The Circadian Rhythm of Plasma Melatonin During the Normal Menstrual Cycle and in Amenorrheic Women*

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ABSTRACT. Plasma melatonin, PRL, and LH levels were measured in samples collected every 2 h for 24 h from 14 normally cycling women during the early follicular, periovulatory, and luteal phases of their menstrual cycles. Plasma melatonin levels also were measured in samples collected at the same interval from 7 patients with hypothalamic amenorrhea. A distinct daily rhythm in plasma melatonin was evident in all subjects, with peaks occurring around 0300 h. Each woman’s rhythm was remarkably consistent throughout the menstrual cycle (in terms of the phase, amplitude, and total melatonin secreted). Plasma PRL levels also exhibited daily rhythms which did not change during the menstrual cycle; the nocturnal peak plasma PRL level tended to occur 1-2 h after that for melatonin. Among the amenorrheic women, both daytime and nighttime melatonin levels were significantly higher (p < 0.009) than in the normal women. Their plasma PRL levels were similar to those in the normal women.

We conclude that, as for PRL, the circadian rhythm of melatonin secretion does not change significantly during the normal menstrual cycle. The elevated plasma melatonin levels in women with hypothalamic amenorrhea suggest that the hormone may be involved in the neuroendocrine pathology underlying this disorder. (J Clin Endocrinol Metab 68: 881, 1988)

EXOGENOUSLY administered melatonin has anti-gonadotropic actions in many mammals (1–3), and endogenous melatonin has been implicated in the control of sexual maturation and reproductive cyclicity in seasonally breeding species (4, 5). The efficacy of melatonin in modifying particular reproductive functions varies markedly depending on species (5), the age of the test animal (3), and the time at which the melatonin is administered, relative to both the prevailing light-dark schedule (6, 7) and the phase of the animal’s estrus cycle (1). In seasonal breeders, the time of the year at which reproductive activity occurs may naturally be determined by the number of hours per day that the pineal secretes melatonin (8).

Recent evidence suggests (9, 10) that melatonin might also be involved in the regulation of human reproductive processes, particularly puberty (11) and the menstrual cycle (12, 13). Only a few investigators have attempted to characterize plasma melatonin patterns throughout the normal menstrual cycle (14–18), and wide discrepancies exist among the findings in these reports. In most of these studies plasma melatonin concentrations were measured only a few times during the 24-h light-dark cycle or in only small numbers of women. Interindividual variations in plasma melatonin levels are substantial, while intraindividual variations tend to be relatively small (19, 20). Hence, subtle time-dependent changes in circadian melatonin secretion are best identified by sampling an individual subject at frequent intervals for 24-h periods. In this study we determined the daily rhythm of plasma melatonin levels and the relationship of this rhythm to that of plasma PRL in normal women at several times during the menstrual cycle and in women with hypothalamic amenorrhea.

Subjects and Methods

Subjects

Fourteen normal women with regular menstrual cycles were studied. They ranged in age from 19–34 yr (mean, 26 yr) and in weight from 52–69 kg (mean 59 kg). All had a biphasic basal body temperature pattern during a preovulatory cycle. All described themselves as having conventional life styles and normal sleep/wake rhythms, and none was taking any medications. They
were requested to refrain from using intrauterine devices or steroidal contraceptives, and to maintain records of daily basal body temperature and menstrual flow patterns during the study cycle. The days of the menstrual cycle were tracked by counting the first day of menses as the first day of the cycle; days 1–7 were designated as the early follicular phase, days 13–16 the periovulatory phase, and days 21–25 the midluteal phase. The length of the study cycle ranged from 27–32 days, and the midluteal phase plasma progesterone levels were more than 5 ng/mL (16 nmol/L).

Seven women (aged 22–35 yr; mean, 28.4) with hypotalamic amenorrhea of at least 6-month duration also were studied (Table 1). All were within 10% of ideal body weight and had normal thyroid function, a positive progestin test, and low (four women) or normal (three women) plasma estradiol levels. None had a history of psychiatric illness or strenuous exercise, one (patient 4; Table 1) had postpil amenorrhea, and another (patient 6) had postpartum amenorrhea.

Experimental design

The normal women were admitted to the Clinical Research Center (CRC) of the Massachusetts Institute of Technology for three periods of 24 h each during the early follicular, periovulatory, and midluteal phases of a single cycle. The amenorrheic women were admitted to the CRC at their convenience for a single 24-h period. Blood samples were obtained via an indwelling venous cannula at 2-h intervals for 24 h, starting at 1300 h. Seven milliliters of blood were withdrawn into a heparinized test tube and centrifuged (3000 × g), and the plasma was frozen and stored at −20 C until assay.

The women were asked to remain within the CRC, but their activity was otherwise unrestricted. They were given similar meals (2350 Cal/day; 45% carbohydrate, 42% protein, and 13% fat). The lights (500–1000 lux) were turned off, and the women slept between 2300 and 0700 h; nighttime blood samples were obtained using a flashlight (10–20 lux) without awakening the women.

Assay methods

Plasma melatonin concentrations were measured by RIA using CIDtech Ultraspecific melatonin antiserum (CIDtech Research Inc., Hamilton, Ontario, Canada) (21). The samples (1 mL) were extracted with 5 mL chloroform; the organic phase was then evaporated to dryness under a stream of nitrogen. The residues were dissolved in 0.5 mL 0.01 mol/L phosphate buffer (pH 7.5) containing 0.1% gelatin, and then washed with 1 mL petroleum ether. The buffer extracts of plasma or buffer samples containing graded concentrations of authentic melatonin were then mixed with 100 μL antiserum (diluted 1:6000) and 100 μL (3000 cpm) 3Hmelatonin (New England Nuclear Corp., Boston, MA). After incubation for 1 h at 37 C, saturated ammonium sulfate solution was added, the mixtures were incubated overnight at 4 C, and the antibody-bound 3Hmelatonin precipitated by (NH4)2SO4 was collected by centrifugation. Radioactivity was measured in a liquid scintillation spectrophotometer, and melatonin concentrations were estimated by 3 means of a log-log plot (22). All samples from an individual woman were analyzed in the same assay, in duplicate. The recovery of 50 or 100 pg/mL (216 or 431 pmol/L) melatonin added to duplicate pooled plasma samples was 96–100%. The intraassay coefficients of variation for plasma melatonin measurements were 8.1% and 10% for 50 and 100 pg/mL (216 and 431 pmol/L), respectively (n = 10). The corresponding interassay coefficients of variation were 17.3% and 7.3%. The sensitivity of the assay (defined as the concentration yielding a value that was twice the 80th of maximum binding) was 5 pg/mL (22 pmol/L).

Plasma PRL levels were measured by double antibody RIA, using a commercial kit (Radioassay System Laboratories, Inc., Carson, CA). The lower limit of sensitivity for this assay was 2.5 ng/mL (2.5 μg/L), and the upper limit was 100 ng/mL (100 μg/L); intra- and interassay variations were 4.2% and 7.9%, respectively (n = 10).

Plasma progesterone was determined using a RIA kit (RSL, Inc. Carson, CA).

Mathematical and statistical analysis

Data on the circadian rhythms of plasma melatonin and PRL were analyzed using two methods: 1) by calculating the area under the concentration-time curve, using the trapezoidal method (23), and 2) by using a microcomputer-based nonlinear least squares cosine analysis, as described by Rummel et al. (24). The advantages of the cosine analysis lie in its objective detection of a given rhythm and, especially, in its quantification of such parameters as amplitude (the measure of half the extent

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yr)</th>
<th>Duration of amenorrhea</th>
<th>Basal LH (mIU/mL)</th>
<th>Basal FSH (mIU/mL)</th>
<th>Basal PRL (ng/mL)</th>
<th>Plasma melatonin (peak nighttime; pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>8 months</td>
<td>8.1</td>
<td>6.1</td>
<td>5.2</td>
<td>162</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>5 yr</td>
<td>3.1</td>
<td>4.2</td>
<td>10.0</td>
<td>160</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>10 yr</td>
<td>7.1</td>
<td>8.5</td>
<td>10.6</td>
<td>88</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>3 yr</td>
<td>5.2</td>
<td>7.4</td>
<td>13.4</td>
<td>139</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>10 months</td>
<td>8.3</td>
<td>10.4</td>
<td>9.6</td>
<td>164</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>5 yr</td>
<td>8.7</td>
<td>11.9</td>
<td>15.0</td>
<td>215</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>7 months</td>
<td>3.0</td>
<td>4.2</td>
<td>12.2</td>
<td>206</td>
</tr>
</tbody>
</table>

To convert picograms per mL melatonin to picomoles per L, multiply by 4.31.

*The mean (±SE) peak plasma melatonin level of 14 normal women was 83 ± 8 pg/mL.*
of the rhythmic change in a cycle), mesor (the average value during the cycle), and acrophase (crest of the sine function used to approximate the rhythm).

For statistical analysis, values below assay sensitivity were assigned a value of zero. All of the data for a specific phase of the menstrual cycle were pooled for analysis. Mean values for each phase were computed and compared with those of the other phases, using repeated measures analysis of variance. The results in the amenorrheic women were compared with those in the normal women using Student's paired t test; P < 0.05 was considered significant.

**Results**

Figure 1 shows the mean 24-h plasma melatonin profiles of the normal women during the early follicular, periovulatory, and luteal phases of their menstrual cycles. Daily rhythms in plasma melatonin concentrations were evident in all women; daytime (0900–2100 h) values ranged from undetectable [≤5 pg/mL (≤22 pmol/L)] to 25 pg/mL (108 pmol/L), and all women had a nocturnal increase, starting around 2100 h and peaking [83 ± 8 (±SEM) pg/mL (358 ± 34 pmol/L)] around 0300 h. The large SEMs of the mean melatonin levels between 0100 and 0500 h reflect the large interindividual variations in peak height, as noted previously (9). However, each individual's melatonin patterns varied little during the three 24-h periods: areas under the curve varied by 5% or less, and each woman's lowest peak levels were within 10% of her greatest peak level.

The curves relating plasma melatonin concentration to time of day at different phases of the menstrual cycle are summarized in Table 2. The mean values of the areas under the plasma melatonin curve and the amplitudes of the melatonin rhythm were slightly, but not significantly, lower [687 ± 124 (±SEM) pg/mL·h (2960 ± 534 pmol/L·h) and 39 ± 7 pg/mL (168 ± 30 pmol/L), respectively] during the follicular phase than during either the periovulatory [723 ± 147 pg/mL·h (3116 ± 639 pmol/L·h)] and luteal [736 ± 139 pg/mL·h (3172 ± 509 pmol/L·h)] phases.

All normal women had a diurnal rhythm in plasma PRL; peak levels occurred around 0500 h (Fig. 1). (A second much smaller rise in plasma PRL occurred around 1900 h, during the follicular and periovulatory phases, but not during the luteal phase.) Unlike the variations in plasma melatonin, there were relatively small interindividual variations in the areas under the PRL curves or in the amplitude of the PRL rhythm. The mean values for the area under the curve relating plasma PRL to time (as well as for the amplitude and the mesor of the PRL rhythm) were slightly but not significantly higher during the periovulatory phase than during the rest of the menstrual cycle (Table 2).

There was a phase difference between the elevations in plasma melatonin and PRL (Fig. 1 and Table 2); the onset of the melatonin surge (around 2100 h) preceded by about 2 h that of the main PRL surge. The rapid fall of plasma melatonin levels, between 0500 and 0700 h, also occurred about 2 h earlier than the morning decline in plasma PRL. This phase difference was evident in each of the women on 3 study days.

**The seven women with hypothalamic amenorrhea had**

**Table 2. Plasma melatonin and PRL rhythms in normal and amenorrheic women**

<table>
<thead>
<tr>
<th>Cycle phase</th>
<th>Area under the curve (pg/ml·h)</th>
<th>Amplitude (pg/mL)</th>
<th>Mesor (pg/mL)</th>
<th>Acrophase (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melatonin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>587 ± 124</td>
<td>39 ± 7</td>
<td>28 ± 5</td>
<td>3:54 ± 0:24</td>
</tr>
<tr>
<td>Periovulatory</td>
<td>723 ± 148</td>
<td>41 ± 9</td>
<td>30 ± 6</td>
<td>3:42 ± 0:18</td>
</tr>
<tr>
<td>Luteal</td>
<td>736 ± 139</td>
<td>40 ± 7</td>
<td>24 ± 6</td>
<td>3:28 ± 0:13</td>
</tr>
<tr>
<td>Amenorrhea</td>
<td>1439 ± 176°</td>
<td>62 ± 9°</td>
<td>52 ± 6°</td>
<td>3:56 ± 0:12</td>
</tr>
</tbody>
</table>

| PRL         |                                 |                   |               |               |
| Normal women|                                 |                   |               |               |
| Follicular  | 302 ± 19                        | 5 ± 1             | 13 ± 1        | 4:32 ± 0:31   |
| Periovulatory | 332 ± 18                       | 6 ± 0             | 14 ± 1        | 5:02 ± 0:28   |
| Luteal      | 314 ± 17                        | 6 ± 1             | 13 ± 1        | 4:46 ± 0:16   |
| Amenorrhea  | 310 ± 17                        | 6 ± 0.8           | 14 ± 1.1      | 4:53 ± 0:25   |

Values are the mean ± SE. Samples were obtained every 2 h for 24 h from 14 normal women and 7 women with hypothalamic amenorrhea. To convert melatonin values to picomoles per L, multiply by 4.31. *P < 0.005 vs. values in normal women.
normal patterns of melatonin secretion (Fig. 2); however, their total daily melatonin secretion (estimated from the curve relating plasma hormone concentrations to time) was significantly higher ($P < 0.005$) than that of the normal women [1440 ± 176 vs. 715 ± 158 pg/mL·h (6206 ± 759 vs. 3082 ± 595 pmol/mL·h), respectively]. The plasma melatonin levels in the amenorrheic women were elevated both during the daytime and at night (Fig. 2).

**Discussion**

These results indicate that the daily rhythm in plasma melatonin levels and the absolute levels themselves change little during the menstrual cycle in normal women, but that plasma melatonin levels, though still rhythmic, may be significantly elevated in some women with hypothalamic amenorrhea. Although distinct differences were found among the normal women in the phase and amplitude of the melatonin rhythm and in the total amounts of the hormone secreted per day, there was, for each woman, remarkable consistency in these parameters throughout the menstrual cycle.

A few investigators have sought changes in plasma or urinary melatonin levels or patterns during the menstrual cycle; their findings have been conflicting (14–18). The wide discrepancies among those reports may reflect technical problems associated with early attempts to assay melatonin as well as variable sampling approaches. The consistency of each individual’s plasma melatonin pattern has also recently been described by Webley and Leidenberger (12). However, these researchers reported an increase in daily melatonin secretion between the follicular and luteal menstrual phases, a finding that was not confirmed in our study. Our conclusions are, however, in agreement with the results of another recent study (25) which failed to detect changes in urinary 6-hydroxymelatonin sulfate (a melatonin metabolite) during the menstrual cycle.

We also found that the daily pattern of PRL secretion changed little through the menstrual cycle. Some women had an increased nocturnal plasma PRL level at midcycle, but the peak level at this time was not significantly different for the group as a whole. This finding is in agreement with other reports (26–29) which reported that some, but not all, women have increased plasma PRL levels at midcycle. The small, early evening peak in plasma PRL levels that was evident in most of our subjects has also been observed previously (29, 30).

Assessment of the temporal relationships between plasma PRL and melatonin levels during the menstrual cycle has not been made previously. Tight point to point correlations between plasma PRL and melatonin levels in samples obtained at 2-h intervals would not be anticipated, given the erratic spurs that characterize PRL secretion (28). However, the consistency of the phase delay between the peak nocturnal plasma melatonin and PRL levels suggests a physiological relationship between the two events. Other indications of such a relationship are the findings that plasma PRL concentrations in rats (31) and humans (32, 33) rise within an hour after melatonin administration. Plasma melatonin levels were reported to be high in patients with hyperprolactinemia (34); however, reliable information is still lacking on the exact relationship, if any, between hyperprolactinemia and pineal secretory activity. Our finding of peak nocturnal plasma melatonin levels before peak plasma PRL levels agrees with the findings in five adult men (35). The mechanism by which melatonin might affect PRL secretion is not known. It could suppress hypothalamic dopamine release (36), thus diminishing dopaminergic inhibition of PRL secretion, or it might act centrally, by enhancing serotonin-mediated neurotransmission (37).

The physiological role of melatonin during the menstrual cycle is unclear. The lack of an obvious temporal relationship between plasma ovarian steroids and melatonin levels does not necessarily negate a role for melatonin in regulating the human menstrual cycle. On the contrary, our preliminary finding of a temporal relationship between the onset of the preovulatory LH surge and the early morning decrease in plasma melatonin (38) is consistent with other reports that the onset of the LH surge is an early morning occurrence (39) and suggests that melatonin might contribute to the timing of the preovulatory LH surge in humans, as it does in other mammalian species (5, 8, 40). Moreover, our finding of increased plasma melatonin levels in amenorrheic women suggests that impaired melatonin secretion may be involved in the neuroendocrine pathology that underlies hypothalamic amenorrhea.

**FIG. 2.** Mean (±SE) plasma melatonin levels in 14 normal women (mean of all 3 study days) and 7 women with hypothalamic amenorrhea. To convert picograms per mL melatonin to picomoles per L, multiply by 4.31.
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