SHORT COMMUNICATION

Estimation of instantaneous secretory rates and intrinsic characteristics of luteinizing hormone secretion in women with Kallmann syndrome before and after estriol administration

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SUMMARY

Three Kallmann syndrome (KS) patients were examined to assess characteristics of LH response to GnRH bolus, with and without GnRH sensitization using Instantaneous Secretory Rate (ISR) computation before and after estriol treatment (60 days, 2 mg/day). Six healthy women were enrolled as controls and underwent GnRH bolus during the early follicular phase (days 3-5 of the menstrual cycle). After estriol treatment, the KS patients showed a higher LH response to GnRH bolus and similar LH pulse duration to healthy controls. These data support the hypothesis that the administration of weak estrogen improves LH response to GnRH in hypogonadotropic women with KS. Reproductive Biology 2011 11 3:284-293.

Key words: Kallmann syndrome, LH pulses, estriol treatment

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INTRODUCTION
Kallmann syndrome (KS) is a relatively frequent type of genetically based primary amenorrhea with hypogonadotropic hypogonadism (HH) characterized by the absence of endogenous GnRH and anosmia [5, 10, 14, 16]. Clinically, the degree of hypogonadism and smell deficiency varies significantly not only among unrelated patients but also within affected families [5, 11]. Kallmann syndrome represents a good physiopathological model to understand the dynamics of LH secretion from the gonadotropes before and after GnRH sensitization, and after mild estrogenic priming. Estrogen administration has been proposed to improve the pituitary function in KS patients undergoing induction of ovulation (fertility purposes) or in anovulatory amenorrheic women [9]. The aim of this study was to evaluate the effects of a mild estrogenic priming on GnRH-induced LH secretion in KS patients. For this purpose a specific algorithm for pulse detection and instantaneous secretory rate (ISR) computation was used [12].

MATERIAL AND METHODS
The study design was approved by the Ethical Committee at the University of Modena and Reggio Emilia, Italy. Among patients attending the outpatient ambulatory at the Centre for Gynaecological Endocrinology (University of Modena and Reggio Emilia, Italy) for their amenorrheic conditions, we found three patients affected by Kallmann syndrome (18, 20 and 30 years old). The patients agreed to participate in the present study, and were clinically and endocrinologically evaluated. They were characterized by: 1/ primary amenorrhea, 2/ hypogonadism with low LH and FSH plasma levels, 3/ anosmia ascertained by means of olfactory screening tests [13], 4/ history of normal fenotypical development and normal occurrence of adrenarcal development, and 5/ absence of any endocrinological abnormalities; no thyroid, prolactin (PRL) or adrenal gland disease. None of the patients was under hormone replacement therapy, and only one had used it in the past (one year before the study).

A magnetic resonance imaging (MRI) of the forebrain demonstrated aplasia (two patients) or hypoplasia (one patient) of the olfactory bulbs and tracts. MRI was performed to exclude hypothalamic or pituitary lesions as the cause of HH. Ultrasound pelvic examination reported the presence of the hypoplastic uterus and small ovaries with no developing follicles in all the patients. Six
healthy, fertile women (age range: 24-33 years) were selected among doctors and students of our unit to form a control group. All of them were selected according to the following criteria: 1/ no pregnancy and regular menstrual cyclicity during the last 12 months, 2/ absence of any endocrinological abnormalities, no thyroid, prolactin (PRL) or adrenal gland disease, and no sign of any other systemic disease, and 3/ no hormonal treatment, and no use of oral contraceptives during the last six months.

All KS patients underwent a pulsatility test. During this test, a GnRH bolus (10 µg/ml of physiological solution; Ferring, Germany) was injected at +60 and +150 minute of the test to assess LH and FSH response. A low dose of GnRH was chosen to closer mimic a physiological stimulation. Starting at 8:00, blood was sampled every 10 min for 4 h to determine LH and FSH plasma profiles. The following day, the patients underwent a pituitary sensitization performed via a subcutaneous administration of GnRH bolus (10 µg) for three days (at 9:00 am). On day 4, the KS patients underwent again the pulsatility test and then were submitted to mild estrogenic treatment (estriol; Ovestin, Organon, The Netherlands; 60 days; 2 mg/day; per os). After the treatment, the patients underwent again the endocrinological evaluation (pulsatility test, pituitary sensitization, pulsatility test) described above. None of the patients referred adverse side effects due to the treatment. The healthy controls underwent the GnRH test during the early follicular phase (days 3-5) of the menstrual cycle, and were sampled 10 min before and 0, 10, 20, 30, 40, 50, 60 min after the GnRH bolus (10 µg/ml; Ferring, Germany) for plasma determinations of LH and FSH.

Plasma LH, FSH and estradiol concentrations were determined as previously described [7, 8]. The sensitivity of the LH assay was 0.1 IU/ml. The cross-reactivities with free α- and β- subunits of LH, FSH and TSH were less than 2% [7]. Intra-assay and inter-assay coefficients of variation were 4.9 and 7.4%, respectively. The LH secretory response to GnRH bolus was studied on raw data and data submitted to the instantaneous secretory rates (ISR) computation using the DETECT program [12] at p=0.01 (1%) for a nominal false positive rate. Plasma hormone levels can be assumed to be the difference between input from the pituitary and output from all the organs and tissues responsible for its metabolic clearance. Since the clearance rate
Kallmann syndrome and LH pulses

constants and t \( \frac{1}{2} \) for LH are known [17], the ISR can be easily computed with the specific algorithm included in DETECT program [12]. The duration of the pulses found on ISR is the duration of the secretory bursts from the gonadotropes. The variance model used for ISR was as previously described [7, 12]. The amplitude of LH pulse in response to GnRH bolus was computed on raw data as the difference between the maximal height of the LH response and LH plasma level observed at the time of the stimulation. When performing ISR computation, the amplitude of LH pulse after GnRH bolus was automatically detected with Detect program on instantaneous secretory rate profile [12]. One-way ANOVA and Student's t-test for paired and unpaired data were used to test significant differences among or between groups, as appropriate. Data are expressed as mean±SD.

RESULTS AND DISCUSSION

As expected, the KS patients were hypogonadotropic, having LH and FSH plasma concentrations below the detectable level (0.1 mIU/ml). After 60 days of estriol administration, no changes were observed in gonadotropin and estradiol plasma concentrations. The LH plasma level of the KS patients was below the detection limit from 0 to 60 minute i.e. to the response to the first GnRH bolus. The LH response to GnRH bolus (fig. 1) corresponded to the difference between the maximal height of the pulse and the LH plasma concentration at the time of the bolus administration. Under basal conditions, the LH response to exogenous GnRH did not differ between the first and second GnRH bolus (fig. 1A; tab. 1). After three days of GnRH sensitization, the LH response to the first GnRH bolus (fig. 1B) was higher than those under basal conditions and after the second GnRH bolus (tab. 1). The LH response to GnRH was higher after estriol administration than before, and the first LH response was higher than the second one (fig. 1C; tab. 1). The three days of sensitization after estriol treatment induced a significantly higher LH response to GnRH bolus than in all the other experimental conditions (tab. 1; fig. 1D). The computation of ISR confirmed observations made on raw data.

Since GnRH receptor expression is related to a direct GnRH modulation of its own RNA synthesis [2, 15], our data suggest that three days of GnRH administration together with the long term estriol priming induced both a higher sensitization/expression of GnRH-
receptor as well as synthesis and storage of LH [1, 2, 15]. This explains the greater response of LH to both GnRH stimulations. These observations differ from those of Zimmer et al. [18] whose patients were affected by functional hypothalamic amenorrhea.

![Figure 1](image)

Figure 1. Luteinizing hormone (LH) response profiles (mean±SD) of Kallman syndrome patients to GnRH test (♦ raw data; ■ ISR data). Panel A: baseline conditions; Panel B: after GnRH sensitization; Panel C: under estriol treatment; Panel D: after GnRH sensitization under estriol treatment. ISR: instantaneous secretory rate; for details: see the text; Arrows depict the times of GnRH administration; *p<0.05 vs. baseline conditions (1A); **p<0.05 vs. all examined conditions (1A, B and C).

As expected, the healthy controls demonstrated a higher LH response to GnRH bolus compared to the KS patients under all experimental conditions (tabs. 1 and 2; figs. 1 and 2). The mean duration of LH pulse was similar under different experimental conditions in the KS patients, and did not differ with the healthy controls (tabs. 1 and 2). In addition, ISR computation showed that the mean duration of LH pulse were shorter than in raw data. These data...
suggest that LH pulse duration and the intrinsic cellular mechanism driving LH secretion from the gonadotropes always acts in the same way and for the same amount of time.

![Figure 2. Luteinizing hormone (LH) response profiles (mean±SD) of healthy controls to GnRH test (♦ raw data; ■ ISR data). Arrow depicts the time of GnRH administration; n=6. ISR: instantaneous secretory rate; for details: see the text.](image)

![Table. 1. Characteristics of GnRH-induced LH pulses (mean±SD) in patients with Kallmann syndrome (KS)](table)

<table>
<thead>
<tr>
<th>LH plasma concentration</th>
<th>First GnRH bolus</th>
<th>Second GnRH bolus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw data</td>
<td>LH pulse amplitude (mIU/ml)</td>
<td>LH pulse duration (min)</td>
</tr>
<tr>
<td>Basal conditions</td>
<td>0.39±0.1</td>
<td>80.0±10.0</td>
</tr>
<tr>
<td>After GnRH sensitization</td>
<td>1.46±0.8*</td>
<td>75.0±5.0</td>
</tr>
<tr>
<td>Basal conditions after estriol treatment</td>
<td>0.57±0.1*</td>
<td>85.0±5.0</td>
</tr>
<tr>
<td>After GnRH sensitization and estriol treatment</td>
<td>2.86±0.7*</td>
<td>75.3±11.5</td>
</tr>
</tbody>
</table>

| ISR data               | LH pulse amplitude (mIU/ml) | LH pulse duration (min) | LH pulse amplitude (mIU/ml) | LH pulse duration (min) |
| Basal conditions       | 0.3±0.1          | 41.0±10.0         | 0.24±0.1          | 43.3±5.7          |
| After GnRH sensitization | 1.1±0.4*       | 41.6±5.5          | 0.46±0.2§       | 46.6±5.5          |
| Basal conditions after estriol treatment | 0.3±0.1          | 50.5±16.0         | 0.18±0.0          | 45.0±5.0          |
| After GnRH sensitization and estriol treatment | 1.5±0.2§       | 46.6±15.0         | 0.80±0.2§       | 43.3±11.5          |

n=3; ISR: data submitted to the instantaneous secretory rates (ISR) computation using the DETECT program [12]; ¹ the difference between the maximal height of the LH response to GnRH and LH plasma level at the time of the stimulation; *p <0.05 vs. basal conditions and vs. second GnRH bolus; §p<0.05 vs. basal conditions and basal conditions after estriol treatment
Table. 2. Characteristics of GnRH-induced LH pulses (mean±SD) in healthy controls

<table>
<thead>
<tr>
<th>LH plasma concentration</th>
<th>LH pulse amplitude</th>
<th>LH pulse duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw data</td>
<td>17.4±2.3</td>
<td>82.0±10.0</td>
</tr>
<tr>
<td>ISR data</td>
<td>9.8±3.2</td>
<td>44.0±11.0</td>
</tr>
</tbody>
</table>

n=6; ISR: data submitted to the instantaneous secretory rates (ISR) computation using the DETECT program [12]; \(^1\) the difference between the maximal height of the LH response to GnRH and LH plasma level at the time of the stimulation

The LH response to GnRH was higher after estriol administration than before the treatment. Our results are in agreement with previously reported data concerning patients affected by secondary amenorrhea treated with 17-epi-estriol-3methyl ether (epimestrol, a weak estrogen; [9]) as well as in KS patients before the induction of ovulation program [1]. We demonstrated that in KS patients, the use of estriol dose as low as 2 mg/day was able to affect the ability of gonadotropes to synthesize and store high amounts of LH. As it was observed in an animal model [4], this LH could be later released in response to GnRH bolus.

In this study, the LH pulse duration estimated after ISR computation had a longer duration than those under other physiological or physiopathological conditions reported previously [6, 8]. It is possible that exogenous GnRH is not similar to the endogenous hypothalamic GnRH discharge, despite the low GnRH dose used. However, our estimates are similar to those reported by De Nicolao et al. [3] who presented a non-parametric deconvolution approach and evaluated the intrinsic LH pulse characteristics after GnRH stimulation in a group of healthy male volunteers. They reported that after GnRH bolus injection, ISR computation provided a duration of LH response ~50 min [3] similar to those presented in this study.

The present study confirms that LH pulse amplitude is related to the amount of GnRH stimulating the gonadotrope cells [1], and supports the hypothesis that the duration of the secretory burst is a constant/built-in parameter of the gonadotropes [3]. The steroid milieu modulates the amplification of LH response to GnRH stimulation (i.e. LH pulse amplitude) inducing a higher GnRH
receptors expression and a higher LH synthesis by the gonadotropes [2]. In conclusion, we demonstrated that estrogentic priming might be essential to induce an adequate GnRH-stimulated LH response in KS female patients. This could be relevant in view of ovulation induction [1] if pregnancy is requested. Further studies are needed to ascertain whether this estrogen priming might improve conception.

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REFERENCES


