URINARY MELATONIN RHYTHMS DURING SLEEP DEPRIVATION IN DEPRESSED PATIENTS AND NORMALS

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Melatonin excretion was measured in 8 hour urine aliquots for eight healthy controls and six depressed patients. Both groups had similar diurnal rhythms, with increased melatonin excretion during the night. When subjects were sleep deprived, remaining awake and active in continuous light from 7 a.m. one morning until 11 p.m. the following day, the diurnal rhythm in melatonin excretion remained unchanged. These data in man appear to be inconsistent with previous studies in rats showing rapid light-induced suppression of the nocturnal rise in pineal melatonin synthesis.

The physiological and neurochemical control of pineal melatonin synthesis in laboratory animals has been explored through in vitro studies of pineal enzyme and melatonin content (reviewed in 1,2), and more recently by assay of plasma (3,4) and urine (4) melatonin levels. The recent demonstration that urinary melatonin excretion in man is quantifiable, and follows a diurnal rhythm of increased night-time excretion similar to that in rats (5), raises the possibility of assessing physiological control of the human rhythm in well states and in illness. Because of considerable evidence that altered biorhythms may be important in affective illness (6,7), we have compared the melatonin rhythm in hospitalized depressed patients and controls. In laboratory animals, exposure to light rapidly suppresses the increased pineal melatonin synthesis characteristic of dark periods (8,9,10,1,2). In conjunction with studies of the antidepressant effect that frequently accompanies one night's sleep deprivation in depressed patients (11,12), we have also assessed constant light and sleep deprivation as possible suppressants of nocturnal melatonin excretion in adult volunteers and in depressed patients.

Methods

Urinary melatonin excretion was measured in six healthy adult volunteers (4 males and 2 females, age range 19-65), and in six moderately to severely depressed patients (1 male and 5 females, age range 19-50) who met research

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criteria for primary affective illness (13). The subjects, who resided on a clinical research unit at the National Institute of Mental Health, gave informed consent for all procedures, followed a low monoamine diet, and were drug-free for at least 14 days prior to the study.

Urine was collected on consecutive days including one or two baseline days, a sleep deprivation day, and a recovery day. During the baseline phase, subjects continued the usual ward routine of awakening at 7 a.m. and retiring at approximately 11 p.m. The sleep deprivation phase of urine collections began at 11 p.m. the following night, with subjects delaying sleep for 24 hours, remaining awake in the light for a total of 40 hours. The recovery day constituted a return to the initial schedule of sleep from 11 p.m. to 7 a.m. Urine samples were collected for the 8-hour periods of 11 p.m. to 7 a.m. (night), 7 a.m. to 3 p.m. (day), and 3 p.m. to 11 p.m. (evening). Because their urines for the recovery night were lost, one volunteer and one patient were excluded from paired comparisons of the three study days.

Throughout the study, subjects continued routine hospital day and evening activities, including occasional passes outdoors for the volunteers, and therapy sessions and an hour’s gym for the patients. Subjects spent the night awake sitting in the day room, occupied by conversation, card games, reading, and television. Constant wakefulness was monitored by the nursing staff. No formal meal was provided during the night, although light snacks were not prohibited.

Lighting in the day room by overhead "cool-white" fluorescent bulbs ranged from approximately 50 to 80 foot candles at eye level (when seated). Mood changes were evaluated by two-hour self and nurse behavioral ratings, as described elsewhere (14). Subjects had standard sleep EEG’s during the baseline and recovery periods; two patients wore wristwatch style activity monitors (15) during the sleep deprivation.

Urine samples were collected without preservative in the 8-hour aliquots and were stored frozen at -20°C until assayed. Urinary melatonin was measured by bioassay (5). Briefly, urine was concentrated on Amberlite XAD-2 resin, eluted with organic solvents, and quantitated by the dermal melanophore response of larval anurans to melatonin in their bathing medium.

Because of evidence that light spectrum may influence light-induced changes in melatonin rhythms (16), two additional male volunteers (V7 and V8) remained awake during a night of continuous exposure to fluorescent light similar in spectrum to daylight ("Vita-Lite," Duro-Test, Inc., North Bergen, N.J.); light intensity was approximately 85 foot candles. The protocol for these two volunteers was also modified to include: 1) three baseline days of urine collection prior to sleep deprivation; 2) EEG verification of night-time wakefulness; 3) a light meal and later snack during the night awake; 4) a daytime nap from 11 a.m. to 7 p.m. with sleep EEG recording (in collaboration with J.C. Gillin, M.D.) in a dark room following the night awake; 5) urine collections every 4 hours during the study; and 6) assay of sequential 4-hour urine melatonin excretion by radio-immunoassay following a thin layer chromatography separation (17,18). This assay yields melatonin levels similar to those of the bioassay. Because of the modifications in the procedure, melatonin data from these two subjects are presented separately below.

Results

Night-time melatonin excretion by the normal subjects during the baseline period was significantly higher than the mean of day and evening output (p < .02 by paired t-test, two tailed), with 69% of the daily total excreted at
night, similar to results in a previous study (5). The six depressed patients showed the same diurnal rhythm, with 67% of urinary melatonin excreted during the night (p < .05 by paired t-test, one-tailed). Mean daily melatonin excretion during the baseline period was similar for the volunteers (9.6 ± 2.7 ng/24 hrs) and for the depressed patients (11.6 ± 2.5 ng/24 hrs).

Comparison of melatonin excretion by patients and controls during the night of wakefulness in constant light revealed no apparent alteration in the pattern of increased night-time excretion of melatonin during sleep deprivation (Figure 1). A three-way analysis of variance across subjects (patients vs.

**MELATONIN RHYTHM AND SLEEP DEPRIVATION IN DEPRESSED PATIENTS AND CONTROLS**

![Graph showing urinary melatonin excretion](image)

**FIG. 1**

Urinary melatonin excretion is similar in magnitude and diurnal rhythm in healthy controls and depressed patients. The nocturnal rise in melatonin excretion is not suppressed by sleep deprivation in the light for either controls or patients. Melatonin levels were measured by bioassay.
### Table 1

**NIGHT-TIME MELATONIN EXCRETION IN VOLUNTEERS AND PATIENTS FOLLOWING SLEEP DEPRIVATION**

<table>
<thead>
<tr>
<th>Controls</th>
<th>Age, Sex</th>
<th>Baseline Night -2</th>
<th>Baseline Night -1</th>
<th>Sleep Dep. Night</th>
<th>Recovery Night</th>
<th>Depression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22 F</td>
<td>5.5</td>
<td>2.7</td>
<td>2.6</td>
<td>4.2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>30 M</td>
<td>–</td>
<td>9.3</td>
<td>8.0</td>
<td>22.0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>20 M</td>
<td>4.9</td>
<td>2.6</td>
<td>8.2</td>
<td>2.7</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>65 F</td>
<td>15.5</td>
<td>14.5</td>
<td>27.0</td>
<td>29.0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>22 M</td>
<td>1.1</td>
<td>2.3</td>
<td>1.1</td>
<td>1.5</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>19 M</td>
<td>6.1</td>
<td>7.1</td>
<td>6.1</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Depressed Patients</th>
<th>Age, Sex</th>
<th>Baseline Night -2</th>
<th>Baseline Night -1</th>
<th>Sleep Dep. Night</th>
<th>Recovery Night</th>
<th>Depression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22 F</td>
<td>–</td>
<td>5.5</td>
<td>2.3</td>
<td>2.7</td>
<td>Worse</td>
</tr>
<tr>
<td></td>
<td>19 F</td>
<td>14.0</td>
<td>15.0</td>
<td>19.0</td>
<td>15.0</td>
<td>No Change</td>
</tr>
<tr>
<td></td>
<td>50 F</td>
<td>–</td>
<td>8.2</td>
<td>5.1</td>
<td>6.0</td>
<td>Improved</td>
</tr>
<tr>
<td></td>
<td>44 F</td>
<td>8.5</td>
<td>10.8</td>
<td>4.7</td>
<td>7.7</td>
<td>Worse</td>
</tr>
<tr>
<td></td>
<td>40 F</td>
<td>1.0</td>
<td>1.6</td>
<td>3.6</td>
<td>0.8</td>
<td>Improved</td>
</tr>
<tr>
<td></td>
<td>27 M</td>
<td>2.8</td>
<td>5.5</td>
<td>6.9</td>
<td>–</td>
<td>Worse</td>
</tr>
</tbody>
</table>

Total Night-time Melatonin Excretion (11 p.m. to 7 a.m.) Measured by Bioassay, Expressed in ng/8 hours.

controls), day (baseline, sleep deprivation, recovery) and time of day (night vs. mean of day and evening), with repeated measures on the last two factors, indicated no significant main effects or interactions, except for significantly increased melatonin excretion at night. Table I illustrates that for both volunteer and patient groups, urinary melatonin excretion remained relatively stable during the night of wakefulness in the light. As in previous data (5), there was a substantial range across subjects in melatonin excretion during the baseline nights (1.0 to 15.5 ng/8 hrs) (Table I), also reflected in total 24-hour output (1.0 to 22.0 ng/24 hrs). Excretion tended to be stable for each subject on consecutive baseline days. Differences between subjects in melatonin excretion were not explained by age, sex, body weight, or urine volume.

In Figure 2, data for the two additional volunteers (V7 and V8) demonstrate a stable baseline rhythm of melatonin excretion, with persistence through the night of exposure to Vita-Lite. The EEG records documented normal sleep during baseline nights and constant wakefulness for both subjects during the
Nocturnal elevation in urinary melatonin excretion persists in two healthy controls sleep deprived under Vita-Lite. EEG-documented sleep from 11 a.m. until 7 p.m. the following day failed to increase melatonin excretion to night-time levels. Melatonin levels were measured by radio-immunoassay.

sleep-deprivation night. Moreover, the combined influence of darkness and sleep during the scheduled nap from 11 a.m. to 7 p.m. the subsequent day did not induce the increase in melatonin excretion typical of night.

Mean activity during the sleep deprivation night (85 counts/hour in the two patients monitored) was much higher than during the subsequent night asleep (12 counts/hour), and in fact approached mean daytime activity levels (126 counts/hour). Similarly, for volunteers V7 and V8, mean activity during the night awake (84 counts/hour) was near that for a control day (87 counts/hour), and greater than for a control night asleep (7 counts/hour). This indicator agrees with nurse observations that subjects remained alert and active during the night awake.

When the depressed patients were evaluated for post-sleep deprivation
mood response as described elsewhere (14), two fit criteria for improved mood (responders), one showed equivocal improvement, and three were not improved. In this limited patient sample, neither baseline nor sleep-deprivation induced change in night-time melatonin excretion (see Table I) is distinctively different for responders and non-responders.

**Discussion**

Studies in laboratory animals, including the rat and several species of birds, have clearly documented a rhythmic increase in pineal content of melatonin (19), and the melatonin-synthesizing enzymes Hydroxyindole-O-methyl transferase (9) and Serotonin N-acetyl transferase (NAT) (20,1) during the dark phase of regular diurnal cycles. The data in this study replicate an earlier demonstration of increased urinary excretion of melatonin during night-time sleep in healthy volunteers (5) and are consistent with the reported increases in melatonin concentration in random samples of night-time plasma (21).

The present study demonstrates the unaltered persistence of increased nocturnal melatonin excretion in healthy volunteers and depressed patients during one night's sleep deprivation in constant light. These results appear to be inconsistent with the rapid and persistent light-induced decrease in melatonin synthetic enzymes in the rat pineal (8,9). While it is possible that short naps in our subjects could have escaped the close nurse observation, animal data suggest that brief lapses of darkness should not obscure the overall light-induced suppression of melatonin excretion. Moreover, similar nonsuppression was observed in conjunction with EEG-documented all night wakefulness in the last two volunteers. While the night-time lighting met the spectral and intensity criteria necessary for synthesis suppression in rats (16,22), it is possible that the human pineal enzymes may respond only to more intense light. Immobilization stress increases pineal melatonin in rats (23); stress influences in the present study were minimized by the routine nature of sleep deprivation studies on the clinical unit, coupled with the psychosocial support of the nursing staff.

The possibility that extrapineal melatonin synthesis in man (24) could have a diurnal rhythm independent of and obscuring that of the pineal seems unlikely, especially in view of the major reduction of urinary melatonin excretion in rats following pinealectomy (18). In a recent preliminary study with rats, one night's exposure to continuous light failed to suppress the nocturnal rise in urinary melatonin excretion (25), in agreement with the human data. There is a lack of direct data on the relationship between the activity of pineal synthetic enzymes, overall pineal melatonin production, and urinary melatonin excretion.

Pineal melatonin production is influenced by extrapineal circadian oscillators, as manifested by the persistence of rhythmicity of melatonin in constant darkness (26); such extrapineal rhythms appear to influence pineal responsiveness to alterations in environmental light. Thus, rats kept in darkness for six hours during the day-time fail to show the rise in NAT characteristic of night-time darkness (9). In this respect, the failure of daytime darkness and EEG-documented sleep to increase basal urinary melatonin excretion in two volunteers in the present study is in agreement with the animal data. Similar results in volunteers were previously observed using random plasma samples (27). Taken as a whole, the data in the present study are consistent with a close link between melatonin rhythms in man and a relatively stable circadian clock. If melatonin rhythm can be altered by
light-dark reversal, the change, like that for cortisol (28), may occur only gradually over a number of days. Preliminary data on two additional volunteers support this possibility.

Previous studies (6,7) have suggested an association between depressive illness and altered circadian rhythms, an example being the altered cortisol rhythm in some depressed patients (29). Melatonin has behavioral effects in animals (30) and may have effects on mood in man. For example, exogenous melatonin administration to depressed patients (31) produced an apparent exacerbation of symptoms. In the present study, however, the quantity of daily melatonin excretion in depressed patients was similar to that for controls studied concurrently, and was not related to symptom severity in the patients. The nocturnal increase in urinary melatonin excretion in the depressed patients was similar to the diurnal rhythm in the volunteers, and was not related to mood improvement in depressed patients following sleep deprivation. To the extent that urinary melatonin levels reflect pineal rhythms, these findings weigh against a major alteration of melatonin function in depression.

References

25. H. J. LYNCH, Unpublished observations.