OUTSTANDING CONTRIBUTION

Episodic leptin release is independent of luteinizing hormone secretion

T. Sir-Petermann1,2, M. Maliqueo1, A. Palomino2, D. Vantman2, S. E. Recabarren3 and L. Wildt4

1Division of Endocrinology, Department of Internal Medicine, School of Medicine, University of Chile, 2Institute of Maternal and Child Research, University of Chile, Santiago, 3Laboratory of Animal Physiology and Endocrinology, School of Veterinary Medicine, University of Concepción, Chillán, Chile, 4Division of Gynecological Endocrinology and Reproductive Medicine, Department of Obstetrics & Gynecology, University of Erlangen, Germany

To whom all correspondence should be addressed at: Las Palmeras 299, Interior Quinta Normal, Casilla 33052, Correo 33, Santiago, Chile

Several studies suggest that leptin modulates hypothalamic-pituitary-gonadal axis functions. Leptin may stimulate release of gonadotrophin releasing hormone (GnRH) from the hypothalamus and of gonadotrophins from the pituitary. A synchronicity of luteinizing hormone (LH) and leptin pulses has been described in healthy women and in patients with polycystic ovarian syndrome, suggesting that leptin may modulate the episodic secretion of LH. However, it has not been established whether LH regulates the episodic secretion of leptin. To further examine LH-leptin interactions, we studied the episodic fluctuations of circulating LH and leptin in two patients with Kallmann’s syndrome (KS) before and on day 7 of pulsatile GnRH administration, and compared these with those observed in the early follicular phase of 10 regularly menstruating women divided into two control groups according to the body mass index of each patient. To assess episodic hormone secretion, blood samples were collected at 10 min intervals for 6 h, before and on day 7 of GnRH administration in KS patients, and during days 3–7 of the follicular phase in normally cycling women. LH and leptin concentrations were measured in all samples. For pulse analysis, the cluster algorithm was used. Before treatment, an apulsatile pattern with no endogenous LH pulsations was observed in both KS patients. However, leptin pulses were assessed in both women. During GnRH administration, pulsatile LH activity was achieved in both patients with pulse characteristics similar to those of the respective control group. Serum leptin concentrations and leptin pulsatile patterns were not modified. These results suggest that circulating leptin is probably not modulated by pulsatile GnRH-LH secretion.

Key words: Kallmann’s syndrome/leptin/LH/pulsatility

Introduction

Recent data in the mouse demonstrate that leptin, the protein hormone of the recently cloned ob gene, which is implicated in the control of body weight and thermogenesis (Zhang et al., 1994), also appears to act as a metabolic signal to the reproductive axis (Barash et al., 1996). In ob/ob mice, leptin deficiency results in hypogonadotrophic hypogonadism, impaired sexual maturation and infertility, which are corrected by leptin administration (Ahima et al., 1996; Chehab et al., 1996). In normal prepubertal mice, leptin administration may reduce the time to first oestrus and mating (Chehab et al., 1997). In humans, a role for circulating leptin in the regulation of reproduction is also suggested by many studies (Magoffin and Huang, 1998; Rosenbaum and Leibel, 1998). In prepubertal children, circulating leptin concentrations increase in parallel to the percentage body fat, acting as a possible metabolic signal for the onset of puberty (Garcia-Mayor et al., 1987; Clayton et al., 1997; Mantzoros et al., 1997). In adults, leptin appears to play a role in promoting hypothalamic function and maintaining adequate amounts of gonadotrophin secretion (Laughlin et al., 1998). According to some studies (Yu et al., 1997a,b), leptin may stimulate gonadotrophin-releasing hormone (GnRH) release from the hypothalamus, and luteinizing hormone (LH) and follicle stimulating hormone (FSH) release from the pituitary, probably by acting on its own receptor and promoting nitric oxide release.

A synchronicity of LH and leptin pulses in the mid-to-late follicular phase of the menstrual cycle of healthy women has been demonstrated (Licinio et al., 1998), suggesting that leptin may regulate the minute-to-minute oscillations in plasma concentrations of LH. Recently we extended these observations to patients with polycystic ovarian syndrome (PCOS), demonstrating that circulating leptin and LH are synchronized in patients with PCOS, but weaker (only 20 of 39 pulses) and with a phase-shift greater than in normal women (Sir-Petermann et al., 1999).

There are several ways to explain the phenomenon of coupling of LH and leptin release. The first is that LH regulates leptin secretion. The second is that leptin regulates LH secretion and the third is that both hormones are driven by a common oscillator whose nature and location are not currently known. To further examine the relationship between LH and leptin, we studied the episodic fluctuations of circulating LH and leptin in patients with hypogonadotrophic hypogonadism and anosmia (Kallmann’s syndrome, KS) before and during pulsatile GnRH administration, and compared them with those
observed in regularly menstruating women. KS offers a unique opportunity to study LH–leptin interactions in a state of natural inactivity of the gonadal axis, caused by the absence of hypothalamic GnRH secretion.

Materials and methods

Subjects
Two women with KS desiring pregnancy were studied. Diagnosis of KS was based on the presence of primary hypogonadotrophic amenorrhea, anosmia, no clinical or radiographic evidence of a hypothalamic or pituitary defect and normal basal and stimulated levels of other anterior pituitary hormones. One patient (KS2) was obese [body mass index (BMI) >30 kg/m²], but showed no other endocrine deficiency.

In addition, 10 normally cycling women of similar age, divided into two groups according to their BMI, acted as the control groups. None of these women had taken oral contraceptives or other medication for at least 6 months before starting the study. Prior to the study, informed consent was obtained from all subjects. This study was approved by the local ethical committee. The clinical characteristics of the two KS patients and the two control groups are presented in Table I.

Study protocol
The study was performed in the University Clinical Research Center beginning at 0900 after a 10 h overnight rest and fast in all women.

KS patients were studied before the initiation of therapy and during the seventh day of pulsatile GnRH administration. Control women were studied in the early follicular phase of the menstrual cycle (day 3–7).

For the study of episodic hormone secretion, blood samples were collected at 10 min intervals for 6 h, using a sampling device that allowed the continuous withdrawal of blood through a heparinized catheter (Sir-Petermann et al., 1995). LH and leptin were determined in all samples. Oestradiol was determined in samples 1, 19 and 37.

Table I. Clinical and endocrine characteristics for Kallmann’s syndrome patients (KS) and control groups (CG)

<table>
<thead>
<tr>
<th></th>
<th>CG1</th>
<th>KS1 (n = 1)</th>
<th>CG2</th>
<th>KS2 (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27</td>
<td>29</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>(21; 35)</td>
<td></td>
<td>(29; 33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.7</td>
<td>21</td>
<td>37.4</td>
<td>31.5</td>
</tr>
<tr>
<td>(21.0; 24.3)</td>
<td></td>
<td>(31.2; 39.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>5.8</td>
<td>0.14</td>
<td>3.9</td>
<td>0.16</td>
</tr>
<tr>
<td>(3.1; 10.6)</td>
<td></td>
<td>(3.1; 5.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>7.6</td>
<td>0.40</td>
<td>5.8</td>
<td>0.6</td>
</tr>
<tr>
<td>(5.2; 10.4)</td>
<td></td>
<td>(4.6; 7.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oestradiol (pg/ml)</td>
<td>68.6</td>
<td>&lt;10</td>
<td>73.6</td>
<td>&lt;10</td>
</tr>
<tr>
<td>(45; 110)</td>
<td></td>
<td>(50; 82)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean and range.

*CG1 body mass index (BMI) >25 kg/m²; CG2 BMI >30 kg/m².

LH = luteinizing hormone; FSH = follicle stimulating hormone.

were studied in the early follicular phase of the menstrual cycle (day 3–7).

For the study of episodic hormone secretion, blood samples were collected at 10 min intervals for 6 h, using a sampling device that allowed the continuous withdrawal of blood through a heparinized catheter (Sir-Petermann et al., 1995). LH and leptin were determined in all samples. Oestradiol was determined in samples 1, 19 and 37.

GnRH administration
In both KS patients a positive withdrawal bleed occurred after the administration of oral contraceptives. Beginning day 2 after with-
Table II. Serum luteinizing hormone (LH) concentrations over 6 h and LH pulse characteristics in Kallmann’s syndrome patients (KS) and control groups (CG), before and during gonadotrophin releasing hormone (GnRH) administration

<table>
<thead>
<tr>
<th></th>
<th>CG1</th>
<th>KS1 basal</th>
<th>KS1 + GnRH</th>
<th>CG2</th>
<th>KS2 basal</th>
<th>KS2 + GnRH</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (IU/l)/6 h</td>
<td>4.8</td>
<td>0.12(^a)</td>
<td>4.7</td>
<td>3.99</td>
<td>0.14(^b)</td>
<td>2.7(^a)</td>
</tr>
<tr>
<td>(3.2; 5.9)</td>
<td>(0.1; 0.2)</td>
<td>(2.8; 6.5)</td>
<td>(3.6; 4.8)</td>
<td>(0.1; 0.32)</td>
<td>(1.3; 3.8)</td>
<td></td>
</tr>
<tr>
<td>LH (number/6 h)</td>
<td>5.6</td>
<td>-</td>
<td>4</td>
<td>6.3</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>(5; 6)</td>
<td></td>
<td></td>
<td>(5; 7)</td>
<td></td>
<td></td>
<td>(1.2; 1.6)</td>
</tr>
<tr>
<td>LH amplitude (IU/l)</td>
<td>1.6</td>
<td>-</td>
<td>2.0</td>
<td>1.1</td>
<td>-</td>
<td>1.2</td>
</tr>
<tr>
<td>(0.9; 2.3)</td>
<td></td>
<td></td>
<td>(1.0; 2.9)</td>
<td>(0.6; 1.9)</td>
<td>(0.2; 0.5)</td>
<td>(2.9)</td>
</tr>
<tr>
<td>LH pulse height (IU/l)</td>
<td>5.9</td>
<td>-</td>
<td>6.14</td>
<td>5.7</td>
<td>-</td>
<td>3.5</td>
</tr>
<tr>
<td>(3.4; 8.3)</td>
<td></td>
<td></td>
<td>(3.4; 13.0)</td>
<td></td>
<td></td>
<td>(2.9)</td>
</tr>
<tr>
<td>LH pulse area (IU/l/min)</td>
<td>50.9</td>
<td>-</td>
<td>111.3</td>
<td>25.3</td>
<td>-</td>
<td>29.5</td>
</tr>
<tr>
<td>(16.4; 83.4)</td>
<td></td>
<td></td>
<td>(72.8; 175.1)</td>
<td>(16.7; 37.9)</td>
<td></td>
<td>(15.5; 59.9)</td>
</tr>
</tbody>
</table>

Values are mean and range.

\(^a\)95% prediction limit (1.07; 9.04).
\(^b\)95% prediction limit (0.26; 6.44).

Table III. Serum leptin concentrations over 6 h and leptin pulse characteristics in Kallmann’s syndrome (KS) patients (KS) and control groups (CG) before and during gonadotrophin releasing hormone (GnRH) administration

<table>
<thead>
<tr>
<th></th>
<th>CG1</th>
<th>KS1 basal</th>
<th>KS1 + GnRH</th>
<th>CG2</th>
<th>KS2 basal</th>
<th>KS2 + GnRH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)/6 h</td>
<td>11.3</td>
<td>13.9</td>
<td>13.7</td>
<td>28.3</td>
<td>16.0</td>
<td>15.6</td>
</tr>
<tr>
<td>(6.4; 19.2)</td>
<td>(11.3; 17.1)</td>
<td>(10.6; 18.1)</td>
<td>(19.9; 36)</td>
<td>(7.9; 21.2)</td>
<td>(9.2; 19.4)</td>
<td></td>
</tr>
<tr>
<td>Leptin pulse (number/6 h)</td>
<td>6.3</td>
<td>7</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>(6; 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(5; 7)</td>
</tr>
<tr>
<td>Leptin amplitude (ng/ml)</td>
<td>3.6</td>
<td>2.1</td>
<td>1.7</td>
<td>8.6</td>
<td>3.9</td>
<td>3.4</td>
</tr>
<tr>
<td>(1.7; 6.9)</td>
<td>(0.5; 5.5)</td>
<td>(0.5; 3.0)</td>
<td>(4.6; 15.5)</td>
<td>(0.9; 6.5)</td>
<td>(0.9; 5.9)</td>
<td></td>
</tr>
<tr>
<td>Leptin pulse height (ng/ml)</td>
<td>13.3</td>
<td>15.4</td>
<td>14.9</td>
<td>31.6</td>
<td>18.5</td>
<td>17.1</td>
</tr>
<tr>
<td>(6.9; 23.3)</td>
<td>(13.9; 17.1)</td>
<td>(12.5; 18)</td>
<td>(22.8; 41.7)</td>
<td>(15.9; 21.2)</td>
<td>(14.4; 19.4)</td>
<td></td>
</tr>
<tr>
<td>Leptin pulse area (ng/ml/min)</td>
<td>76.2</td>
<td>28.7</td>
<td>23.9</td>
<td>111.7</td>
<td>89.4</td>
<td>63.3</td>
</tr>
<tr>
<td>(35.3; 113.5)</td>
<td>(6.4; 156)</td>
<td>(1.2; 77.7)</td>
<td>(95.5; 325)</td>
<td>(25.8; 163)</td>
<td>(11; 140)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean and range.

Hormone assays

Serum LH and oestradiol were determined by electrochemiluminescence (Boehringer Mannheim, Mannheim, Germany; range: 0.1-200 IU/l for LH and 10-4000 pg/ml for oestradiol), total leptin was measured by radioimmunoassay (Linco-Research Inc., St Louis, MO, USA). The intra- and inter-assay coefficients of variation respectively were 1.1 and 2.1% for LH; 2.5 and 3.6% for leptin and 2.7 and 8% for oestradiol.

Pulse analysis and statistical evaluation

For pulse analysis, the computerized version of the cluster pulse algorithm, (Veldhuis and Johnson, 1996), was used. We selected a cluster configuration of 1x2 (one sample for the test peak and two for the test nadir), and a t-value of 2.121 to constrain the likelihood of false positive pulse determination to <5%. The following mean properties of pulsatile hormone concentrations were analysed: pulse frequency (number of significant peaks/6 h), pulse amplitude, pulse height and pulse area.

The mean value of each hormone parameter in each subject was calculated (control subjects and KS patients). Because this was a clinical study, where one single subject was compared with reference values obtained from a group, the 95% prediction limits were determined (Whitmore, 1986) to establish whether or not a given new value (mean value of the respective KS patient) was within the range observed in the control group (data obtained from mean values of five control subjects). Results are expressed as means and ranges.

Results

Figure 1 shows the LH and leptin profiles of both KS patients, before (A) and during (B) GnRH administration.

Before treatment, LH concentrations were at the limit of detection; an apulsatile pattern with no endogenous LH pulsations was observed in both patients. However, evident pulses of leptin were demonstrated in both women.

During GnRH administration (seventh day), pulsatile LH activity was induced in both KS patients. The characteristics of the leptin pulsatile pattern were not modified.
Table II shows the serum concentration of LH and LH pulse characteristics in KS patients and the control groups. Before treatment, mean LH concentrations were lower (outside the prediction limits) in KS patients compared to the controls. During GnRH administration, both patients exhibited a LH pulsatile pattern with pulse characteristics similar to those of the respective control group. Serum leptin concentrations and leptin pulse characteristics are presented in Table III. Basal leptin concentrations and leptin pulse characteristics did not differ between KS patients and the respective control groups. During GnRH administration, serum leptin concentrations and leptin pulse characteristics were not modified.

**Hormone concentrations**

Before therapy, oestradiol concentrations were lower in KS patients as compared with control women (Table I). During GnRH administration, oestradiol concentrations rose, reaching values comparable to those observed in the early follicular phase of the menstrual cycle [KS1 <10 versus 68 (48; 83) pg/ml; KS2 <10 versus 72 (50; 85) pg/ml].

**Discussion**

In this study we evaluated the episodic fluctuations of circulating LH and leptin in patients with KS before and during GnRH administration, as compared with normally cycling women studied in the early follicular phase of the menstrual cycle (day 3–7). In the absence of GnRH–LH pulse activity, the pulsatile pattern of leptin was present in these patients. Exogenous GnRH administration did not modify the leptin concentration or the leptin pulse characteristics.

In healthy women and in patients with PCOS, a synchronicity of LH and leptin pulses has been observed, suggesting a coupling of LH and leptin release (Licinio et al., 1998; Sir-Petermann et al., 1999). The stimulatory effect of leptin on the neuroendocrine-reproductive axis has been established in some species, including rat (Cagampang et al., 1990), monkey (Finn et al., 1998, Nagatani et al., 1998) and human (Laughlin et al., 1997, 1998). However, on the contrary, it has not been demonstrated whether LH regulates leptin secretion.

The pattern of pituitary hormone secretion may be viewed as being the consequence of the activity of a central oscillator modulated by internal or external factors, and could account for the modulatory effect of leptin on GnRH–LH secretion. In this respect, it appears to be more likely that LH regulates GnRH–LH secretion than vice versa. This assumption is based on a study in girls with precocious puberty treated with GnRH analogues in which leptin pulsatility persists despite the inhibition of the gonadal axis (Palmert et al., 1998), as well as on the observation made in our previous study (Sir-Petermann et al., 1999), in relation to the dynamics of hormone secretion. Leptin concentrations increased in blood before or concomitantly with but seldom after LH peaks. According to these observations and the data of the present study, it is proposed that circulating leptin might regulate GnRH–LH secretion, but GnRH–LH secretion is apparently not involved in modulating episodic leptin release, thus suggesting that the episodic leptin release is not driven by the GnRH pulse generator.

The mechanisms that account for episodic leptin release are not completely understood. Ultradian rhythmicities are common characteristics of many systems (Lavie and Kripke, 1981), including the hormonal system (Brandenberger, 1992). According to this point of view, ultradian leptin release could be seen as part of a general phenomenon, this assumption being based on the fact that in our KS patients the pulse generator of leptin was intact. However, Simon et al. (1998) established recently that 50% of the leptin pulses were preceded by an insulin or glucose pulse. These results suggest that glucose and insulin ultradian oscillations may affect subsequent leptin release, implying that the ultradian leptin pulses may be driven by a peripheral oscillator, probably related to pancreatic activity (Sirek et al., 1985; Simon et al., 1987). Taking all these observations into account, we propose that fluctuations in insulin and glucose concentrations drive the ultradian leptin secretion, which in turn is coupled to the LH pulsatile secretion. The real significance of the coupling of these hormones must be elucidated; it probably reflects a general regulatory phenomenon in which the secretion of a regulatory factor precedes or is concomitant with the secretion of the target factor.

In summary, we demonstrated that there are no differences between normal and KS women in the pulsatile characteristics of circulating leptin, and finally that circulating leptin is not modified by exogenous GnRH administration.

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**References**


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