Depression of FSH and LH secretion following pulsatile
GnRH administration in ovariectomized women

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To investigate the mechanism by which pulsatile adminis-
tration of gonadotrophin-releasing hormone (GnRH) modi-
ﬁes secretion of luteinizing hormone (LH) and follicle-stimu-
lating hormone (FSH), we studied three
groups of ﬁve women who had been ovariectomized for
non-malignant gynaecological conditions at least 6 months
previously, none of whom had received substitutional
hormone therapy. Before and after 15 day treatment
with subcutaneous pulsatile GnRH (one 20 µg dose every
90 min in group A, one 10 µg dose every 90 min in
group B and one 20 µg dose every 120 min in group
C), pulsatile secretion of LH and FSH was characterized
by determining these hormones in 4 ml blood samples
taken every 10 min for 8 h (9.00 a.m. to 5.00 p.m.). For
both LH and FSH, mean serum concentration and pulse
amplitude were lower after GnRH treatment than before
(and in the case of LH the decrease depended upon both
the size and frequency of exogenous GnRH pulses) but
in no group was there a signiﬁcant change in LH or
FSH pulse frequency. We conclude that exogenous
pulsatile GnRH probably acts by partially desensitizing
the pituitary rather than by depressing endogenous
GnRH secretion. Such partial desensitization would
explain reports that exogenous pulsatile GnRH improves
ovulation by women with polycystic ovary syndrome.

Key words: exogenous pulsatile GnRH/GnRH analogues/
induction of ovulation/IH and FSH rhythms/pituitary desensit-
arization

Introduction

The amplitude and frequency of the gonadotrophin pulses
secreted by the pituitary of women of fertile age depend on
the phase of the menstrual cycle (Flicori et al., 1986). Secretion
is stimulated by pulses of gonadotrophin-releasing hormone
(GnRH) that are emitted by the hypothalamus under the control
of the ‘GnRH pulse generator’ and other signals relayed via
diverse neuron nuclei (Leranth et al., 1992), and is regulated
by feedback from the gonads.

We have previously reported that pulsatile subcutaneous
administration of GnRH induces ovulation more rapidly in
women with low or zero gonadotrophin levels due to hypogona-
dotropic hypogonadism of hypothalamic origin, than in
women with normal gonadotrophin levels receiving GnRH for
oligomenorrhoea (Graña, 1997). We interpreted this ﬁnding as
showing that exogenous GnRH requires a certain time to
prevail over the action of endogenous GnRH. Two suggestions
have been put forward as to the mechanism by which exogenous
GnRH prevails over the endogenous hormone: partial desensit-
ization of the pituitary (Lambalk et al., 1986, 1987) and
depression of secretion of GnRH by the hypothalamus
(Lambalk et al., 1991).

To clarify the mechanism of action of exogenous pulsatile
GnRH, we have now studied the effects of three different
administration regimens on secretion of follicle stimulating
hormone (FSH) and luteinizing hormone (LH) in ovariectom-
ized women.

Material and methods

Subjects

We studied 15 women aged 44–55 years who had been ovariectom-
ized on account of non-malignant gynaecological conditions at
least 6 months previously (seven for uterine leiomyoma, four for
ovarian endometriosis and four for ovarian cystadenoma). None
had received substitutional hormone treatment, and none had taken
psychotropic, anti-epileptic or neuroleptic drugs in the 3 months
prior to this study. All volunteered to take part in the study after
being duly informed of its nature, and gave written consent.
Before their inclusion, determination of FSH, LH, oestradiol,
triiodothyronine (T3), thyroxine (T4) and thyroid-stimulating hor-
mone (TSH) conﬁrmed surgical menopause (FSH >40 IU/l,
oestradiol <50 pg/ml) and ruled out thyroid dysfunction and other
alterations, and standard haematological and biochemical parameters
were found to be normal.

Experimental protocol

The subjects were assigned to three groups: the ﬁrst ﬁve to group A,
the second ﬁve to group B and the third ﬁve to group C. In each
subject, pulsatile secretion of LH and FSH was characterized before
(day 0) and after (day 16) a group-speciﬁc 15 day treatment with
subcutaneous pulsatile GnRH (Luforan® 500, Serono, Madrid, Spain),
administered by means of a Zyklomat Pulse infusion pump (Ferring,
Kiel, Germany). Group A subjects received one 20 µg dose every
90 min, group B subjects a 10 µg dose every 90 min and group C
subjects a 20 µg dose every 120 min. Characterization of LH and
FSH secretion was based on their determination in 4 ml blood samples
taken every 10 min over an 8 h period (09.00–17.00 h), during which
time the subject remained seated or supine and took a continental
breakfast and a light lunch, together totalling approximately 2000
Figure 1. Serum LH (○-○) and FSH (●-●) concentrations during the pre-treatment (left) and post-treatment (right) studies in subjects from groups A (top), B (middle) and C (bottom). The pulses detected are marked as arrows (↓).

calories. The last GnRH pulse was administered 1 min before the third blood sample of the post-treatment characterization session was taken.

**Determination of hormones**

Blood samples were centrifuged immediately and the resulting sera were stored at −20°C pending analysis. FSH, LH and oestradiol were determined in triplicate (in each case with the three subsamples in a single run) using an ACS-180 Plus automatic analyser (Chiron Diagnostics, Medfield, MA, USA) and reagents from the same supplier; FSH and LH were determined by sandwich immunoluminescence assays, and oestradiol by a competitive immunoluminescence assay using monoclonal antibody against position 6 of the oestradiol molecule. The FSH method, calibrated with WHO 2nd International Reference Preparation (1980) (NIBSC 78/549), had a detection limit of 0.03 IU/l and conditional coefficients of variation (CVs) of 6.81% at 5.9 IU/l and 6.35% at 64.7 IU/l. The LH method, calibrated with WHO 2nd International Reference Preparation (1988) (NIBSC 80/552), had a detection limit of 0.09 IU/l and conditional CVs of 3.86% at 5.5 IU/l and 4.14% at 36.1 IU/l.

**Pulse detection**

LH and FSH pulses in serum were detected by a non-parametric statistical method (Lado-Abeal *et al.*, 1991) that in validatory trials with simulated LH series had achieved a sensitivity and positive accuracy (Urban *et al.*, 1989) of 0.88 and 1.00 respectively, with a sum of 1.88 similar to that reported for the cluster method (Urban *et al.*, 1988). The parameters calculated were pulse amplitude, interpulse
Exogenous pulsatile GnRH desensitizes the pituitary

Table I. Mean serum LH concentrations (IU/l)\(^a\) in three groups of five ovariectomized women before and after 15 days pulsatile exogenous GnRH\(^b\), and the median of the percentage fall in mean serum LH concentration in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
<th>(P) value</th>
<th>Fall in LH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>22.12 ± 3.77 (27.10)</td>
<td>5.74 ± 1.94 (5.40)</td>
<td>&lt; 0.001</td>
<td>75.00</td>
</tr>
<tr>
<td>B</td>
<td>36.88 ± 19.03 (29.80)</td>
<td>13.72 ± 5.71 (13.00)</td>
<td>&lt; 0.001</td>
<td>52.03</td>
</tr>
<tr>
<td>C</td>
<td>22.17 ± 2.22 (21.95)</td>
<td>8.80 ± 0.59 (8.70)</td>
<td>&lt; 0.001</td>
<td>62.64</td>
</tr>
</tbody>
</table>

A versus B versus C A versus B \(P < 0.05\) A versus B \(P < 0.05\)

\(\)NS A versus C \(P < 0.05\) A versus C \(P < 0.05\)

\(\)NS B versus C NS

\(^a\)Means ± SD with medians in parentheses.
\(^b\)Group A, one 20 µg pulse every 90 min; group B, one 10 µg pulse every 90 min; group C, one 20 µg pulse every 120 min.

NS = not significant.

Table II. LH pulse amplitude (IU/l)\(^a\) and interpulse intervals (min)

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitudes</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>A</td>
<td>4.52 ± 2.25 (4.60)</td>
<td>1.25 ± 0.74 (1.10)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>B</td>
<td>5.55 ± 2.53 (5.50)</td>
<td>3.04 ± 2.69 (2.10)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C</td>
<td>5.58 ± 2.55 (5.65)</td>
<td>2.33 ± 1.27 (1.91)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

A versus B versus C A versus B \(P < 0.001\) A versus B \(P < 0.001\)

\(\)NS A versus C \(P < 0.001\) A versus C \(P < 0.001\)

\(\)NS B versus C NS

Interpulse intervals

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>56.41 ± 17.55 (60)</td>
<td>60.69 ± 19.81 (60)</td>
<td>(NS)</td>
</tr>
<tr>
<td>B</td>
<td>63.44 ± 25.60 (60)</td>
<td>62.65 ± 26.32 (60)</td>
<td>(NS)</td>
</tr>
<tr>
<td>C</td>
<td>71.29 ± 24.19 (70)</td>
<td>74.61 ± 25.96 (70)</td>
<td>(NS)</td>
</tr>
</tbody>
</table>

A versus B NS A versus B \(\)NS A versus B \(\)NS

\(\)NS B versus C NS B versus C NS

\(^a\)Means ± SD with medians in parentheses; NS = not significant.

Table III. Mean serum FSH concentrations (IU/l)\(^a\) and the median of the percentage fall in mean serum FSH concentration in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
<th>(P) value</th>
<th>Fall in FSH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>58.44 ± 20.26 (67.90)</td>
<td>35.47 ± 13.42 (40.35)</td>
<td>&lt; 0.001</td>
<td>34.90 %</td>
</tr>
<tr>
<td>B</td>
<td>59.50 ± 19.22 (64.00)</td>
<td>38.14 ± 18.09 (32.70)</td>
<td>&lt; 0.001</td>
<td>44.70 %</td>
</tr>
<tr>
<td>C</td>
<td>38.90 ± 7.51 (37.70)</td>
<td>29.86 ± 5.48 (27.80)</td>
<td>&lt; 0.001</td>
<td>23.42 %</td>
</tr>
</tbody>
</table>

A versus B versus C A versus B versus C A versus B versus C

\(\)NS A versus B versus C NS A versus B versus C NS

\(^a\)Means ± SD with medians in parentheses; NS = not significant.

Expression of results and statistical analysis

Pulse amplitude, interpulse interval and mean concentration results are expressed in the tables below as means ± SD for each group and session, with medians in parentheses. The statistical significance of differences among groups was estimated with the Kruskal–Wallis test; when significant differences were detected, pairwise comparisons were effected using the Mann–Whitney test. Within-group differences between pre- and post-treatment values were assessed with the Wilcoxon matching pairs test.

Results

Figure 1 shows how serum LH and FSH concentrations varied during the pre-treatment and post-treatment studies in three subjects (one from each group).

Mean serum LH concentration

During the first (pre-treatment) evaluation, there were no statistically significant differences among the mean serum LH concentrations in the three subject groups (Table I). The mean levels in the post-treatment session were decreased. The fall was significantly larger for group A than for groups B and C,
in keeping with which the post-treatment value for group A was significantly lower than those for groups B and C, which did not differ significantly from each other.

**LH pulses**

Pre-treatment, there were no significant differences among the three groups as regards LH pulse amplitude (Table II). The post-treatment values were significantly lower than the pretreatment values in all three groups, and were significantly lower in group A than in group B or group C. Both pre- and post-treatment, the interval between LH pulse peaks was significantly shorter in group A than in group C, but in no group was there a significant difference between pre- and post-treatment values (Table II).

**Mean serum FSH concentration**

In each group, mean serum FSH concentration was significantly lower after GnRH treatment than before, but neither before nor after treatment were there significant differences among the three groups (Table III).

**FSH pulses**

Pre-treatment FSH pulse amplitude was significantly smaller in group C than in the other groups, but the three groups did not differ significantly in this respect after treatment (Table IV). Post-treatment FSH pulse amplitude was only significantly different from the pre-treatment value in the groups receiving 20 µg GnRH pulses. Before GnRH treatment, the interval between FSH pulse peaks was significantly shorter in group A than in group B or group C. For no group was the post-treatment FSH interpulse interval significantly different from the pre-treatment value, nor were there any significant differences in post-treatment FSH interpulse interval among the three groups.

**Discussion**

Menopausal and ovariectomized women are ideal subjects for the study of pituitary gonadotrophin secretion and its modification because of their large FSH and LH pulses and the absence of ovarian feedback to which these large pulse amplitudes are due.

In this study, 15 day subcutaneous pulsatile administration of GnRH to ovariectomized women was followed by a fall in their mean serum LH concentrations and LH pulse amplitudes to values typical of the medium-late follicular phase (Lado-Abeal et al., 1991). The extent of the reduction depended in both cases on both the size and the frequency of the GnRH pulses, the most effective of the three regimens used being one 20 µg dose every 90 min. These findings agree with those of other authors (Uemura et al., 1992; Scheele et al., 1996).

The GnRH treatment was also followed by a fall in mean serum FSH concentrations, but the percentage reduction was less than for LH and did not differ significantly among groups (which is compatible with the possibility of a mechanism that is independent of GnRH pulse size and frequency). FSH pulse amplitude was significantly reduced by administering GnRH at a level of 20 µg per pulse, regardless of pulse frequency, but not by 10 µg pulses.

The intervals between gonadotrophin pulses were not significantly altered by the GnRH treatment in any group. This suggests that the GnRH-induced reduction in gonadotrophin levels is less likely to have been due to suppression of hypothalamic emission of GnRH (one of the mechanisms mooted by Lambalk et al., 1991) than to partial desensitization of the pituitary by GnRH, a well-documented effect of exogenous GnRH (Lambalk et al., 1986; Conn and Crowley, 1991).

The differences between the responses of LH secretion and FSH secretion to exogenous pulsatile GnRH suggest that synthesis and/or release of FSH may not be so dependent on GnRH as in the case of LH, an observation previously made by Genazzani and co-workers (Genazzani et al., 1994, 1996). This would explain why GnRH analogues reduce LH and FSH levels to markedly different extents, inhibiting LH secretion almost completely but reducing serum FSH only to medium follicular phase levels (Broekmans et al., 1993). There is controversy as to whether the use of GnRH analogues is always advantageous in reproductive medicine (Kingsland et al., 1992) or guarantees a reduction in the incidence of serious complications of gonadotrophin treatment.

<p>| Table IV. FSH pulse amplitude (IU/l)* and interpulse intervals (min) |
|------------------------|------------------------|------------------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitudes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>7.67 ± 5.19 (5.40)</td>
<td>3.48 ± 2.49 (2.70)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>5.95 ± 3.04 (5.80)</td>
<td>6.65 ± 6.87 (4.60)</td>
<td>NS</td>
</tr>
<tr>
<td>Group C</td>
<td>4.23 ± 2.15 (4.20)</td>
<td>2.88 ± 1.51 (2.80)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Interpulse intervals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>62.19 ± 49.30 (40)</td>
<td>66.67 ± 43.35 (55)</td>
<td>NS</td>
</tr>
<tr>
<td>Group B</td>
<td>88.33 ± 55.92 (80)</td>
<td>67.74 ± 41.37 (60)</td>
<td>NS</td>
</tr>
<tr>
<td>Group C</td>
<td>86.25 ± 40.09 (80)</td>
<td>85.00 ± 45.40 (85)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*a Means ± SDs with medians in parentheses.”
such as multiple pregnancy or ovarian hyperstimulation syndrome (OHS) (Ron-El et al., 1991). It has been suggested that GnRH analogues may even favour the development of OHS, especially in women with polycystic ovary syndrome (PCOS) (Navot et al., 1992), and that they may harm the oocyte (Pellicer et al., 1992; Testart et al., 1993). PCOS involves high serum LH concentrations (Burger et al., 1989) due to both the frequency and amplitude of LH pulses being increased by a hyperactive hypothalamo–pituitary axis (Waldstreicher et al., 1986; Kazer et al., 1987), but it seems plausible that to treat PCOS and other anovulatory conditions involving high LH levels, it may suffice to depress pituitary activity partially rather than entirely (Monroe et al., 1986; Filicori et al., 1993).

The partial suppression of LH and FSH levels in the present study of ovariectomized women suggests that this can be achieved by pulsatile GnRH treatment, and this notion seems to be in consonance with reports that the ovulation of women with PCOS is improved by pulsatile administration of GnRH both when this treatment is given following administration of GnRH analogues (Filicori et al., 1991) and also when it is given in successive menstrual cycles (Corenthal et al., 1994). We postulate that in both cases pulsatile GnRH causes partial suppression of gonadotrophin secretion: when given following GnRH analogues, this results in its being associated with an immediate rise in gonadotrophin levels (given the total suppression brought about by the analogues), and when given in successive cycles it brings about a fall in gonadotrophin levels (an effect that only becomes manifest in the second cycle). It is therefore possible that the best GnRH ‘analogue’ is exogenous GnRH, in the sense that GnRH achieves the desired effects without completely suppressing LH secretion and without giving rise to climacteric symptoms.

The dependence of follicle maturation on the presence of low levels of LH as well as FSH has recently been stressed by Hillier (1996), who stated that in women with WHO Type 1 infertility, the response to pure FSH will depend on simultaneous administration of LH. The induction of pituitary production of both gonadotrophins by pulsatile exogenous GnRH may be a more natural alternative to direct administration of gonadotrophins.

To sum up, in this work we found that 15 day subcutaneous pulsatile administration of GnRH to ovariectomized women brought about a significant dose- and frequency-dependent reduction in the mean serum concentration and pulse amplitude of LH, together with a reduction in mean FSH levels that, in this study, did not depend on dose or frequency to a statistically significant extent; the intervals between LH or FSH pulses were not altered by the GnRH treatment. We conclude that pulsatile administration of GnRH at an appropriate pulse frequency probably reduces gonadotrophin levels by decreasing the sensitivity of the pituitary to GnRH rather than by depressing hypothalamic GnRH emission, and reason that this effect may prove useful for hormonal control in reproductive medicine.

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References


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