Activity of gonadotrophin surge-attenuating factor during the luteal phase in superovulated women

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Gonadotrophin surge-attenuating factor (GnSAF) is a putative nonsteroidal ovarian factor that attenuates the luteinizing hormone (LH) surge in superovulated women. GnSAF bioactivity was studied during the luteal phase by investigating six normally ovulating women in two cycles—a spontaneous and a follicle-stimulating hormone (FSH)-treated cycle. In both cycles, the pituitary response to an acute intravenous injection (10 μg) of luteinizing-hormone-releasing hormone (LHRH) was investigated in late follicular (follicle size 16 mm), early luteal (day 5 after human chorionic gonadotrophin, hCG), midluteal (day 9 after hCG) and late luteal phase (day 12 or 13 after hCG). FSH was injected daily at the dose of 225 iu on cycle days 2, 3 and 4, and 150 iu thereafter. The increase in LH and FSH (mean ± SEM) 30 min after LHRH in the spontaneous cycles decreased significantly from early to late luteal phase and remained unchanged in the FSH-treated cycles. Increases in LH and FSH 30 min after LHRH were significantly attenuated in the FSH-treated compared with the spontaneous cycles in late follicular and luteal phases. Serum oestradiol and progesterone concentrations were significantly higher in the FSH than in the spontaneous cycles only in early, but not in mid- and late luteal phase. The pattern of serum oestradiol and progesterone changes during the luteal phase did not correlate with the increases in LH and FSH 30 min after hCG both in the spontaneous and the FSH cycles. These results suggest that GnSAF bioactivity is high during the luteal phase of superovulated cycles.

Introduction

In superovulated women, the endogenous luteinizing hormone (LH) surge is markedly attenuated (Messinis et al., 1985). The attenuation has been related to a putative nonsteroidal ovarian factor called gonadotrophin surge-attenuating factor (GnSAF) (Messinis and Templeton, 1989). The effect of GnSAF is exerted at least at the pituitary level where it reduces luteinizing-hormone-releasing hormone (LHRH)-stimulated gonadotrophin secretion (Messinis and Templeton, 1990a). Activity of GnSAF has been detected during the follicular phase of superovulated cycles (Messinis and Templeton, 1990b). In that respect, the role of GnSAF is not limited to the time of the midcycle LH surge, but is also extended at least to the first half of the cycle when it controls LHRH-induced gonadotrophin release by a negative effect (Messinis and Templeton, 1991a). Several studies using various in vitro bioassay systems have shown activity of GnSAF, distinct from inhibin, in steroid-free human follicular fluid from superovulated and spontaneous cycles (Busbridge et al., 1990; Fowler et al., 1990a; Knight et al., 1990).

During the luteal phase of superovulated cycles basal gonadotrophin secretion is markedly reduced (Messinis and Templeton, 1987). In one study, the midluteal pituitary response to a pharmacological dose of LHRH was also decreased (Martikainen et al., 1987). However, it is unclear whether GnSAF is produced during the luteal phase of superovulated cycles. Previous studies using submaximal doses of LHRH have investigated pituitary sensitivity (first pool) and reserve (second pool) throughout the human menstrual cycle (Wang et al., 1976; Yen and Lein, 1976). In superovulated cycles, the two pools of gonadotrophin secretion are markedly attenuated by GnSAF and this can be used as an in vivo bioassay to assess the activity of GnSAF in superovulated women (Messinis and Templeton, 1991b; Messinis et al., 1991).

In the present study a similar approach was used to investigate GnSAF bioactivity during the luteal phase of FSH superovulated cycles.

Materials and Methods

Patients

The study included six normally cycling volunteer women with longstanding unexplained infertility. Approval for the study was obtained from the local Ethical Committee and the patients gave written informed consent. Ovulation was confirmed in all women by serum progesterone measurements and ultrasound scans of the ovaries before admission to the study. The patients were investigated during two cycles—a spontaneous and a follicle-stimulating hormone (FSH)-treated cycle. FSH (75 iu FSH per ampoule; Metrodin: Serono Laboratories, Welwyn Garden City, Herts) was injected intramuscularly once
a day at the dose of 225 iu on cycle days 2, 3 and 4, and 150 iu thereafter, until the administration of human chorionic gonadotrophin (hCG). The latter was given as a single intramuscular injection of 5000 iu when the largest follicle exceeded 18 mm (19–20 mm) by ultrasound. In each cycle the pituitary response to an acute intravenous injection of 10 μg LHRH was investigated four times – in late follicular phase (largest follicle 16 mm by ultrasound), early luteal phase (day 5 after hCG), midluteal phase (day 9 after hCG) and late luteal phase (days 12 and 13 after hCG, respectively, in the FSH and the spontaneous cycles).

Blood samples in relation to each LHRH injection (time 0) were taken at −15, 0 and 30 min. The point of 30 min after LHRH (pituitary sensitivity) was chosen, as in a recent study (Messinis et al., 1991), because at that time the LH response to a single submaximal dose of LHRH is maximal both during spontaneous and FSH-treated cycles (Wang et al., 1976; Messinis and Templeton, 1991b). LH and FSH were measured in all blood samples taken during the LHRH experiments. Basal concentrations of oestradiol and progesterone (time 0) were also measured in both cycles.

**Hormone assays**

FSH and LH were measured in blood using immunoradiometric assays incorporating two high-affinity monoclonal antibodies. Kits were purchased from Serono Diagnostics Ltd, Woking (FSH MAIA clone and LH MAIA clone, respectively). The results are expressed as iu l⁻¹ of standards calibrated against the WHO 2nd IRP for human FSH (78/549) and the WHO 1st IRP for human LH (68/40). For oestradiol measurement in serum a solid-phase radioimmunoassay was used. Kits (Coat-A-Count Estradiol) were purchased from DPC (Diagnostics Products Corporation, Los Angeles). Values are expressed as pmol l⁻¹. Progesterone was measured in serum using the Amerlex-M Progesterone RIA kit (Amer sham International plc, Amersham) and results are expressed as nmol l⁻¹. The lower limits of detection for FSH, LH, oestradiol and progesterone were 0.5 iu l⁻¹, 0.3 iu l⁻¹, 30 pmol l⁻¹ and 0.25 nmol l⁻¹, respectively, while interassay and intra-assay coefficients of variation were 7.9 and 7.8%, 6.3 and 4.4%, 6.7 and 4.2% and 7.0 and 6.4%, respectively.

**Statistical analysis**

Statistical analysis of the results was performed using one-way analysis of variance. Variations in gonadotrophin response to LHRH were assessed from the spontaneous to the FSH cycles by calculating the variance ratio (F). Within the same group of cycles, differences in the response to LHRH pulses were assessed using Student’s paired t test. Although the arithmetic means of the hormone values are presented, in the statistical calculations hormone results were transformed into logarithms.

**Results**

In each LHRH experiment, basal LH and FSH values were calculated as the mean of the values at −15 and 0 min. The response to LHRH was calculated as the net increase at 30 min in LH (△ LH30) and FSH (△ FSH30) above the basal value (zero level). Basal LH and FSH values (mean ± SEM) (Fig. 1) in the spontaneous cycles decreased significantly from early (8.2 ± 0.7 and 6.1 ± 0.3 iu l⁻¹, respectively) to midluteal phase (5.0 ± 0.6 and 3.7 ± 0.3 iu l⁻¹, respectively, P < 0.01), with no significant change in late luteal phase. In the FSH cycles, basal LH values were significantly lower than in the spontaneous cycles in early (1.0 ± 0.2 iu l⁻¹, P < 0.001) and midluteal phase (2.6 ± 0.6 iu l⁻¹, P < 0.05), with no significant difference in late luteal phase (2.5 ± 0.4 iu l⁻¹). A significant increase in basal LH values from early to midluteal phase was seen in the FSH cycles (P < 0.05). In contrast to LH values, basal FSH values in the FSH cycles decreased significantly from early (2.1 ± 0.4 iu l⁻¹) to midluteal phase (0.8 ± 0.1 iu l⁻¹, P < 0.01) with a trend to increase in late luteal phase (1.2 ± 0.2 iu l⁻¹, P = 0.06). When, however, only paired samples were compared (n = 4), this increase was significant (0.6 ± 0.1 versus 1.2 ± 0.2 iu l⁻¹, P < 0.05). FSH values were significantly lower in the FSH than in the spontaneous cycles throughout the luteal phase (Fig. 1).

In the late follicular phase (Fig. 2), basal values of LH (mean ± SEM) in the FSH cycles (2.6 ± 0.5 iu l⁻¹) were significantly lower than in the spontaneous cycles (5.1 ± 0.4 iu l⁻¹),
Fig. 2. (a) Basal LH and FSH concentrations and (b) increases in LH and FSH concentrations (ΔLH and ΔFSH) response (30 min) to an acute intravenous injection (10 µg) of LHRH in late follicular phase of (■) spontaneous and (□) FSH-treated cycles in six normally ovulating women. Values are means ± SEM and represent net increase above the basal value. ***P < 0.05. **P < 0.01. *P < 0.001 compared with corresponding spontaneous cycles.

*P < 0.05*, while basal FSH values in the FSH cycles increased significantly owing to the exogenous administration of FSH. ΔLH30 in late follicular phase decreased significantly in the FSH-treated (5.6 ± 1.3 iu l⁻¹) compared with the spontaneous cycles (17.1 ± 3.6 iu l⁻¹, P < 0.05). ΔFSH30 was also significantly reduced in the FSH cycles (Fig. 2); however, this must be interpreted with caution because of the exogenous administration of FSH. The size of the leading follicle (mean ± SEM) on the day of the LHRH experiment in late follicular phase was similar in the spontaneous (16.5 ± 0.2 mm) and FSH cycles (16.7 ± 0.3 mm), while serum oestradiol concentrations were significantly higher in the FSH cycles owing to multiple follicular development (1540 ± 353 versus 526 ± 34 pmol l⁻¹, P < 0.01) (mean ± SEM number of follicles ≥ 12 mm 3.8 ± 0.6).

ΔLH30 and ΔFSH30 decreased significantly from early (28.4 ± 3.2 and 2.9 ± 0.4 iu l⁻¹, respectively) to mid- (21.0 ± 2.2 and 2.0 ± 0.2 iu l⁻¹, respectively, P < 0.05) and late luteal phase of the spontaneous cycles (16.0 ± 1.5 iu l⁻¹, P < 0.01 and 1.4 ± 0.1 iu l⁻¹, P < 0.05, respectively) (Fig. 3). The decrease from mid- to late luteal phase was also significant for both ΔLH30 and ΔFSH30 (P < 0.05). In the FSH cycles, ΔLH30 and ΔFSH30 were significantly attenuated compared with the spontaneous cycles in early (6.2 ± 1.1 and 0.5 ± 0.1 iu l⁻¹, respectively, P < 0.001), mid- (6.3 ± 1.3 iu l⁻¹, P < 0.01 and 0.5 ± 0.1 iu l⁻¹, P < 0.001, respectively) and late luteal (6.0 ± 0.7 iu l⁻¹, P < 0.05 and 0.4 ± 0.1 iu l⁻¹, P < 0.01, respectively) phases. ΔLH30 and ΔFSH30 did not change significantly in the FSH cycles from early to late luteal phase. Serum oestradiol and progesterone concentrations in the spontaneous cycles increased significantly from early to midluteal phase (P < 0.05) and decreased from mid- to late luteal phase (P < 0.05) in the FSH cycles. Serum oestradiol and progesterone concentrations in the spontaneous cycles were significantly higher in the FSH-treated (1642 ± 188 pmol l⁻¹ and 80.7 ± 15.7 nmol l⁻¹, respectively) than in the spontaneous cycles (444 ± 55 pmol l⁻¹, P < 0.001 and
23.8 ± 2.3 nmol l⁻¹, P < 0.01, respectively). In mid- and late luteal phases, serum values of these two steroids did not differ significantly between the two cycles.

The duration of the luteal phase (mean ± SEM) was significantly shorter in the FSH (11.8 ± 0.5 days) than in the spontaneous cycles (13.5 ± 0.2 days, P < 0.05). In two women, the late luteal phase LHRRH experiments was not performed owing to <12 day duration of the luteal phase.

Discussion

The present study confirms previous suggestions that the response of the pituitary to LHRH in the late follicular phase of superovulated cycles is markedly attenuated, supporting the contention that GnSAF is produced at this stage of the cycle (Messinis and Templeton, 1989, 1990a, b). This study, however, further demonstrates that pituitary sensitivity to LHRH is also markedly reduced throughout the luteal phase of superovulated cycles. Although oestriadiol itself or oestradiol in combination with progesterone can affect pituitary sensitivity and reserve in women (Yen et al., 1974; Lasley et al., 1975; Chang and Jaffe, 1978), in the present study the attenuation of LHRH-induced gonadotrophin release does not seem to be related to such a steroidal effect for the following reasons. First, the higher concentrations of oestriadiol and progesterone in the early luteal phase of the FSH-treated compared with the spontaneous cycles could maintain the pituitary in a state of low responsiveness to LHRH (Nippoldt et al., 1989). However, in the present study concentrations of these steroids in mid- and late luteal phase did not differ significantly between the two cycles, although at the same time pituitary sensitivity to LHRH was still markedly reduced. Second, the pattern of pituitary response to LHRH during the luteal phase of the FSH cycles did not correlate with that of steroidal changes, i.e. unchanged response from early to late luteal phase versus gradually decreasing steroidal concentrations. It, therefore, seems unlikely that the decreased pituitary sensitivity to LHRH in the FSH cycles is related to a steroidal effect during the luteal phase and it is probable that the attenuation of LHRH-induced gonadotrophin release represents increased GnSAF bioactivity.

It is difficult to assess whether inhibin produced during the luteal phase contributed to this attenuation. This nonsteroidal substance, which by definition causes the suppression of FSH secretion, under specific conditions also reduces the secretion of LH. However, animal studies in vitro and in vivo have shown that the secretion of LH, either basal or stimulated by LHRH, is less sensitive to the suppressing effect of inhibin than is the secretion of FSH (Farnworth et al., 1988; Knight et al., 1991). In addition, higher concentrations of inhibin are required for the suppression of LHRH-stimulated than for basal FSH release in rat pituitary cultures (Farnworth et al., 1988). The extent to which these data apply to humans is not known. Nevertheless, in the present study, basal concentrations of FSH in the FSH cycles increased in all women from mid- to late luteal phase (Fig. 1), while at the same time LHRH-induced gonadotrophin release remained markedly attenuated. These findings taken together with the above observations in animals suggest that inhibin is not the principal regulator of this attenuation during the luteal phase. In addition, several studies have shown that GnSAF contained in steroid-free human follicular fluid is different from inhibin (Busbridge et al., 1990; Fowler et al., 1990a; Knight et al., 1990) and has a molecular mass in the range of 10–30 kDa (Fowler et al., 1992). It is possible, therefore, that the attenuation of LHRH-induced gonadotrophin release during the luteal phase of superovulated cycles is the result of the synergistic effect of GnSAF and inhibin. Furthermore, hCG is probably excluded as a potential inhibitor of gonadotrophin secretion in the present study, since this hormone was administered to all women both in the spontaneous and the FSH-treated cycles.

Different patterns of pituitary response to LHRH and steroidal concentrations were also seen during the luteal phase of the spontaneous cycles. In these cycles, pituitary sensitivity to LHRH decreased significantly from early to late luteal phase and this has previously been reported by others (Wang et al., 1976). Progesterone is known to augment oestriadiol-stimulated pituitary sensitivity to LHRH shortly after its administration to women pretreated with oestriadiol (Lasley et al., 1975; Chang and Jaffe, 1978), and this can explain the enhanced gonadotrophin secretion in response to LHRH during the midcycle LH surge (Wang et al., 1976). Although the combined effect of oestriadiol and progesterone on gonadotrophin secretion becomes suppressive when applied for several days (Soules et al., 1984; Nippoldt et al., 1989), it is difficult to explain how both the increasing concentrations of these steroids during the first half and the decreasing concentrations during the second half of the luteal phase could have similar suppressing effects on the pituitary response to LHRH. It would be expected that pituitary sensitivity would either increase or, as with basal gonadotrophin concentrations, at least remain unchanged towards the end of the cycle when concentrations of these two steroids decline. It is likely, therefore, that apart from steroids, GnSAF is another regulator of LHRH-induced gonadotrophin release during the luteal phase of spontaneous cycles, but this requires further investigation. A previous study has provided preliminary evidence that GnSAF may be produced during the early stages of the human menstrual cycle (Messinis et al., 1991).

The source of GnSAF production during the luteal phase of superovulated cycles is not known. It is possible that, in a similar way to inhibin (McLachlan et al., 1987a, b; Tsonis et al., 1987), GnSAF is produced by the corpus luteum. However, another possibility is that some of the smaller follicles that do not have the capacity to respond to hCG and luteinize continue producing GnSAF during the early luteal phase. A previous study in vitro has shown higher activity of GnSAF in the follicular fluid of smaller compared with larger follicles (Fowler et al., 1990b). However, more studies are required to clarify the source of GnSAF production during the luteal phase of the cycle.

Furthermore, the present study confirms previous data that basal LH secretion is suppressed during the luteal phase of FSH superovulated cycles and demonstrates that endogenous FSH is also decreased. However, recovery of basal LH secretion occurred earlier than recovery of FSH secretion (Fig. 1) and this supports a differential control of the two gonadotrophins by the ovaries during the luteal phase. Previous studies have shown that basal LH secretion during the follicular phase is mainly controlled by steroidal factors, while both steroidal and non-steroidal substances are important for the control of FSH secretion (Messinis and Templeton, 1988, 1989).
In conclusion, the present study demonstrated that LHRRH-stimulated gonadotrophin release during the luteal phase of FSH superovulated cycles is markedly reduced. It is suggested that GnSAF is produced both during the follicular and the luteal phase of the human menstrual cycle.

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