Circadian pattern of circulating plasma ACTH, cortisol, and aldosterone in patients with β-thalassemia

Paolo Pasqualetti¹, Domenico Colantonio¹, Antonio Collacciani², Raffaele Casale¹ and Gianfranco Natali¹

Department of Internal Medicine¹, School of Medicine and Surgery, Chair of Medical Pathology, University of L’Aquila, and Division of Internal Medicine², Section of Hematology, Hospital of Avezzano, L’Aquila, Italy

Abstract Plasma levels of ACTH, cortisol, and aldosterone were measured for an entire day every 2 h, starting from midnight, in 4 healthy subjects, and in 4 patients with β-thalassemia, without evidence for any endocrine disease. The subjects, after synchronized standard life conditions for 10 days, were held in constant supine position during the study. The data were analysed by the "cosinor" method. The results show significant circadian rhythms for the three biological variables in healthy subjects. In the thalassemic patients a significant circadian rhythm was detected only for cortisol and aldosterone. No rhythm was demonstrated for ACTH in the patient group. While no differences were found in mesors and acrophases for the three hormones between the two groups, a significant difference was observed regarding amplitudes. These data suggest that in β-thalassemia, the secretion rhythmicity of ACTH is modified, whereas the adrenal cortex maintains its own physiologic rhythmicity in hormone secretion.

Patients with β-thalassemia frequently exhibit several endocrine abnormalities. These include hypothalamic-pituitary dysfunction, hypothyroidism, adrenal insufficiency, hypoparathyroidism, and pancreatic dysfunction (1-6). In the course of thalassemia, clinically evident alterations in adrenal function are infrequent. However, despite normal basal levels of ACTH (7), cortisol (2,7-10), and aldosterone (8), a partial adrenal insufficiency has been reported, with high basal levels of plasma ACTH and decreased response of cortisol after ACTH stimulus (7,9-12). ACTH, cortisol, and aldosterone present time-dependent variations in their circulating concentrations (13), referred to as "circadian rhythm". Chronobiological modifications in these circadian patterns could precede the homeostatic alterations and the clinical evidence of endocrine disease (13).

The aim of the present study was to elucidate the circadian rhythms of these hormones in patients with β-thalassemia, without clinical evidence of endocrine abnormalities, compared with a group of clinically healthy subjects.

Subjects and Methods

Two groups of subjects were studied: 4 clinically healthy subjects, 3 males and 1 female, aged from 18 to 24 years, and 4 patients, 3 males and 1 female, aged from 18 to 22 years, with β-thalassemia (3 major and 1 intermedia). The diagnosis of β-thalassemia was based on clinical and biochemical data. The clinical and laboratory features of thalassemic patients are summarized in Table 1. The patients with β-thalassemia major required repeated blood transfusions whereas the patient with β-thalassemia intermedia received only occasional transfusions. All patients were treated at intervals with desferrioxamine. No patients had clinical and laboratory evidence of any endocrine disease.

Both the control group and the patients were given a normocaloric diet, with 120 mmol/day of sodium (normal sodium intake), with meals at 8.00, 13.00, and 18.30 h. Sleep and/or rest period lasted from 22.00 to 6.00 h. In all cases, no drugs and/or blood transfusions were administered in the synchronization period. The synchronization period lasted 10 days, and the study was performed on
Table 1.
Clinical and laboratory features in the 4 patients with β-thalassemia.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI</th>
<th>Type of thalassemia</th>
<th>Age of first transfusion</th>
<th>Hb (g/l)</th>
<th>Ferritin (µg/l)</th>
<th>ASP (µkat/l)</th>
<th>ALT (µkat/l)</th>
<th>LDH (µkat/l)</th>
<th>Total bilirubin (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>18</td>
<td>163</td>
<td>65</td>
<td>24.5</td>
<td>major</td>
<td>4</td>
<td>86</td>
<td>2450</td>
<td>1.73</td>
<td>2.07</td>
<td>3.12</td>
<td>32.75</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>19</td>
<td>168</td>
<td>63</td>
<td>22.3</td>
<td>major</td>
<td>1</td>
<td>98</td>
<td>4300</td>
<td>1.23</td>
<td>1.40</td>
<td>3.63</td>
<td>42.66</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>22</td>
<td>171</td>
<td>65</td>
<td>22.2</td>
<td>major</td>
<td>2</td>
<td>102</td>
<td>3620</td>
<td>1.68</td>
<td>1.58</td>
<td>2.89</td>
<td>32.20</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>20</td>
<td>158</td>
<td>47</td>
<td>18.8</td>
<td>intermediate</td>
<td>13</td>
<td>113</td>
<td>1280</td>
<td>0.82</td>
<td>0.93</td>
<td>2.44</td>
<td>18.81</td>
</tr>
</tbody>
</table>

the eleventh day. With the subjects held in constant supine position, blood samples were drawn from a peripheral vein every 2 h, starting from midnight, during the whole day in order to determine plasma ACTH, cortisol, and aldosterone circulating levels. For hormonal determination, measurement of circulating levels were performed by radioimmunoassay method, using commercial kits. In particular, plasma ACTH was determined by "ACTHK RIA kit" (CEA-Sorin, Saluggia, Vercelli, Italy), with a sensitivity of 2.2 pmol/l, with a coefficient of variation of 9.0% at a mean value of 18.71 pmol/l, and with a cross-reactivity of 3.0% with β-MSH. Plasma cortisol was measured by "CORT-CTK 125 RIA kit" (CEA-Sorin), with a sensitivity of 6.9 nmol/l, with a coefficient of variation of 6.2% at a mean value of 568.35 nmol/l, and without any cross-reactivity. Plasma aldosterone was determined by "ALDOCTK-2 RIA kit" (CEA-Sorin), with a sensitivity of 4.16 pmol/l, with a coefficient of variation of 7.0% at a mean value of 471.25 pmol/l, and with cross-reactivity of 4.2% with 3β-α-tetrahydroaldosterone.

The time-correlated values were subjected to rhythmicometric circadian analysis, using the "cosinor" method, in order to establish the presence of a statistically significant circadian rhythm, and to evaluate the parameters (mesor, amplitude, and acrophase) (14).

The mesors of each variable were compared in the two groups by means of the "mesor test" (14); the amplitudes and the acrophase using the "Hotelling's statistic test" (14). An analysis using chronograms (average ± 1σ) has also been employed.

The study was performed according to the Helsinki II Declaration, and informed consent was obtained from each subject.

Results

Fig. 1 illustrates the circadian variation of cortisol, aldosterone, and ACTH in each patient with β-thalassemia. While cortisol and aldosterone have their peaks in the morning hours, ACTH fluctuates widely during the day in each patient.

Table 2.
Rhythmicometric circadian analysis by the "cosinor" methods of the circulating levels of ACTH, cortisol, and aldosterone in the two groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>No. of observations</th>
<th>PR</th>
<th>p</th>
<th>Mesor ± SEM</th>
<th>p mesor</th>
<th>Amplitude (95% CL)</th>
<th>p amplitude</th>
<th>Acrophase (95% CL)</th>
<th>p acrophase</th>
<th>Hours and minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH (pmol/l)</td>
<td>controls</td>
<td>48</td>
<td>81</td>
<td>0.007</td>
<td>17.59±0.53</td>
<td>&gt;0.05</td>
<td>4.32 (1.61±7.13)</td>
<td>&lt;0.05</td>
<td>-64° (-46°—-118°)</td>
<td>&gt;0.05</td>
<td>4.16 (3.04±7.52)</td>
</tr>
<tr>
<td></td>
<td>patients</td>
<td>48</td>
<td>29</td>
<td>0.09</td>
<td>16.03±1.03</td>
<td>0.05</td>
<td>0.79 (0.70±2.46)</td>
<td>&gt;0.05</td>
<td>-59° (- - + -)</td>
<td>&gt;0.05</td>
<td>3.56 (- - + -)</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>controls</td>
<td>48</td>
<td>80</td>
<td>0.003</td>
<td>355.9±22.11</td>
<td>&gt;0.05</td>
<td>160.9 (107.6±281.4)</td>
<td>&lt;0.05</td>
<td>-101° (-77°--148°)</td>
<td>&gt;0.05</td>
<td>6.44 (5.08±9.52)</td>
</tr>
<tr>
<td></td>
<td>patients</td>
<td>48</td>
<td>69</td>
<td>0.016</td>
<td>289.7±8.3</td>
<td>&gt;0.05</td>
<td>115.9 (11.1±276.3)</td>
<td>&lt;0.05</td>
<td>-110° (-10°+162°)</td>
<td>&gt;0.05</td>
<td>7.29 (9.40±10.48)</td>
</tr>
<tr>
<td>Aldosterone (pmol/l)</td>
<td>controls</td>
<td>48</td>
<td>66</td>
<td>0.009</td>
<td>194.5±8.1</td>
<td>&gt;0.05</td>
<td>57.7 (28.9±93.2)</td>
<td>&lt;0.05</td>
<td>-112° (-82°--158°)</td>
<td>&gt;0.05</td>
<td>7.28 (5.28±10.32)</td>
</tr>
<tr>
<td></td>
<td>patients</td>
<td>48</td>
<td>43</td>
<td>0.043</td>
<td>190.9±7.8</td>
<td>&gt;0.05</td>
<td>27.7 (10.1±71.3)</td>
<td>&lt;0.05</td>
<td>-134° (-52° + -190°)</td>
<td>&gt;0.05</td>
<td>8.56 (3.28±12.40)</td>
</tr>
</tbody>
</table>

PR = percentage of rhythm (percentage of variability accounted for by the fitted model of the rhythm); CL = confidence limits at 95% of probability.
Fig. 1.
Circadian variation in circulating plasma levels of ACTH, cortisol, and aldosterone in each patient with β-thalassemia. Numbers of the patients correspond to those in Table 1.

Fig. 2.
Circadian fluctuation in circulating plasma levels of ACTH, cortisol, and aldosterone (mean ± 1sd) in the group of healthy subjects (■), and in patients with β-thalassemia (●).
Fig. 2 illustrates the circadian fluctuations of plasma ACTH, cortisol, and aldosterone in the two groups under study.

Table 2 summarizes the time-related values of the variables, analysed with the "cosinor" methods. The results show that, while the healthy subjects present a statistically significant (p<0.05) circadian rhythm for all hormones considered this rhythm is not present for ACTH in thalassemic patients (p>0.05). No significant differences (p>0.05) are found between the two groups in mesors and acrophases of the three variables; the amplitudes of ACTH, cortisol, and aldosterone are significant (p<0.05) lower in the patient group than in control group.

Discussion

The data on pituitary-adrenal function in thalassemia are very controversial. In fact, some studies have reported normal basal levels of ACTH, cortisol, and aldosterone (2,7-10), and a normal response to metyrapone (15) and to stimulation with ACTH (2,16). On the other hand, other studies have demonstrated hypofunction or reduced reserve of adrenal cortex in thalassemic patients, with high basal ACTH levels (11,12), and an abnormal increased response to insulin-induced-hypoglycemia (17). Regarding mineral-corticoid secretion, urinary levels of aldosterone have been found within the normal range (8).

At present, no data are available on the circadian rhythm of the pituitary-adrenal axis in thalassemia. Our data indicate that, in patients with ß-thalassemia without clinical evidence of endocrine abnormalities, a lack in the circadian rhythmicity of ACTH could be present, whereas adrenal cortex seems to maintain its own diurnal periodicity.

The pathophysiology of endocrine abnormalities in ß-thalassemia is a very complex problem. In fact, numerous mechanisms are involved, such as decreased adrenal cortex response to ACTH stimulus, simultaneous hypothalamic-pituitary-adrenal defects, slow hormonal metabolic clearance owing to concomitant chronic liver disease, and endocrine deficiencies owing to iron overload in glands (3,7,17). It is possible that some of these factors, especially hypothalamic-pituitary defects, could contribute to the loss of the ACTH circadian rhythm. On the contrary, despite iron overload in the adrenal cortex (18), reduced clearance of steroids, reduced synthesis of transport proteins by the liver, and altered neuronal control of the hypothalamic-pituitary-adrenal axis (7), the adrenal cortex maintains its own physiologic circadian periodicity of hormonal secretion, even if at lower levels, as demonstrated by reduced rhythm amplitudes.

A similar circadian pattern as in thalassemia could be observed in other pathological conditions, such as in cirrhosis of the liver (19-21), and in Cushing's disease (22-24). In these conditions, there is a progressive derangement in the circadian periodicity of the pituitary-adrenal axis, related to the degree of the disease. In other words, the sequential loss in the circadian rhythmicity of the hormones can take part in the natural history of cirrhosis of the liver and Cushing's disease. Also in ß-thalassemia, the presence of a preclinical state is hypothesized, characterized only by the lack of ACTH circadian periodicity, which does not seem to modify the adrenal cortex circadian pattern. It is possible that the appearance of clinically evident endocrine manifestations could depend on the loss of all hormonal circadian rhythms.

The chronobiological approach to disease could offer new information and better understanding of the pathophysiology of the endocrine abnormalities in ß-thalassemia. Further studies are required to confirm these data, and to define better their implications for clinical practice.

References