Pituitary response to exogenous LHRH in superovulated women

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Summary. The response of the pituitary to exogenous LHRH was investigated in 9 normally ovulating women during the late follicular phase of a spontaneous (control) cycle, a cycle during treatment with clomiphene and a cycle during treatment with 'pure' FSH. During clomiphene treatment, basal FSH concentrations increased significantly up to Day 6 of the cycle and then decreased progressively while basal LH values showed a continuous rise. During treatment with FSH, basal LH concentrations decreased significantly. The response of both FSH and LH to LHRH showed a significant and quantitatively similar decrease during clomiphene or FSH administration as compared to the spontaneous cycles. It is suggested that basal secretion of FSH and LH is regulated by two separate mechanisms, and that an ovarian inhibitory factor(s) attenuates the response of both FSH and LH to exogenous LHRH and possibly the endogenous LH surge in superovulated cycles.

Keywords: LHRH; LH; FSH; pituitary; ovulation; man

Introduction

It has been reported that in superovulated monkeys the endogenous LH surge is blocked by an unknown factor produced by the hyperstimulated ovaries (Schenken & Hodgen, 1983; Littman & Hodgen, 1984). Studies in women have shown that the occurrence of an endogenous LH surge during superovulation induction is dependent on the treatment regimen; both clomiphene and unknown ovarian substances are important regulators (Messinis et al., 1985, 1986a; Messinis & Templeton, 1987a). However, when an endogenous LH surge occurs in superovulated women, it is markedly attenuated both in amplitude and duration as compared to spontaneous cycles (Messinis et al., 1985, 1986a; Messinis & Templeton, 1986).

Although the factors which are responsible for the attenuation of the LH surge are not known, evidence has been provided that they are produced by the hyperstimulated ovaries (Messinis et al., 1986b). Supraphysiological levels of oestradiol, achieved by exogenous administration of oestrogen to women, are not able to suppress LH secretion during the mid-cycle LH surge in spontaneous cycles (Messinis & Templeton, 1987b). Therefore, it seems probable that ovarian substances other than oestradiol are responsible for the attenuation of the LH surge in superovulated women, but the nature and mechanism of action are not known.

It has been established that the midcycle LH surge is the result of the positive feedback effect of oestradiol on the hypothalamic–pituitary system and that hypothalamic LHRH is an important component of that mechanism (Yen & Lein, 1976). The present study was undertaken to investigate the response of the pituitary to exogenous LHRH during induction of multiple follicular development, in an attempt to explain the mechanism of the attenuation of the endogenous LH surge in superovulated cycles.
Materials and Methods

Patients. Nine women with long-standing unexplained infertility volunteered for the study and gave written informed consent. All had normal ovulatory cycles as assessed by serum progesterone measurements and ultrasound scans of the ovaries for at least the previous 3 months before admission to the study. They were studied in 3 cycles, i.e. a spontaneous cycle (N = 9), a cycle during treatment with clomiphene (N = 9) and a cycle during treatment with ‘pure’ FSH (N = 8). Between the 2nd and the 3rd cycle the women had a break of at least 1 month. During the spontaneous cycle all patients were given 100 µg LHRH as an acute intravenous injection (between 09:00 and 12:00 h) in the late follicular phase. Monitoring of the cycle was performed with oestradiol measurements in serum and ultrasound scans of the ovaries. Blood samples (09:00 h) were taken on cycle Days 2, 4, 6, and 8 of the cycle and daily thereafter. Ultrasound scans of the ovaries were started on Day 8 of the cycle. Day 1 of the cycle was considered as the day of onset of the menstrual period.

During the 2nd cycle, the women were treated with clomiphene citrate, 100 mg/day, from cycle Days 2 to 10. On Day 10, LHRH was injected as in the spontaneous cycle. Day 10 was chosen based on information obtained in a previous study (Messinis & Templeton, 1988). Blood samples and ultrasound scans were performed as in the spontaneous cycle.

During the 3rd cycle, the women (N = 8) were given single intramuscular injections of ‘pure’ LHRH (75 i.u. FSH per ampoule: Metrodin, Serono Laboratories, Welwyn Garden City, Herts, UK) at a dose of 3 ampoules (225 i.u.) per day starting on Day 2 of the cycle. Monitoring of treatment was performed with oestradiol measurements in serum on Days 2 and 4 and daily thereafter and with daily ultrasound scans starting on Day 5. LHRH was injected as above when the largest follicle reached the size of at least 16 mm in diameter. On that day FSH injections were stopped.

In all 3 cycles, hCG was given as a single intramuscular injection of 5000 i.u. on the day after the LHRH injection. The objective during treatment with clomiphene and FSH was to increase the number of ovulations and therefore the chance of conception. In 4 cycles (2 clomiphene and 2 ‘FSH’), due to the development of 5 follicles >12 mm in diameter, hCG was withheld and the patients were advised to avoid intercourse. Ovulation was confirmed by ultrasound in the cycles in which hCG was injected. In all women, the daily blood samples were stopped on the day of hCG injection. In all morning blood samples, FSH, LH and oestradiol were measured. Progesterone and testosterone were also measured on the day of the LHRH injection. Blood samples in relation to LHRH injection (time 0) were taken at -15, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min. In these blood samples FSH and LH were measured.

Hormone assays. FSH was measured in serum by an immunoradiometric assay incorporating 2 high-affinity monoclonal antibodies. Kits were purchased from Serono Diagnostics Ltd, Woking, Surrey, UK (FSH MAIA clone). The results are expressed as mIU/ml of standards calibrated against the WHO 2nd IRP for human FSH (78/549). Cross-reaction of FSH injected to women with the antibody was 36%. LH was measured in serum by a similar immunoradiometric assay with two high-affinity monoclonal antibodies using kits purchased from Serono Diagnostics Ltd (LH MAIA clone). The results are expressed as mIU/ml of standards calibrated against the WHO 1st IRP for human LH (68/40). Measurement of oestradiol-17β in serum was done by a direct radioimmunoassay using a kit purchased from Biodata (Serono Diagnostics Ltd). Values are expressed as pmol/l. Progesterone was measured in serum using the Amerlex-M Progesterone RIA kit (Amersham International plc, Amersham, Bucks, UK) and results are expressed as nmol/l. For testosterone measurement the TESTO-CT radioimmunoassay kit purchased from International-CIS, High Wycombe, Bucks (UK) was used. Results are expressed as nmol/l. The lower limits of detection for FSH, LH, oestradiol, progesterone and testosterone were 0.25 mIU/ml, 0.15 miu/ml, 55 pmol/l, 0.25 nmol/l and 0.28 nmol/l respectively, while interassay and intra-assay coefficients of variation were 5-2% and 3-5%, 12-5% and 10-0%, 8-0% and 5-0%, 9-4% and 5-1% and 10-0% and 6-0%, respectively.

Statistical analysis. Statistical evaluation of the results was performed by one-way analysis of variance. Comparisons were performed between the untreated group of cycles (spontaneous) and each one of the treatment groups (clomiphene or FSH) separately as well as between clomiphene and FSH cycles. Variations in basal FSH and LH levels or in pituitary response to LHRH (Δ area) were assessed from one group to the other by calculating the variance ratio (F).

Results

In this study, basal values of LH and FSH in each LHRH experiment were calculated as the mean of the values at -15 and 0 min for each hormone. The response of the pituitary to LHRH was assessed by calculating the net increase in LH (ΔLH) and FSH (ΔFSH) concentrations above the basal value (zero level).

Response to LHRH

In all women the response of the pituitary to exogenous LHRH was investigated during the late follicular phase of the 3 cycles as assessed by follicle size and serum oestradiol concentrations. The
size of the largest follicle (mean ± s.e.m.) on the day of LHRH injection did not differ significantly among the three groups of cycles (spontaneous 17-1 ± 0·3 mm, range 16–19 mm, N = 9; clomiphene 17-2 ± 0·4 mm, range 16–19 mm, N = 9; FSH 17-5 ± 0·3 mm, range 16–19 mm, N = 8). However, serum oestradiol concentrations on the same day were significantly higher in the clomiphene (1952 ± 183 pmol/l) and the FSH cycles (1707 ± 226 pmol/l) than in the spontaneous cycles (677 ± 62 pmol/l) (P < 0·001), with no significant difference between the clomiphene and the FSH cycles. Treatment with clomiphene or FSH induced multiple follicular development in all women. The mean (± s.e.m.) number of follicles > 12 mm in diameter on the day of the LHRH injection did not differ significantly between the clomiphene (3-4 ± 0-4) and the FSH cycles (3-7 ± 0-4). Serum progesterone and testosterone concentrations (mean ± s.e.m.) on the day of the LHRH injection did not differ significantly among the three groups of cycles (spontaneous 2-6 ± 0·5 nmol/l and 2·0 ± 0·3 nmol/l, respectively; clomiphene 2-5 ± 0·3 nmol/l and 2-3 ± 0·2 nmol/l, respectively; FSH 2·6 ± 0·6 nmol/l and 1·8 ± 0·3 nmol/l, respectively).

**Basal FSH and LH concentrations**

Serum concentrations of hormones on Day 2 in the clomiphene and the FSH cycles did not differ significantly from those on Day 2 in the spontaneous cycles (Fig. 1). During treatment with clomiphene, FSH values increased up to Day 6 (F2,24 = 3·5, P < 0·05), but decreased thereafter up to Day 10 (F3,32 = 2-9, P < 0·05; Fig. 1a). Serum FSH concentrations on Day 6 were significantly higher in the clomiphene than in the spontaneous cycles (Fig. 1a). In contrast, LH concentrations during treatment with clomiphene showed a continuous increase up to Day 10 (F5,48 = 5·5, P < 0·01; Fig. 1a) and were significantly higher than in the spontaneous cycles from Days 6 to 10. Serum oestradiol concentrations increased more rapidly during clomiphene treatment and were significantly higher than in the spontaneous cycles from Days 6 to 10 (Fig. 1a).

The significant increase in serum FSH concentrations during FSH treatment, as compared to the spontaneous cycles, was due to the fact that this drug cross-reacted in the FSH assay and, therefore, evaluation of endogenous FSH secretion was not possible in this study. Serum LH values decreased significantly during treatment with FSH (F4,33 = 20·7, P < 0·001; Fig. 1b) in agreement with our previous data (Messinis & Templeton, 1987a). From Day 4, LH concentrations were significantly lower than in the corresponding spontaneous cycles (Fig. 1b). Serum oestradiol concentrations increased more rapidly in the FSH cycles and were significantly higher than in the spontaneous cycles from Day 4 (Fig. 1b).

**LH and FSH response to LHRH**

A markedly attenuated response of both LH and FSH to exogenous LHRH was seen in all 9 women during treatment with clomiphene as compared to the spontaneous cycles (Fig. 2). ΔLH area and ΔFSH area under the curve (mean ± s.e.m.) were significantly reduced in the clomiphene cycles (1768 ± 237 and 427 ± 48 mi.u./ml/180 min, respectively) as compared to the spontaneous cycles (6698 ± 1156, F1,16 = 17·4, P < 0·01; and 1728 ± 397 mi.u./ml/180 min, F1,16 = 10·5, P < 0·01, respectively). The percentage decrease in the area under the curve during treatment with clomiphene was similar for both LH (63·8 ± 8·1%) and FSH (67·0 ± 5·8%).

Similar to the clomiphene cycles, the response of pituitary LH to exogenous LHRH during treatment with FSH was also markedly attenuated in all 8 women as compared to the corresponding spontaneous cycles (Fig. 3). LH area under the curve was reduced significantly (2364 ± 696 vs 6737 ± 1311 mi.u./ml/180 min) as a result of the FSH treatment (F1,14 = 8·6, P < 0·05). The percentage decrease in the ΔLH area was 60·0 ± 6·6% and did not differ significantly from that seen in the corresponding 8 clomiphene cycles of the same women (62·1 ± 9·0%). The response of pituitary FSH to exogenous LHRH during treatment with FSH was not evaluated due to the exogenous administration of FSH to the women.
Fig. 1. Serum concentrations of FSH, LH and oestradiol during the follicular phase of treated cycles (●) with (a) clomiphene and (b) FSH in 9 normally ovulating women. Comparison with untreated spontaneous cycles (○). Values are mean ± s.e.m. for 9 women in (a) and 8 in (b) except for Day 8 in the FSH cycles for which N = 5. ***P < 0.05, **P < 0.01, *P < 0.001 (compared to corresponding spontaneous cycles).

On the day of the LHRH injection, basal LH concentrations were significantly lower in the FSH (1.4 ± 0.1 mi.u./ml, N = 8) than in the spontaneous (5.0 ± 1.0 mi.u./ml, N = 9) (P < 0.01) and the clomiphene (8.1 ± 0.5 mi.u./ml, N = 9) (P < 0.01) cycles and significantly higher in the clomiphene than the spontaneous cycles (P < 0.05). Despite these differences, when the absolute values instead of the net increase in LH and FSH were taken into account, the pattern of pituitary response to LHRH in each cycle was similar to that described above (data not shown).

Discussion

In the present study, clomiphene was given to normally cyclic women for more than 5 days because such a treatment induces a substantial degree of ovarian hyperstimulation and results in sustained blockage of both the negative and positive feedback effects of oestradiol (Messinis & Templeton, 1988). The disparity between basal FSH and LH concentrations during treatment with clomiphene is in agreement with our previous data and suggests that basal secretion of these two gonadotrophins is regulated by two separate mechanisms (Messinis & Templeton, 1987a, 1988). The results in the clomiphene and FSH cycles (Fig. 1) are compatible with the concept that during the
Fig. 2. LH and FSH responses to an acute intravenous injection (time 0 min) of 100 µg LHRH in the late follicular phase of spontaneous cycles (○) and clomiphene-treated cycles (●) in normally ovulating women. Values are mean ± s.e.m. for 9 women and represent net increase above the basal level.

Fig. 3. LH response to an acute intravenous injection (time 0 min) of 100 µg LHRH in the late follicular phase of spontaneous cycles (○) and FSH treated cycles (●) in normally ovulating women. Values are mean ± s.e.m. for 8 women and represent net increase above the basal level.
early follicular phase of the menstrual cycle the negative feedback effect of the ovaries on both FSH and LH secretion is mediated by oestradiol, while from the mid-follicular phase FSH secretion is mainly controlled by a non-oestrogenic ovarian substance. It is possible that this FSH-suppressing factor is inhibin, a selective inhibitor of FSH secretion (McLachlan et al., 1988).

The present results show that superovulation induction with clomiphene or FSH results in a marked attenuation of the pituitary response to exogenous LHRH. Since LHRH is an important component of the oestradiol positive-feedback effect, it is possible that this is at least part of the mechanism which attenuates the endogenous LH surge in cycles superovulated with similar treatment regimens (Messinis et al., 1985; Messinis & Templeton, 1986). Since clomiphene exerts antioestrogenic effects on the hypothalamic–pituitary system (Adashi, 1984), it is suggested that supraphysiological concentrations of oestradiol are not responsible for the decreased pituitary response to LHRH. On the contrary, oestradiol is known to sensitize the pituitary to LHRH from the early to late follicular phase of the normal menstrual cycle (Yen et al., 1972; Jaffe & Keye, 1974) and artificially induced supraphysiological concentrations of oestradiol in spontaneous cycles did not affect the characteristics of the midcycle LH surge (Messinis & Templeton, 1987b). It is possible therefore that the attenuation of the pituitary response to LHRH is due to a non-oestrogenic ovarian substance(s) secreted under the hyperstimulation process.

A similar degree of ovarian hyperstimulation was achieved in the clomiphene and FSH cycles. Although in the clomiphene treatment cycles the attenuation of the response to LHRH could be due to the antioestrogenic effect of this compound (Wang & Yen, 1975), this possibility is unlikely due to the similar degree of attenuation in these and the FSH cycles. It seems therefore that the attenuation is mainly related to the degree of ovarian hyperstimulation rather than to the kind of treatment regimen and this is compatible with our previous data in superovulated cycles with an attenuated endogenous LH surge (Messinis et al., 1986b).

The fact that the decrease in the response to LHRH was quantitatively similar for both LH and FSH demonstrates that, in contrast to basal values, the suppression of the two gonadotrophins during LHRH stimulation is due to the same ovarian factor(s). It is not clear, however, whether this factor(s) exerts its effects at the pituitary or the hypothalamic level. Although still unspecified, the fact that this factor(s) seems to be different from oestradiol and that serum progesterone and testosterone concentrations were similar in spontaneous and hyperstimulated cycles suggests that the factor(s) is non-steroidal. This is further supported by data showing that pig follicular fluid contains a non-steroidal substance, different from inhibin, which suppresses the LH response to LHRH by rat pituitary cells in vitro (Danforth et al., 1987).

Since the responsible factor(s) affects equally the secretion of LH and FSH, we refer to this as gonadotrophin surge-attenuating factor (GnSAF). It is not known whether this putative factor(s) is the same as a gonadotrophin surge-inhibiting factor in monkeys (Sopelak & Hodgen, 1984). Species differences may be important. Although in monkeys the endogenous LH surge during superovulation induction is blocked invariably (Schenken & Hodgen, 1983), it is not clear whether in superovulated women the blockage and the attenuation of the LH surge are controlled by the same mechanism (Messinis & Templeton, 1987a).

The dose of LHRH used in this study, although pharmacological, is similar to that in previous studies (Yen et al., 1972; Jaffe & Keye, 1974). A dose–response relationship of LH to LHRH has been reported in normal men (Kastin et al., 1971). However, the possibility that pharmacological doses of LHRH induced an abnormal LH secretion burst is not excluded. In this study, FSH and LH measurements were performed by immunoassay. Whether bioassay of the same samples would provide different results is not known. Finally, for the interpretation of the results of the present study we assumed that the half-lives of LH and FSH do not change in the various conditions.

In conclusion, the present study shows that during superovulation induction in normal cyclic women, basal secretion of LH and FSH is regulated by two separate mechanisms. However, the response of both FSH and LH to LHRH is suppressed by the same ovarian inhibitory factor(s). This is possibly part of the mechanism which attenuates the endogenous LH surge in superovulated cycles.
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References


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