Intrinsic secretory characteristics of luteinizing hormone and prolactin episodic release during pubertal development

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The intrinsic characteristics of LH and prolactin (PRL) episodic secretion were evaluated in a group of 18 children (8M and 10F). The children were divided into two groups according to the Tanner stage: Group A (Tanner ≤ 1, N = 7, 3M and 4F, 6–10 years of age) and group B (Tanner 2–3, N = 11, 5M and 6F, 9–11 years of age). A pulsatility study of 4 h, sampling every 10 min, was carried out in all children. LH and PRL plasma levels were assayed by IFMA and RIA respectively. LH and PRL secretory episodes were then identified on plasma determinations using the program DETECT. Instantaneous secretory rates (ISR) were then computed for both LH and PRL using the specific algorithm within the DETECT program. Plasma LH levels were different between the two groups of children. Group A children showed undetectable LH plasma levels (below the minimal detectable dose of 0.1 mIU/ml), while group B demonstrated LH plasma levels in the normal range of values for age and sexual development (1.5 ± 0.3 mIU/ml, mean ± SEM). LH pulse frequency for group B was 3.2 ± 0.4 pulses/h. No significant differences in mean plasma PRL levels, pulse frequency and pulse amplitude were observed between the two groups of children. Computation of ISR for LH (group B only) and PRL (both groups) identified the intrinsic episodic characteristics of the two hormones. No significant differences in LH and PRL pulse frequencies were observed when comparing the results estimated on ISR with those estimated on plasma concentrations. No significant changes in PRL pulse amplitude were observed between the two groups. Conversely, a shorter duration of LH and PRL secretory episodes was found. In conclusion, in children PRL secretory bursts from lactotropes lasted the same number of minutes independently of the Tanner stages. Moreover, the LH secretory events were clearly detectable during the daytime only when puberty had already started. The duration of PRL and LH secretory events was similar to adult fertile subjects. These data indicate that the gonadal maturation does not modify LH and PRL secretory events from the pituitary.

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Normal pubertal development in humans is associated with the activation of the hypothalamic-pituitary-gonadal axis, which matures between the ages of 9 and 13 years in girls and between 10 and 14 in boys. During puberty, plasma gonadotropins have a diurnal rhythm, with higher levels during the night (1, 2). Additionally, the response of exogenous GnRH changes from a FSH-predominant response during prepuberty to a LH-predominant response at puberty (3). A secretory pattern of plasma prolactin (PRL) has recently been characterized throughout pubertal maturation (4). PRL pulse frequency and plasma concentrations do not change till adulthood.

Even though the outlines of LH and PRL pubertal changes have been established previously, the detailed intrinsic secretory characteristics have never been described. Instantaneous secretory rates (ISR) for LH were studied in fertile women (7, 19), in several forms of secondary amenorrhea (7), in men (19) and PRL in fertile and postmenopausal women (20). ISR computation unmasks the secretory bursts from hypophyseal cells showing data comparable to those present in pituitary portal vessels, by eliminating all variables that can effect plasma levels (secretion and metabolic clearance). Since the value of gonadal maturation on these characteristics is not known, the present study evaluated LH and PRL secretory characteristics before and during pubertal maturation.

Materials and methods

Subjects

Eighteen prepubertal children (8M and 10F) were enrolled for this study after obtaining the informed consent of the child and of at least one parent. The study
Table 1. Characteristics of pubertal development of children under study. Age is expressed as years + months.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>BMI</th>
<th>Breast</th>
<th>Pubic hair</th>
<th>Testis (ml)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>6.00</td>
<td>15</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>6.02</td>
<td>13</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
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<td>15</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>6.06</td>
<td>15</td>
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<td>I</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>7.10</td>
<td>14</td>
<td>I</td>
<td>I</td>
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<tr>
<td>6</td>
<td>F</td>
<td>9.02</td>
<td>18</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>10.08</td>
<td>15</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Pubertal</td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>9.02</td>
<td>15</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
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<td>15</td>
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<td>II</td>
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<tr>
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<tr>
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<td>II</td>
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<tr>
<td>5</td>
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<td>16</td>
<td>II</td>
<td>II</td>
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<tr>
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<td>III</td>
<td>III</td>
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<td>11</td>
<td>F</td>
<td>11.08</td>
<td>18</td>
<td>III</td>
<td>II</td>
</tr>
</tbody>
</table>

The protocol was approved by the Human Studies Committee of the University of Modena, Italy.

All children (6–11 years of age) studied were hospitalized in the Pediatric Department for routine control.

The children were subdivided into two groups: (A) at Tanner stage 1 for pubic hair (PH), breast and testicular volume (N = 7, 3M and 4F, 6–10 years of age) and (B) at Tanner stages 2–3 for PH, breast and testicular volume (N = 11, 5M and 6F, 9–11 years of age) (Table 1). Gonadal steroid plasma levels were within the range for their age (estradiol < 125 pmol/l; testosterone < 1.71 nmol/l) and none of the girls of group B had an occurrence of the menarche. All children were studied in the morning for 4 h (from 0800 to 1200), sampling was done every 10 min and a maximum of 1.5 ml of blood withdrawn for each sample.

Hormonal assay

Plasma LH concentrations were determined using an immunofluorimetric assay (Pharmacia, Uppsala, Sweden) previously described (5–7). The plasma volume used for the assay was 50 µl and the sensitivity of the assay, expressed as the minimal detectable dose, was 0.1 mIU/l. The cross-reactivity with free α- and β-subunits of LH, FSH, and TSH was less than 2%, as previously described (6). Intra- and inter-assay coefficients of variation based on two different quality control samples were 4.8 and 7.5%, respectively. Plasma PRL concentrations were determined using a commercially available immunoradiometric kit (Radim, Pomezia, Rome, Italy). This assay showed intra- and interassay coefficients of variation equal to 3.1% and 6.2%, respectively.

All samples from the same subjects were analyzed in duplicate in the same assay for both LH and PRL. To obtain a precise evaluation of measurement error of both the assays, we also assayed at least 25 replicates from a serum pool of the same subject (obtained combining 50 µl aliquots from each of the 25 plasma samples).

Estradiol and testosterone plasma concentrations were determined on the first two samples for each time series of every subject using a commercially available kit (Radim, Pomezia, Rome, Italy). Intra-assay and interassay coefficients of variation based on two different quality control samples were 5.9% and 9% for estradiol, 6.1% and 9.3% for testosterone.

Pulse detection

LH and PRL secretory episodes were identified using the program DETECT (8), which is a statistically based and validated program for pulse detection (9). The program identifies significant secretory events using both the analysis of first derivatives of data for the identification of rapid events and the linear fitting for the detection of slow events. The percent of variability (coefficient of variation) computed on the serum pool of each patient was entered as variance for the DETECT program analysis. Time series were analyzed with a p-value equal to 0.01 (1%) for the nominal false positive rate. As previously described (7), the actual false positive rate was determined analyzing the data of the plasma pool of each subject assayed together with the time series. At the nominal p level of 0.01 (1%) for false positive errors the observed false positive rate was 1.25%, which was satisfactorily close to the expected value of 1%.

Instantaneous secretory rates (ISR) were then computed for both LH and PRL using the specific
Algorithm within the DETECT program (8). Clearance rate constants and t1/2 for LH and PRL are known, so the ISR can easily be computed. Rate constants for LH and PRL clearance were set as previously determined (10, 11) at t1/2 of 17.8 and 90 min, with fractional amplitudes of 0.62 and 0.38 for LH, and at t1/2 of 25 min for PRL (11). The variance model used for ISR was computed as follows $s_{ISR}^2 = 2 \times s^2$, where $s$ is the standard deviation or measurement error, and $s^2$ is the variance (12, 13).

Statistical analysis
The presence of significance between groups was tested, after analysis of variance (one-way ANOVA), using Student’s t-test for paired and unpaired data, as appropriate.

Results
Table 2 summarizes the hormonal characteristics of the children. No differences in estradiol and testosterone plasma concentrations were observed when considering the groups according to the Tanner stage.

LH and PRL secretory patterns of two prepubertal children (one boy and one girl) for each group are shown in Figs. 1 and 2. Since there were no statistically significant differences for LH and PRL pulse frequency between boys and girls of the same pubertal stage, all the following data are presented as mean (boys plus girls) for each pubertal stage. Plasma LH levels were significantly different in the two groups of children, group A children showing undetectable LH plasma levels (below the minimal detectable dose of 0.1 mIU/ml), while in group B plasma LH levels were in the range for age and sexual development (1.5 ± 0.3 mIU/ml, mean ± SEM). LH pulse frequency for group B was 3.2 ± 0.4 peaks/4 h (Table 3).

No significant differences were observed for PRL between the two groups of children in terms of mean plasma levels, pulse frequency and pulse amplitude (Table 2 and 3).

Computation of ISR for both LH (group B only) and PRL (both groups) identified the intrinsic episodic characteristics of the two hormones. No significant differences in LH and PRL pulse frequencies and pulse amplitudes were observed when comparing results estimated on ISR with those estimated on plasma concentrations. Conversely, a shorter duration of LH and PRL secretory episodes was found for both LH and PRL when comparing analysis of plasma determinations with ISR computations (Group A: PRL 74.2 ± 21.5 and 27 ± 2.66 min, respectively, p < 0.01) (Group B: LH 64.1 ± 13.1 and 25.2 ± 2 min, respectively, p < 0.01; PRL 83.2 ± 11.3 and 21.7 ± 1.5 min, respectively, p < 0.01).

Discussion
The present study describes the intrinsic characteristics of LH and PRL episodic release revealed by ISR...
computation and shows that during pubertal development the pituitary does not change the duration of the secretory burst of PRL from the lactotropes, while the episodic release of LH is observed only when pubertal maturation is started.

Several studies have assessed LH (14–18) and PRL (4) episodic secretion in children of both sexes all along their sexual development. Our recent data confirm that plasma LH levels increase during pubertal maturation (4, 16), while PRL levels do not change significantly (4). The use of the sensitive DELFIA and IRMA assay methods permitted us to evaluate LH and PRL plasma concentrations with a limited measurement error, reducing to the minimum the possible random scatter when computing the instantaneous secretory rates. However, plasma LH levels of children in group A were below the assay detection limit (lower than 0.1 mIU/ml). In children at a higher Tanner stage, plasma LH were clearly characterized by detectable discrete secretory episodes, definite in their amplitude. The transition throughout the various Tanner stages is evidently characterized by a progressive increase of LH pulse amplitude and mean LH plasma levels more than in pulse frequency, as previously reported (4). Children at Tanner stages 2–3 showed a LH episodic release which probably reflected the increased sensitivity of gonadotropes to endogenous GnRH (4). In fact, exogenous GnRH administration evoked a higher LH response at Tanner stage 3 than at stage 1 (4).

In all children, independently of sex, when LH was measurable in plasma, secretory bursts were shorter in duration than estimated on plasma concentrations. Interestingly, the duration of LH secretory bursts was similar for all children (males and females) and was in...
Table 3. LH and PRL pulse characteristics of children under study. Note that LH and PRL pulses’ duration after computation of ISR are similar for all Tanner stages investigated.

<table>
<thead>
<tr>
<th>Tanner stages</th>
<th>Plasma LH peaks/4 h</th>
<th>Plasma Duration (min)</th>
<th>ISR LH peaks/4 h</th>
<th>ISR Duration (min)</th>
<th>ISR Amplitude U1/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanner 1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tanner 2–3</td>
<td>3.2 ± 0.4</td>
<td>64.1 ± 13.1</td>
<td>3.8 ± 0.5</td>
<td>25.2 ± 2</td>
<td>0.5 ± 0.03</td>
</tr>
<tr>
<td>Tanner Stages</td>
<td>PRL peaks/4 h</td>
<td>Duration (min)</td>
<td>PRL peaks/4 h</td>
<td>Duration (min)</td>
<td>Amplitude ng/ml</td>
</tr>
<tr>
<td>Tanner 1</td>
<td>3.4 ± 0.5</td>
<td>74.2 ± 21.5</td>
<td>3.5 ± 0.5</td>
<td>27.0 ± 2.6</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>Tanner 2–3</td>
<td>2.7 ± 0.3</td>
<td>83.2 ± 11.3</td>
<td>2.7 ± 0.3</td>
<td>21.7 ± 1.5</td>
<td>3.4 ± 0.7</td>
</tr>
</tbody>
</table>

§ Mean ± SEM.

agreement with what has previously been estimated in healthy adults of both sexes (7, 19). Since gonadal steroid plasma levels are very low in all children, the secretory burst of LH in children seemed not to be modulated by steroids as well as in patients with menstrual disturbances (7, 21). However, it cannot be excluded that hypothalamic neurons are somehow sensitive to the almost undetectable plasma levels of gonadal steroids of children at Tanner stage 1.

As previously reported (4), PRL episodic release from lactotropes maintains an unmodified secretory pattern throughout puberty, similar to that observed in adulthood (22). No significant changes of PRL pulses occurred in the children of either groups. However, when ISR were computed a significant reduction in pulse duration was observed. As for LH, children demonstrated a pulse duration of PRL secretory events in the same range of the estimates reported for fertile and postmenopausal women (20). These data demonstrate that lactotropes maintain their secretory ability unchanged throughout puberty and that lactotropes secrete PRL in discrete secretory episodes which are not affected by the changes of gonadal steroid plasma levels occurring at the various ages of reproductive life.

Since LH and PRL are secreted by two distinct cell types under completely different regulatory pathways, this study suggests that in prepubertal children as well as in adults a common intrinsic mechanism may drive both gonadotropes and lactotropes in determining LH and PRL secretory bursts. The possible presence of a common modulatory pathway regulating LH and/or PRL secretion is supported by the fact that both LH and PRL showed a similar duration of secretory events, irrespective of the fact that LH increases its plasma concentrations and modifies its episodic discharge frequency all along pubertal maturation, while PRL episodic release did not show any change.

In conclusion, at puberty LH and PRL secretory bursts from gonadotropes and lactotropes resulted constant in duration and similar to adult healthy subjects.

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