Melatonin Suppression by Light in Euthymic Bipolar and Unipolar Patients

John I. Nurnberger, Jr, MD, PhD; Sherril Adkins, RN; Debomoy K. Lahiri, PhD; Aimee Mayeda, MD; Kuolung Hu, MS; Alfred Lewy, MD, PhD; Aaron Miller, MD; Elizabeth S. Bowman, MD; Marvin J. Miller, MD; N. Leela Rau, MD; Carrie Smiley, RN; Dawn Davis-Singh, BS

Background: Previous studies have suggested that bipolar patients are supersensitive to light suppression of melatonin and that this may be a trait marker for genetic vulnerability. The present study was an attempt to replicate and extend this observation. Propranolol hydrochloride effects were compared with light effects because of the documented influence of β-adrenergic receptors on melatonin production. Nighttime levels of corticotropin and cortisol were also examined as potential trait vulnerability markers.

Methods: Melatonin levels in euthymic bipolar patients (n = 29) were tested before and after 500-lux light was administered between 2 and 4 AM and on a separate night in the dark. Results were compared with those of a group of patients with unipolar depression (n = 24) and with those of a group of non-psychiatrically ill control subjects (n = 50). Lithium effects and propranolol effects were tested in subgroups.

Results: No group differences were seen in light suppression among bipolar patients, unipolar patients, and controls; an analysis of the whole group did not reveal differences in propranolol effect, differences in corticotropin or cortisol levels, or evidence for a lithium effect. However, patients with bipolar I affective disorder showed the following: (1) significantly lower melatonin levels on the light night, at baseline and following light exposure; and (2) a later peak time for melatonin on the dark night.

Conclusions: The general hypothesis of increased light sensitivity in bipolar patients was not supported. However, melatonin secretion abnormalities were confirmed in the subgroup with bipolar I disorder. Further assessments of circadian rhythm disruption as a vulnerability marker in bipolar illness are indicated.

Arch Gen Psychiatry. 2000;57:572-579

From the Department of Psychiatry, Indiana University School of Medicine (Drs Nurnberger, Lahiri, Mayeda, A. Miller, and Bowman and Mas Adkins, Hu, Smiley, and Davis-Singh), Department of Psychiatry, Roudebush Veterans Affairs Medical Center (Dr Mayeda), the Institute of Psychiatric Research, Larue Carter Hospital (Dr M. J. Miller), and Department of Psychiatry, Wishard Hospital (Dr Rau), Indianapolis, Ind; and the Department of Psychiatry, Oregon Health Sciences University, Portland (Dr Lewy).

Bipolar affective disorder involves disruption of intrinsic biological rhythms, including the sleep-wake cycle, hormonal rhythms, and temperature regulation. A phase advance in rhythms explains some of these observations; a general phase instability has also been hypothesized.⁴⁻⁷

The hormone melatonin has a time-keeping function in many mammals and appears to adjust the timing of circadian rhythm information transmitted from the suprachiasmatic nucleus of the hypothalamus to entrain physiologic rhythms.⁸⁻¹⁰ A physiologic role for melatonin in humans has yet to be clearly established. Some apparently normal humans have low to undetectable levels of melatonin.¹¹ Patients undergoing long-term treatment with β-blockers show suppressed melatonin secretion without ill effects.¹⁶,¹⁹ Melatonin levels decrease with chronological age.¹²⁻¹³ Administered melatonin is known to lower body temperature at night²² and may induce sleepiness.²³(192-208)

Bright light can shift the phase of melatonin secretion in humans.²⁴ The photoperiodic control of melatonin makes it a potential marker of circadian rhythms. Lewy²⁵ has suggested use of the dim-light melatonin onset as a marker of circadian rhythm phase and period.

Melatonin is an indolamine derivative of serotonin and is produced by the action of the enzymes N-acetyltransferase and hydroxyindole-O-methyltransferase, with the first step being rate limiting. Production is primarily within the pineal body; the hormone is secreted into the blood and into the cerebrospinal fluid.²⁶(60-66) Specific receptors for melatonin are found in multiple brain areas, including the hypothalamus, the cerebellum, and the pineal body itself. Melatonin is secreted into plasma at high levels (60-100 pg/mL) at night and at low levels (3-10 pg/mL) during the daytime.

The anatomic pathway mediating melatonin secretion is complex. Retinal stimulation by light produces a tonic inhibitory signal to the suprachiasmatic nucleus of the hypothalamus via the reti-
SUBJECTS AND METHODS

SUBJECTS

Subjects consisted of 29 patients with bipolar I (n=21) or bipolar II (n=8) affective disorder, 24 patients with unipolar affective disorder, and 50 controls. Patients were recruited from an Indiana University, Indianapolis, outpatient clinic, and additional patients and controls were recruited by advertisement (primarily on campus). A Structured Clinical Interview for DSM-III-R was administered by a nurse-clinician (S.A.). Two psychiatrists (J.I.N., M.J.M., E.S.B., N.L.R. and A.M.) then made the diagnosis in patients who met the criteria by DSM-III-R and by modified Research Diagnostic Criteria for the disorder indicated. Patients with bipolar I disorder (bipolar disorder, not otherwise specified by DSM-III-R) and unipolar patients had recurrent episodes of depression. Controls had no ongoing psychiatric illness and no history of major affective disorder, psychosis, or any other psychiatric impairment by Structured Clinical Interview for DSM-III-R. All subjects gave informed consent in writing.

Patients were tested in the euthymic state, i.e., functioning at their usual interepisode level for several months and scoring less than 3 on the Raskin Mood Scale. Twenty-two bipolar patients and 24 unipolar patients were tested after remaining free from psychoactive medication for at least 2 weeks (5 weeks for fluoxetine and 3 weeks for carbamazepine and for antidepressants other than fluoxetine). No patient took other medications known to affect melatonin levels. Alcohol was not permitted for 3 days before the test. A urine drug and alcohol screen was performed on the day of testing. Smoking and eating were not permitted after admission to the research unit. A subgroup of bipolar patients was tested on their ongoing therapeutic regimen of lithium (n=20), including 13 patients who were also tested while not taking lithium and 7 additional patients tested while taking lithium only. A subgroup of bipolar patients not taking lithium (n=13) and controls (n=41) were tested with a single 10-mg oral dose of propranolol or placebo given at 11 PM on the night of testing. Twenty-two controls were also retested on a second occasion to provide a measure of reliability.

Female subjects were required to be using a reliable method of birth control (but not oral contraceptives) and to not be pregnant (determined by a urine screen). Premenopausal women were not tested during their menstrual period. Subjects were stratified by age, race, sex, and season of testing. Each bipolar and unipolar patient was matched with a control of the same sex, race, age (within 3 years), and season of testing (within 6 weeks). Women were also matched for menstrual status (premenopausal or postmenopausal). The clinical characteristics of patients and matched controls are summarized in Table 1 and Table 2. Three additional unmatched controls were included in the total control sample.

CLINICAL PROCEDURE

Subjects sitting up in bed and looking at a 500-lux light positioned at the foot of the bed can vary the amount of light they receive by 20% by leaning forward or backward (J.I.N., unpublished data, 1986). Nurnberger et al reduced this variation by placing a seated subject in the center of an arc of lights. In the present study, we further modified the procedure by placing a bank of full-spectrum shielded fluorescent lights behind the subject in a small room, such that light was reflected from the wall, floor, and ceiling. The participant was thus exposed to a dispersed source of light, the intensity of which was calibrated by handheld photometer to be 500 lux, ±50 lux. Subjects were carefully monitored to ensure that they were awake, with eyes open, gazing in an appropriate direction, during the 2-hour period of the testing.

Subjects arrived at the Indiana University General Clinical Research Center, Indianapolis, on the evening of testing, and retired between 11 PM and midnight. For the dark night condition, subjects were allowed to sleep through the night; blood samples were drawn through an indwelling catheter with the aid of a small flashlight with a red filter. For the light night condition, subjects were awakened at 2 AM and asked to sit in a cardiac chair between 2 and 4 AM. Blood was drawn at 1, 1:30, 2, 2:20, 2:40, 3, 3:20, 3:40, 4, 4:30, 5, and 6 AM. After the 4 AM sample, the subject was allowed to return to bed; thus, the first 3 samples and the last 3 samples were drawn from subjects recumbent in a dark room. Pilot studies showed that posture and sleep per se did not significantly affect melatonin values. Each subject was invited to participate in a dark night and a light night study; study continued on next page.
nights were separated by at least 3 days. The order of study nights was randomized. Sleep deprivation did not generally affect mood in these euthymic patients.

HORMONE ASSAYS

A radioimmunoassay (RIA) for melatonin was developed following previously published methods. Samples of plasma were drawn into heparinized tubes, held on ice before centrifugation (≤2 hours), and stored at −70°C until testing. For primary antibody, the sheep antimelanotin antiserum was obtained from Guildhay Antisera Co, Surrey, England, and supplied as a freeze-dried sample. The antiserum was developed against N-acetyl-5-methoxytryptophan or bovine thyroglobulin. Cross-reactivity with N-acetyltryptamine, 6-hydroxy-melatonin, and N-acetyltryptophan was less than 1%. The assay was linear to 60 pg/mL and sensitive from 5 to 500 pg/mL. Intra-assay variability, based on duplicate measurement of 28 samples, was 2.14%. Interassay variability averaged 7.3% (n=28). The correlation of values between the RIA and a chromatographic–mass spectrometric assay was 0.92 (n=14).

Cortisol was assayed using a double-antibody iodine 125 (125I) RIA (Diagnostic Products Corporation, Los Angeles, Calif). Intra-assay variability was 2.5% when the value was between 140 and 1400 nmol/L. Interassay variability was 6.3%, and the detection limit was 5.6 nmol/L.

Plasma content of ACTH was determined using an immunoradiometric assay (Nichols Institute, San Juan Capistrano, Calif.). The antibody is specific for ACTH(1–39) and exhibits less than 0.001% cross-reactivity with peptides of similar structure, including ACTH fragments 1 to 24, 11 to 24, and 18 to 39. A 200-µL aliquot of sample or standard was combined with 100 µL of the antibody complex (125I-labeled monoclonal ACTH antibody and biotin-coupled polyclonal ACTH antibody in HEPES buffer containing protein stabilizers and 0.1% sodium azide) in polypropylene tubes and vortex mixed. An avidin-coated polystyrene bead was added to each tube, and the tubes were incubated at room temperature for 20±2 hours. Beads were washed twice, and ACTH content was quantified by counting samples concurrently with standards containing known quantities of human ACTH(1–39). The minimum detectable quantity of ACTH that could be distinguished from zero with a 95% confidence limit was 0.22 pmol/L. The estimated concentration of melatonin at 20% binding was 105.7 pg/mL; the estimated concentration of melatonin at 50% binding, 281.8 pg/mL; and the estimated concentration of melatonin at 80% binding, 536.6 pg/mL of plasma. The intra-assay and interassay coefficients of variation were 4.67% and 8.48%, respectively.

DATA ANALYSIS

Melatonin suppression was calculated as in Lewy et al: 100%−[(average melatonin level between 3 and 4 AM/average melatonin level between 1 and 2 AM)×100%]. A dark-adjusted melatonin suppression index was then calculated by subtracting the value of the suppression measured on the dark night from that on the light night. Suppression was compared between patient groups and their matched controls using matched-pair 2-tailed t tests. The power was 93% to detect an effect of size 0.8 and 63% to detect an effect of size 0.5. Each group of patients was also compared with the entire control group using independent 2-tailed t tests and Wilcoxon 2-sample tests. The α level of significance was .05.

A 3-way analysis of variance was applied to test the effects of diagnosis, treatment (light or dark), and drug (propranolol or no drug), with 3 to 4 AM average melatonin level as the dependent variable (as in a previous analysis). The power was 83% to detect an effect of size 0.4 and 45% to detect an effect of size 0.25. A separate analysis of variance was used to test lithium effects in bipolar patients. Separate analyses were performed to assess the effects of age (Pearson product moment correlation) and sex and season of testing (3-way analysis of variance). In each case, possible interactions were also considered. Paired and independent t tests were also used for secondary assessment of lithium and propranolol effects.

For each subject, the amplitude of variation of the melatonin signal was assessed using the average deviation from the total testing period mean at each point; the sum of the absolute value of these deviations was then divided by the total testing period mean. Peak time was compared as a surrogate for phase. All computations were calculated by SAS statistical software, version 6.12 (SAS Institute Inc, Cary, NC). Data are given as mean±SE unless otherwise indicated.

RESULTS

Mean suppression levels on the light night were 29.8%±5.5%, 32.2%±6.2%, and 34.6%±2.6% in bipolar patients, unipolar patients, and controls, respectively (Figure 1). Matched-pair comparisons did not show differences for the light night alone or for the dark-adjusted melatonin suppression value (data not shown).
A test of light suppression in 22 controls on 2 separate occasions showed modest but significant reproducibility (\(r=0.46; 95\%\) confidence interval, 0.01-0.91; \(P=0.03\), Spearman rank correlation). Baseline (average 1-2 AM) melatonin levels were similar among the 3 groups. Effects of sex and season of testing on light suppression level were not seen in controls or in patients. Controls showed a negative correlation between age and light suppression level (\(r=-0.35; 95\%\) confidence interval, \(-0.64\) to \(-0.06; n=50; P=0.01\)), whereas bipolar patients did not (\(r=0.05; 95\%\) confidence interval, \(-0.48\) to 0.38; \(n=23; P=0.41\)). Patients with light suppression greater than the bipolar mean did not differ from those with suppression less than the bipolar mean on age at onset, number of episodes, recent medication regimen, longest medication regimen, history of hospitalization, or presence of comorbid psychiatric conditions.

Propranolol decreased melatonin levels significantly, but this effect did not differentiate bipolar patients from controls (Figure 2, top and bottom). A significant relation between light effect and propranolol effect was seen in the controls (\(r=0.32; 95\%\) confidence interval, 0.01-
but not in bipolar patients ($r = -0.19; 95\%$ confidence interval, $-0.82$ to $0.44; n=13; P= .38$).

No effect of lithium treatment was seen in bipolar patients tested while not taking and while taking medication (Figure 3).

Cortisol and ACTH levels increased during the light administration procedure. Cortisol values did not distinguish the diagnostic groups (Figure 4 and Figure 5), and neither did ACTH values (Figure 6) or the melatonin-cortisol ratio (data not shown).

When bipolar patients were subdivided, patients with bipolar I disorder (Figure 7) showed a trend to increased dark-adjusted melatonin suppression compared with matched controls ($62.7\%\pm 15.6\%$ vs $40.0\%\pm 9.1\%$). The patients with bipolar I disorder demonstrated significantly lower baseline melatonin levels and nadir (the 3-4 AM average) melatonin levels on the light night. Patients with bipolar I disorder also showed a trend to greater amplitude of variation in melatonin secretion than controls ($20.70\%\pm 12.09\%$ vs $13.80\%\pm 8.30\%; t= 1.98; P=.07$) on the dark night. Peak time was noted to be significantly later in patients with bipolar I disorder compared with matched controls.

Among patients with bipolar I disorder, there was a significant interaction between medication status and dark-adjusted melatonin suppression (Figure 8), with the greatest suppression scores among those not taking any psychotropic medications for 5 weeks or longer ($98.8\%\pm 24.1\%$) compared with modest suppression among those taking lithium alone ($36.6\%\pm 43.8\%$) or not taking any medications for less than 5 weeks ($35.7\%\pm 43.1\%$).

**COMMENT**

The present results provide some support for the hypothesis of melatonin secretion abnormalities among patients with bipolar I illness. Patients showed decreased melatonin levels before and after light administration and a later peak on the night without light. Patients with bipolar I disorder showed $62\%$ dark-adjusted melatonin suppression compared with $48\%$ in the matched controls. The greatest suppression scores were noted in the patients not taking medication for the longest period. This study is similar to
that of Lam et al36 in the low baseline melatonin levels noted in patients. Our results tend to agree with those of Lewy et al34 and Nathan et al38 when the subgroup with bipolar I disorder is considered. However, a general difference between bipolar patients and controls is not seen.

Baseline melatonin levels were in the 40 pg/mL range for subjects in the Lewy et al34 study and in the 60 pg/mL range in the present study. This may be due to nonspe-
Qualities of the light stimulus differed in the present study compared with the study by Lewy et al and could conceivably account for the disparate results. For instance, although full-spectrum fluorescent light was used in both studies, the light in the present study was reflected from a greenish beige wall and thus the incident light was not full spectrum. Pursuing this explanation would require the hypothesis that bipolar patients differ in neural systems linked to one or more sets of retinal cones but not all.

Other characteristics of the procedure may account for observed differences. In the Lewy et al study, subjects were not directly monitored during the procedure. It is possible that bipolar patients may have been more highly motivated than controls to look consistently and directly at the light source in front of them. We attempted to minimize such behavioral differences in the present study with close monitoring. The subject was seated in a chair and was asked to be awake throughout the procedure, facing forward with eyes open. Nursing staff monitored compliance with these instructions.

Another possible explanation involves random variability in modest samples within a heterogeneous subject pool. The Lewy et al study involved observations of 11 bipolar patients. The present study includes 29 patients (21 with bipolar I disorder). It may be that increased sensitivity is a shared characteristic of only a subset of bipolar patients. This argument gains cogency by recent studies in the genetics of complex disease. Results from a recent genomic survey by the NIMH Genetics Initiative Bipolar Group reduce the likelihood that any single locus accounts for 50% or greater variance in a large data set. In fact, no single locus putatively identified thus far appears to account for more than 15% of the variance. The hypothesis of a unitary cause for bipolar illness has thus lost substantial support and no longer seems reasonable as a premise. Suarez et al have analyzed the statistical characteristics of such complex inheritance. A true finding may not be replicated until many subsequent groups of similar size have been tested. A similar argument may apply to trait markers.

The major limitations of this study are the limited sample size and the age distribution of the subjects. We were also unable to control for the circadian phase of each subject on the light-testing night (eg, by assessing dim-light melatonin onset). The inclusion of a dark night did show that melatonin onset and offset times were similar in patients and controls (Figure 2, top and bottom). However, patients with bipolar I disorder show a delayed peak in melatonin level on the dark night. This suggests that further investigation of phase delay in euthymic patients with bipolar I disorder may be indicated.

There is reason for some optimism regarding future studies. Patients with bipolar I disorder showed melatonin secretion abnormalities, as previously noted. This subgroup has generally not been separated in previous melatonin suppression studies. It is also possible that medication effects might mask a true difference between groups. Increased suppression in the subgroup not taking medication for 5 weeks or longer is consistent with this interpretation. However, it may be difficult to pursue further investigation of phase delay in euthymic patients with bipolar I disorder.
hypotheses that require extensive periods of not taking medication and overnight challenge tests, if large populations must be tested. More economical and efficient methods might be devised to explore hypotheses related to circadian rhythm disruption in bipolar affective disorder.

Accepted for publication January 24, 2000.

This study was supported by grant R01 MH43325 from the National Institute of Mental Health, Rockville, MD, and by a grant from the Indiana Division of Mental Health, Indianapolis. We acknowledge grant PHS MO1 RR750 from the Indiana University General Clinical Research Center, Indianapolis. We thank Janice Froehlich, PhD, Department of Medicine, Indiana University School of Medicine, Indianapolis, for determining the corticotropin levels.

Reprints: John I. Nurnberger, Jr, MD, PhD, Department of Psychiatry, Indiana University School of Medicine, 71 Union Dr, Indianapolis, IN 46202-4887 (e-mail: jnurnber@iupui.edu).

REFERENCES


