Enhanced circadian rhythm of melatonin in anorexia nervosa

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Abstract. Plasma melatonin circadian profiles were investigated in a group of 4 patients with anorexia nervosa and 4 healthy regularly cycling women. There were no differences in the mean age of both groups, whereas the anorexia nervosa patients had lower mean body weight (37.8 ± 2.0 vs 57.0 ± 4.9 kg) and body mass index (13.9 ± 1.1 vs 20.8 ± 2.0). Samples were collected every 2 h and plasma melatonin was measured by using a RIA with an iodinated tracer. Anorexia nervosa patients exhibited higher diurnal (60.7 ± 1.8 vs 25.4 ± 1.72 pmol/l, P < 0.02) and nocturnal (419.2 ± 37.4 vs 108.0 ± 33.6 pmol/l), P < 0.001) mean plasma melatonin concentrations. There were no differences in the time peak for nocturnal melatonin secretion in both groups, detected at 02.00 h. In anorexia nervosa, the melatonin circadian profile paralleled that observed in the control group, indicating that the increased melatonin values for anorexia nervosa were probably due to an enhanced secretory pineal function rather than an impaired melatonin metabolism. These results suggest a participation of the pineal gland in the pathophysiology of anorexia nervosa.

Anorexia nervosa is a psychoneuroendocrine disease characterized by an isolated hypogonadotropic hypogonadism (Boyar et al. 1974; Halmi & Sherman 1977; Beaumont & Abraham 1981), which is believed to be of hypothalamic origin (Vigersky & Loriaux 1977). An inhibitory influence of melatonin, the major pineal secretory product, on hypothalamic GnRH pulsatility (Ying & Greep 1973; Bittman et al. 1985; Robinson et al. 1986;

Glass & Knotts 1987) has been extensively demonstrated in experimental animals, but solid data on humans are lacking. Thus, we decided to investigate pineal function by determining plasma melatonin circadian profiles in patients with anorexia nervosa, in order to clarify a possible participation of the pineal gland in the impairment of gonadal function observed in this disease.

Subjects and Methods

Plasma samples were obtained during a 24-h period from 4 healthy women aged 23 to 27 years and 4 women with anorexia nervosa aged 17 to 27 years. Mean age of both groups was not statistically different (25.2 ± 1.7 vs 20.2 ± 5.7). None of the subjects had other associated diseases or had taken oral contraceptives or medications in the 3 months preceding the study. The controls were regularly menstruating women in the follicular phase of the menstrual cycle. None of the anorexia nervosa patients had primary amenorrhea. The American Psychiatric Association criteria were used for the diagnosis of anorexia nervosa. Mean body weight for controls was within the normal range (57.0 ± 4.9 kg), with a mean body mass index of 20.0 ± 2.0. The anorexia nervosa patients had body weights ranging from 35.0 to 39.6 kg (mean ± SEM = 37.8 ± 2.0 kg) with a mean body mass index of 13.9 ± 1.1; both mean body weight (P < 0.05) and body mass index (P < 0.01) were statistically different between groups.

The protocol was performed after obtaining informed consent. Controls were admitted to the Clinical Research Unit for a 24-h period, whereas in anorexia nervosa pa-
During daytime, control values were in all cases and at all the time points studied, close to, or below the sensitivity of the method (20 pmol/l), whereas in the anorexia nervosa patients the values were in all cases within the detectable range (Table 1). During the nighttime period, anorexia nervosa patients also exhibited higher concentrations of plasma melatonin as evidenced by a 3-fold increase in the integrated nocturnal melatonin value (53 758 ± 14 895 vs 180 565 ± 28 160, P < 0.001). Moreover, the initiation of the nocturnal melatonin increase in the anorexia nervosa group anticipated that observed in controls, without differences in the time of detection of the peak values (at 02.00 h). There were no differences in the offset time of melatonin secretion, with similar plasma melatonin concentrations for both groups at the time when lights came on (Fig. 1). The mean diurnal and nocturnal melatonin values, and the anthropometric parameters of both groups are expressed in Table 2.

Results

Melatonin profiles for both groups are represented in Fig. 1. Anorexia nervosa patients showed higher levels of plasma melatonin both during the daytime and nighttime when compared with controls. During nighttime.

Patients, the 24-h blood sampling was performed during their stay in the Endocrine ward during an acute phase of the disease. Ambient indoor lighting was monitored during the whole day of the study by using a Gossen (FRG) luxometer. Light intensity ranged from 500 to 3000 lux during the daytime and was not detectable during the nighttime. Lights were off from 23.00 to 07.00 h. Blood samples were collected via an indwelling catheter from a forearm vein at 2-h intervals.

Plasma melatonin was measured by a RIA which uses radioiodinated melatonin as tracer (Tiefenauer & Andres 1984) obtained from a commercial source (Eurodiagnostics, Appeldorn, Holland). The sensitivity of this method is 20 pmol/l, with an intra-assay coefficient of variation of 8.5% at 137.7 pmol/l and 13.3% at 34.4 pmol/l, and an inter-assay coefficient of variation 15.2%. All the samples of an individual were run within the same assay in duplicate.

Differences between groups were investigated by using an analysis of variance (ANOVA), with significance assumed for P < 0.05. Melatonin values lower than the sensitivity of the RIA were considered to be 20 pmol/l for calculations. Results are expressed as mean ± SEM.

Discussion

The results we report herein are not in agreement with those of Birau et al. (1984), but are very similar to those recently reported by Ferrari et al. (1988) who used the same RIA as we did. Berga et al.
Table 1.
Plasma melatonin values of anorexia nervosa patients vs healthy women, during a period of 24 h. Results are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Controls (N = 4)</th>
<th>Anorexia (N = 4)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>08.00</td>
<td>38.3 ± 13.3</td>
<td>73.6 ± 16.8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>10.00</td>
<td>29.3 ± 4.73</td>
<td>71.9 ± 11.2</td>
<td>n. s.</td>
</tr>
<tr>
<td>12.00</td>
<td>&lt; 20</td>
<td>107.5 ± 60.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>14.00</td>
<td>21.9 ± 0.43</td>
<td>58.1 ± 36.6</td>
<td>n. s.</td>
</tr>
<tr>
<td>16.00</td>
<td>&lt; 20</td>
<td>36.6 ± 5.16</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>18.00</td>
<td>&lt; 20</td>
<td>25.8 ± 3.01</td>
<td>n. s.</td>
</tr>
<tr>
<td>20.00</td>
<td>&lt; 20</td>
<td>35.3 ± 13.8</td>
<td>n. s.</td>
</tr>
<tr>
<td>22.00</td>
<td>31.8 ± 5.60</td>
<td>85.2 ± 34.0</td>
<td>n. s.</td>
</tr>
<tr>
<td>24.00</td>
<td>81.4 ± 16.8</td>
<td>332.3 ± 74.5</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>02.00</td>
<td>207.9 ± 98.6</td>
<td>499.3 ± 176.9</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>04.00</td>
<td>127.4 ± 85.2</td>
<td>373.2 ± 49.1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>06.00</td>
<td>101.6 ± 76.2</td>
<td>221.7 ± 42.6</td>
<td>n. s.</td>
</tr>
</tbody>
</table>

(1988) too found higher nocturnal levels of circulating melatonin in patients with hypothalamic amenorrhea, but did not evidence any differences in the diurnal values of patients and controls; these findings are not contradictory to our results if we take into account that the methodology used by Berga et al. is slightly less sensitive (43 pmol/l) than ours (20 pmol/l), and, on the other hand, the patients they studied had a less severe hypothalamic disorder than the anorexia nervosa patients. Both diseases, hypothalamic amenorrhea and anorexia nervosa, may be considered to have a common physiopathological mechanism, the latter being a more intense and complex form of the disorder. Thus, it would not be surprising that plasma melatonin is higher in anorexia nervosa than in isolated hypothalamic amenorrhea. Therefore, melatonin can be used as a marker, among others, of the severity of the disease in these patients. Whether the increased concentrations of plasma melatonin are the cause or the consequence of the hypothalamic hypogonadism of anorexia nervosa patients remains to be investigated. The existence of a lower blood volume in anorexia nervosa patients, which could explain higher melatonin values by a mechanism of hemoconcentration, does not seem convincing since we have also observed higher diurnal and nocturnal plasma melatonin in patients with anorchia (Tortosa et al. 1988) who were weight-matched with their controls.

Taken together, these observations suggest the existence of an inhibitory modulation of gonadal steroids on pineal melatonin synthesis in humans. The feedback control of reproductive hormones on pineal function has been already demonstrated in rodents (Cardinali & Vacas 1978) and ovariectomy is followed by an increment in the amplitude of melatonin secretion in the ewe (Arendt et al. 1983). Moreover, androgen and estrogen receptors are present in the rat pineal gland (Cardinali et al. 1975). It is also remarkable that plasma melatonin is higher during infancy and prepuberty with a decline towards the adult values at puberty, when gonadal steroids increase (Waldhauser et al. 1984); additionally, pineal function and plasma melatonin are depressed during the first year of life, when circulating androgens and estrogens are

Table 2.
Integrated plasma melatonin concentrations and anthropometric parameters in anorexia nervosa patients and healthy women. Integrated melatonin values are expressed in arbitrary units. All values are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 4)</th>
<th>Anorexia (N = 4)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime melatonin</td>
<td>25.4 ± 6.0</td>
<td>60.7 ± 10.8</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Nighttime melatonin</td>
<td>108.0 ± 33.6</td>
<td>419.3 ± 37.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Integrated nocturnal melatonin</td>
<td>53 758 ± 14 895</td>
<td>180 565 ± 28 160</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25.2 ± 1.7</td>
<td>20.2 ± 5.7</td>
<td>n. s.</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.5 ± 4.2</td>
<td>165.2 ± 6.7</td>
<td>n. s.</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.0 ± 4.9</td>
<td>37.8 ± 2.0</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Body mass index</td>
<td>20.0 ± 2.0</td>
<td>13.9 ± 1.1</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>
high (Reiter 1986). Finally, women living in long dark winters of high latitude countries show higher values of melatonin secretion and decreased ovarian and androgenic activities (Kaupila et al. 1987).

On the other hand, it could be argued that plasma melatonin reflects both its synthesis by the pineal and its rate of metabolism; thus, a lower metabolic capacity in seriously affected anorexia nervosa patients may explain the higher levels of this indoleamine. However, this possibility seems unlikely because, despite having anorexia nervosa, none of these patients had signs of liver disease that suggested a reduced hepatic melatonin metabolism. This possibility cannot be completely excluded if plasma or urinary 6-OH-melatonin sulphate, the main metabolite of melatonin (Fellenberg et al. 1981), is not measured. A low value of 6-OH-melatonin sulphate in these patients in the presence of high plasma melatonin concentrations would indicate a reduced hepatic melatonin metabolism. Our observation of high plasma melatonin levels in other hypogonadal patients not suffering a severe illness, as anorhmic patients, also suggests an increased pineal indoleamine synthesis rather than a decreased melatonin degradation. Moreover, the values for plasma melatonin concentration were similar in both groups of anorexic and anorhmic patients, suggesting that the low levels of gonadal hormones may be the chief factor determining the high plasma melatonin values. The maintenance of a circadian pattern for plasma melatonin with a nyctohemeral increase also indicates that the amplified melatonin profiles in anorexia nervosa reflect an enhanced pineal secretory activity. In anorexia nervosa, a primary dysregulation of the hypothalamic control on GnRH action may be followed by an enhanced melatonin synthesis and secretion as a consequence of the impaired gonadal steroid pineal input, and finally these higher concentrations of circulating melatonin may potentiate the hypogonadal situation by acting at the hypothalamic level, where melatonin binding sites have been described (Cardinali et al. 1979).

It remains to be investigated if any therapeutic manipulation of pineal melatonin synthesis in anorexia nervosa, i.e. suppression of melatonin synthesis by beta-blocker treatment, and its consequent reduction of melatonin exposure, would improve or accelerate the recovery of gonadal function in these patients.

In conclusion, the existence of an enhanced circadian melatonin pattern suggesting pineal hyperfunction in patients with anorexia nervosa is described, and their physiopathological implications are discussed.

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