Non-responsiveness of serum gonadotropins and testosterone to pulsatile GnRH in hemochromatosis suggesting a pituitary defect

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We investigated the potential pituitary origin of gonadal insufficiency in hemochromatosis. Gonadotropin secretion was studied in seven patients with hemochromatosis and hypogonadism, before and after chronic pulsatile GnRH therapy. Pulsatile LH secretion was studied before (sampling every 10 min for 6 h) and after 15—30 days of chronic pulsatile GnRH therapy (10—12 µg per pulse). Prior to GnRH therapy, all the patients had low serum testosterone, FSH and LH levels. LH secretion was non-pulsatile in four patients, while a single pulse was detected in the remaining three. Chronic pulsatile GnRH administration did not increase serum testosterone levels; similarly, serum LH levels remained low: neither pulse frequency nor pulse amplitude was modified. We conclude that hypogonadism in hemochromatosis is due to pituitary lesions.

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Hemochromatosis is a rare disorder characterized by iron deposition in various tissues and organs. Hypogonadism is the most common endocrine dysfunction in men with this condition (1, 2) and, although several authors have suggested that it may be due to iron-induced damage to testicular Leydig cells (3, 4), most studies support a central origin; indeed, the majority of patients have low plasma levels of LH and FSH. A pituitary dysfunction has been suggested by indirect findings (2, 3, 5), but some authors have raised the possibility of a hypothalamic defect (6). Recently, Wang et al. (7) treated three male patients suffering from severe thalassemia and chronic iron overload with pulsatile gonadotropin-releasing hormone (GnRH) and obtained no improvement in testosterone levels.

To determine the hypothalamic or pituitary nature of the defect in hemochromatosis, we studied seven men with hemochromatosis and hypogonadism by evaluating gonadotropin secretion before and after long-term GnRH administration.

Patients and methods

Patients

Seven men aged 17 to 55 years with proven idiopathic hemochromatosis (N = 5) or chronic iron overload secondary to multiple transfusions for beta-thalassemia major (N = 2) were studied. All gave their informed consent. Hemochromatosis was diagnosed on the basis of the following criteria: serum iron above 20 µmol/l, transferrin saturation above 50%, serum ferritin above 800 µg/l and increased iron content in liver biopsy specimens. All the patients had evidence of clinical hypogonadism: two had testicular atrophy, while puberty had occurred late in four. Single or repeated intramuscular injections of human chorionic gonadotropin (hCG) 5000 IU were given, and serum testosterone was measured daily for four days to assess the testicular response. All the patients were also evaluated for the remaining pituitary functions, using standardized multiple stimulation test (200 µg TRH iv, arginine chloride 25 g in 400 ml solution iv, lysine vasopressin (LVP) 10 IU sc) and measurements of PRL, GH, TSH, cortisol before and at regular intervals after stimulation. None of our seven patients had evidence of other pituitary deficiency. Table 1 summarizes the main biological and hormonal characteristics of the patients.

Study design

Investigations were performed at the time of diagnosis, before iron depletion, as follows. After obtaining at least three baseline blood samples, a GnRH test (100 µg iv) was performed at 09.00 on day 1, with blood sampling at 0, 10, 20, 30, 40, 60 and 90 min. Pulsatile LH secretion was analysed on the second day by sampling blood every 10 min from 08.00 to 14.00. Then GnRH was administered iv (N = 6) or sc (N = 1) in a pulsatile fashion over 15 to 30 days, one pulse every 90 min (10—12 µg), using a portable pump (Autosyringe, Travenol,
parametric were expressed and was each gramme of time. All Pulse Pulse with sensitivity variation comercially Plasma Institute, or Plasma. No. Patient Age (yr) Weight (kg) Height (cm) Diagnosis HLA Serum ferritin (µg/l) Hepatic iron* (µmol/g of tissue) Serum T basal † (nmol/l) Serum T peak/hCG‡ (nmol/l) 1 23 66 174 IH§ A1 A11 B14 B1 3720 133 0.3 2.7 2 32 60 180 IH not done 1750 101 1.7 7.1 3 42 73 176 IH A2 B38 2480 96 1.4 10.2 4 55 59 174 IH A2 A32 B7 B14 2564 74 0.1 5.9 5 17 67 161 B. thaï 884 0.7 13.6 6 31 86 178 IH A9 A32 B12 B1 4260 421 1.8 13.4 7 17 39 155 B. thaï* 2080 31 3.1 5.8

* Normal hepatic iron concentration < 9 µmol/g of tissue.
† Normal male testosterone levels range from 13.6 to 34.0 nmol/l.
‡ IH = idiopathic hemochromatosis.
§ B. thaï = Beta thalassemia major.

or Zyklomat. Ferring). Just before the end of GnRH administration, pulsatile LH secretion was again analysed, followed by a repeat GnRH test (100 µg iv).

Methods

Hormone assays

Plasma FSH concentrations were measured using commercially available kit (RIA-gnost®hFSH, Behring Institute, Germany). Intra- and interassay coefficients of variation were 4% and 5%, respectively. FSH concentrations are expressed as IU/l (MRC 78/549 standard). The sensitivity of the assay is 0.13 IU/l. Plasma testosterone concentrations were determined by means of a radioimmunoassay kit (Behring Institute, Germany). Intra and interassay coefficients of variation were 4.5 and 11%, respectively. Plasma LH was determined in duplicate, with a radioimmunoassay technique using the principle of a one-stage sandwich assay with monoclonal antibodies (RIA-gnost® hLH, Behring Institute, Germany). All samples from each subject were assayed at the same time. The intra- and interassay coefficients of variation were 8 and 9%, respectively. LH concentrations are expressed as IU/l (MRC 68/40 standard). The sensitivity of the assay is 0.15 IU/l. Results are expressed as median and range.

Pulse analysis

Pulse analysis was performed with the Cluster programme written by Veldhuis and Johnson (8), using cluster sizes of 2 by 2 and at a statistic value of 2.0. At each time point the actual experimental error was estimated from the two replicate assays. Pulse amplitude was defined as the difference between the pre-peak nadir and the peak concentration. The pulse characteristics were compared for each subject by means of the non-parametric Wilcoxon rank sum test for matched pairs to avoid assumptions concerning the probability distribution of the variables.

Results

Prior to chronic pulsatile GnRH therapy, the seven patients had low serum testosterone levels (1.4-0.1-3.1 nmol/l) and low basal serum gonadotropin levels (0.35-0.1-1.1 IU/l for LH and 0.35-0.15-0.5 IU/l for FSH). Serum LH values did not increase after a single GnRH injection (Table 2). HCG administration produced an increase in serum testosterone levels in all patients, to a median level of 7.1 (2.7-13.6) nmol/l (p < 0.05). LH secretion was not-pulsatile in four patients, while the remaining three showed a single pulse during the 6 h analysis.

Chronic pulsatile GnRH therapy did not induce a significant increase in serum testosterone levels (median
Table 3. Pulsatile LH response to chronic pulsatile GnRH therapy in seven patients with hemochromatosis. Blood samples were drawn at 10 min intervals during 6 h for LH determination; LH pulses were studied using the Cluster programme.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Treatment</th>
<th>Mean ± sD (IU/l)</th>
<th>LH pulse amplitude (IU/l)</th>
<th>LH pulse frequency ( pulses/6 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Before</td>
<td>0.18 ± 0.10</td>
<td>0.31</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>GnRH</td>
<td>0.19 ± 0.12</td>
<td>0.31</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Before</td>
<td>0.20 ± 0.11</td>
<td>0.15</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>GnRH</td>
<td>0.22 ± 0.16</td>
<td>0.68</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Before</td>
<td>0.22 ± 0.17</td>
<td>0.15</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>GnRH</td>
<td>0.22 ± 0.11</td>
<td>0.15</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Before</td>
<td>0.16 ± 0.07</td>
<td>0.45</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 1. Time profiles of LH concentrations in patient nos. 4 and 5 before () and at the end (●) of pulsatile GnRH therapy. Arrows denote GnRH pulses administered via a portable pump during the 6 h sampling period; the stars (*) indicate significant LH pulses identified by the Cluster programme.

Discussion

Hypogonadism is the most frequent endocrine disturbance produced by iron overload in hemochromatosis. The low levels of gonadotropins and the gonadal insufficiency in our patients were consistent with the hypogonadotrophic hypogonadism generally observed in this setting (2, 3, 5). The results of several studies have suggested that testicular function (even that of atrophic testes) is intact, since testicular biopsy specimens either show no excess iron deposits or deposition limited to vessel walls and, thus, respecting interstitial Leydig cells (9).

All our patients responded to hCG: the initial testosterone level increased at least twofold, the same order of increment usually observed in healthy men. One patient required repeated hCG injections to obtain this response. The lowest testosterone responses (<6 nmol/l) occurred in the patients with incomplete sexual maturation. The testosterone response to hCG was similar to that observed in hypothalamic hypogonadotrophic hypogonadism (10, 11), probably reflecting the degree of previous exposure to gonadotropins rather than the actual degree of gonadotropin deficiency.

These findings support a central origin of gonadal failure in patients with hemochromatosis, but whether the site is hypothalamic or pituitary is controversial. Indirect evidence for pituitary lesions has been provided by the co-existence of pituitary deficiencies (particularly prolactin in thalassemic patients) and gonadotropin insufficiency (12). However, one may emphasize that in our patients, as in the majority of the reported patients, pituitary deficiency was limited to gonadotropins, raising the question of knowing why lesion of hemochromatosis is limited to only one or two types of pituitary cell. In addition, autopsy studies have shown that the anterior pituitary contains substantial iron deposits, particularly in gonadotrope cells (13). Our findings now provide direct evidence of pituitary unresponsiveness to GnRH, on the basis of the weak or absent pulsatile LH activity before pulsatile GnRH treatment for two to four weeks, together with the absence of a significant increase in LH levels during treatment. Pulsatile GnRH administration is usually used to discriminate between pituitary and hypothalamic lesions, since patients with a chronic GnRH deficiency do not respond to a single dose of GnRH (14). With this regimen, neither basal nor GnRH-
stimulated gonadotropin levels reached the normal range in our patients with hemochromatosis and hypogonadism, and LH secretion remained non-pulsatile, while in our experience such a regimen has proved to restore GnRH pulsatility and normal testosterone levels in males with hypogonadotrophic hypogonadism due to hyperprolactinaemia (15). One may ask whether the LH assay, the blood sampling interval of 10 min and the 6 h duration of sampling we used were accurate enough to detect LH pulses. In fact, using the same assay we were able to demonstrate modifications in LH pulse frequency in other clinical conditions (16), and 10 min time-interval or 6 h duration appeared sufficient in our experience (15) as in others (17) to detect significant pulses in particular when a GnRH pulse regimen is provided at a given frequency (of one pulse every 90 min) by a portable pump.

Chelation therapy before puberty can improve growth and sexual maturation in beta-thalassemia major (18). In contrast, the reversibility of iron-induced damage to the pituitary appears to be inconsistent, regardless of the etiology of hemochromatosis, although several authors have reported a recovery of normal gonadotropin and gonadal steroid secretion in certain patients after intensive iron depletion (12, 19, 20). Follow-up studies of treatment by iron chelation will be necessary to evaluate the reversibility of pituitary damage in terms of pulsatile gonadotropin secretion.

In conclusion, gonadotropin unresponsiveness to GnRH in patients with hemochromatosis appears to be unchanged by chronic pulsatile GnRH therapy, suggesting that a pituitary rather than a hypothalamic defect is responsible for hypogonadism in hemochromatosis.

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