy is based on the light-induced formation of hydrophilic molecules from the lipophilic bilirubin.

There is minimal information available about the effects of light on normobilirubinemic adults. According to a case study, molecular changes similar to those in newborns occurred in the structure of bilirubin in an adult subject wearing only shorts and exposed to natural sunlight for 90 min (McDonagh 1986); however, no changes in the serum level of total bilirubin could be found. This may be due to the short exposure time and the fact that the bilirubin concentration changes caused by the excretion of photodegradation products may be detectable only after a longer period. Therefore, we studied whether a 6-hour skin light exposure might cause changes in total serum bilirubin levels or in the amounts of bilirubin excreted in urine of healthy adult volunteers.

Methods and Materials

Seven healthy unmedicated subjects (two female and five male, all white, age range 19–43 years) gave informed consent after the nature of the study had been explained. The study protocol was accepted by the ethical committee of the institute. During the 5 days preceding the study the subjects were told to avoid bright lights at night, strenuous physical exercise, and beverages containing alcohol.

For the sham light laboratory session the subjects arrived between 5:00 PM and 6:30 PM and were asked to empty their bladders, after which a venous cannula for hourly blood samples was inserted in the antecubital vein. Thereafter the subjects were in a dimly lit room (<10 lux, Chroma Meter CL-100, Minolta, Osaka, Japan). During the period from 7:00 PM to 11:00 PM the subjects were allowed to play games and listen to music but not to lie down. A standard snack was served from 10:00 PM to 11:00 PM. Urine was collected at 11:00 PM (not at 12:00 AM) for practical reasons.

At 11:00 PM the subjects lay down under the light sources and their eyes were covered with black cloth (tied four times around the head and extending from the upper forehead to cheeks) impermeable to the illuminance of 10,000 lux (tested by the authors). The subjects were also specifically asked to report immediately if any light entered their eyes during the light exposure. No such report was given by any one of the subjects. In addition, the light sources were covered with multiple dark blankets to prevent light from escaping to the surroundings. During the sham light session, the lights of the panels were off, but fans were on from 12:00 AM to 6:00 AM to make the sound conditions similar to those during the light exposure session. At 7:00 AM the last blood sample was drawn, the eyes were uncovered, and the urine was collected.

On the same day between 5:00 PM and 6:30 PM the subjects returned to the laboratory for the skin light session, which was carried out in a manner similar to that of the sham light session except that from 12:00 AM to 6:00 AM the lights were on and the fans were directed under the panels to cool the air. The temperature under the panels was continuously monitored by the researchers during both sessions. Most of the time it was 24–26°C, but rose transiently to 29°C during the light exposure session. The subjects were allowed to sleep from 11:00 PM to 7:00 AM while lying, but their sleep was disturbed when the researchers monitored the posture, eye covers, and temperature under the light panels and collected the hourly blood samples. The sleep was similarly disturbed during both nights.

Three light sources, each suitable for the light exposure of two subjects lying side by side, consisted of two fluorescent broadband spectrum (Figure 1A) white light tubes (Philips TLD 18/950, Rosendahl, Holland; 5300 K, 58 W, length 152 cm) assembled in a wooden structure (length 168 cm, height 60 cm, width 48 cm), giving an illuminance of approximately 10,000 lux and an integrated irradiance of 32 W/m² (measured spectroradiometri-
naked skin between the neck and hip levels (the size of the
illuminated body area was estimated to be approximately 50
\( \text{cm}^2 \)), distance from the lamps ca. 20 cm). For one subject
a commercial fluorescent blue light device (Figure 1B) was used
as the light source (Phototherapy lamp Medela Ag, Baar,
Switzerland, 90 W, 3000 lux, 15 W/m\(^2\), distance 40 cm from the
subject). The other conditions and protocol for this subject were
similar to those of the other subjects. All calculations and
statistical evaluations were performed both by including and by
excluding the subject exposed to blue light. Because the results
and interpretations were the same for both sets of subjects, only
the results from all seven subjects are presented.

The blood samples were centrifuged and the serum stored at
\(-24^\circ\text{C}\). Melatonin was extracted from 1.0 mL of serum with
chloroform and measured in duplicate by radioimmunoassay
(Vakkuri et al 1984). The nonspecific binding of the tracer was
5–6%. The least detectable concentration, defined as apparent
concentration at 2 SDs from the counts at maximum binding
\((n = 6 \text{ in each assay})\), was smaller than the lowest standard (1.95
ng/L). Intra-assay variability was <10%. The interassay variabil-
ity during 24 months in 16 assays including the assays of this
study was 15–18%, depending on the concentration. All samples
of each pattern were measured in the same assay.

The assay used for the analysis of total bilirubin was a
modified diazo method (Doumas et al 1982; Jendrassik and Grön
1938). Total bilirubin was measured in both sessions from serum
samples taken at 12:00 AM and 6:00 AM and from urinary
specimens taken as described above. The intra-assay variability
was <7%. All bilirubin samples were placed in dark tubes,
protected from light during all procedures, and measured in the
same assay.

Two-way analysis of variance (ANOVA) for repeated mea-
sures was used in statistical evaluations of the serum melatonin
and bilirubin levels, the excretion rate of urine, and the excretion
of bilirubin in urine. In addition, the secretion of melatonin
during the 2 nights was compared by applying the area under the
curve (AUC) analysis. The “prelight” and “during light” AUCs
and the peak and postlight levels of melatonin were expressed as
percentages of the corresponding values in the sham light session
and evaluated by two-tailed one-sample \( t \) test (deviation from
100%). The minimum detectable deviation from 100% was
calculated by a method described previously (Rosner 1986). The
same method was used for the calculations of the minimum
detectable difference in serum total bilirubin levels between the
samples taken at 12:00 AM and 6:00 AM.

Results

All seven subjects had a clear melatonin rhythm with peak
values during the night (Figure 2, B–H). The average
serum level profiles did not differ significantly between
the sham light and skin light sessions (two-way ANOVA;
Figure 2A). In most subjects the profiles were very similar
during both nights. However, two of the seven subjects
(Figure 2, B and C) had somewhat lower serum melatonin
concentrations during the light exposure than during the
sham light, one from the beginning of the treatment for
3–4 hours and the other during the latter part of the light
exposure from 3:00 AM to the end of the experiment.

In AUC analysis it was found that subject B had
similarly decreased melatonin levels before and during
the light treatment as compared with the corresponding
intervals in the sham light night (85% vs. 86%; Table 1).
The decrease of serum melatonin in subject C during light
exposure seemed more pronounced (AUC during light
78% of sham light vs. AUC prelight 102% of sham light;
Table 1); however, statistical evaluation of the mean
AUCs of all seven subjects did not disclose any differ-
cences between the sessions. Furthermore, the melatonin
peak level, found at any time from 12:00 AM to 6:00 AM,
did not differ between the sessions, nor did the postlight
melatonin concentration (Table 1).

The mean serum bilirubin levels were equal in both
sessions at 12:00 AM and of the same magnitude at 6:00 AM
(Table 2). The standard deviation of the individual differ-
cences between the values at 12:00 AM and 6:00 AM was
\( \pm 3.4 \mu\text{mol/L} \). Thus, the minimum detectable difference
was 3.7 \( \mu\text{mol/L} \ (n = 7, p = .05, \text{power } 80\%) \). Bilirubin

![Figure 1. Spectral power distributions of the light sources used in the experiment. (A) White light. (B) Blue light.](image)
excretion in urine was equally low during both sampling intervals and similar in both sessions. Thus, no effect of skin light exposure was found on serum total bilirubin concentration or excretion rate in urine. There were no significant correlations between the individual serum bilirubin levels and the excretion rates in urine, probably due to the large variation of urine bilirubin excretion rates.

**Discussion**

The 6-hour bright light exposure on a large skin area had no significant influence on the average melatonin secretion profile in our subjects. The result is in line with the findings that illumination of the popliteal skin with bright white light (Lockley et al 1998) or blue light (400–550 nm; Hébert et al 1999) for 3 hours did not suppress melatonin in healthy adults. The illuminated skin areas in the previous studies were 10 and 50–100 cm², respectively. In our study, a rough estimation for the skin area illuminated effectively was about 50 cm × 40 cm. Thus, prolonging the exposure period twofold and extending the illuminated area at least 20-fold did not make the treatment more effective.

Furthermore, the conclusion that melatonin suppression
in humans does not occur through skin illumination is supported by the finding that in hyperbilirubinemic newborns exposed to phototherapy with the eyes covered, the serum melatonin levels were elevated rather than suppressed (Jaldo-Alba et al 1993). In addition, there is evidence for the inefficacy of facial illumination to suppress melatonin in completely blind people (Czeisler et al 1995) and for its relative inefficiency to suppress melatonin in sighted people with the eyes closed (Hatonen et al 1999); however, the lack of melatonin suppression by extraocular light does not exclude the possibility that other brain functions can be influenced by extraocular light.

The decreased serum melatonin concentrations in two of the seven subjects during the light exposure session raises the question of whether there might be interindividual differences in the sensitivity to extraocular light and whether melatonin suppression might be seen in a subpopulation. Usually, the individual melatonin profiles are very similar from night to night (Arato et al 1985; Arendt 1988; Laakso et al 1990), as they were in the other five subjects in this study. Because the low melatonin levels in the two subjects were not found evenly during the light exposure and the decrease occurred during different times within the light exposure period, it seems improbable that the suppressions were caused by light.

When we tried to find the explanation for the suppression, we noticed that these two persons deviated from the other five subjects in having an exceptionally high urine flow during the light exposure session from 11:00 PM to 7:00 AM (B sham light/light 36/64 mL/hour, C 22/69 mL/hour; other five subjects 29/22, 30/29, 24/25, 21/26, and 58/69 mL/hour). Thus, although the mean urine flows did not differ significantly between the sessions, the two persons with the decreased serum melatonin levels excreted urine during the light exposure approximately two or three times the amount they excreted during the sham light exposure. When interviewed afterwards, subject B said that she felt thirsty in the evening preceding the light exposure and drank a lot of water, which was freely available during the sessions. Subject C was given water to drink several times during the light exposure because he complained of “unbearable thirst.”

Thus, the water balance of the two subjects most probably differed between the two sessions. It has been shown that changes in hemodynamics by posture alterations can result in significant changes in serum melatonin

Table 1. Characteristics of Serum Melatonin Profiles in Seven Healthy Subjects during the Skin Light Session

<table>
<thead>
<tr>
<th>Subject</th>
<th>Area under the curve</th>
<th>During light (12:00 AM–6:00 AM)</th>
<th>Peak level (any time)</th>
<th>Postlight level (6:00 AM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>93</td>
<td>97</td>
<td>90</td>
<td>117</td>
</tr>
<tr>
<td>B</td>
<td>85</td>
<td>86</td>
<td>93</td>
<td>103</td>
</tr>
<tr>
<td>C</td>
<td>102</td>
<td>78</td>
<td>74</td>
<td>65</td>
</tr>
<tr>
<td>D</td>
<td>130</td>
<td>110</td>
<td>90</td>
<td>135</td>
</tr>
<tr>
<td>F</td>
<td>92</td>
<td>103</td>
<td>114</td>
<td>119</td>
</tr>
<tr>
<td>G</td>
<td>96</td>
<td>103</td>
<td>107</td>
<td>99</td>
</tr>
<tr>
<td>H</td>
<td>83</td>
<td>97</td>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>97 ± 16</td>
<td>96 ± 11</td>
<td>95 ± 13</td>
<td>105 ± 22</td>
</tr>
<tr>
<td>MDD</td>
<td>20</td>
<td>14</td>
<td>16</td>
<td>27</td>
</tr>
</tbody>
</table>

Light was directed for 6 hours (12:00 AM–6:00 AM) to the chest and abdomen of the subjects lying under the light sources with their eyes covered. Subjects B–D and F–H: fluorescent white light, illuminance 10,000 lux, integrated irradiance 32 W/m². Subject E: blue light, 3000 lux, 15 W/m². All values are given as percentages of the corresponding values of the control profiles determined in the sham light session without turning the lights on. None of the mean percentages was different from 100% (two-tailed one-sample t test). MDD, minimum detectable deviation from 100% (n = 7, p = .05, power 90%).

Table 2. Concentration of Serum Total Bilirubin, Excretion of Urine, and Excretion Rate of Urine Bilirubin (mean ± SEM) in Seven Healthy Subjects during the Sham Light and the Skin Light Sessions

<table>
<thead>
<tr>
<th>Time</th>
<th>Serum bilirubin (µmol/L)</th>
<th>Period</th>
<th>Volume (mL/hour)</th>
<th>Bilirubin (nmol/hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham light</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:00 AM</td>
<td>12.6 ± 3.0</td>
<td>6:00 PM–11:00 PM</td>
<td>65 ± 17</td>
<td>284 ± 70</td>
</tr>
<tr>
<td>6:00 AM</td>
<td>13.4 ± 3.5</td>
<td>11:00 PM–7:00 AM</td>
<td>31 ± 5</td>
<td>233 ± 23</td>
</tr>
<tr>
<td>Skin light</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:00 AM</td>
<td>12.9 ± 3.0</td>
<td>6:00 PM–11:00 PM</td>
<td>61 ± 7</td>
<td>316 ± 84</td>
</tr>
<tr>
<td>6:00 AM</td>
<td>13.8 ± 2.6</td>
<td>11:00 PM–7:00 AM</td>
<td>43 ± 9</td>
<td>204 ± 56</td>
</tr>
<tr>
<td>Two-way analysis of variance</td>
<td></td>
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<td></td>
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<tr>
<td>Session</td>
<td>ns</td>
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<tr>
<td>Time</td>
<td>ns</td>
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<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Interaction</td>
<td>ns</td>
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<td>ns</td>
</tr>
</tbody>
</table>

Light exposure (10,000-lux, 32 W/m² cool white in six subjects and 3000-lux, 15 W/m² blue light in one) of chest and abdomen from 12:00 AM to 6:00 AM.
concentrations (Deacon and Arendt 1994). It is possible that alterations in fluid balance for other reasons also cause changes in serum melatonin concentrations. In addition, an increased urine flow has been reported to increase the rate of melatonin excretion in sheep, probably due to decreased tubular reabsorption (Valtonen et al. 1993). In the sheep study, the serum melatonin concentration did not decrease within 90 min; however, a decrease can appear if the abundant urine flow continues for a long time and the loss of melatonin is not compensated for by increased melatonin synthesis. We consider the variations in fluid balance the most probable reason for the decreased serum melatonin levels in subjects B and C.

The 6-hour bright light exposure on the chest and abdomen did not change the serum total bilirubin concentrations or the urinary excretion rate of bilirubin in our subjects. When bilirubin absorbs a photon, three types of chemical reactions can occur: slow photo-oxidation (e.g., to monopyrroles and dipyrroles), structural isomerization to lumiurbin, or configurational isomerization by conversion from double to single bonding of one of the bridges joining the pyrrole rings (Ennever 1990; McDonagh and Lightner 1985). The reaction products are more water soluble than the original substrate and can be excreted in bile and urine.

The diazo method used in the measurements does not detect the oxidation products or ubiquirin, but it does detect all the configurational isomers (Ennever 1990). Our results suggest that significant skin light-induced photo-oxidation or structural isomerization of bilirubin does not occur in normobilirubinemic adults during a 6-hour skin light exposure. As in the previous case study (McDonagh 1986), the configurational isomerization could have occurred in our subjects, but the reactions did not lead to any detectable decrease of total bilirubin levels. The possibility remains that different isomers can have different effects in the central nervous system. In addition to the heme-related compounds, other molecules such as vitamin D, known to be sensitive to ultraviolet radiation (Holick 1995), may serve as messengers from the skin to the brain (Stumpf and Privette 1991).

In summary, the secretion of the pineal hormone melatonin, known to be very sensitive to ocular light, was not significantly affected by skin light exposure on a larger body area (chest and abdomen) and for a longer period (6 hours) than previously tested; however, possible effects of extraocular light on other hypothalamic functions cannot be excluded by this study. Moreover, the present results do not support a role for short-term fluctuations of serum total bilirubin levels in mediating the light information from the skin to the central nervous system. The possibility remains that more subtle changes in the structure of bilirubin are involved in extraocular phototransduction.

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References


Ennever JF (1990): Blue light, green light, white light: more than previously tested; however, possible effects of extraocular light on other hypothalamic functions cannot be excluded by this study. Moreover, the present results do not support a role for short-term fluctuations of serum total bilirubin levels in mediating the light information from the skin to the central nervous system. The possibility remains that more subtle changes in the structure of bilirubin are involved in extraocular phototransduction.


