Evidence of differential control of FSH and LH responses to GnRH by ovarian steroids in the luteal phase of the cycle

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BACKGROUND: It is known that during the follicular phase of the cycle, estradiol sensitizes the pituitary to GnRH. The aim of this study was to determine the role of ovarian steroids in the control of GnRH-induced gonadotrophin secretion in the luteal phase of the cycle. METHODS: Eighteen normally cycling women were studied during the week following bilateral ovariectomy plus hysterectomy performed in early to mid-luteal phase. Six of the women received no hormonal treatment post-operatively (group 1, control), six received estradiol through skin patches (group 2) and the remaining six received estradiol plus progesterone (group 3). In all women the response at 30 min of LH (ΔLH) and FSH (ΔFSH) to GnRH (10 μg i.v.) was investigated on a daily basis. RESULTS: In group 1, serum FSH, LH and ΔFSH values increased progressively following ovariectomy, while in groups 2 and 3 this increase was postponed or abolished. In contrast to ΔFSH, ΔLH values showed the same pattern of changes in all three groups with a significant decline up to post-operative day 4 and a gradual increase thereafter. CONCLUSIONS: These results demonstrate, for the first time, that in the early to mid-luteal phase of the cycle, estradiol and progesterone participate in the control of GnRH-induced FSH, but not LH, secretion. It is possible that in the luteal phase, the response of LH to GnRH is partly regulated by gonadotrophin surge attenuating factor.

Key words: estradiol/GnRH/gonadotrophins/ovary/progesterone

Introduction

It has been established that ovarian steroids play an important role in the control of gonadotrophin secretion from the pituitary. Clinical experiments have shown that exogenous estrogen is able to suppress basal levels of LH and FSH during the follicular phase of the cycle (Tsai and Yen, 1971; Monroe *et al.*, 1972a; Young and Jaffe, 1976; Messinis and Templeton, 1990; Messinis *et al.*, 1992). On the other hand, changes in the production of endogenous estrogen, such as after ovarian stimulation with FSH or after bilateral ovariectomy, result respectively in a decrease or increase of endogenous gonadotrophin values (Yen and Tsai, 1971; Monroe *et al.*, 1972b; Barlow *et al.*, 1981; Messinis and Templeton, 1989; Kamel *et al.*, 1991; Messinis *et al.*, 1992; Alexandris *et al.*, 1997). In the case of ovariectomy, the pattern of LH increase following the operation is similar to that of FSH, but the values for both gonadotrophins are persistently lower in women oophorectomized in the luteal rather than the follicular phase of the cycle (Yen and Tsai, 1971; Alexandris *et al.*, 1997). Although the difference in gonadotrophin values between the two phases of the cycle can be attributed to the increased concentrations

of progesterone during the luteal phase, information regarding the contribution of this steroid to the negative feedback mechanism at that stage is limited (Nippoldt *et al.*, 1989).

In-vivo experiments have shown marked changes in the responsiveness of the pituitary to GnRH during the normal menstrual cycle, with a significant increase from the early follicular phase to mid-cycle and a progressive decline thereafter (Wang *et al.*, 1976). Although estradiol is the primary factor that sensitizes the pituitary to GnRH during the follicular phase (Lasley *et al.*, 1975), the role of ovarian steroids in the control of pituitary sensitivity to GnRH during the luteal phase has not been investigated. In a recent study in women, we have demonstrated that following ovariectomy in the luteal phase of the cycle, the response of FSH to GnRH increased progressively, while that of LH declined markedly. This indicates a differential control of FSH and LH by the ovaries (Alexandris *et al.*, 1997), but the mechanism is not clear.

The present study was undertaken to investigate the mechanism through which the ovaries control GnRH-induced LH and FSH secretion during the luteal phase of the menstrual cycle by treating normal premenopausal women with estradiol and

progesterone in order to prevent the ovariectomy-induced decline in the concentrations of these two steroids.

Materials and methods

Patients

The study included 18 normally cycling women, aged 42–46 years, with normal FSH values in the early follicular phase (<10 IU/l) and ovulatory progesterone levels on cycle day 21 (>20 nmol/l). Approval for the study was obtained from the local ethics committee and the women gave written informed consent. All women were studied during the week following bilateral ovariectomy plus total hysterectomy performed by laparotomy under general anaesthesia (09:00 h). The ovaries were normal and the indications for the operation were benign uterine lesions, such as fibroids and menorrhagia. The women were divided into three groups based on whether they were treated or not with ovarian steroids. In group 1 ($n = 6$) no hormonal treatment was given to the women post-operatively. In group 2 ($n = 6$) the women received estradiol through skin patches (Estraderm TTS; Novartis, Athens, Greece). The first patch was applied on the day of ovariectomy immediately after the operation at the dose of 100 µg/24 h. Further patches were applied on post-operative days 3 and 6. In group 3 ($n = 6$) the women received estradiol, as in group 2, plus progesterone (Utrogestan capsules 100 mg/capsule; Faran, Athens, Greece) intravaginally at the dose of 300 mg/day (100 mg every 8 h). The first dose of progesterone was applied after the end of the operation and the last dose on post-operative day 6. In women receiving hormonal treatment, no contraindications for the administration of these steroids were identified. The operation was performed in the early to mid-luteal phase of the cycle, i.e. 5 days after the endogenous LH peak detected by LH measurement in daily blood samples taken from the time the follicle size was 16 mm in diameter as assessed by ultrasound. In all women, the pituitary response to GnRH (10 µg i.v.) was investigated on a daily basis, starting in the morning before the operation until post-operative day 7, i.e. the day of discharge. Blood samples in relation to each GnRH injection (time 0) were obtained at –15, 0 and 30 min. The 30 min point was chosen because at that time a maximal response to GnRH has been reported and this represents pituitary sensitivity to GnRH (Wang *et al.*, 1976). FSH and LH were measured in all blood samples, while basal values of estradiol and progesterone were measured in the samples taken at –15 and 0 min. During the operation, the presence of a corpus luteum was confirmed. Before the operation, all women had normal haemoglobin levels (>12 g/dl) and the operations were performed without any complications. The blood loss was <300 ml in all patients and the post-operative period was uneventful.

Hormone assays

For the measurement of FSH and LH in serum, a microparticle enzyme immunoassay (MEIA) was used (AxSYM FSH and AxSYM LH respectively; Abbott Laboratories, Abbott Park, IL, USA). The lower limits of detection for FSH and LH were 0.37 and 0.50 IU/l respectively. Estradiol was measured using MEIA (AxSYM Estradiol; Abbott Laboratories). The lower limit of detection for estradiol was 73 pmol/l. For progesterone measurement, a solid-phase chemiluminescent enzyme immunoassay (Immulite progesterone; DPC, Los Angeles, CA, USA) was used. The lower limit of detection for progesterone was 0.6 nmol/l. The inter- and intra-assay coefficients of variation for FSH, LH, estradiol and progesterone were 3.2 and 4.1, 2.6 and 4.2, 2.3 and 5.5, and 8.0 and 4.1% respectively.

Statistical analysis

Before the statistical analysis the results were transformed into logarithms, but the arithmetic means of values are presented. For comparisons within the same group statistical analysis of the results was performed using one-way analysis of variance (ANOVA) followed by Dunnet's post-hoc test, while for comparisons between groups two-way ANOVA was used. The Statview 5 program (Abacus Concepts Inc., Berkley, CA, USA) was used.

Results

For each hormone, a basal value was calculated as the mean of the values at –15 and 0 min. Figure 1 shows basal FSH, LH, estradiol and progesterone concentrations before and after the operation in the three groups of women. Pre-operative basal values of these hormones did not differ significantly between the three groups. Following the operation, serum FSH values in group 1 increased gradually up to day 7 ($P < 0.01$). In group 2, serum FSH values initially remained unchanged up to post-operative day 4, but started to increase significantly thereafter up to day 7 ($P < 0.01$). Finally, in group 3, serum FSH concentrations did not increase at all post-operatively. FSH values were significantly higher in group 1 than in the other two groups on days 3–7 and in group 2 significantly higher than in group 3 on days 5–7 (Figure 1). Serum LH values in group 1 showed a gradual increase up to post-operative day 7 ($P < 0.01$). In group 2, LH values remained unchanged up to day 4 and increased significantly thereafter ($P < 0.05$), while in group 3 they did not increase at all post-operatively. Serum LH values in group 1 were significantly higher than in the other two groups on days 3–7 and in group 2 significantly higher than in group 3 on days 5–7 (Figure 1). At the end of observation (day 7), in group 1 serum FSH concentrations had increased 3.5-fold, and those of LH 2-fold.

Serum estradiol concentrations in group 1 declined significantly after the operation ($P < 0.05$) (Figure 1). In contrast, in groups 2 and 3, serum estradiol values (mean ± SEM) did not decrease after ovariectomy, but were maintained at levels (between 247 ± 45 and 345 ± 69 pmol/l) that were not significantly different from those before the operation (259 ± 51 and 250 ± 49 pmol/l respectively) and significantly higher than in group 1 on days 1–7 (Figure 1). The concentrations of progesterone showed an abrupt decrease in both groups 1 and 2 on day 1 following the operation ($P < 0.001$). In group 3, serum progesterone concentrations (mean ± SEM) did not change significantly post-operatively, remaining at levels (between 20.3 ± 2.1 and 24.6 ± 2.5 nmol/l) that were not significantly different from those before the operation (21.8 ± 4.3 nmol/l), but significantly higher than in groups 2 and 3 on days 1–7 (Figure 1).

In each GnRH experiment, the response of LH and FSH to GnRH was calculated as the net increase at 30 min above the basal value (ΔLH and ΔFSH respectively). Serum ΔLH and ΔFSH values before the operation did not differ significantly between the three groups (Figure 2). In group 1, ΔFSH values after the operation increased progressively up to post-operative day 7 ($P < 0.001$). In contrast, in groups 2 and 3, ΔFSH values remained unchanged up to days 5 and 6 respectively, after which the values increased significantly ($P < 0.05$). ΔFSH values were significantly higher in group 1 than in the other two groups on days 1–7 and in group 2 significantly
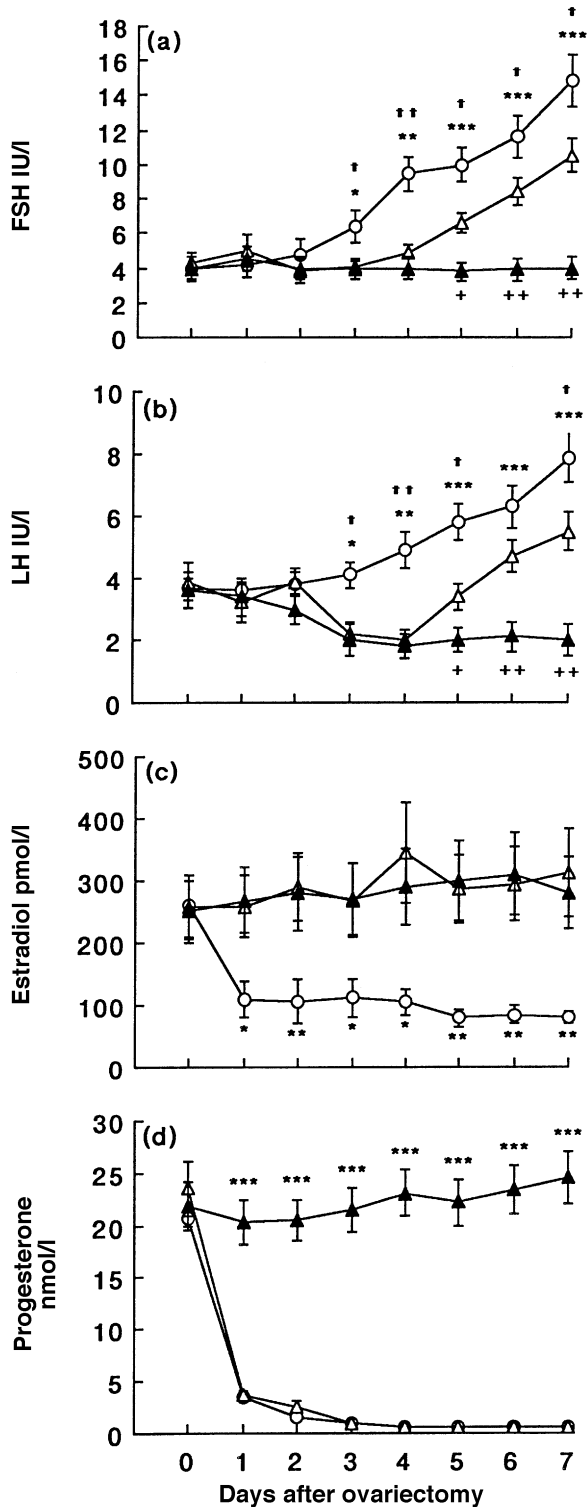
Figure 1. Serum FSH, LH, estradiol and progesterone values before and after bilateral ovariectomy plus hysterectomy performed in early to mid-luteal phase (day 0) in 18 normally ovulating women. Six of the women (O) received no hormonal treatment post-operatively (group 1), six (Δ) received estradiol through skin patches on days 0, 3 and 6 (group 2) and the remaining six (▲) received estradiol, as in group 2, plus progesterone intravaginally from days 0–6 (group 3). (**a**) and (**b**) *P < 0.05; **P < 0.01; ***P < 0.001 (difference from group 3). †P < 0.05; ††P < 0.01 (difference from group 2). +P < 0.05; ++P < 0.01 (difference from group 2). (**c**) *P < 0.05; **P < 0.01 (difference from groups 2 and 3). (**d**) ***P < 0.001 (difference from groups 1 and 2).
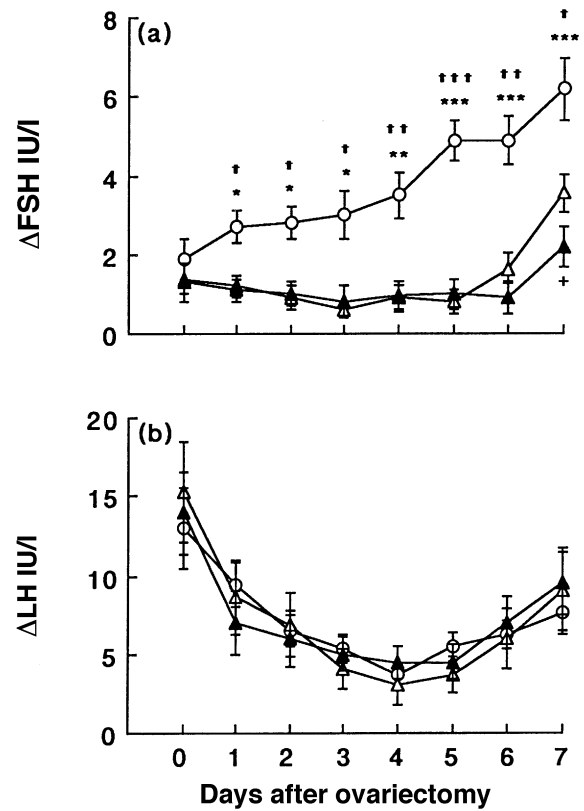
Figure 2. Responses of FSH (ΔFSH) and LH (ΔLH) at 30 min to GnRH (10 µg i.v.) before and after bilateral ovariectomy plus hysterectomy performed in early to mid-luteal phase (day 0) in 18 normally ovulating women. Six of the women (O) received no hormonal treatment post-operatively (group 1), six (Δ) received estradiol through skin patches on days 0, 3 and 6 (group 2) and the remaining six (▲) received estradiol, as in group 2, plus progesterone intravaginally from days 0–6 (group 3). *P < 0.05; **P < 0.01; ***P < 0.001 (difference from group 3). †P < 0.05; ††P < 0.01; †††P < 0.001 (difference from group 1). +P < 0.05 (difference from group 2).

higher than in group 3 on day 7 (Figure 2). In contrast to ΔFSH, ΔLH values showed the same pattern of changes in all three groups during the post-operative period (Figure 2). In particular, ΔLH values declined gradually up to day 4 (P < 0.01), increasing significantly thereafter up to day 7 (P < 0.05). At all points, ΔLH values did not differ significantly between the three groups.

Discussion

In the present study, the increasing basal values of FSH and LH following ovariectomy in the women who did not receive hormonal treatment are in agreement with our previous data (Alexandris et al., 1997). The greater increase in serum FSH values compared with LH is probably related to the lower metabolic clearance rate and higher production rate of FSH (Coble et al., 1969). In the women who were treated with estradiol, this increase was only postponed for a few days, thus indicating that estradiol alone contributes to, but is not sufficient to maintain, the ovarian suppressing effect on gonadotrophin secretion towards the mid-luteal phase of the cycle. There is only one study in the literature in which women

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were treated immediately after ovariectomy with estradiol that, similarly to the present study, prevented the increase in FSH and LH levels, but serial blood samples were taken only for the first four post-operative days (Kamel *et al.*, 1991). Low plasma FSH and LH concentrations were also maintained in women undergoing abdominal surgery, in whom, however, estradiol levels remained high post-operatively, not with exogenous estrogen, but with the conservation of the ovaries (Barlow *et al.*, 1981). When in the present study estradiol was combined with progesterone, there was no increase in FSH and LH levels for at least a week after ovariectomy. Since with these treatments the high luteal concentrations of estradiol and progesterone were maintained following ovariectomy, it is evident that both steroids are required to keep low secretion of gonadotrophins in the early to mid-luteal phase of the cycle. Whether progesterone alone might be able to suppress FSH and LH secretion is not known. However, previous studies have shown that although exogenous estradiol was able to postpone the inter-cycle rise of FSH during the luteal–follicular transition (Le Nestour *et al.*, 1993), progesterone suppressed LH or FSH levels in the follicular or luteal phases or in women with inactive ovaries only in the presence of estradiol (Soules *et al.*, 1984; Nippoldt *et al.*, 1989; de Ziegler *et al.*, 1992). The present results do not exclude the possibility that non-steroidal substances, such as inhibin A, participate in the control of gonadotrophin secretion, since the levels of this protein in women are increased during the luteal phase (Groome *et al.*, 1996), while data in monkeys have shown that inhibin A is able to suppress serum FSH levels *in vivo* (Molskness *et al.*, 1996). Although inhibin levels were not measured in the present study, they would be expected to decrease markedly following ovariectomy (Alexandris *et al.*, 1997).

The present study is the first to investigate the effect of estradiol and progesterone on pituitary sensitivity to GnRH in premenopausal women following bilateral ovariectomy. In terms of changes in GnRH-induced FSH secretion in the untreated (control) group of women, the pattern was similar to that previously reported, i.e. a continuous rise following ovariectomy (Alexandris *et al.*, 1997), thus illustrating a suppressing effect of the ovaries on the pituitary at that stage of the cycle. We infer that estradiol contributes to, but is not solely responsible for, this suppressing effect, since in the women who were treated with estradiol alone the increase in ΔFSH values was delayed but not abolished. Although with the addition of progesterone the period of the estradiol-induced suppression was extended, the rise in ΔFSH eventually occurred, suggesting that the two steroids together are not sufficient to mediate completely the ovarian suppressing effect on FSH and that other ovarian factors also play a role. A factor that could negatively affect GnRH-induced FSH secretion, at least *in vitro*, is inhibin A (Burger, 1992), the levels of which are normally high in the mid-luteal phase of the cycle (Groome *et al.*, 1996).

Our data confirm previous findings that following ovariectomy the pattern of changes in LH response to GnRH is different from that of FSH response (Alexandris *et al.*, 1997). The decreasing values of ΔLH in the women who did not receive hormonal treatment could be interpreted as indicating

that the ovaries exerted a sensitizing effect on LH secretion before the operation. However, the fact that the pattern of changes in ΔLH values was unaffected by treatment with the steroids suggests that estradiol and progesterone are not mediators of such an ovarian effect on LH response to GnRH in the mid-luteal phase. It is possible, therefore, that either a sensitizing effect of the ovaries on the pituitary is exerted through unspecified substances, or that the decrease in ΔLH values following ovariectomy is controlled by extra-ovarian mechanisms. Such mechanisms could be related to depleted stores of pituitary gonadotrophins as a result of the preceding mid-cycle LH surge that affected LH reserves more than those of FSH. The latter possibility is more likely based on previous data that a declining pattern of LH response to GnRH during the luteal phase of the cycle has been also reported in women with intact ovaries (Messinis *et al.*, 1993). The fact, however, that following ovariectomy the decline in ΔLH was interrupted shortly after the operation, i.e. ~4 days from the mid-luteal stage (Figure 2), while in women with intact ovaries the decline continues until the end of the luteal phase (Messinis *et al.*, 1993), indicates an earlier recovery of the pituitary in the ovariectomized than in the non-ovariectomized women. This suggests that GnRH-induced LH secretion in the luteal phase is not entirely unaffected by the ovaries. It is possible that a factor, different from estradiol and progesterone, maintains a low responsiveness of LH to GnRH towards the end of the cycle. Such a factor that specifically reduces LH response to GnRH is gonadotrophin surge attenuating factor (GnSAF) (Messinis and Templeton, 1989), but its role at that stage of the cycle needs to be further investigated.

So far, this factor has been purified from human follicular fluid as a protein of 12.5 kDa that shows identity to the carboxyl terminal fragment of human serum albumin (HSA) (Pappa *et al.*, 1999). More recently, we have demonstrated that human luteinized granulosa cells express the mRNA of HSA, thus supporting the initial characterization of GnSAF (Karligiotou *et al.*, 2001). Previous studies have shown that exogenous FSH stimulates the production of GnSAF *in vivo* both in the follicular and the luteal phases of the cycle (Messinis *et al.*, 1996, 1998). The evidence from these studies is that in the luteal phase GnSAF is produced by small antral follicles rather than by the corpus luteum. From a physiological point of view, it is possible that the activity of GnSAF increases in the late luteal phase of the cycle as a result of the effect of the inter-cycle rise of FSH.

Although estradiol and progesterone do not seem to play an important role in GnRH-induced LH secretion in the early to mid-luteal phase, an alternative approach to the explanation of the present data, particularly regarding basal gonadotrophin secretion, could be through a regulating effect of estradiol on progesterone receptors in the pituitary (Sprangers *et al.*, 1991). Such an effect might be mediated via estrogen receptor α, based on data in mice demonstrating the presence of high levels of receptor α mRNA and the absence of receptor β mRNA in the pituitary (Couse *et al.*, 1997).

In conclusion, the present study provides evidence that in the early to mid-luteal phase of the cycle, estradiol and progesterone are important components of the suppressing

effect of the ovaries on basal FSH and LH secretion. However, in terms of gonadotrophin response to GnRH, the study demonstrates for the first time that these two steroids participate in the control of FSH, but not of LH, secretion. It is possible that in the luteal phase the response of LH to GnRH is partly controlled by GnSAF.

Acknowledgement

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