The effect of long-term pulsatile GnRH administration on the 24-hour integrated concentration of GH in hypogonadotropic hypogonadal patients

M. Giusti, R. Torre, P. Cavagnaro, R. Attanasio, L. Traverso and G. Giordano

ISMI, Cattedra di Endocrinologia, Università di Genova, Genoa, Italy

Abstract. Measurement of integrated concentration of GH by means of continuous withdrawal sampling is a method of evaluating physiological hormonal secretion. Integrated concentration of GH was evaluated in 5 subjects with idiopathic hypogonadal hypogonadism (range 19–27 years) and in a 17-year-old male with idiopathic delay of puberty (5 males, 1 female) before and 30–240 days after the start of pulsatile GnRH administration. Gonadotropins and testosterone or 17β-estradiol were restored, whereas 24-h integrated concentration of GH (before therapy 5.4 ± 1.3 IU/1; during GnRH 8.1 ± 2.0 IU/1; P < 0.05) was increased by GnRH therapy. However, no correlation was found between GH levels and sex steroid concentrations during GnRH pulsatile administration. These data further confirm that a physiological increase in gonadotropins and sex steroids can modulate GH synthesis and/or release.

Normal pubertal growth spurt requires the concerted action of sex steroids, GH and somatomedin-C. However, previous reports regarding the effects of sex steroids on 24-h GH levels during spontaneous puberty or exogenous sex hormones treatment have described either no change (Plotnick et al. 1974; Thompson et al. 1972) or an increase (Finkelstein et al. 1972; Link et al. 1986; Mauras et al. 1987; Miller et al. 1982) in GH levels. Recently, mean 24-h GH concentrations were evaluated in three subjects with idiopathic hypogonadotropic hypogonadism (IHH) during pulsatile GnRH administration. Stanhope et al. (1985) reported an increase in the pulsatile release of GH from the first week of GnRH therapy in a 16-year-old girl with IHH. Liu et al. (1987) observed a rise in both the mean 24-h GH levels and GH pulse amplitude without any change in GH pulse frequency in two IHH adult males treated with GnRH in a pulsatile fashion for 3 and 10 months, respectively.

To further evaluate GH release during long-term GnRH pulsatile administration, we studied the 24-h integrated GH concentration (IG-GH) in five patients with IHH and also in one subject with idiopathic delayed puberty (IDP). IC-GH measurement by continuous withdrawal technique allows a more physiological and consistent assessment of spontaneous GH secretion than multiple sampling during day and night (Zadik et al. 1985).

Patients and Methods

Six patients from whom informed consent was obtained, were studied. Four males and one female fulfilled the diagnostic criteria of IHH (Liu et al. 1987); one male patient had IDP according to the definition of Wagner et al. (1986). Pretreatment clinical characteristics of the subjects are summarized in Table 1. Clinical stages of puberty were evaluated according to Tanner (1962). Testicular volume, assessed by Prader’s orchidometer ranged from 4 to 8 ml. Prior to initial evaluation, 3 out of 6 patients had been treated with androgens or gonadotropins. However, previous treatments were discontinued at least 3 months before the start of the study.

The 24-h IC-GH was measured before and 30–240 days after the start of GnRH administration. GnRH therapy was given in a pulsatile fashion every 120 min by a
miniature infusion pump (Zyklomat, Ferring, West Germany). Both the amount of GnRH per pulse and the route employed in delivering the drug in each subject are reported in Table 1. The studies were performed after 3 days in order to get the patients accustomed to hospital life. Activity and diet were not restricted during the sampling period. Lights were turned off between 22.00–07.00 h. Sleep was evaluated by visual inspection of the subjects.

Blood was sampled by means of a small pump adjusted to withdraw blood at a constant rate through a non-thrombogenic catheter inserted into an antecubital vein. Blood collection tubes were replaced every 60 min. Aliquots were taken from each tube and combined to obtain the 24-h pool from which the IC-GH was measured. In addition, IC-GH was evaluated both during sleeping and waking periods. Mean 24-h concentration of LH, FSH, testosterone (T) and 17β-estradiol (E₂) were also measured during this study.

To avoid inter-assay variability, all measurements for

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>PH*</th>
<th>G/B**</th>
<th>Diagnosis</th>
<th>Previous treatment</th>
<th>GnRH (days of therapy)</th>
<th>Dosage per pulse (ng · kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>M</td>
<td>2</td>
<td>1</td>
<td>IDP</td>
<td>None</td>
<td>30</td>
<td>60 · iv</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td>IHH</td>
<td>None</td>
<td>240</td>
<td>320 · sc</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>M</td>
<td>1</td>
<td>1</td>
<td>IHH</td>
<td>None</td>
<td>30</td>
<td>150 · sc</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>M</td>
<td>4</td>
<td>3</td>
<td>IHH</td>
<td>hCG</td>
<td>30</td>
<td>80 · iv</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>M</td>
<td>3</td>
<td>3</td>
<td>IHH</td>
<td>hCG, T</td>
<td>50</td>
<td>60 · iv</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>M</td>
<td>3</td>
<td>2</td>
<td>IHH</td>
<td>hCG, T</td>
<td>40</td>
<td>160 · sc</td>
</tr>
</tbody>
</table>

* PH: pubertal hair. ** B: breast, or G: genitalia state according to Tanner (1962).

<table>
<thead>
<tr>
<th>Table 1. Clinical data of patients.</th>
</tr>
</thead>
</table>

Table 2. Hormonal data before and during pulsatile GnRH therapy.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Integrated concentration GH (mIU/l)</th>
<th>LH (IU/l)</th>
<th>FSH (IU/l)</th>
<th>T (nmol/l)</th>
<th>E₂ ** (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24-h</td>
<td>Sleep</td>
<td>Waking state</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Before</td>
<td>10.6</td>
<td>22.6</td>
<td>3.4</td>
<td>12.7</td>
<td>11.7</td>
</tr>
<tr>
<td>During</td>
<td>13.4</td>
<td>23.8</td>
<td>7.0</td>
<td>25.9</td>
<td>12.8</td>
</tr>
<tr>
<td>2 Before</td>
<td>6.2</td>
<td>8.8</td>
<td>4.6</td>
<td>1.3</td>
<td>1</td>
</tr>
<tr>
<td>During</td>
<td>6.4</td>
<td>12.2</td>
<td>7.8</td>
<td>5.0</td>
<td>4.7</td>
</tr>
<tr>
<td>3 Before</td>
<td>9.0</td>
<td>9.6</td>
<td>3.8</td>
<td>4.2</td>
<td>1.3</td>
</tr>
<tr>
<td>During</td>
<td>13.4</td>
<td>16.2</td>
<td>11.6</td>
<td>13.4</td>
<td>5.3</td>
</tr>
<tr>
<td>4 Before</td>
<td>2.4</td>
<td>3.4</td>
<td>2.0</td>
<td>1.1</td>
<td>2.8</td>
</tr>
<tr>
<td>During</td>
<td>2.6</td>
<td>4.6</td>
<td>1.4</td>
<td>18.4</td>
<td>2.4</td>
</tr>
<tr>
<td>5 Before</td>
<td>5.8</td>
<td>8.6</td>
<td>4.0</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td>During</td>
<td>6.8</td>
<td>10.6</td>
<td>4.4</td>
<td>33.7</td>
<td>19.0</td>
</tr>
<tr>
<td>6 Before</td>
<td>1.4</td>
<td>1.8</td>
<td>1.2</td>
<td>1.1</td>
<td>1</td>
</tr>
<tr>
<td>During</td>
<td>2.8</td>
<td>3.2</td>
<td>2.6</td>
<td>12.6</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Before x: 5.4 | 9.1 | 3.2 | 3.6 | 3.1 | 2.95 | 42
± SEM: 1.3 | 3.0 | 0.5 | 1.9 | 1.7 | 1.05 | 5
During x: 8.1* | 11.8* | 5.8 | 18.2* | 8.9* | 26.55* | 87*
± SEM: 2.0 | 3.1 | 1.5 | 4.2 | 2.5 | 7.04 | 17

* P < 0.05 before vs during; ** mean values evaluated only in males.
each subject were performed in the same assay. Reagents for GH assay were obtained from CIS (Saluggia, Italy); standards were calibrated against WHO 66/217. The minimal detectable GH level was 0.4 mIU/l and the intra-assay variability was 3.1%. Gonadotropins were measured using reagents provided by Biodata (Milan, Italy). LH and FSH values were expressed in IU/l as equivalent of the 2nd IRP-hMG; the sensitivity of the method was 1 IU/l for both hormones. The intra-assay variability was 4.3 and 7.1% for LH and FSH, respectively. T and E\(_2\) (DPC, Los Angeles, CA) were evaluated without extraction. Sensitivity of the methods was 0.5 nmol/l for T and 37 pmol/l for E\(_2\). Intra-assay variability was 4.0% and 6.2%, respectively. The value of the assay detection limit was assigned to undetectable hormonal levels in order to calculate mean values. The 24-h GH behaviour was compared before and during GnRH pulsatile administration using the analysis of variance for repeated measurements (Armitage 1982). The Wilcoxon rank test was used to compare mean value obtained before and during therapy. Correlations between different parameters were examined by Spearman’s correlation coefficient. Data with \(P < 0.05\) were considered statistically significant. All the results were expressed as mean ± SEM.

Results

Gonadotropins and sex steroids were restored by pulsatile GnRH administration in male patients (Table 2). In the IHH female, GnRH therapy caused menarche and the 2nd study of the 24-h IC-GH was performed on day 4 after onset of the menstrual cycle. Fig. 1 reports the mean 24-h GH behaviour found before and during GnRH administration. GH levels were significantly higher during the pulsatile therapy (\(P < 0.01\)). In all subjects the 24-h IC-GH was significantly increased after GnRH therapy (\(P < 0.05\) (Table 2). The increase in IC-GH was found to be more evident during the sleeping period (\(P < 0.05\) than during the waking period (NS) (Table 2). The increase in the IC-GH levels was not found to be related to the changes in gonadotropin or sex steroid levels induced by GnRH therapy.

Discussion

Many studies indicate that pharmacological amounts of sex steroids increase GH secretion. It is well known that testosterone increases GH responsiveness to arginine- and insulin-induced hypoglycemia in prepubertal and pubertal subjects (Illig & Prader 1970; Martin et al. 1968). Likewise, estrogens increase GH release after physical exercise (Wideman et al. 1976) and pharmacological stimuli (Merimee & Fineberg 1971). On the other hand, spontaneous (Link et al. 1986; Mauras et al. 1987) or stimulated (Marimee & Fineberg 1971) GH secretion can be increased by testosterone administration in children of short stature and/or delayed sexual development.
IH and IDP are due to different degrees of impaired neuroendocrine control of GnRH biosynthesis and release from the hypothalamus (Clayton 1987). Both these pathological conditions can be compared with experimental conditions allowing the study of physiological activation of the pituitary-gonadal axis by exogenous GnRH and its effect on GH secretion. Recently, in patients with delayed puberty during pulsatile GnRH administration, Stanhope et al. (1988) showed an increase in GH pulsatility without change in GH pulse frequency which was coincident with the onset of growth acceleration in both girls and boys. Our experience demonstrated that in IH and IDP patients there is a significant increase in 24-h IC-GH, after 30 to 240 days of pulsatile GnRH administration in the presence of a clear gonadal steroido-genetic response. The beginning of puberty, induced by pulsatile GnRH therapy, amplifies 24-h GH secretion as previously shown during the physiological progress of puberty (Giusti et al. 1978). Integrated daily GH concentration confirms data drawn from multiple sampling in IHH subjects during GnRH therapy (Liu et al. 1987; Stanhope et al. 1985). Increased sex hormone levels in body fluids seem to be a decisive factor of the increasing integrated GH secretion, because of the rapid onset of GnRH-induced gonadotropin and gonadal steroid secretion.

However, GH changes are not directly related to absolute T or E_2 levels induced by GnRH neither to their increase or treatment duration. Moreover, no correlation was shown in adult males with IHH between either free-T or mean GH levels after androgenization (Liu et al. 1987). Similarly no correlation was found between T levels and 24-h IC-GH in normal pubertal subjects (Thompson et al. 1972).

Mechanism(s) and site(s) of action of sex steroids on the modulation of GH secretion are still not understood. Moreover, experimental data in animals are also conflicting (Fukata & Martin 1986; Hoeffler & Frawley 1986; Ohlsson et al. 1987). The interaction of neurotransmitters in the hypothalamus (Stanhope et al. 1985) and/or T/E_2 (or their metabolites) mediated amplification of GH synthesis/release could be hypothesized. The rise of GHRH-induced GH responsiveness during hGG-induced testosterone response in IHH subjects (Giusti et al. 1987) is possibly consistent with this latter hypothesis.

References
Ohlsson L, Isaksson O & Jansson J O (1987): Endogenous testosterone enhances growth hormone (GH)-re-


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Dr Massimo Giusti,
ISMI Cattedra di Endocrinologia,
Viale Benedetto XV, 6,
1-16132 Genoa,
Italy.