NOTE

Studies on GnRH Agonist Suppression of Estrogen Production in Patients with Endometriosis

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Abstract

The chronic administration of GnRH agonists to women results in the reversible suppression of estrogen production by the ovary. In the present study, the mechanism of the GnRH agonist suppression of estrogen production was investigated in patients with endometriosis. During the treatment with intranasal buserelin spray, the concentration of serum estradiol-17β (E2) was suppressed to near-castrate levels. Despite this marked suppression of serum E2, immunoreactive LH and FSH levels in serum were not changed. On the other hand, serum bioactive LH was markedly reduced. It was also observed during the treatment that the pituitary LH pulse disappeared and pituitary response to exogenous GnRH was significantly suppressed. In contrast, ovarian response to human menopausal gonadotropin (hMG) was not altered during the treatment. These findings suggest that the GnRH agonist suppression of estrogen production in the patients with endometriosis is through both suppression of the secretion of biologically active LH and the reduction of the LH pulse, but not through a direct inhibitory effect on ovarian estrogen biosynthesis.

The chronic administration of potent agonistic analogs of GnRH suppresses gonadal steroid production. This has led to the clinical use of these compounds as a form of hormonal treatment for sex steroid-dependent diseases including endometriosis. In fact, the clinical usefulness of GnRH agonists in the treatment of endometriosis is well established (Lemay et al., 1984). Although a variety of theories have been proposed to explain the GnRH agonist suppression of estrogen production, its exact mechanism(s) of action in women has not been fully elucidated. Some of the mechanisms proposed are: 1) suppression of pituitary gonadotrope responsiveness to endogenous GnRH resulting in decreased secretion of gonadotropins (Schriock et al., 1985; West et al., 1987), 2) alteration in the pituitary LH pulse.
pattern, 3) secretion of a qualitatively different and biologically less active LH (Meldrum et al., 1984), and 4) direct inhibition of ovarian steroid biosynthesis (Tureck et al., 1982).

In the preliminary study, patients with endometriosis were treated for 6 months with nasal administration of the GnRH agonist buserelin. We found that, in the patients who responded to buserelin, the concentration of serum estradiol-17β (E2) was decreased to near-castrate levels by 2 months of the treatment. Despite the marked reduction in serum E2, the levels of serum gonadotropins were not changed during the treatment. These findings prompted us to examine in detail the mechanism(s) of action of GnRH agonists in the treatment of endometriosis. It also seems to be of great value to clarify the mode of action of GnRH agonists when the patients with endometriosis are managed with hormonal therapy. In the present study, therefore, the secretion of immunological gonadotropins, secretion of biological LH, pulsatile secretion of pituitary LH, and ability to biosynthesize ovarian estrogen before and during the treatment with intranasal spray of buserelin were investigated in patients with endometriosis.

Materials and Methods

Patients and protocol

Five patients who were 24 to 36 years old and had a regular menstrual cycle were included in the present study. They all had a long-standing history of dysmenorrhea. Endometriosis was documented by laparoscopy in all patients. They had no previous hormonal treatment for their endometriosis. The nature of the study was explained to each subject in detail, and consent was obtained.

All patients recorded their daily basal body temperature during a pretreatment cycle and consecutive treatment period. All patients were treated with 300 μg of buserelin by intranasal administration every 8 h for 6 months. The treatment was started within 5 days of the menstrual cycle. Serum LH, FSH and E2 determinations were performed on blood samples obtained in the early follicular phase of a pretreatment cycle and in every two weeks during the treatment. Blood was always drawn prior to the nasal spray. The mode of pituitary LH secretion, response of pituitary gonadotropins to GnRH, and the estrogen producing ability of the ovary were studied in the early follicular phase of a pretreatment cycle and in the twelfth week after the beginning of treatment. The presence of a pulsatile secretion of LH was examined in blood samples obtained at 15 min intervals for a period of 4 h. A pulse was defined as an LH level which exceeded the mean level by 2 standard deviations of the intra-assay variation and reached a level above 10 mIU/ml (Buckman et al., 1981). In order to evaluate the response of pituitary LH and FSH, 100 μg of GnRH (gonadorelin acetate, Tanabe Seiyaku Co., Ltd., Japan) was administered iv, and blood was obtained at 30 min after the injection. Human menopausal gonadotropin (hMG, Organon, Oss, the Netherlands) was loaded to estimate the ovarian estrogen biosynthesizing ability. One hundred and fifty IU of hMG was administered im for 3 consecutive days, and blood was obtained at 2 and 4 days after the beginning of injection. The serum concentrations of bioactive LH were also measured in blood samples obtained in the early follicular phase of a pretreatment cycle and treatment cycles.

Hormone assays

Serum concentrations of immunoreactive LH, FSH and E2 were measured by radioimmunoassay (RIA) as described previously (Miyake et al., 1980). The minimum detectable levels of these hormones in the assays were as follows: LH, 0.1 mIU Second International Reference Preparation of hMG (2nd IRP-hMG)/tube; FSH, 0.2 mIU 2nd IRP-hMG/tube; E2, 3 pg/tube. The intra-assay coefficients of variation in these assays were 8.3% for LH, 9.0% for FSH and 8.0% for E2, respectively. Serum concentrations of bioactive LH were measured by quantitating the testosterone secreted by dispersed rat Leydig cells in vitro. The assay was performed according to the technique of Dufau et al. (1976). Leydig cells were prepared from adult rat testes by collagenase dispersion of decapsulated gonads, as previously described (Terakawa et al., 1982).
Serum samples were assayed by the incubation of 10 µl aliquots with dispersed Leydig cells at 37°C for 3 h. Testosterone production of the samples was measured by RIA and the minimum detectable level in the assay was 12.5 pg/tube. The intra-assay coefficient of variation in the testosterone assay was 5.7%. The standard used for bioactive LH assay was a first International Reference Preparation (code 6840), kindly supplied by the National Institute for Biological Standards and Control in England. The sensitivity of the LH RIA was 1 mIU/ml.

All values were expressed as mean ± SE. Student’s paired t-test was used for the statistical analysis.

Results

All five patients remained anovulatory during the treatment, with the exception of one patient who ovulated once at 2 weeks after the beginning of the treatment. The serum E₂ concentration gradually decreased to approximately the menopausal concentration, less than 30 pg/ml, by the twelfth week of the treatment in all patients (Fig. 1). Once the E₂ concentration reached the near-castrate level, there

Fig. 1. Serum E₂ concentration in 5 patients during 24 weeks of buserelin treatment. Blood samples were obtained in the early follicular phase of a pretreatment cycle (0 week) and every 2 weeks during the treatment. The serum E₂ concentration was measured by RIA.

Fig. 2. Serum concentration of immunoreactive LH and FSH in 5 patients during 24 weeks of buserelin treatment. Blood samples were obtained in the early follicular phase of a pretreatment cycle (0 week) and every 2 weeks during the treatment. The serum concentration of gonadotropins was measured by RIA.
was no further increase during the treatment. In contrast to the marked reduction in serum E₂, both serum LH and FSH remained unchanged in all patients throughout the treatment. Fig. 2 shows the basal gonadotropin concentration in each individual during 24 weeks of treatment. Neither LH nor FSH in serum decreased throughout the treatment. Thus, the suppression of serum E₂ by intranasal buserelin spray could not be explained at all by the serum immunoreactive gonadotropin level. Although basal gonadotropin secretion was not changed, gonadotrope response to exogenous GnRH was suppressed during the treatment (Fig. 3). In particular, pituitary LH release after a single dose of GnRH injection, judged by a net increase in the serum LH concentration, was markedly reduced.

We therefore examined the mechanism(s) of action of buserelin which induces suppression of estrogen production by the ovary. The mode of pituitary LH secretion before and during the treatment was studied by frequent blood sampling. Fig. 4 shows

![Fig. 3](image-url)

*Fig. 3. Responsiveness of pituitary gonadotrope to exogenous GnRH in the patients before (0 week) and during (12 weeks) buserelin treatment. A single dose of 100 μg GnRH was injected iv, and blood was obtained before and at 30 min after the injection. A net increase in immunoreactive serum LH and FSH (mean ± SE of 4 patients) after the injection is shown. *Difference from the value obtained before the treatment (P): <0.05.*

![Fig. 4](image-url)

*Fig. 4. Mode of pituitary LH secretion in 4 patients before and during buserelin treatment. Blood samples were obtained at 15 min intervals for a period of 4 h. Arrows indicate an LH pulse. A pulsatile secretion of LH (two pulses per 4 h) observed before the treatment disappeared during the treatment.*
individual data obtained from four cases. In all four patients, a pulsatile secretion of LH (two pulses per 4 h) demonstrated in the pretreatment cycle had disappeared by the twelfth week after the beginning of treatment. These findings suggest that the disappearance of the pituitary LH pulse pattern following chronic treatment with the GnRH agonist may be responsible for decreased estrogen production in patients with endometriosis. The concentrations of bioactive LH in the serum of patients were reduced following the treatment period. Of the two cases examined 4 weeks after the beginning of treatment, serum bioactive LH was reduced in one but not in the other. Of the four cases examined 12 weeks after the beginning of treatment, the concentration of bioactive LH in all cases was remarkably reduced. Fig. 5 shows the mean concentration of immunoreactive and bioactive LH in the four cases. In contrast to the sustained concentration of immunoreactive LH (17.5±3.9 and 19.3±4.0 mIU/ml before and during the treatment, respectively), the concentration of bioactive LH (19.6±7.3 mIU/ml) was significantly (P<0.05) decreased during the treatment (2.3±0.9 mIU/ml). These observations suggest that the secretion of biologically less active LH following the treatment with GnRH agonist may also cause reduced estrogen production in the patients with endometriosis.

Finally, the ovarian estrogen biosyn-
thesizing ability of the patients before and during the treatment was evaluated. During the treatment, the serum $E_2$ concentration in the patients was increased to the same extent in response to hMG administration as before the treatment. Fig. 6 shows individual data for four cases obtained before and at the twelfth week after the beginning of treatment. Thus, there was no difference between the ovarian response to hMG in a pretreatment cycle and in a treatment cycle, suggesting that the GnRH agonist, buserelin, does not have a direct inhibitory effect on ovarian estrogen biosynthesis in patients with endometriosis.

**Discussion**

The present study was designed to elucidate the mode of action of GnRH agonists in patients with endometriosis. All five patients included in the present study responded to nasal administration of buserelin, and their serum $E_2$ levels fell to near-castrate levels by the twelfth week of the treatment. This made it possible to evaluate the proposed mechanisms by which GnRH agonists decrease serum $E_2$ in patients with endometriosis. Previous studies in patients with endometriosis (Schriock et al., 1985) and uterine fibroids (West et al., 1987) showed that the serum immunoreactive gonadotropin concentration declined and was suppressed significantly during treatment period with either nasal or subcutaneous administration of GnRH agonist. It was also demonstrated that there is a relationship between the degree of estrogen suppression and the serum immunoreactive LH concentration (Schriock et al., 1985; West et al., 1987). In the present study, however, despite the marked reduction in serum $E_2$ during the treatment, neither immunoreactive LH nor FSH in serum significantly declined below the base level. These data suggest that the inhibitory effect of GnRH agonist on estrogen production in patients with endometriosis cannot be explained on the basis of down-regulation of pituitary immunoreactive gonadotropin secretion. Although either subcutaneous or intranasal administration of buserelin to patients with prostatic cancer markedly suppressed serum testosterone to the near-castrate level, the magnitude of testosterone suppression could not be entirely explained by the nature of the decline in plasma LH which fell much less (Rajfer et al., 1986). These results support our finding that a decreased secretion of immunoreactive gonadotropins is not responsible for the suppression of estrogen production.

A review of the literature shows that there is no previous report of chronic GnRH treatment on pituitary LH pulses in women, although a marked decrease in the frequency and amplitude of LH pulses following the constant infusion of GnRH agonist has been reported in men (Bhasin et al., 1987). In the present study, a pulsatile LH secretion observed in a pretreatment cycle disappeared in all patients examined, whose estrogen levels fell to the near-castrate level following chronic buserelin treatment. It is believed that ovarian follicular development occurs with pulsatile gonadotropin secretion. It is, therefore, likely that the absence of, or a marked decrease in, the frequency of LH pulses results in decreased estrogen production in women. A significant decrease in the pituitary response to GnRH during the treatment was demonstrated in the patients with endometriosis. However, the pulsatile frequency of LH is governed by hypothalamic influences (Knobil, 1980). There is no evidence that GnRH agonist causes a direct ultrashort feedback on the hypothalamic GnRH pulse generator. The mechanism by which chronic GnRH treatment inhibits pulsatile gonadotropin secretion is not yet known.
A marked decrease in the serum bioassayable LH concentration during chronic buserelin treatment suggests secretion of a molecularly altered LH species with diminished biological activity. Evans et al. (1984) provided evidence of molecular heterogeneity of circulating LH during GnRH agonist treatment. The reduction in serum bioactive LH in the patients with endometriosis correlated with the reduction in serum E₂ during buserelin administration. Similar data demonstrating a significant reduction in serum bioactive LH after GnRH agonist treatment have been reported in women with endometriosis and those with polycystic ovarian disease (Meldrum et al., 1984). GnRH agonist may inhibit ovarian function in patients with endometriosis by multiple mechanisms. These include secretion of a molecularly altered LH with diminished biological activity. It may be speculated that buserelin treatment also decreased serum bioassayable FSH and consequently suppressed estrogen production in the patients with endometriosis. Recent studies, however, have demonstrated that chronic GnRH agonist administration does not alter the qualitative characteristics of FSH in normal men (Pavlou et al., 1988). In contrast, GnRH antagonists seem to have a unique ability to decrease the biological potency of FSH in normal men (Dahl et al., 1986) and normal women (Kessel et al., 1988). To clarify whether chronic GnRH agonist treatment reduces serum bioactive FSH concentrations, a study in patients with endometriosis is under way in our laboratory.

One of the proposed mechanisms by which GnRH agonist suppresses estrogen production is a direct inhibition of ovarian steroid biosynthesis. In the present study, however, no direct inhibitory effect of GnRH agonist on ovarian estrogen biosynthesis was observed, since all the patients examined retained the ability to produce ovarian estrogen during chronic buserelin treatment. Direct inhibitory effects of GnRH agonists on ovarian steroidogenesis have been demonstrated in rodent models (Hsueh and Erickson, 1979). In a study with cultured human granulosa cells, GnRH agonist was also found to inhibit the secretion of progesterone in a dose-dependent manner (Tureck et al., 1982). However, an ovarian site of action of GnRH agonist in the inhibition of gonadal function is less likely to be important in women, since GnRH receptors are not detectable in human ovaries (Clayton and Huhtaniemi, 1982). Several studies have failed to demonstrate that GnRH or GnRH agonist has an effect on human luteal cells in vitro (Casper et al., 1984). The intratesticular steroid concentration and the enzymatic activity were significantly reduced in GnRH agonist-treated men, whereas the addition of human chorionic gonadotropin reversed these inhibition due to GnRH agonist (Rajfer et al., 1987). These results also suggest that the inhibitory effect of GnRH agonist does not act directly on the testis but is mediated via the pituitary gland. Our conclusions regarding the ability to produce ovarian estrogen during GnRH agonist treatment are based on data obtained from only 4 cases. Nevertheless, a similar ovarian response to hMG load between before and during the treatment was sufficient to allow us to conclude that GnRH agonist does not have a direct inhibitory effect on ovarian steroidogenesis.

The most probable mechanism of action of GnRH agonist-induced resorption of endometriosis is estrogen deprivation and subsequent atrophy. GnRH agonist may inhibit ovarian estrogen production in patients with endometriosis by multiple mechanisms. Indeed, a variety of theories have been proposed to explain the GnRH agonist suppression of estrogen production in women. Based on the results obtained in the present study, however, it is strongly
suggested that GnRH agonist suppression of estrogen production in patients with endometriosis is through both suppression of the secretion of bioactive LH and reduction of the LH pulse, but not through the suppression of immunoreactive gonadotropins and direct inhibition of ovarian estrogen biosynthesis.

References


