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GONADOTROPHIN RESPONSES TO GnRH PULSES IN HYPOGONADOTROPHIC HYPOGONADISM: LH RESPONSIVENESS IS MAINTAINED IN THE PRESENCE OF LUTEAL PHASE CONCENTRATIONS OF OESTROGEN AND PROGESTERONE

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SUMMARY

LH pulse secretion changes during the menstrual cycle from a rapid regular pattern in the follicular phase to a slower and irregular pattern in the luteal phase. To determine whether the irregular LH pulse pattern in the luteal phase reflects altered GnRH secretion or altered pituitary responsiveness to GnRH, we gave low dose GnRH pulses (25 ng/kg i.v.) every 2 h or every hour for 10 or 12 d to three women with isolated GnRH deficiency. After 4 d of GnRH alone, oestradiol (E_2) was given and after 6 d progesterone (P) was added to mimic the hormonal milieu of the luteal phase. LH and FSH were measured every 4 h throughout and also every 20 min for 6 or 12 h, before and after GnRH alone (day 0 and day 4), after E_2 (day 6), and after E_2+P (day 10 and day 12). Both GnRH pulse frequencies resulted in a rapid increase in plasma FSH to peaks on day 4 (every 2 h) and day 2 and 3 (every hour). FSH concentrations then declined as plasma E_2 rose to 50–80 pg/ml reflecting the selective inhibitory effect of E_2 on FSH release. Plasma LH was also increased after the hourly GnRH injections and this regimen was associated with a more rapid rise in E_2 reflecting follicular maturation. In contrast to the differences in mean hormone concentrations, administration of GnRH at both frequencies resulted in sustained one-on-one responsiveness of LH that was maintained in the presence of both oestrogen and progesterone at mid-luteal phase concentrations. We conclude that the slow frequency of LH pulses observed during the luteal phase reflects decreased GnRH pulse frequency rather than impaired pituitary responsiveness to GnRH.

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Plasma concentrations of pituitary gonadotrophins (FSH and LH) fluctuate in a pulsatile manner throughout the menstrual and oestrous cycles of humans and other species (Cargille *et al.*, 1969; Johansson *et al.*, 1971; Midgley & Jaffe, 1971; Yen *et al.*, 1972; Santen & Bardin, 1973; Baird, 1978; Backstrom *et al.*, 1982; Reame *et al.*, 1984; Filicori *et al.*, 1986). Marked differences have been found in the patterns of LH secretion during the follicular and luteal phases of the cycle: in the former, LH pulses are regular, frequent (every 50–100 min), and of uniform amplitude; whereas the luteal phase is characterized by infrequent, irregular pulses of variable amplitude. The mechanisms of these different secretory patterns are unclear but they have been presumed to reflect altered GnRH secretion produced by the changing steroid milieu. This view stems mainly from studies showing preserved pituitary responsiveness to a single (Nillius & Wide, 1972) or up to five pharmacological pulses of GnRH given at 2-h intervals during the luteal phase of the cycle (Wang *et al.*, 1976). The possibility remains, however, that luteal phase gonadal steroid concentrations may alter gonadotrophin responses to repeated physiological pulses of GnRH by a direct effect at the pituitary level.

The present study was designed to determine whether pituitary responsiveness to GnRH was maintained on a one-to-one basis in the presence of a steroid milieu similar to that present during the luteal phase of the cycle. If uniform LH responsiveness were maintained, the data would provide strong support for the supposition that the slow, irregular LH pulse patterns normally observed in the luteal phase are representative of altered hypothalamic GnRH secretion, rather than changed pituitary sensitivity to GnRH. To avoid problems of interpreting the results in the presence of endogenous GnRH secretion, we studied three female patients with isolated gonadotrophin deficiency who received 'physiological' doses of GnRH (25 ng/kg per pulse) at hourly or two-hourly intervals throughout a 10–12 d period.

MATERIALS AND METHODS

Patients studied

Patient 1 was an 18-year-old woman with primary amenorrhoea and hyposmia. The patient was adopted and no family history was available. Physical examination revealed lack of pubertal development (Tanner stage 1) and an otherwise healthy woman (height 155·6 cm, weight 47·4 kg).

Patient 2 was a 21-year-old woman who presented initially with primary amenorrhoea and lack of pubertal development. She had an intact sense of smell. Her 18-year-old sister also had absent sexual development and primary amenorrhoea. The patient had previously received oestrogen therapy for 2 years which resulted in the development of secondary sexual characteristics (Tanner stage 4). Physical examination was normal (height 154 cm, weight 54·4 kg). She had not received any medication for 1 year before the study.

Patient 3 was a 20-year-old woman with primary amenorrhoea and delayed pubertal development. Her sense of smell was intact. There was no family history of infertility, however one sister was under treatment for irregular menses. Physical examination revealed minimal breast development (Tanner stage 2). The remainder of the examination was normal (height 165·1 cm, weight 47·9 kg).

All three patients had normal prolactin concentrations, thyroid function tests and imaging of the sella turcica (sella roentgenograms in patients 1 and 2, and CT scan in patient 3). Patient 1 also had normal GH, cortisol and TSH response to a combined insulin hypoglycaemia/TRH test.

Study protocol

The studies were approved by the Institutional Review Board and performed at the Clinical Research Center of The University of Michigan Hospitals. Written informed consent was obtained from the patients before the study.

Patients 1 and 2 were studied for 10 d and patient 3 for 12 d. Pulse injections of GnRH (25 ng/kg body weight) in normal saline were given intravenously beginning on day 1 and continued throughout the study. Injections were given every 2 h to patient 1 and every hour to patients 2 and 3. On day 0 (control day) saline pulses were given at the same frequency.

At 2000 h on day 4, micronized 17β-oestradiol (Estrace, Mead Johnson Laboratories, Evansville, Indiana, USA) was begun orally with a loading dose of 2 mg and then 1 mg was continued every 8 h throughout the study (days 5–10 in patients 1 and 2, days 5–12 in patient 3). At 2000 h on day 6 progesterone in oil was begun with a loading dose of 0·4 mg/kg i.m. and 0·2 mg/kg was given every 12 h on subsequent days.

Blood for measurement of LH and FSH was obtained every 4 h throughout the study and every 20 min for variable periods of time on each study day to assess the gonadotrophin response to the GnRH injections. In patients 1 and 2 the periods of frequent sampling were for 12 consecutive hours on day 0 (2400–1200 h), and on days 4, 6, and 10 (0800–2000 h), and for two consecutive hours on each other day. For patient 3 the periods of frequent sampling were for 8 h (2400–0400 h, 0800–1200 h) on day 0, six consecutive hours (0800–1400 h) on days 4, 6, 10 and 12, and for two consecutive hours (0800–1000 h) on each other day.

Serum levels of oestradiol (E_2), progesterone (P) and oestrone (E_1; patients 1 and 2 only) were measured every 12 h (0800 and 2000 h) throughout the study. All blood samples were centrifuged and the plasma was separated and stored at −20°C until assayed.

Hormone assays and data analysis

Plasma LH (Midgley, 1966), FSH (Midgley, 1967), E_1, E_2 (England *et al.*, 1974), and P (Niswender, 1973) were measured by established radioimmunoassay methods. Gonadotrophin concentrations are reported in mIU of the Second International Reference Preparation of hMG after conversion from LER 907, which was used as the assay standard. For LH and FSH the intra-assay coefficient of variation (CV) was inversely proportional to the measured amount and for concentrations of 2·5, 7·5, and 15 mIU/ml averaged 20%, 9·5% and 6·6%, respectively. The interassay CV was 11% for the LH and FSH assays and 15% for the E_2 and P assays. For statistical analyses, values below assay sensitivity were assigned a value of assay sensitivity. E_1 and E_2 concentrations are expressed as pg/ml (1 pg/ml oestrone = 3·7 pmol/l; 1 pg/ml oestradiol = 3·7 pmol/l). Progesterone concentrations are expressed as ng/ml (1 ng/ml progesterone = 3·2 nmol/l).

A significant LH pulse was defined as a rise from nadir to peak within 40 min by a

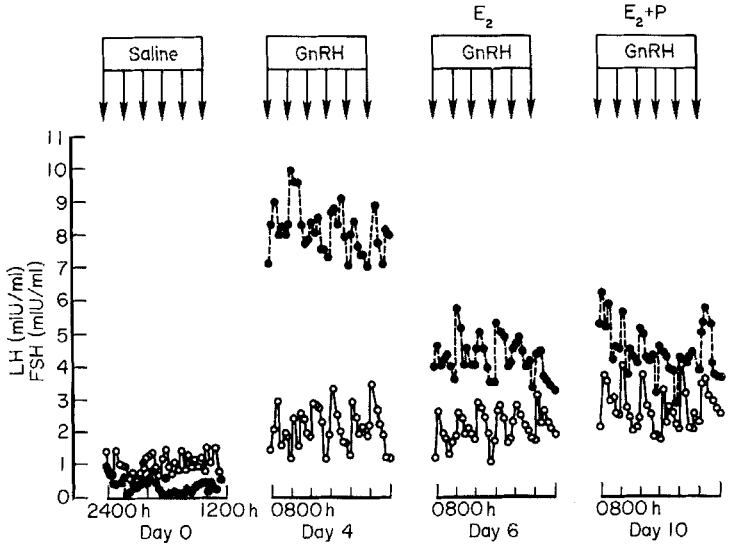

Fig. 1. FSH (●) and LH (○) responses to GnRH pulses in patient 1 during 12 h frequent (q20min) sampling periods on days 0, 4, 6 and 10.

minimal detectable increment (MDI) as determined by twice the intra-assay coefficient of variation (CV) of replicate samples from each individual patient (MDI = mean LH value × CV × 2) (Reame *et al.*, 1984). The MDI calculated in this manner is very similar to that obtained by using the criterion of three times the assay CV above the LH nadir value. LH and FSH pulse amplitudes were determined by the difference between nadir and peak values for each significant gonadotrophin pulse. Gonadotrophin pulse amplitudes were compared using Student's *t*-test.

RESULTS

Figures 1, 2 and 3 show plasma FSH and LH concentrations during the periods of 20 min sampling in patients 1, 2 and 3, respectively. Frequent sampling periods were selected on day 0 (saline pulses), day 4 (after 4 d of GnRH pulses), day 6 (2 d after the addition of Estrace), day 10 (after 4 d of combined E_2 and P administration) and in patient 3, on day 12 (after 6 d of combined E_2 and P administration).

Significant LH and FSH pulses were not present on day 0 (saline injections) in all patients, however a significant LH response occurred after every GnRH injection on days 4, 6, 10 and 12. The amplitude of LH pulses on these days were comparable over time in patient 1 ($1{\cdot}5 \pm 0{\cdot}4$, $1{\cdot}1 \pm 0{\cdot}2$ and $1{\cdot}6 \pm 0{\cdot}2$ mIU/ml, mean ± SEM) and in patient 3 ($3{\cdot}2 \pm 0{\cdot}6$, $3{\cdot}5 \pm 0{\cdot}8$, $4{\cdot}2 \pm 0{\cdot}5$, $4{\cdot}7 \pm 1{\cdot}0$ mIU/ml). In patient 2, LH pulse amplitude was similar on days 4 and 6 ($3{\cdot}4 \pm 1{\cdot}0$ and $4{\cdot}4 \pm 1{\cdot}3$ mIU/ml) but was higher on day 10 ($6{\cdot}1 \pm 2{\cdot}4$ mIU/ml, $P < 0{\cdot}0005$ vs day 4; $P < 0{\cdot}05$ vs day 6). FSH responses occurred after each GnRH injection in patient 1 and the amplitudes of each FSH pulse were comparable ($1{\cdot}2 \pm 0{\cdot}2$, $1{\cdot}2 \pm 0{\cdot}3$, $1{\cdot}0 \pm 0{\cdot}1$ mIU/ml). In patients 2 and 3, FSH pulses occurred irregularly in response to hourly GnRH injections on days 4, 6, 10 and 12. On day 10 in

patient 2, FSH was below assay detectability for six of the 12 h studied and significant FSH responses did not occur.

The mean plasma gonadotrophin concentrations (mean of every 4 h samples between 0800–2000 h and 2000–0800 h) and the oestradiol and progesterone concentrations during the 10–12 d study period for all three patients are shown in Fig. 4. In patient 1, (GnRH injections every 2 h) gonadotrophin levels were low (< 1 mIU/ml) on the control day. Preinjection levels of FSH began to increase after only two or three GnRH pulse injections and continued to rise to a peak (8·3 mIU/ml) on day 4. After the first dose of Estrace, plasma FSH declined through day 6 after which time FSH concentrations remained stable (approximately 4 mIU/ml). Plasma LH concentrations increased slowly and continued to rise through day 10 reaching a maximum of 2·3 mIU/ml. In patient 2 and 3, (hourly GnRH injections) mean gonadotrophin concentrations were also low during saline injections on the control day (< 1·6 mIU/ml). The increase in preinjection levels of FSH occurred more rapidly than in patient 1, reaching a peak concentration of 9·1 mIU/ml at 0800 h on day 2 in patient 2 and 13·9 mIU/ml at 0800 h on day 3 in patient 3. FSH concentrations began to decline before administration of Estrace and continued to decrease for the remainder of the study reaching undetectable levels by day 8 in patient 2, and 1·9 mIU/ml by day 12 in patient 3. Preinjection levels of LH increased rapidly in these two patients and continued to rise to day 6. At this time in patient 2, a few hours after the first P injection was given, a large LH peak occurred (27 mIU/ml) and persisted for 6 h. For the remainder of the study mean LH concentrations varied between 7 and 14·4 mIU/ml.

Plasma oestradiol rose little in patient 1 and was only 45 pg/ml before Estrace administration on day 4. Subsequently, E_2 rose to a maximum of 200 pg/ml on days 8 and 9. The low E_2 value on day 10 (40 pg/ml) was related to the omission of one Estrace dose.

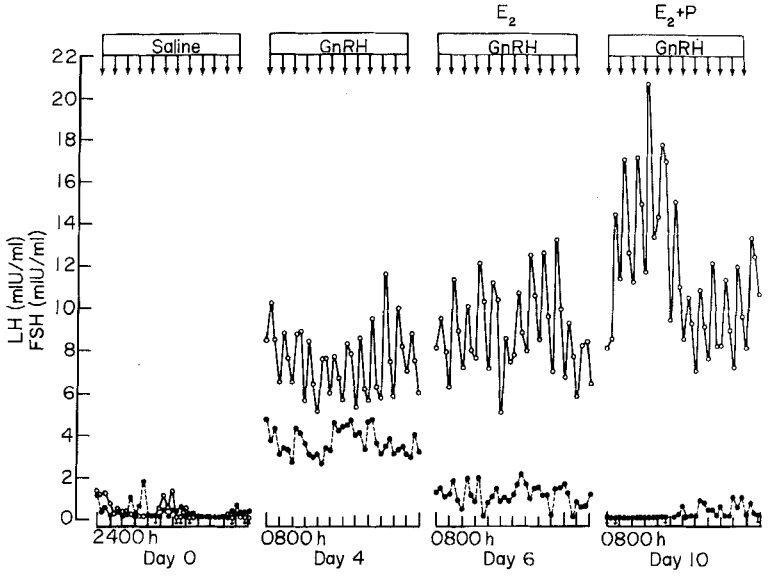

Fig. 2. FSH (●) and LH (○) responses to GnRH pulses in patient 2 during 12 h frequent (q20min) sampling periods on days 0, 4, 6 and 10.

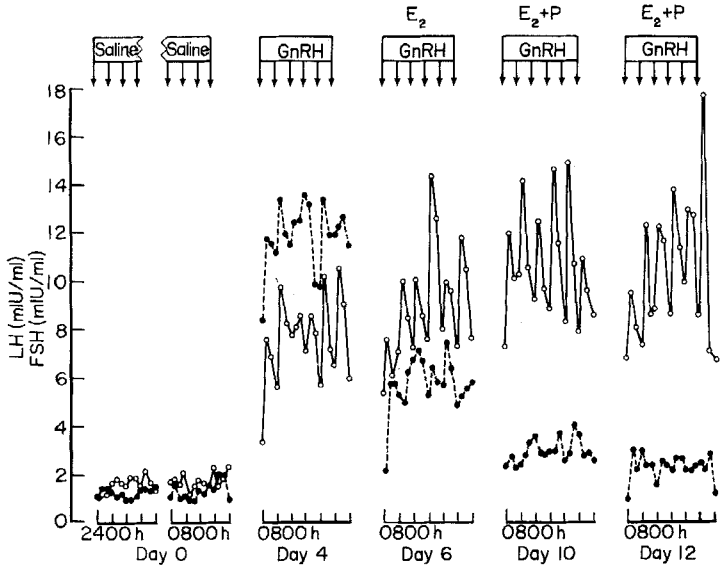

Fig. 3. FSH (●) and LH (○) responses to GnRH pulses in patient 3 during 6 h frequent (q20min) sampling periods on days 0, 4, 6, 10 and 12.

Plasma E_2 rose to values greater than 80 pg/ml in patients 2 and 3 before the first Estrace dose. E_2 continued to rise and reached 800 pg/ml on day 10 in patient 2 and 550 pg/ml in patient 3. In patients 1 and 2 plasma E_1 values were unchanged throughout the first four days of the study and averaged 88 ± 14 pg/ml in patient 1 and 135 ± 9 pg/ml in patient 2 (mean $\pm$ SEM). As expected, plasma E_1 levels were increased after Estrace administration and averaged 707 ± 84 and 529 ± 69 pg/ml in patients 1 and 2, respectively.

Plasma progesterone was initially low ($\leqslant 0{\cdot}4$ ng/ml) in all patients and remained so before P administration in patients 1 and 3. In patient 2 plasma P rose to 0·7–2 ng/ml on days 5 and 6 before P administration. After P injections were begun on day 6, similar concentration of P were present in all three patients, and varied between 6 and 15 ng/ml.

DISCUSSION

In this study, we aimed to define the effect of E_2 and P on the patterns of gonadotrophin secretion in response to pulsatile administration of GnRH in three patients with GnRH deficiency. Specifically, the study was designed to determine whether consistent LH responsiveness to GnRH pulses was maintained in the presence of E_2 and P concentrations to mimic the luteal phase steroid milieu. The GnRH dose (25 ng/kg body weight) was chosen to simulate the presumed pituitary portal concentrations of GnRH (Kelch *et al.*, 1975; Carmel *et al.*, 1976; Neill *et al.*, 1977; Eskay *et al.*, 1977; Sarkar *et al.*, 1978; Marshall & Kelch, 1979; Crowley & McArthur, 1980; Valk *et al.*, 1980, 1981) and the injection intervals used (60–120 min) were similar to LH pulse frequencies observed during the follicular phase of normal menstrual cycles (Midgley & Jaffe, 1971; Yen *et al.*, 1972; Filicori *et al.*, 1982; Reame *et al.*, 1984; Soules *et al.*, 1984).

LH and FSH concentrations tended to rise more rapidly after the faster frequency of GnRH stimulation. The peak level and decline in plasma FSH was not influenced by

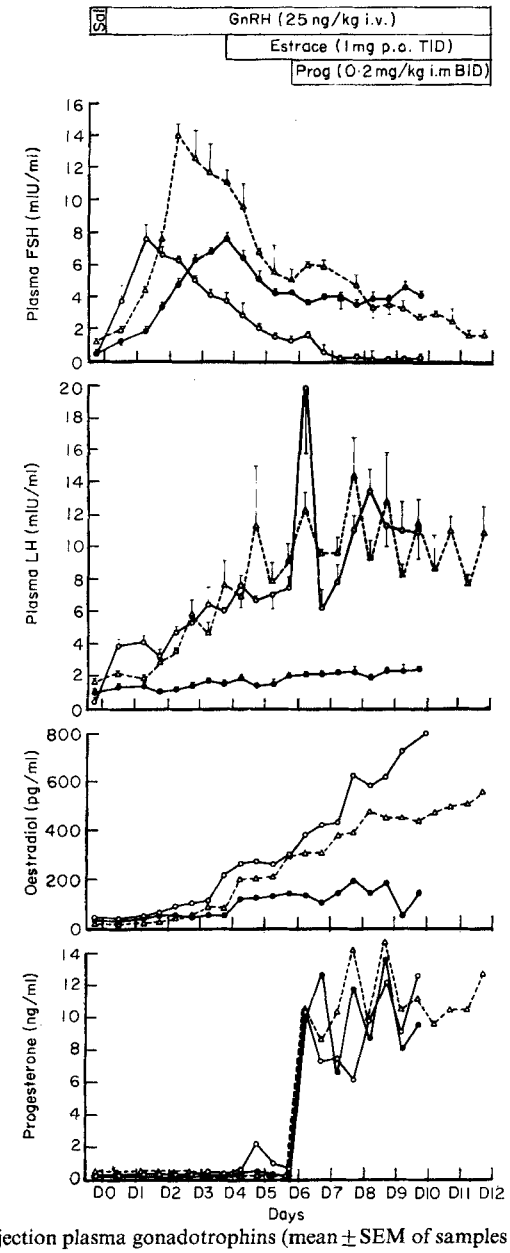

Fig. 4. Mean preinjection plasma gonadotrophins (mean ± SEM of samples every 4 h from 0800 to 2000 h and 2000 to 0800 h) are shown in the upper two panels, and gonadal steroid concentrations at 0800 h and 2000 h are shown in the lower two panels. Closed symbols are data from patient 1 (GnRH q2h) and open symbols are data from patients 2 (○) and 3 (△) (GnRH q1h). TID, Three times daily; BID, twice daily.

GnRH pulse frequency and FSH began to decline in all patients when plasma E_2 levels exceeded 50–80 pg/ml. This is in agreement with our previous observations that E_2 selectively inhibits FSH secretion (Marshall *et al.*, 1983), and a further increase in E_2 in

patient 2 (from endogenous and exogenous sources, as reflected in the lower E_1/E_2 ratio observed on days 5–10) resulted in a fall in FSH to unmeasurable levels.

Consistent one-to-one responses of LH to GnRH pulses given at both frequencies were observed in the presence of luteal phase concentrations of P and in the presence of a wide range of plasma E_2 levels (45–800 pg/ml). These findings support and extend previous observations which showed preserved pituitary responses to a single (Nillius & Wide, 1972) or up to five successive pharmacological doses of GnRH during the luteal phase in normal cycling women (Wang *et al.*, 1976). Observations in normal cycling women suggest that the duration of pituitary exposure to P used in the present study (4 and 6 d of mid-luteal phase concentrations of P) should be adequate for any effect of P on pituitary responsiveness to be manifest. In normal women, LH secretion shows a slow frequency, irregular pattern by day 18–19 of the cycle (Reame *et al.*, 1984) and this pattern has been noted 2 d after the mid-cycle LH surge (Filicori *et al.*, 1984). Similarly, LH pulse frequency is reduced after 5 d of P administration to oestrogen-replaced ovariectomized ewes (Goodman *et al.*, 1981). Overall, these data indicate that the decrease in LH pulse frequency seen during the normal luteal phase of the cycle reflects an effect of ovarian steroids on the frequency of GnRH secretion and not an alteration in the ability of the pituitary to maintain responsiveness to GnRH.

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