Episodic nyctohemeral secretion of melatonin in adult humans: Lack of relation with LH pulsatile pattern

Alberto de Leiva¹, Federico Tortosa¹, Miguel A. Peinado¹, José Serrano¹, José Rodriguez-Espinosa² and Manuel Puig-Domingo¹

Departments of Endocrinology¹ and Biochemistry², Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Spain

Abstract  The concentration of melatonin and LH were determined in plasma samples obtained at 10-min intervals during 4 h of darkness (00.00-04.00 h) from 4 normal women, age 23-27 years, in the early follicular phase of the menstrual cycle and in 6 normal men, age 23-31 years. Additionally, melatonin concentration was determined in samples obtained from the men at 10-min intervals for 4 h during the day (10.00-14.00 h). A pulsatile pattern of melatonin secretion was found for all the subjects during darkness. There was no significant difference between women and men as to the number of pulses (2.8 ± 0.5 vs 5.2 ± 1.0 per 4 h), amplitude of pulses (51.3 ± 28 vs 27.2 ± 6 ng/l), concentration per 4 h (32.5 ± 13 vs 31.0 ± 5 ng/l), or apparent half-life of melatonin (19.3 ± 2.3 vs 15.3 ± 7.5 min). The mean amplitude of the melatonin pulse correlated (r = 0.865, p<0.001) with the mean melatonin concentration per 4 h. A pulsatile LH secretion pattern was found for the 10 subjects and did not correlate significantly with the melatonin secretion pattern. The results are consistent with an independent signal for the demonstrated nyctohemeral pulsatile melatonin and LH secretions.

The pineal gland is the main site of melatonin synthesis and secretion in humans (1,2). Plasma melatonin concentrations reflect faithfully pineal activity (3,4). In mammals, melatonin shows a circadian pattern of synthesis and secretion entrained by the light-dark period, pineal activity being maximal during the dark period; thus, melatonin levels are low during the day and high at night (5). Studies carried out in healthy volunteers after frequent blood samples suggest that melatonin is secreted episodically (6,7). The variability of the reported results may be explained by differences in the frequency and hour of the sample collection, cycle analyses criteria, age of the subjects, and technical differences in the measurement of melatonin, among others. The apparent half-life (t½) of circulating melatonin is also a matter of controversy, with no agreement in the data reported until now (7-9). Moreover, no physiological role has yet been defined for melatonin in humans. Although a strong influence of melatonin in the reproductive function in rodents (5) has been clearly established, there are no unequivocal data of this gonadal modulation of melatonin in humans.

The purpose of this work was to investigate the possibility of an episodic nature of melatonin secretion, to determine the apparent t½ of endogenous melatonin, and to study the possible correlation of melatonin levels with LH surges in a group of healthy adult humans.

Subjects and Methods

Plasma samples were obtained from 6 healthy men, aged 23 to 31 years and 4 healthy women, aged 23 to 27 years. Neither mean age (20.7 ± 1.82 vs 25.2 ± 1.7 years) (mean ± SEM), nor mean Body Mass Index (23 ± 1.8 vs 20.8 ± 2.0) were statistically different between sexes. Women were studied in the first half of the follicular phase of the menstrual cycle (4-8 days after onset of the menstrual bleeding). No subjects received medications, nor experi-
mented weight reduction, nor were engaged in strenuous sport. Each volunteer provided informed consent before the initiation of the study. The subjects were admitted to the research ward at 09.00 h after an overnight fast of 12 h. A standard diet of 2500 kcal was given during the 24-h period of study.

The protocol was performed during the months of April, May and June. Light intensity was monitored during the whole trial by using a luxometer (Gossen, FRG). Environmental illumination fluctuated between 500 and 5000 lux during the lighting period (07.00-23.00 h) and was undetectable during the dark period (23.00-07.00 h). A dim red light was used for the collection of samples during the dark period.

Blood samples were collected in EDTA-containing plastic tubes at 2-h intervals during the light period and hourly during the dark period from a peripheral vein via an indwelling catheter which had an attached stopcock; subjects were in the supine position during blood collection. A heparin solution was used for washing the iv line after each collection to maintain the permeability of the cannula. The first millilitre of each sample was discarded.

In order to investigate a possible episodic secretion of melatonin, samples were collected at 10-min intervals during a 4-h period from 00.00 to 04.00 h in the 10 subjects. Additionally, a diurnal 10-min interval blood sampling (from 10.00 to 14.00 h) was performed in the 6 men.

Half-life of endogenous melatonin was calculated after a semilogarithmic plot when 3 or more descending points were detected. The expected melatonin concentration after a descending value was calculated only in those cases in which melatonin t½ had been previously determined in other cycles. The expected melatonin concentration was calculated using a formula for substances with monocompartmental distribution (10)

\[ C = C_0 \cdot e^{-kt} \]

where \( C = \) expected concentration, \( C_0 = \) concentration before a descending value, \( K = \) elimination constant and \( t = \) time of the studied interval in minutes.

After collection, blood samples were immediately spun at 1500 × g for 15 min and plasma was separated, frozen and stored at −20°C until assayed. Before determination of melatonin, samples were extracted with 100% diethyl ether (3 ml of diethyl ether shaken for 1 min with 0.5 ml sample plasma); the mixture was frozen at −20°C and the ether extract was decanted and the aqueous phase discarded; then, the solvent phase was evaporated under a 100% nitrogen stream. The residue was reconstituted in RIA buffer (0.1 mol/l PBS pH 7.4, containing 0.1% BSA) and assayed for melatonin determination. The average percent recovery calculated for this extraction procedure is above 90%. Plasma melatonin was measured by radioimmunoassay (Eurodiagnostics, Appeldorn, Holland), using \(^{125}\)I-melatonin as tracer and a polyclonal antibody raised in rabbit by immunization with bovine thyroglobulin conjugated with N-acetylmethoxytryptophan (11). The cross-reactivity of the primary antibody with the most important indoleamines is the following: <0.01% for 5-methoxytryptophan, serotonin creatinine sulphate, serotonin, L-tryptophan, tryptamine, N-acetylytryptophan, 0.02% for N-acetylsertotonin, 0.05% for 5-methoxytryptamine, and 1.00% for 6-OH-melatonin. This RIA has a sensitivity of 5 ng/l, with an intra-assay coefficient of variation of 8.5% at 32 ng/l and 13.3% at 8 ng/l, and an inter-assay coefficient of variation of 13.9% at 17.5 ng/l and 16% at 89.6 ng/l.

LH was measured by using a double-antibody RIA (Serono Diagnostic SA, Coinsins, Switzerland) with an intra-assay coefficient of variation of 5.6% at 65.9 IU/l and 5% at 3.7 IU/l; the inter-assay coefficient of variation was 5.2% at 52 IU/l and 8% at 4 IU/l. All the samples of an individual were run in duplicate within the same assay, either for melatonin or for LH determination.

The analysis of melatonin and LH fluctuations was performed using a computerized programme described by Clifton & Steiner (12). One pulse was defined as an increase higher than the threshold which is followed by a decrease also greater than the threshold value. For an initial evaluation of frequency and amplitude of the fluctuations, the threshold was set at 2.7 times the noise. Noise defines the random errors in the sample value owing to assay variability and technique of collection. On subsequent evaluations the threshold is readjusted depending on the detected frequency and amplitude. This iterative readjustment and estimation process is repeated until the same result is obtained in two consecutive evaluations (12).

Differences between groups in hormonal values and secretory parameters were investigated by performing an analysis of variance (ANOVA). The correlation between plasma melatonin and LH nocturnal values (00.00-04.00 h) was statistically analysed by determination of the Spearman coefficient. Correlation between calculated vs detected plasma melatonin values in the descending phase of the cycles was performed by linear regression analysis (Pearson coefficient). Melatonin samples that were below the sensitivity of the assay were assumed to be of 5 ng/l for calculations. Results are expressed as mean ± SEM.

Results

Melatonin circadian rhythm
A nycthemeral pattern for plasma melatonin was observed in all subjects (Table 1). Mean melatonin concentrations were near or below the limit of sensitivity of the assay (5 ng/l) during the light period, whereas they were elevated during the dark period (6.3 ± 1.8 vs 30.1 ± 7.9 ng/l; p<0.001). Maximal melatonin concentrations were reached at 03.00 h (44.8 ± 8 ng/l) in men and at 02.00 h in women.
(48.3 ± 19 ng/l). A progressive decrease to low diurnal levels was observed 2 h after the onset of light. Mean melatonin values during light (6.7 ± 1 vs 5.6 ± 0.9 ng/l) and dark (33.4 ± 10.3 vs 24.5 ± 12.3 ng/l) periods were not statistically different between sexes.

Melatonin episodic secretion
Representative individual nocturnal profiles of plasma melatonin (samples collected every 10 min between 00.00-04.00 h) of both sexes are shown in Fig. 1. After the computerized analysis of individual data, a pattern of nocturnal episodic melatonin secretion was detected in each subject. There was a considerable variability in the peak values observed among subjects. Fluctuations that were considered as pulses showed a mean cycle length or period of 60 ± 15 min for men and 95 ± 17 min for women, and a mean amplitude of 27.2 ± 6 ng/l in men and 51.3 ± 28 ng/l in women (Table 2). During the 00.00-04.00 h period no significant differences were observed between sexes, either in the mean plasma melatonin concentration (31.0 ± 5 ng/l in men vs 32.5 ± 13 ng/l in women), mean value of the period or amplitude and area under the curve of plasma melatonin (7839 ± 1266 for men vs 7862 ± 3280 for women). Mean plasma melatonin concentration (31.7 ± 5 ng/l) for the whole group during the 4 h of darkness with samples collected every 10 min showed a significant direct correlation (r = 0.863, p<0.001) with the mean peak melatonin concentration (59.6 ± 8 ng/l). During the light period when samples were collected in men at 10-min intervals, no cycles or pulses of melatonin were detected, although most values of the majority of the subjects were below the sensitivity of the assay, as previously mentioned.

Apparent t½ of endogenous melatonin
The t½ of endogenous melatonin was calculated by semilogarithmic plots of 3 or more consecutive descending values. Only in subjects No. 1, 3, 6, 7, 8, and 9 (3 men and 3 women) did this occur. With these data, the apparent t½ of endogenous melatonin was around 18 min (Table 2). No significant differences between sexes were observed. In those subjects in whom melatonin t½ could be calculated, the expected melatonin concentration of all the descending segments showed a significant direct correlation (r = 0.817, p<0.001) with the detected melatonin values.

LH pulsatility
Representative individual profiles of LH between 00.00-04.00 h in both sexes are shown in Fig. 1. With samples obtained at 10-min intervals, a pulsatile secretion of LH was detected in both sexes. Computerized analyses of the data indicated that the mean cycle length or period of LH secretory episodes was 71 ± 17 min for men and 75 ± 11 min for women (Table 1). When the whole group was analysed no significant correlation was observed between respective melatonin and LH plasma values. Only a direct correlation between melatonin

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (hours)</th>
<th>11</th>
<th>13</th>
<th>15</th>
<th>17</th>
<th>19</th>
<th>21</th>
<th>23</th>
<th>24</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>09</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td>1</td>
<td>&lt;5</td>
<td>6</td>
<td>11.3</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>24.9</td>
<td>29.9</td>
<td>41.6</td>
<td>32.4</td>
<td>67</td>
<td>36.8</td>
<td>19</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>7.5</td>
<td>18</td>
<td>34.2</td>
<td>24.1</td>
<td>30</td>
<td>15.6</td>
<td>22</td>
<td>25</td>
<td>12.3</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>44.6</td>
<td>48</td>
<td>103.6</td>
<td>91</td>
<td>98.8</td>
<td>68.3</td>
<td>22.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>93</td>
<td>19.7</td>
<td>16.3</td>
<td>65.7</td>
<td>50.7</td>
<td>43.5</td>
<td>63.5</td>
<td>30.7</td>
<td>27</td>
<td>15.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>15.1</td>
<td>12.5</td>
<td>6.7</td>
<td>11.4</td>
<td>22</td>
<td>18.9</td>
<td>22</td>
<td>28.1</td>
<td>50</td>
<td>24.1</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td>6</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>29</td>
<td>15.6</td>
<td>11.8</td>
<td>12.6</td>
<td>21.8</td>
<td>13.8</td>
<td>6</td>
<td>5.9</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

Table 1
Plasma melatonin 24-h individual profiles.
Plasma melatonin (ng/l)
and LH was observed for subjects No. 1, 2 and 8 and an inverse correlation for subject No. 7 (Table 2).

Discussion
This study confirms previous investigations on the circadian pattern of melatonin secretion in humans (13–21). Our data strongly suggest that melatonin exhibits a detectable pulsatile pattern in healthy humans which is restricted to the dark period. We did not find any statistical differences between sexes in the values of secretory parameters. Other investigators have also suggested that melatonin is secreted episodically in humans (6, 22); however, the high diurnal melatonin concentrations reported in their studies, probably owing to a less specific assay than those presently available, make their conclusions questionable. Under our technical conditions we were unable to detect a pulsatile diurnal pattern for melatonin secretion in adult volunteers, contrasting with the previous observation by Weinberg et al. (6). Penny (7) detected diurnal pulses of melatonin in a group of pre- and postpubertal girls and boys; in his work no nocturnal data were reported. Differences in both the age of the investigated group and the RIA procedure may explain his findings. Also, in animals like the ewe, Bittman et al. (23) and English et al. (24) have
reported that melatonin is secreted in a pulsatile manner during the night.

The computerized analysis performed in our series permits definition of the parameters of melatonin cyclic secretion in healthy adult humans. The mean value for the period of plasma melatonin cycles is around 60 min in men and 95 min in women, and the mean value of the cycle amplitude is 37 ng/l for the whole group. Despite considerable interindividual variability in the length and amplitude of the cycles, the maximal melatonin concentrations during the 4-h dark period correlated directly with the mean melatonin concentrations, indicating that the magnitude of the pulses is the principal determinant of the plasma concentration of melatonin. Webley & Leidenberger (8) did not detect episodic secretion of melatonin in 10 women, and argued that the frequency of pulses like those detected by Penny (7) is not consistent with an apparent serum melatonin t½ of 47 min. Webley & Leidenberger (8) calculated t½ after exogenous melatonin administration, therefore, not in a physiological manner, and it is possible that the huge amount of melatonin given in their study modified the distribution and elimination phases and the rate of metabolism of melatonin, leading to a higher value of the apparent t½. We believe that in our study the calculated apparent t½ of serum melatonin reflects the physiological situation and agrees with the value reported by Penny (7), 20 min, in pubertal children and with the studies by Gibbs & Vriend (9) in rats. The wide range of melatonin t½ observed in our work may be explained by interindividual metabolic differences.

The demonstration of a significant correlation between the expected and the detected plasma melatonin concentrations indicates that the descending segments between two time points reflect the disappearance of melatonin from the plasma, which supports the episodic character of melatonin secretion. The inverse correlation between the highest plasma melatonin levels and the shortest

---

Table 2
Nocturnal melatonin and LH episodic secretion parameters.

<table>
<thead>
<tr>
<th>Group</th>
<th>Melatonin</th>
<th>LH</th>
<th>Melatonin: LH correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amplitude (ng/l)</td>
<td>Mean conc. (ng/l)</td>
<td>Period (min)</td>
</tr>
<tr>
<td>Men</td>
<td>1</td>
<td>24.8</td>
<td>37.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>23.2</td>
<td>23.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>43.3</td>
<td>37.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>46.0</td>
<td>47.1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8.8</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>16.9</td>
<td>25.1</td>
</tr>
<tr>
<td>Women</td>
<td>7</td>
<td>33.5</td>
<td>24.9</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>40.0</td>
<td>25.4</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>122.8</td>
<td>66.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8.9</td>
<td>15.2</td>
</tr>
</tbody>
</table>

Mean ± SEM

- Men: 36.8 ± 11, 31.7 ± 5, 73.8 ± 12, 17.7 ± 3, 4.1 ± 0.8, 8.3 ± 0.9, 72.8 ± 10
- Women: 27.2 ± 6, 31.0 ± 5, 60 ± 15, 15.3 ± 9, 5.3 ± 1.4, 7.5 ± 0.8, 71.3 ± 17

NS = no statistically significant differences

---

80
apparent melatonin $t^{1/2}$ postulated by Gibbs & Vriend (9) has not been confirmed in our study. Despite a marked tendency, we could not find a significant inverse correlation ($r = -0.595$, NS) between melatonin levels and its $t^{1/2}$.

The absence of detected cycles during the light period confirms that melatonin secretion is inhibited by light or that plasma melatonin fluctuations are below the sensitivity of the assay. However, it should be pointed out that our study was performed in a clinical research unit, whereas Penny's (7) subjects were studied in an outpatient department; as it has been postulated that activity increases daytime melatonin secretion (6), this factor should be taken into account to explain the higher melatonin levels found by Penny. On the other hand, these higher melatonin plasma concentrations might also be related with a particular melatonin secretory pattern of pre-pubertal subjects. Detectable diurnal melatonin levels might be of relevance in some pathological situations associated with increased day time melatonin concentrations, such as anorexia nervosa (25) in which a regression to a prepubertal-like situation takes place.

Until now it has not been possible unequivocally to demonstrate a physiological role for melatonin in humans. However, it has been suggested that melatonin might have a permissive effect on the maturation of the hypothalamo-pituitary-adrenal and gonadal axis (7, 17, 26), and that low plasma melatonin levels could be responsible for the macrogenitosomia observed in the fragile X chromosome syndrome (27). The LH pulsatile pattern that we detected in women during the follicular phase of the menstrual cycle is in agreement with previous reports (28, 29). In our study the melatonin and LH pulsatile patterns were not superimposable and did not show a clear statistical correlation, appearing as two independently regulated hormonal secretions. However, a decrease in the frequency of LH pulses between 03.00-05.00 h (30), the time when plasma melatonin rises to its maximal levels, has been described, with an inverse correlation between the integrated melatonin and LH levels; conversely, Fevre et al. (22) have found a positive correlation between melatonin and LH secretion in 5 pubertal boys. Although no significant correlation was found in our study when the whole group was considered, three of the subjects (2 men and one woman) presented positive correlations between melatonin and LH plasma values. Thus, we believe that more studies are needed to clarify this question. The physiological importance of melatonin pulses remains to be defined. The absence of pulses, or cyclic variations in plasma melatonin does not seem to affect its biological effects, as observed by Bittman et al. (23) studying pinealectomized ewes treated with melatonin infusions. This point requires further investigations in humans.

In conclusion, a definite pattern of episodic secretion of melatonin, restricted to the nocturnal period, is reported in healthy adult humans, with an apparent lack of relationship with the LH episodic secretion.

Acknowledgments

The authors are indebted to Dr Donald K. Clifton for the gift of the cycle detection analysis program, to Dr Josep Mª Pou for his advice in the discussion of data, to Josep Oriola for laboratory assistance, and to Mrs Paquita Cañas and Angeles Rodríguez for secretarial assistance.

This work was supported by a grant from FISS No. 86/1050 (Spain).

References

8. Webley GE, Leidenberger F. The circadian pattern of