EFFECT OF LHRH AGONIST (BUSERELIN) ON PULSATILE SECRETION OF LHRH AND LH

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Synopsis The LHRH agonist buserelin was administered intranasally to eight patients with endometriosis in doses of 300, 600 or 900μg/day for six months. In all patients buserelin clearly suppressed ovulation. In the patients treated with 900μg/day, estradiol levels declined to less than 30pg/ml, and LH release in response to LHRH testing decreased markedly after 2 months of treatment. A mild decrease in LH release was noted in the premarin test at the end of the treatment. At the end of buserelin treatment baseline levels of plasma LHRH, LHRH pulse amplitude and frequency were not lowered. However, baseline levels of plasma LH and LH pulse amplitude decreased.

These results indicate that buserelin may act to decrease pituitary response to LHRH.

Key words: Buserelin • Pulsatile secretion • LHRH • LH

Introduction

The ability of gonadotropin releasing hormone and its analog to regulate fertility and to treat disorders of the reproductive system has been actively investigated1617. LHRH ethylamide, a potent and long-acting analog of LHRH, has been the subject of several investigations910. In normal women treated with LHRH agonists luteolysis911 and anovulation113 may occur. LHRH ethylamide (D-Ser (TBU)-des-Gly-NH₂; buserelin) has been demonstrated to inhibit follicular maturation and ovulation by a down-regulation mechanism of the pituitary-gonadal axis1214.

A recent study of buserelin in patients with endometriosis suggested that it may be useful in inhibiting ovarian activity and relieving symptoms1218.

In this study, we examined the pulsatile pattern of LH and LHRH in patients with endometriosis treated with LHRH ethylamide.

Materials and Methods

Eight women aged 20 to 36 years with a diagnosis of pelvic endometriosis confirmed by laparoscopy or laparotomy, agreed to participate in this study. All had regular menstrual cycles (mean interval 31 days and duration 5 days) and had received no medical treatment for endometriosis during the 3 months before the study started.

Treatment was started on day 2 to 5 of the cycle. Buserelin was administered intranasally in a dose of 300, 600 or 900μg/day for about 6 months. Blood was drawn for LH, FSH, estradiol (E₂) and progesterone (P) determination every 7 days before and during the period of administration. Plasma was stored at −60°C until the time of hormone assay.

LHRH tests were performed once before and every 2 months until 2 months after the end of buserelin administration. Blood samples for LH and FSH were obtained once before and 15, 30, 60 and 180 minutes after the intravenous injection of 100μg of LHRH. Pulsatile secretion of plasma LHRH and LH were analyzed before and at the end of administration. Blood samples were collected every 15 minutes for 180 min.

Preparation of plasma LHRH

Three volumes of acetic methanol solution were added to the plasma sample (2ml). After centrifugation (3,000 × g for 15 min), the supernatant was collected and dried. The dried material was dissolved with 0.01M phosphate buffer saline (pH 7.4) containing 0.05M EDTA, 0.1% BSA and 0.01% NaN₃. Duplicate aliquots (200μl) were used for the assay.

Radioimmunoassay of each hormone

LHRH concentrations were measured with double antibody radioimmunoassay by Takahashi's method21. Anti-LHRH and purified LHRH were obtained from Ohotsuka Assay (Tokushima, Japan). Labeling of LHRH with 125I was done by the conventional chloramin-T technique9. The levels of plasma LH and FSH were determined by double
antibody radioimmunoassay kits (DAIICHI Radioisotope Laboratory). The minimum detectable concentrations of these hormones were 0.5mIU/ml for LH, 0.4mIU/ml for FSH and 1.78pg/ml for LHRH. The intraassay coefficient of variation for LHRH, LH and FSH was 9.6%, 11.0% and 8.0%, respectively. The levels of plasma estradiol and progesterone were determined by double antibody radioimmunoassay kits (Green Cross Co. Ltd., Osaka). The intraassay coefficient of variation for estradiol and progesterone was 6.0% and 7.0%, respectively. Statistical analysis was performed by Student’s t test.

Results

The menstrual pattern, basal body temperature, and plasma concentration of LH, FSH, P and E₂ during treatment with buserelin are shown in Fig. 1. A prompt and significant decrease in E₂ levels occurred during treatment in all three groups (300, 600, 900μg/day). Plasma LH and FSH levels were significantly elevated in those treated with 300 to 600 μg/day even after 2~4 months, while those receiving 900μg had low plasma LH and FSH levels.

After 6 months of Buserelin therapy LH release in response to the LHRH test decreased in patients M.U. and A.K. treated with 300 and 600μg/day, respectively (Fig. 2). However, in patient M.K. who received 900μg/day LH release decreased markedly after two months. In the other patients treated with 600μg/day, whose plasma E₂ levels were higher than 30pg/ml, LH release in response to the LHRH test did not decrease during treatment (data not shown).

Pulsatile secretion of plasma LH at the end of buserelin treatment was compared with that in the early follicular phase of the normal cycle (Fig. 3). A “pulse” was defined as an increase from nadir to peak that was two standard deviations greater than the assay variability. Two or three LH pulses occurred in 3h at the end of treatment, as in the normal early follicular phase, but the amplitude of the LH pulses was lower in those treated with 600 or 900μg/day of buserelin than those in the normal early follicular phase. The amplitude of the LH pulses in case A.K. and M.K. was 8.13±3.13mIU/ml and 6.07±1.83mIU/ml, respectively before buserelin treatment. At the end of treatment each value in case A.K. (600μg/day) and M.K. (900μg/day) was 3.80±2.25mIU/ml and 3.45±1.49mIU/ml, respectively.

The pulsatile secretion of plasma LHRH at the end of buserelin treatment was compared with that in the normal early follicular phase (Fig. 4). LHRH pulses occurred about every 75 min both in the early follicular phase in 2 of the 3 patients before treatment and in 3 patients after buserelin treatment. The baseline values of LHRH were 2
### PULSATILE SECRETION OF LHRH AND LH

<table>
<thead>
<tr>
<th>Patient</th>
<th>Treatment</th>
<th>Before Treatment</th>
<th>After 2 Months</th>
<th>After 4 Months</th>
<th>After 6 Months</th>
<th>Months after cessation of treatment</th>
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<tbody>
<tr>
<td>M.U.</td>
<td>300 μg/day</td>
<td>○ LH, □ FSH</td>
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<tr>
<td>A.K.</td>
<td>600 μg/day</td>
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<tr>
<td>M.K.</td>
<td>900 μg/day</td>
<td>○ LH, □ FSH</td>
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Fig. 2. Response of pituitary LH and FSH to an injection of 100μg of LHRH before and 2, 4 and 6 months during and 2 months after treatment with various dose of buserelin. LH ○, FSH ●.

![Graph showing LH and FSH levels](image)

Fig. 3. Pulsatile secretion of plasma LH in early follicular phase and at the end of buserelin treatment. Blood was drawn at 15 min intervals for 180 min from 5 patients in the early follicular phase (day 4–7 of cycle) before buserelin treatment (top) and from 3 patients at the end of treatment (300, 600 or 900μg/day of buserelin) (bottom).

~3pg/ml both in the early follicular phase and after buserelin treatment.

The amplitude of the LHRH pulses before buserelin treatment and at the end of treatment was 1.38±1.10pg/ml and 1.31±0.68pg/ml, respectively. Buserelin treatment did not suppress the baseline values, pulse frequencies of LHRH and the LHRH pulse amplitude, but the baseline value of LH and the LH pulse amplitude decreased. In the patients treated with 900μg/day of buserelin the suppression of LH release in the LHRH test was observed by 2 months (data not shown).

### Discussion

Endometriosis is a disease which develops slowly in women during the reproductive period and regresses after menopause. Danazol therapy, which is commonly called a pseudomenopause therapy, has become the first choice because of clinical effectiveness. Recently, LHRH ethylamide (buserelin), a potent and long-acting analog of LHRH, has been reported to be useful in the treatment of endometriosis. Buserelin suppresses ovarian function by reducing gonadotropin release by the pituitary. This ovarian suppression has been
explained by a down regulation of gonadotropin\textsuperscript{10}, through the effect of buserelin on the hypothalamus and pituitary have not been fully clarified. Carmel et al. demonstrated a pulsatile release of LHRH into the portal circulation in the rhesus monkey\textsuperscript{2}.

Recently, several investigators have reported that LHRH in human peripheral blood can be detected by radioimmunoassay\textsuperscript{11}. Knobil showed that pulsatile stimulation of the pituitary by LHRH was essential for normal gonadal function\textsuperscript{16}. To clarify the mechanism of ovarian suppression, we examined the LH response to LHRH and the pulsatile secretion of LH and LHRH during treatment with buserelin. In this study we administered buserelin intranasally for six months to patients with endometriosis. We showed that this drug clearly suppressed ovulation. LH release in the LHRH test was markedly suppressed by 2 months of treatment with 900\(\mu\)g/day of buserelin. Higher doses of buserelin decreased the baseline value of LH and the pulse amplitude of LH, although the pulse frequency did not change in 67\% of the patients. On the other hand, neither the amplitude nor the frequency of the pulses of LHRH was suppressed even by higher doses of buserelin.

This suggests that buserelin probably reduce the pituitary response to LHRH and suppress the pulsatile secretion of LH. Further studies of the possible regulators of LH pulsatile secretion are needed to clarify the relationship between LH pulsatile secretion and pituitary LH receptor response to LHRH.

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References


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