Metoclopramide test in the diagnosis of isolated hypogonadotrophic hypogonadism

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Abstract. The responses of serum prolactin (Prl) to metoclopramide and LH and FSH to GnRH were studied simultaneously in 9 boys with hypogonadotrophic hypogonadism (HH), 7 boys with constitutional delay of puberty (D) and 15 controls. Metoclopramide increased the Prl levels in all groups. The boys with HH had lower Prl responses than the controls, whereas the boys with D had similar responses to the controls. Of the 9 boys with HH, 8 had subnormal Prl responses, 3 subnormal LH and none subnormal FSH. A metoclopramide test is clearly more sensitive than a GnRH test in differentiating HH and D and appears to make the differentiation between these two conditions clearer.

Differentiation between hypogonadotrophic hypogonadism (HH) and constitutional delay of puberty (D) is often impossible in prepubertal boys and those in early puberty. Serum and urine concentrations of testosterone and gonadotrophins are not informative because in both conditions the levels are low. Furthermore, the GnRH test is of limited value, since about one third of hypogonadotrophic boys have normal responses (Marshall et al. 1972; Bourguignon et al. 1982).

Pubertal boys with D have normal serum prolactin (Prl) responses to direct stimulation of the pituitary lactotrophes with TRH, whereas adult men with HH respond subnormally (Spitz et al. 1983). This subnormal responses is assumed to result from lack of sex steroid exposure rather than from hypogonadotrophism. In addition to direct stimulation of the lactotrophes by TRH, increases in serum Prl levels can be induced by dopaminergic antagonists, e.g. metoclopramide or chlorpromazine (Spitz et al. 1981).

The aim of the present study was to determine 1) whether subnormal serum Prl responsiveness in male HH is a result of pubertal changes in sex steroid milieu or whether it can be found in prepuberty as well and 2) whether a metoclopramide test has any diagnostic value in the differentiation between HH and D.

Materials and Methods

Nine boys with HH were studied (Table 1). Six were prepubertal (patients 1–6) and 3 pubertal (patients 7–9). Of the pubertal boys, one had reached genital stage G (Tanner 1962) spontaneously (patient 8) and the other two (patients 7 and 9) genital stage G2 and pubic hair stage PH4 after therapy with testosterone enanthate (Primoten depot®, Schering AG, Berlin, FRG) 1–5 mg/kg, max. 250 mg monthly. This therapy was discontinued at least 3 months before the study. The diagnosis of HH in the 2 youngest boys, who had Prader-Willi syndrome, had previously been confirmed by subnormal LH response to GnRH. In the other 7 boys the diagnosis was confirmed during a 3.1–3.9-year follow-up after the study by two clinical criteria: 1) clearly prepubertal (<2.2 ml) testis size at bone age 13 years or older and subnormal testis size for older bone age (Zachmann et al. 1974) and 2) no appearance of pubertal penis growth at bone age 13 years or older. In 5 of the boys the diagnosis was further supported by the presence of a syndrome known to include HH (patients 1–3, 6, 7). None of the boys showed elevated basal or post-GnRH gonadotrophin levels in the follow-up period. Apart form gonadotrophin deficiency, no other hormonal deficiencies were found in any of the boys. Seven boys with D (14.8–15.1 years) were studied.
Table 1.
Clinical data on the boys with hypogonadotrophic hypogonadism.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age¹ (year)</th>
<th>Bone age¹ (year)</th>
<th>Follow-up² (year)</th>
<th>Testis vol³ (ml)</th>
<th>Diagnostic criterion</th>
<th>Aetiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.4</td>
<td>6.0</td>
<td>3.1</td>
<td>&lt;1</td>
<td>subnormal LH⁴</td>
<td>Prader-Willi syndrome</td>
</tr>
<tr>
<td>2</td>
<td>8.8</td>
<td>8.0</td>
<td>3.2</td>
<td>&lt;1</td>
<td>subnormal LH</td>
<td>Prader-Willi syndrome</td>
</tr>
<tr>
<td>3</td>
<td>12.0</td>
<td>11.5</td>
<td>3.5</td>
<td>1.1</td>
<td>clinical</td>
<td>Kallmann syndrome⁴</td>
</tr>
<tr>
<td>4</td>
<td>13.0</td>
<td>13.0</td>
<td>3.5</td>
<td>1.0</td>
<td>clinical</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>5</td>
<td>13.9</td>
<td>13.0</td>
<td>3.4</td>
<td>1.4</td>
<td>clinical</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>6</td>
<td>14.3</td>
<td>13.0</td>
<td>3.1</td>
<td>0.4</td>
<td>clinical</td>
<td>Kallmann syndrome⁵</td>
</tr>
<tr>
<td>7</td>
<td>15.6</td>
<td>15.0</td>
<td>3.5</td>
<td>2.8</td>
<td>clinical</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>8</td>
<td>16.6</td>
<td>12.5</td>
<td>3.9</td>
<td>2.6</td>
<td>clinical</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>9</td>
<td>16.6</td>
<td>15.0</td>
<td>3.6</td>
<td>2.5</td>
<td>clinical</td>
<td>Idiopathic</td>
</tr>
</tbody>
</table>

¹ Calendar age and bone age at the time of the study. ² Follow-up time after the study. ³ According to Hansen (1952). ⁴ In previous GnRH tests. ⁵ All patients had anosmia and craniofacial or metacarpal anomaly.

All had entered puberty spontaneously but at least 2 years later than average. They had testicular size appropriate for bone age and were studied at genital stage G2. They were followed for 3.0—3.5 years after the study until the spontaneous appearance of stage G4 (including normal testicular growth) confirmed diagnosis. The present data were not used diagnostically. The controls consisted of 15 boys with incomplete testicular descent; 2 had retracted testes and 13 unilateral incomplete descent. Eight were prepubertal (mean ± SD, 7.9 ± 2.8 years; range 4.6—12.2 years) and 7 pubertal at genital stage G2 (mean ± SD, 13.8 ± 0.9 years; range 12.4—14.7 years).

The Ethical Committee of the Hospital approved this study and informed consents were obtained from the parents.

Methods
All the boys were given, at 08.30—09.00 h a one min iv injection of GnRH (Relafact®, Hoechst AG, Frankfurt, FRG) 3.5 μg/kg (max. 100 μg) and, immediately thereafter, metoclopramide (Metopram®, Leiras, Turku, Finland) 10 mg/1.7 m² (max. 10 mg). Venous blood samples for serum PRL, LH and FSH assays were obtained at −20, 0, 20, 30, 60, 90 and 120 min. Blood samples for testosterone and oestradiol assays were obtained at 0 min. The sera were stored at −20°C until analyzed. All samples of the same patient were analyzed at the same time.

RIAs were used for the determination of PRL (Sorin Biomedica, Saluggia, Italy), LH and FSH (LER-907, LER 690, LER 1801-3, anti-LH batch 2 and anti-FSH batch 4 were donated by the National Institute of Arthritis, Diabetes, Digestive and Kidney Disease, USA). The values were converted to IU/l by multiplying the FSH values (in ng LER 907/ml) by 0.0185 and the LH values (in ng LER 907/ml) by 0.344. Steroid RIAs were made after chromatography on Lipidex-5000 (testosterone) (Apter et al. 1976) or Sephadex-L20 (oestradiol) (Adlercreutz et al. 1982). The maximal serum levels of PRL, LH and FSH were used as measures of response.

Because of positive skewness of the distributions, calculations were made after logarithmic transformation, and geometric means are given throughout. All values of skewness/SEM were then < 2.0. The results of the controls were used to calculate 90% and 95% confidence ranges for the normal PRL response. Previous results from 41 prepubertal and 27 pubertal (genital stage G2) boys who had been given a similar GnRH test were used to calculate 95% confidence ranges for normal gonadotrophin responses (Dunkel et al. 1985). BMDP computer programs were used for statistical analysis (Dixon 1981).

The concentration values of PRL values in mIU/l may be converted to ng/ml b dividing by 30.1.

Results
No significant differences were seen in the mean basal serum levels of testosterone and oestradiol between the boys with HH and the controls or between the boys with HH and D. The mean oestradiol/testosterone ratios were also similar between the controls and the boys with HH both in prepuberty and in puberty. The pubertal controls had higher mean level of testosterone ($P < 0.001$)
Prolactin was and oestradiol.

Metoclopramide levels in pubertal control (solid line, solid circles) and pubertal boys with D (broken line, open circles). Note logarithmic scale.

and lower mean oestradiol/testosterone ratio values \((P < 0.001)\) than the prepubertal but there was no difference in the mean basal levels of oestradiol.

**Prolactin response to metoclopramide** (Fig. 1)

Metoclopramide increased the serum levels of Prl in all groups \((P < 0.001)\). The mean maximal levels did not differ between the prepubertal and pubertal controls nor between the prepubertal and pubertal boys with HH. Both prepubertal and pubertal boys with HH had lower maximal levels \((P < 0.001)\) than the corresponding controls. The boys with D had a mean maximal level which was similar to that of the prepubertal and pubertal controls.

**Gonadotrophin responses to GnRH** (Table 2)

GnRH increased the levels of LH and FSH significantly \((P < 0.05-0.001)\) in all groups except the pubertal boys with HH. The boys with HH had lower mean maximal levels of LH than the controls both in prepuberty \((P < 0.05)\) and in puberty \((P < 0.01)\). The boys with D had higher maximal levels of LH than the pubertal boys with HH \((P < 0.01)\). No differences existed in the mean levels of FSH.

**Discriminating powers of the different test variables** (Fig. 2)

As the mean basal and maximal levels of Prl in the metoclopramide test were similar between the prepubertal and pubertal controls, the Prl results were pooled for the discrimination analysis. In the boys with HH, the maximal serum levels of Prl were completely separate from the levels of the controls. Nevertheless, the lower limits of the 90% confidence ranges of the controls detected only 8 of the 9 boys with HH. The maximal serum levels of LH or FSH were less sensitive discriminators. The maximal level of LH detected only 3 of the 9 boys with HH, whereas that of FSH did not detect a single one. All the control boys had gonadotrophin responses within the normal range (data not shown).

**Table 2.**

Maximal post-GnRH gonadotrophin levels by group and pubertal development.

<table>
<thead>
<tr>
<th></th>
<th>LH (IU/l) mean (± SEM)</th>
<th>FSH (IU/l) mean (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prepubertal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>20.9 (18.1–24.2)</td>
<td>3.3 (2.7–4.0)</td>
</tr>
<tr>
<td>Hypogonadotrophic hypogonadism</td>
<td>12.5 (10.0–15.8)</td>
<td>3.9 (3.2–4.8)</td>
</tr>
<tr>
<td><strong>Pubertal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed puberty</td>
<td>37.2 (34.0–40.7)</td>
<td>3.5 (2.7–4.5)</td>
</tr>
<tr>
<td>Control group</td>
<td>35.6 (32.1–39.4)</td>
<td>3.1 (2.5–3.9)</td>
</tr>
<tr>
<td>Hypogonadotrophic hypogonadism</td>
<td>14.2 (9.0–22.3)</td>
<td>2.2 (1.6–3.0)</td>
</tr>
</tbody>
</table>

1 \(P < 0.05\) vs control group. 2 \(P < 0.01\) vs control group.
Discussion

Our research group has previously shown the superiority of the hCG test to the GnRH test in the diagnosis of HH (Dunkel et al. 1985). The present results indicate that, as a discriminator, the maximal serum Prl level following metoclopramide administration is equal in sensitivity to the hCG test and more sensitive than the maximal levels of LH or FSH following GnRH administration.

Although the control subjects had incomplete testicular descent, which is known to be associated with partial LH deficiency (Job et al. 1974), their results were homogenous. Cases with definite endocrine abnormalities could be expected to give abnormal test responses. Also, if the metoclopramide test differentiated between boys with unequivocal HH and this control group, it should even better distinguish HH from the normal state.

The categorization of the subjects with D and HH was made by well established clinical criteria during a follow-up period of several years after the study, independently of the endocrinological findings. However, in the 2 youngest boys with Prader-Willi syndrome, the follow-up period was not long enough to confirm HH by the clinical criteria, and thus subnormal LH responses to GnRH were considered to establish the diagnosis of HH. According to the previous results of our group, subnormal LH response is a very specific finding in the diagnosis of HH (Dunkel et al. 1985).

In adult men with HH, serum Prl responses to both TRH and chlorpromazine have been reported to be subnormal compared with normal adult men or pubertal boys with D (Winters et al. 1982; Spitz et al. 1983). This has been attributed to an altered sex steroid milieu, especially to low oestradiol levels. The finding in the present study of significantly low Prl responses to metoclopramide in HH in prepuberty is of particular interest, since the patients were similar to the controls in their basal levels of sex steroids. Furthermore, prepubertal and pubertal controls gave identical Prl responses, despite differences in basal and stimulated LH and basal steroid levels. Thus sex steroids at early pubertal levels appear to have little effect on the basal and also on metoclopramide-releasable Prl levels in boys. This is further supported by the finding that the basal Prl levels do not change during puberty in boys (Lee et al. 1974). In HH a low Prl response is possibly not a consequence of an altered sex steroid milieu but an inherent component of the syndrome, caused by differences in the hypothalamic regulation of
Prl synthesis and/or release. Another explanation for the reduced Prl response may, however, be that the testis is not totally quiescent in normal prepubertal boys and e.g. nocturnal differences in sex steroid concentrations may contribute to the observed differences.

That the boys with D and the controls had similar serum Prl responses, indicates that this response can also be used to differentiate boys with D from those with HH. The results of the discriminating power of the GnRH test were similar to previous reports (Marshall et al. 1972; Bourguignon et al. 1982). The metoclopramide test appeared to be more sensitive than the GnRH test in differentiating D and HH, but its final value needs to be decided by larger numbers of patients.

Acknowledgments

This work was supported by the Sigrid Juselius Foundation, the Foundation for Pediatric Research, Helsinki, Finland, and the Medical Research Council of the Academy of Finland.

References


Received on April 16th, 1985.