Effects of acute stimulation with luteinizing hormone-releasing hormone (LRH) on biologically active and immunoreactive serum luteinizing hormone (LH) in pubertal boys

Vanna Montanini, Marco Francesco Celani, Gian Franco Baraghini, Cesare Carani and Paolo Marrama

Department of Endocrinology, University of Modena, Modena, Italy

Abstract. The responses of biologically active LH (BIO-LH) and immunoreactive LH (RIA-LH) to acute stimulation with LRH (0.1 mg iv) were studied in 8 pubertal boys (9–15 years, 2nd–4th Tanner’s stage), and in 10 healthy adult men (20–46 years). Serum levels of BIO-LH were assessed by an in vitro bioassay method based upon testosterone production by mechanically dispersed mouse Leydig cell preparations. In pubertal boys the mean BIO-LH/RIA-LH (B/I) ratio of basally secreted LH was significantly lower than in adult men (1.2 ± 0.2 (SEM) and 2.2 ± 0.2 respectively, P < 0.01). After acute administration of LRH the mean B/I ratio of circulating LH showed a significant increase from the basal value in pubertal boys (2.6 ± 0.2, P < 0.01 vs basal values), whereas no significant difference in LH B/I ratios were demonstrated throughout the study period in adult men (2.1 ± 0.1, P = NS vs basal values). In agreement with this finding, the mean relative maximum response for BIO-LH (BIO-LH Δ%) was higher in pubertal boys than in adult men (1702.7 ± 500.3 and 499.6 ± 65.4% respectively, P < 0.05), whereas the mean RIA-LH Δ% was similar in both groups (609.1 ± 85.1 and 534.1 ± 75.5% respectively, P = NS). No significant differences were shown in the BIO-LH Δ area between pubertal boys (4.9 ± 0.9 area units × 10^3) and adult men (6.7 ± 1.2 area units × 10^3, P = NS), whereas the mean RIA-LH Δ area was significantly lower in the former group (1.9 ± 0.4 area units × 10^3 vs 3.2 ± 0.5 area units × 10^3, P < 0.05). Our study emphasizes that the pubertal pituitary possesses a greater responsiveness for BIO-LH than the adult pituitary, and that in pubertal boys acute stimulation with LRH evokes the release of a more bioactive form of LH.

Although a number of studies have shown a progressive rise in basal levels of radioimmunoassayable LH (RIA-LH), as well as increasing RIA-LH responses to luteinizing hormone-releasing hormone (LRH) administration during sexual maturation (Grumbach et al. 1974; Kelch et al. 1975; Reiter & Root 1980), only a few reports have pointed out the changes in basal levels of biologically active LH (BIO-LH) accompanying the pubertal development (Lucky et al. 1980; Reiter et al. 1982). Moreover, no data are at present available concerning the BIO-LH response pattern to acute administration of LRH in pubertal subjects.

Recently, in agreement with other reports (Lucky et al. 1980; Reiter et al. 1982), we have demonstrated in male subjects that the basal biological activity of serum LH increases more than its immunoreactivity during puberty (Celani et al. 1983a); in addition, we have shown a significant positive correlation between the bioactivity to immunoreactivity (B/I) ratios of LH and the basal levels of serum testosterone during this period of life (Marrama et al. 1983).

Since both the increase in serum testosterone concentrations and the rise in gonadotrophin secretion rate, known to accompany male pubertal maturation, seem to affect the biological activity of circulating LH positively (Solano et al. 1980; Dufau et al. 1976), we have investigated the influences of the acute increase of gonadotrophin secretion rate.
evoked by LRH administration, upon the biological and immunological activities of circulating LH in normal pubertal boys. The results have been compared with those obtained in a control group of healthy adult men.

Materials and Methods

Subjects

The study population consisted of 8 pubertal boys, aged 9 to 15 years (mean age ± SEM = 12.1 ± 0.8 years), and 10 adult volunteers men, aged 20 to 46 years (mean age ± SEM = 29.3 ± 2.9 years). According to the system of Tanner (1962) for gonadal and pubic hair development, pubertal boys were at or between the 2nd and 4th pubertal stage (2/8 2nd pubertal stage, 4/8 3rd pubertal stage and 2/8 4th pubertal stage).

All subjects were found to be healthy at the time of the study, both by clinical and endocrinological evaluation. None of the subjects had had any pharmacological treatment for at least 3 months before the study, and none were obese.

LRH test

Informed consent of parents being obtained, each pubertal boy received a single iv bolus of 0.1 mg LRH (Biodata, Rome, Italy) between 8.00 and 9.00 h a.m. after an overnight fast. Basal blood samples were taken from the antecubital vein at -30, -15, and -1 min prior to administration of LRH. Multiple samples were then withdrawn for the next 2 h at 30, 45, 60 and 120 min, respectively. Blood samples were centrifuged and sera were kept frozen at -20°C until assayed.

The same study protocol was used for the group of healthy adult volunteers.

Assays

LH biological activity was assessed by the in vitro bioassay procedure originally described by Van Damme et al. (1974) based upon testosterone production by mechanically dispersed mouse Leydig cell preparations in response to graded doses of LH. Our several steps of optimization introduced in the original method of Van Damme et al. (1974) have already been described (Baraghini et al. 1983). Briefly, for each bioassay, interstitial cells prepared by mechanical dispersion from adult mouse testes were preincubated for 1 h at 34°C, then centrifuged and resuspended at a concentration of about 0.2 × 10^6 cell/0.1 ml of incubation medium, with a mean cell breakage of 16%. To each incubation vial containing the interstitial cell suspension (0.1 ml), LH standard preparation (0.1 ml, 8 dose levels in duplicate) or serum samples (0.1 ml, 3 dose levels in duplicate) were added.

The incubation was performed at 34°C for 3 h and stopped by addition of 1.0 ml/tube ice cold phosphate buffer. After centrifugation, the immunoreactive testosterone was measured in 0.025–0.050 ml aliquots of the diluted incubation medium. Testosterone production in the sample vials was compared with that of the standard preparation by the 3 + 3 points parallel assay design of Finney (1978).

To measure the low basal levels of BIO-LH in the sera of children, a square root transformation of the amount of testosterone produced (pg1/2) was used for linearizing the lower regions of the response curves to the standard and serum LH (Celani et al. 1983a). Using this procedure, all children had detectable values of basal BIO-LH.

In addition, following Lichtenberg et al. (1982), all serum samples were preheated for 15 min at 50°C before the assay, to eliminate the interfering factors described in male sera (Rajalakshmi et al. 1979). After preheating, satisfactory evidence for linearity and parallelism was observed for 100% of serum samples assayed, without any significant loss of LH bioactivity, as shown by the high recovery (98%) of the biopotency of the preheated LH standard preparation.

The 2nd international reference preparation of human pituitary gonadotrophins (FSH/LH) for bioassay (2nd IRP bio) coded 78/549 (NIHSC, London) was used as standard. The bioassay was sensitive to 0.029 mlU/tube of 78/549. The intra- and inter-assay coefficients of variation were 3.9 and 11.5% respectively, whereas the mean index of precision (k) was 0.042.

Immunoreactive LH was measured by a double antibody radioimmunoassay technique (Romani et al. 1977) using a pituitary preparation, calibrated by RIA against the 2nd IRP HMG, as standard (Biodata, Rome, Italy). The sensitivity, intra- and inter-assay coefficients of variation were respectively: 0.5 mlU/tube, 7.2 and 12.4%.

In accordance with Romani et al. (1977), to convert the bioassay results, expressed in terms of 78/549, to equivalents of 2nd IRP HMG, a conversion factor of 1.06, obtained by repeated determinations of the biopotencies between the two reference preparations, was employed.

Statistics

In order to evaluate the responses of BIO-LH and RIA-LH independent of factors influencing basal levels, the relative maximum response (mIU peak – mIU basal × 100/mIU basal = Δ%) and the integrated area under LH response curves corrected for the basal area (Δ area) were calculated for each subject (Pinto et al. 1979; Mortimer et al. 1976).

The BIO-LH/RIA-LH (B/I) ratio was calculated for each individual serum sample. Both in pubertal boys and in adult men the mean B/I ratio of LRH-stimulated LH and the mean B/I ratio at the bioactive peak were compared with the mean B/I ratio observed under basal conditions.
The results are expressed as mean values ± standard error (SEM). Statistical evaluation of differences was performed by analysis of variance; in addition, the changes of LH B/I ratio throughout the study period were analysed by paired Student's t-test. 

Results

**Basal values**

Mean basal values of BIO-LH, RIA-LH and LH B/I ratio were significantly higher in adult men than in pubertal boys (4.6-, 2.2- and 1.9-fold, respectively).

**Responses to LRH** (Fig. 1 and Table 1)

**BIO-LH.** The mean peak BIO-LH concentration after LRH administration was significantly lower in pubertal boys than in adult men; in contrast, the mean relative maximum response of BIO-LH above basal levels (BIO-LH Δ%) was significantly higher in the former group, showing a greater pituitary responsiveness for BIO-LH during puberty (pubertal boys: Δ% = 1720.7 ± 500.3; adult men: Δ% = 499.6 ± 65.4, P < 0.05). No significant differences were noticed for the mean BIO-LH Δ area between the two groups (pubertal boys: 4.9 ± 0.9 × 10⁻³ area units; adult men: 6.7 ± 1.2 × 10⁻³ area units, P = NS).

**RIA-LH.** Although the mean peak response of RIA-LH was significantly lower in pubertal boys than in adult men, the mean RIA-LH Δ% was not significantly different in the two groups (pubertal boys: Δ% = 609.1 ± 85.1; adult men: Δ% = 534.1 ± 75.5, P = NS), whereas the mean RIA-LH Δ area was significantly lower in pubertal boys (pubertal boys: 1.9 ± 0.4 × 10⁻³ area units; adult men: 3.2 ± 0.5 × 10⁻³ area units, P < 0.05).

**LH B/I ratio.** As shown in Fig. 2, in pubertal boys the mean B/I ratio of LRH-stimulated LH rose significantly from the basal value (2.6 ± 0.2, P < 0.01 vs basal B/I), reaching a peak at 45 min. In particular, the increase of B/I ratio over control values was significant at the peak of LH bioactivity (2.9 ± 0.5, P < 0.01 vs basal B/I). In adult men the mean B/I ratio of serum LH did not change after LRH stimulation (2.1 ± 0.1, P = NS vs basal B/I), and the B/I ratio at the bioactive peak was significantly lower than in pubertal boys.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>BIO-LH (mIU/ml)</th>
<th>RIA-LH (mIU/ml)</th>
<th>B/I ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Peak</td>
<td>Basal</td>
</tr>
<tr>
<td>Pubertal boys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pubertal stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td>1.5</td>
<td>16.6</td>
<td>1.9</td>
</tr>
<tr>
<td>2nd</td>
<td>1.7</td>
<td>16.9</td>
<td>1.1</td>
</tr>
<tr>
<td>3rd</td>
<td>3.7</td>
<td>76.2</td>
<td>3.0</td>
</tr>
<tr>
<td>3rd</td>
<td>3.5</td>
<td>74.0</td>
<td>3.0</td>
</tr>
<tr>
<td>3rd</td>
<td>1.7</td>
<td>87.0</td>
<td>6.0</td>
</tr>
<tr>
<td>3rd</td>
<td>7.1</td>
<td>61.6</td>
<td>4.8</td>
</tr>
<tr>
<td>4th</td>
<td>6.3</td>
<td>77.6</td>
<td>6.0</td>
</tr>
<tr>
<td>4th</td>
<td>9.0</td>
<td>96.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Means ± SEM</td>
<td>4.3 ± 1.0**</td>
<td>63.3 ± 10.9*</td>
<td>3.8 ± 0.6**</td>
</tr>
</tbody>
</table>

| Adult men |       |      |       |      |       |               |
| Means ± SEM | 19.6 ± 3.2 | 100.0 ± 15.2 | 8.4 ± 0.6 | 50.1 ± 5.6 | 2.2 ± 0.2 | 2.2 ± 0.1 |

* P < 0.05 and ** P < 0.01 vs adult men.
Fig. 1.
Responses of biologically active LH (BIO-LH) and immunoreactive LH (RIA-LH) to acute administration of LRH in 8 pubertal boys (mean values ± SEM). The BIO-LH and RIA-LH responses of 10 adult men to the LRH administration are shown in the shaded area.

Fig. 2.
Response of LH bioactivity to immunoreactivity (B/I) ratio to acute administration of LRH in 8 pubertal boys (mean values ± SEM). The B/I ratio response of 10 adult men to the LRH administration is shown in the shaded area. * = P < 0.01 vs basal values (paired Student's t-test).

Discussion
The present study has confirmed our previous evidence that the mean B/I ratio of basally secreted LH is lower in pubertal boys than in adult men (Marrama et al. 1983). Moreover, in agreement with our preliminary data (Celani et al. 1983b), we have shown a significant and rapid increase of LH B/I ratio after acute stimulation with LRH in the pubertal groups. On the other hand, consistent with the data reported by Dufau et al. (1976), the B/I ratio of serum LH remained unchanged in adult men throughout the study period.

In accordance with the 'two pool theory' proposed by Hoff et al. (1977), we could suppose that in pubertal boys the relatively low basal gonadotrophin secretion rate may induce the release of LH only from the 'early pituitary pool' (readily releasable pool), consisting of LH molecules with a low B/I ratio (Dufau et al. 1976; Sawyer-Steffan et al. 1982). On the other hand, when the pubertal pituitary is maximally stimulated by LRH, the 'later pool' (newly synthesised pool) may also be utilized,
leading to the secretion of a more bioactive form of LH. The large bolus dose of LRH used in our study may in fact release LH from both pituitary pools (Dufau et al. 1976; Mortimer et al. 1976).

Experimental evidence in support of this suggestion is that LH released from the rat pituitary stimulated with high concentrations of LRH has a higher B/I ratio than pituitary LH or LH secreted in response to low doses of LRH (Sharpe et al. 1975). Moreover, LRH stimulates the introduction of carbohydrates into polypeptide chains of LH, thereby increasing the number of sialic acid residues in LH molecules (Menon et al. 1977). Since the biological activity of LH at target cell level seems to be related to the sialic acid content of LH molecules (Dufau et al. 1976; Sawyer-Steffan et al. 1982), the increased sialylation of LH after LRH acute administration may be responsible for the rise in the B/I ratio of circulating LH shown in pubertal boys.

We have also demonstrated that the mean relative maximum response for BIO-LH (BIO-LH Δ%), but not for RIA-LH, was significantly higher in pubertal boys than in adult men. In agreement with this finding, no differences in BIO-LH Δ areas were observed between pubertal boys and adult men, whereas a lower RIA-LH Δ area was shown in the former group.

The greater responsiveness for BIO-LH of the pubertal pituitary, when compared with that observed in adult men, is consistent with the mid-pubertal peak of BIO-LH reserve demonstrated by Rich et al. (1982) using a constant infusion of LRH at low doses (2 μg/k/h for 4 h); it should be pointed out, however, that this study failed to show any increase in the B/I ratio of LRH-stimulated LH.

In conclusion, our previous finding that the B/I ratio of serum LH increases from prepuberty to adulthood, as well as the high BIO-LH responsiveness during puberty shown in the present study, suggest that the changes in the central nervous system at male puberty may be associated with the synthesis and release from the pituitary gland of a more bioactive form of LH, probably contributing to the progression of pubertal sexual maturation.

References


Received on September 14th, 1983.