EFFECTS OF CHANGING GONADOTROPHIN-RELEASING HORMONE PULSE FREQUENCY ON GONADOTROPHIN SECRETION IN MEN

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SUMMARY

To investigate the effects of alterations in GnRH pulse frequency on gonadotrophin secretion, we administered low dose GnRH pulses (25 ng/kg) at hourly or 2-hourly frequencies to eight normal men. All subjects received GnRH pulses i.v. every 2 h for 88 h. Following this, exogenous GnRH was discontinued in four normal men (Group A, GnRH withdrawal), and the frequency of GnRH injections was increased to one pulse every hour for 24 h in the other four normal men (Group B, hourly GnRH). Blood samples were obtained every 20 min for LH and FSH and every 12 h for testosterone (T) and oestradiol (E_2). Plasma LH increased in all subjects during injection of GnRH pulses every 2h. Withdrawal of GnRH pulses in Group A men was accompanied by a fall in mean LH, reductions in LH pulse amplitude ($\bar{x} \pm$ SEM: control $6 \cdot 5 \pm 1 \cdot 0$; GnRH withdrawal $4 \cdot 0 \pm 0 \cdot 5$ mIU/ml) and pulse frequency (control $5 \cdot 5 \pm 0 \cdot 2$; GnRH withdrawal $3 \cdot 5 \pm 0 \cdot 7$ pulses/12 h), and an increase in plasma E_2 (control 122 ± 15; GnRH withdrawal 340 ± 37 pmol/l). Gonadotrophin responses to GnRH (25 ng/kg) were normal when tested 32 h after GnRH withdrawal. Injection of hourly GnRH pulses in Group B men was accompanied by a time-dependent change in mean LH, which transiently rose, then fell, and subsequently rose to a plateau during the second 12 h period of hourly GnRH. The final rise in LH was accompanied by an increase in LH frequency to $11 \cdot 8 \pm 0 \cdot 3$ pulses/12 h. These data suggest that: (1) increases in gonadal steroids decrease LH secretion by reducing the amplitude and frequency of endogenous GnRH pulses; and (2) the normal adult male pituitary requires approximately 12 h to initiate a sustained increase in LH secretion in response to a doubling in GnRH pulse frequency.

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Previous studies have shown that the frequency of LH pulses changes during human sexual maturation and the menstrual cycle. Studies in early pubertal children have suggested that LH pulse frequency and amplitude increase transiently during sleep (Corley et al., 1981; Kelch et al., 1985; 1987). During the menstrual cycle, LH pulse frequency increases significantly between the early and late stages of the follicular phase and slows to one pulse every 90–360 min in the luteal phase (Santen & Bardin, 1973; Backstrom et al., 1982; Reame et al., 1984; Filicori et al., 1986). Studies in animals have also shown changes in the periodicity of pulsatile gonadotrophin secretion. Immediately before the LH peak in ewes, LH pulse frequency increases markedly and pulse amplitude is reduced (Goodman & Karsch, 1981). In rats, LH pulse frequency increases during the second day of the oestrous cycle (Gallo, 1981).

Changes in the frequency of pulsatile LH secretion most likely represent alterations in GnRH pulse frequency. Carmel et al. (1976) have shown that GnRH is secreted by the hypothalamus in an episodic manner in monkeys, and studies in sheep (Clarke & Cummins, 1982) have documented that each LH pulse in peripheral venous blood is associated with a GnRH pulse in hypophyseal portal blood.

These observations have led to the hypothesis that alterations in GnRH pulse frequency may be important in the regulation of gonadotrophin secretion. Indeed, data from studies in rats support this hypothesis by showing that the number of pituitary GnRH receptors and acute LH release are modulated by changes in GnRH pulse frequency (Katt et al., 1985). In the present study, we administered low dose pulses of synthetic GnRH i.v. at hourly or 2-hourly frequencies to normal men and evaluated the effects on LH, FSH, testosterone, and oestradiol secretion. The dose of GnRH pulses was chosen to mimic physiological secretion of GnRH.

MATERIALS AND METHODS

Subjects

Eight normal adult male volunteers, aged 19–27 years, were randomly assigned to study group A or B. All had experienced normal growth and maturation by history and had normal adult male sexual development; no subject was receiving medications.

Study protocol

The studies were approved by the Human Investigation Committee of the University of Michigan and were performed after written informed consent had been obtained.

Subjects were admitted to the Clinical Research Center on the day before blood sampling to permit acclimatization to the unit where they were allowed to walk about throughout the study. On admission, an indwelling i.v. cannula was placed in a forearm vein for blood withdrawal and injection of saline or GnRH pulses. On the day after admission (day O), pulses of normal saline (2 ml) were injected i.v. every 2 h for 24 h to serve as a control for subsequent GnRH injections. Beginning at 0800 h on day 1, all subjects received low dose pulses of synthetic GnRH (25 ng/kg) which were administered every 2 h by rapid i.v. bolus injection; this was continued for 88 h until 2400 h on day 4. Thereafter, subjects entered into one of two study designs: GnRH withdrawal or hourly GnRH.

Group A: GnRH withdrawal

Four normal men in this group received i.v. pulses of saline every 2 h from 0200–2400 h on day 5, and a single low dose pulse of GnRH (25 ng/kg) was injected at 0800 h on day 6.

Group B: hourly GnRH

The four normal men in group B received i.v. pulses of GnRH (25 ng/kg) every 1 h from 0100 h on day 5 until 0100 h on day 6 and a single pulse of GnRH (25 ng/kg) at 0800 h on day 6.

In all subjects, plasma LH and FSH were determined from blood samples obtained every 20 min from 0800 h (day 0) to 0740 h (day 1) and again from 1200 h (day 4) to 0200 h (day 6). Plasma LH and FSH were also measured at 0800 h, 0820 h, and 0840 h on days 1–4 and day 6 and at hourly intervals from 0200–0800 h on day 6. Plasma testosterone and oestradiol concentrations were determined each day at 0800 h and 2000 h.

GnRH dosage

The dosage of i.v. synthetic GnRH used in this study (25 ng/kg) was based on earlier estimates that pituitary portal plasma concentrations of GnRH in men vary between 30–300 pg/ml (Kelch *et al.*, 1975; Huseman & Kelch, 1978). After administration of this GnRH dosage to normal men, peripheral plasma GnRH concentrations average 299 ± 70 pg/ml (Valk *et al.*, 1981). Moreover, increments in plasma LH after i.v. injection of 25 ng/kg GnRH in early pubertal boys closely approximate spontaneous nocturnal LH pulses in these children (Corley *et al.*, 1981), and administration of this dose of GnRH to normal men elicits LH responses similar to endogenous LH pulses (Valk *et al.*, 1981).

Assays and analysis of results

Plasma LH, FSH, testosterone, and oestradiol were measured by RIA (Midgley, 1966; 1967; Ismail *et al.*, 1972; England *et al.*, 1974). Gonadotrophin concentrations are reported as milli international units of the Second International Reference Preparation of human menopausal gonadotrophin urinary standard after conversion from First International Reference Preparation of pituitary FSH/LH (Medical Research Council 69/104), which was used as the assay standard. For plasma LH concentrations of 2·5, 7·5, and 15 mIU/ml, the intra-assay coefficients of variation (CV) averaged 20%, 9·5% and 6·6%, respectively. The inter-assay CV was 11% for the LH and FSH assays and approximately 15% for the steroid assays. Samples from each subject were analyzed in two consecutive gonadotrophin assays and in one steroid assay. Testosterone concentrations are expressed as nmol/l (1 ng/ml testosterone = 3·5 nmol/l). Oestradiol concentrations are expressed as pmol/l (1 pg/ml oestradiol = 3·7 pmol/l).

Mean gonadotrophin concentrations were determined by calculating the arithmetic means of LH and FSH values obtained at 20 min intervals during 4 h periods. A significant LH pulse was defined as a rise from nadir to peak within 40 min that was equal to or greater than a mean detectable increment (MDI) which was determined by twice the intra-assay coefficient of variation of replicate samples from each subject (MDI = mean LH concentration × CV × 2). The rationale for this method and comparison with other computerized methods have been reported previously (Reame *et al.*, 1984).
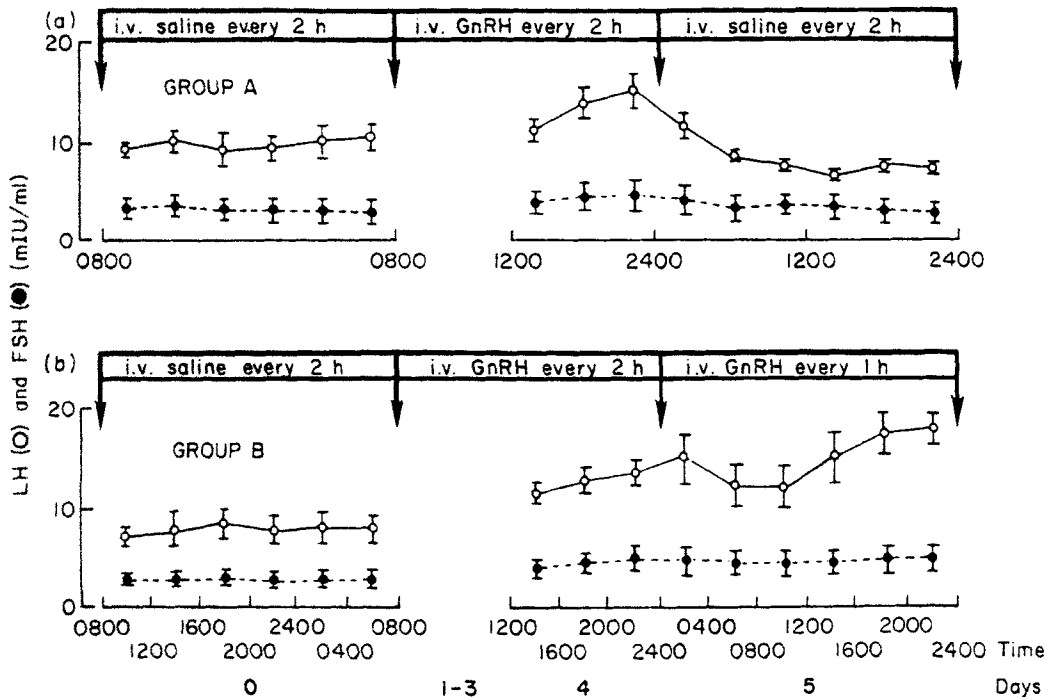
Fig. 1. Mean ± SEM plasma LH (O) and FSH (●) concentrations during 4 h periods in normal men: (a) Group A, GnRH withdrawal (n = 4); (b) Group B, hourly GnRH (n = 4).

Within each group of normal men, comparisons of gonadotrophin and gonadal steroid concentrations before, during and after pulsatile GnRH administration were performed by analysis of variance for repeated measures, followed by the Newman-Keuls' post-hoc comparison test (Bruning & Kintz, 1977). In addition, the Chi-Square (Goodness of Fit) test was used to compare the patterns of change in mean LH on days 0 and 5 (Hopkins & Glass, 1978). Results are expressed as mean ± SEM.

RESULTS

Group A (normal men; GnRH withdrawal)

Administration of GnRH pulses every 2 h resulted in a significant increase in the mean LH concentration at the end of day 4 (2000–2340 h) compared to that on day 0 ($P < 0.01$; Fig. 1). Withdrawal of GnRH pulses was accompanied by a significant fall in mean LH on day 5 compared to day 4 ($P < 0.01$), and values were similar to or below (day 5, 1200–1540 h; $P < 0.05$) those measured during day 0 (Fig. 1). Spontaneous LH pulse amplitude and frequency were decreased significantly on day 5 compared to those on days 0 and 4 ($P < 0.05$, Table 1). LH responses to GnRH at 0800 h on day 6 were comparable to those measured at 0800 h on days 1, 2, and 4 and greater than ($P < 0.05$) those on day 3 (data not shown).

Table 1. Effects of exogenous GnRH pulses on LH pulses in normal men: (A) GnRH withdrawal; (B) hourly GnRH (mean ± SEM)

	Day 0*	Day 4†	Day 5‡		Day 6§
	(0800–0740 h, Day 1)	(1200–2340 h)	(2400–1140 h)	(1200–2340 h)	(0800 h)
LH pulse amplitude (mIU/ml)					
A	6·5±1·0	8·0±1·6	4·0±0·5¶**	3·7±0·8**	14·7±4·9
B	6·1±1·2	9·2±1·3¶	5·4±0·5**	6·8±0·5**	10·4±1·7
LH pulse frequency (pulses/12 h)					
A	5·5±0·2	6·5±0·3	3·5±0·7¶**	5·3±0·3††	—
B	5·8±0·8	6·3±0·3	9·3±0·8¶**	11·8±0·3¶**††	—

* Saline every 2 h i.v.
† GnRH every 2 h i.v.
‡ Saline every 2 h i.v. — Group A; GnRH every 1 h i.v. — Group B.
§ GnRH at 0800 h i.v.
¶ $P<0.05$ *vs* Day 0
** $P<0.05$ *vs* Day 4 (1200–2340 h).
†† $P<0.05$ *vs* Day 5 (2400–1140 h).

Figure 2 illustrates pulsatile gonadotrophin secretion in one subject from group A. Note that LH responses to GnRH on day 4 (10·7 ± 0·8 mIU/ml) were similar in magnitude to amplitudes of endogenous LH pulses on day 0 (9·1 ± 1·1 mIU/ml). The decline in LH secretion during GnRH withdrawal was associated with a reduction in endogenous LH pulse amplitude on day 5 (5·3 ± 0·8 mIU/ml).

Plasma oestradiol concentrations rose significantly in group A subjects on day 5 following pulsatile GnRH administration ($P<0.05$, Table 2). Although mean plasma testosterone values appeared to increase during and after exogenous GnRH pulses, differences were not significant when compared to values on day 0 (Table 2).

Group B (normal men; hourly GnRH)

As in group A, administration of GnRH pulses every 2 h to group B subjects resulted in increased mean LH concentrations on day 4 (2000–2340 h) compared to those on day 0 ($P<0.01$; Fig. 1); this was associated with an augmentation in LH pulse amplitude ($P<0.05$, Table 1). The increase in the frequency of GnRH injections to one pulse every hour was accompanied by a time-dependent pattern of change in mean plasma LH during day 5 which was significantly different from that during day 0 ($P<0.001$; Fig. 1). Mean LH concentrations rose transiently and then fell during the first 12 h of hourly GnRH (day 5, 2400–1140 h). Subsequently, plasma LH values rose to a plateau during the second 12 h period of hourly GnRH on day 5 and were higher than values on days 0 and 4 ($P<0.01$). During the initial fall in mean LH (day 5, 2400–1140 h), LH pulse amplitude was reduced compared to day 4 ($P<0.05$, Table 1), and LH responses were detected after only 37 of 48 GnRH pulses. The subsequent rise in plasma LH (day 5, 1200–2340 h) was associated with an increase in LH pulse frequency ($P<0.05$, Table 1); during the second
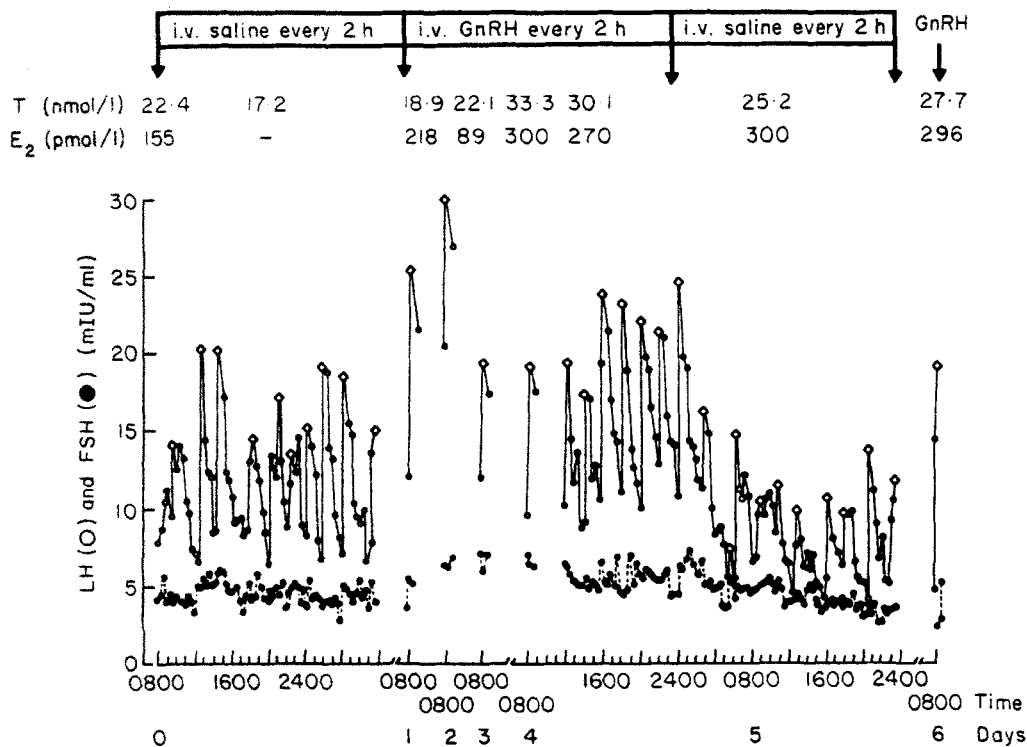
Fig. 2. Endogenous plasma LH (O) and FSH (●) secretory patterns (Days 0 and 5) and gonadotrophin responses to i.v. injections of low dose GnRH pulses every 2 h (days 1–4) in a normal male subject from Group A. (◊) Significant LH pulses. Plasma testosterone (T) and oestradiol (E₂) concentrations, obtained throughout the study, are shown at the top.

12 h period of hourly GnRH injections, significant LH responses occurred after 47 of 48 GnRH pulses.

Data from one subject in group B (Fig. 3) illustrate the decline in plasma LH concentrations during the initial 12 h period of hourly GnRH pulses on day 5. The fall in mean LH was accompanied by a reduction in LH pulse amplitude from $12·6 \pm 2·1$ mIU/ml on day 4 (1200–2340 h) to $6·3 \pm 1·0$ mIU/ml on day 5 (2400–1140 h). Following this, plasma LH rose abruptly, accompanied by an increase in LH pulse frequency. Ten pulses were detectable during the initial 12 h of hourly GnRH injections, and 12 pulses were seen during the second 12 h period.

Plasma testosterone and oestradiol concentrations in group B subjects were increased significantly during GnRH injections ($P < 0·05$; Table 2).

DISCUSSION

Changes in the frequency of pulsatile LH secretion, and presumably GnRH secretion, have been clearly documented in previous studies in animals and humans. During the human female reproductive cycle, LH pulse frequency increases significantly between the early and late stages of the follicular phase (Goodman & Karsch, 1981; Backstrom *et al.*,

Table 2. Effects of exogenous GnRH pulses on plasma gonadal steroid concentrations in normal men: (A) GnRH withdrawal; (B) hourly GnRH (mean ± SEM)

	Day 0*	Day 4†	Day 5‡		Day 6§
	(0800–0740 h, Day 1)	(0800 h)	(2400–1140 h)	(1200–2340 h)	(0800 h)
Testosterone (nmol/l)					
A	20·0 ± 2·5	27·3 ± 1·8	29·8 ± 5·3	21·7 ± 3·9	29·1 ± 5·6
B	21·7 ± 3·2	28·4 ± 3·5¶	30·1 ± 3·5¶	28·4 ± 2·5¶	34·3 ± 2·8¶
Oestradiol (pmol/l)					
A	122 ± 15	181 ± 37	278 ± 48¶**	340 ± 37¶**	—
B	148 ± 7	241 ± 22¶	307 ± 26¶	340 ± 44¶	281 ± 41¶

* Saline every 2 h i.v.
† GnRH every 2 h i.v.
‡ Saline every 2 h i.v. — Group A; GnRH every 1 h i.v. — Group B.
§ GnRH at 0800 h i.v.
¶ $P < 0.05$ *vs* Day 0.
** $P < 0.05$ *vs* Day 4.

1982; Reame *et al.*, 1984) and decreases significantly during the luteal phase (Santen & Bardin, 1973; Hauger *et al.*, 1977; Backstrom *et al.*, 1982; Reame *et al.*, 1984; Filicori *et al.*, 1986). These data suggest that alterations in GnRH pulse frequency are important in the regulation of pulsatile gonadotrophin secretion. In support of this view, Wildt *et al.* (1981) have reported that, in ovariectomized rhesus monkeys with hypothalamic lesions which eliminated endogenous GnRH secretion, a reduction in the frequency of exogenous GnRH pulses from every hour to every 3 h resulted in a significant increase in plasma FSH and a decrease in plasma LH concentrations. Similarly, Gross *et al.* (1987) have demonstrated recently that, in men with isolated gonadotrophin deficiency and presumed endogenous GnRH deficiency, serum FSH concentrations increased progressively relative to LH concentrations as the frequency of exogenous GnRH pulses was decreased. The present study was designed to evaluate the effects of changing the frequency of exogenous GnRH pulses on pulsatile gonadotrophin secretion in normal men.

Injection of GnRH pulses every 2 h for 88 h resulted in increased plasma LH concentrations in all eight normal men. This was presumably due to increased pituitary exposure to GnRH from the additive effects of exogenous GnRH pulses and endogenous GnRH secretion. Pulsatile GnRH administration was also associated with increased plasma oestradiol concentrations, which rose to a greater degree than testosterone. These findings are consistent with results of GnRH infusions in normal men, in whom serum LH rose significantly and serum oestradiol was increased relative to testosterone during exogenous GnRH administration (McNeil *et al.*, 1979).

Withdrawal of exogenous GnRH pulses resulted in a fall in mean plasma LH. This was accompanied by reductions in endogenous LH pulse amplitude and frequency. Factors responsible for the changes in pulsatile LH secretion during exogenous GnRH withdrawal cannot be determined with certainty from the present data. However, one
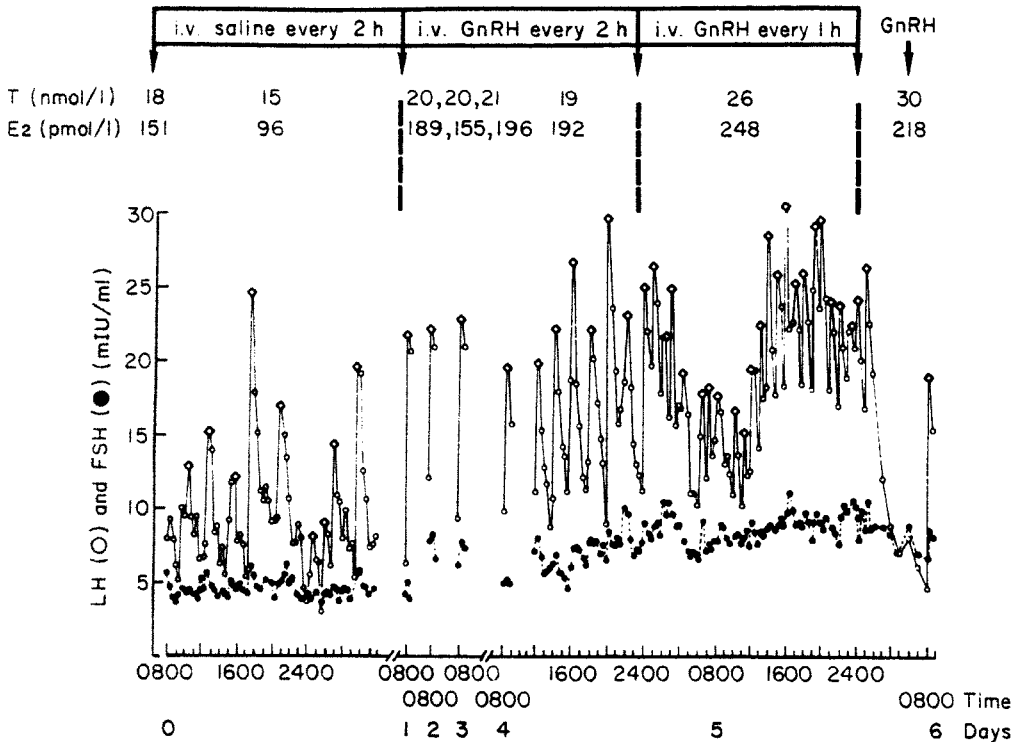
Fig. 3. Endogenous plasma LH (O) and FSH (●) secretory patterns (day 0) and gonadotrophin responses to i.v. injections of low dose GnRH pulses every 2 h (days 1–4) or every 1 h (day 5) in a normal male subject from Group B. (◊) Significant LH pulses. Plasma testosterone (T) and oestradiol (E₂) concentrations are shown at the top.

possibility is that the observed changes were due to modulation of endogenous GnRH and/or gonadotrophin secretion by increased plasma concentrations of gonadal steroids. In support of this assumption, studies in normal men have shown that infusions of oestradiol decrease LH pulse amplitude and pituitary sensitivity to GnRH and infusions of testosterone decrease LH pulse frequency (Santen, 1975). Moreover, Matsumoto and Bremner (1984) have reported that endogenous LH pulse amplitude and frequency are significantly higher in men with primary hypogonadism than in normal men, and testosterone therapy is accompanied by significant reductions in both amplitude and frequency. In the present study, the decrease in LH pulse frequency during GnRH withdrawal was presumably due to a reduction in endogenous GnRH pulse frequency. The decrease in endogenous LH pulse amplitude on day 5 could have been due to reductions in endogenous GnRH pulse amplitude and/or decreased pituitary sensitivity to GnRH; however, the demonstration of normal LH responsiveness to GnRH on day 6 in group A subjects makes the latter possibility less likely.

The acute increase in exogenous GnRH pulse frequency on day 5 in group B subjects was accompanied by a time-dependent pattern of change in LH secretion, characterized by a transient rise, a subsequent fall, and a final rise in mean LH. During the decline in mean LH, pulse amplitude was significantly reduced, and LH response, were not

detectable after approximately one-fourth of the exogenous GnRH pulses. The final rise in LH was accompanied by an increase in LH pulse frequency; indeed, LH responses were identified following 98% of GnRH pulses administered during the latter half of day 5. These data suggest that, in men with intact hypothalamic–pituitary–gonadal axes, it takes the pituitary gland approximately 12 h to become fully responsive to a doubling in GnRH pulse frequency. This temporal delay in pituitary responsiveness presumably represents the time required by the gonadotroph to alter its mode of LH synthesis and/or secretion subsequent to an acute increase in GnRH pulse frequency. However, in-vivo studies, such as the present one, do not allow for determination of the exact intracellular mechanism responsible for this adaptation. Although acute changes in endogenous LH pulse frequency have not been identified in normal men, several investigators have documented an increase in LH, and presumably GnRH, pulse frequency between the early and late stages of the follicular phase of the menstrual cycle in women (Backstrom *et al.*, 1982; Reame *et al.*, 1984; Filicori *et al.*, 1986). During this phase of the cycle, a similar pattern of gonadotrophin secretion is seen immediately before the midcycle LH surge, namely a transient fall in mean LH (Midgley & Jaffe, 1971) followed by an acute increase in mean LH and LH pulse amplitude (Reame *et al.*, 1984).

ACKNOWLEDGEMENTS

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