0021-972X/86/6206-1136$02.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 62, No. 6
Printed in U.S.A.

Characterization of the Physiological Pattern of Episodic Gonadotropin Secretion throughout the Human Menstrual Cycle*

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ABSTRACT. To characterize the spectrum of pulsatile gonadotropin secretion during the course of the normal menstrual cycle, we studied normal women during 51 ovulatory cycles. Plasma gonadotropin concentrations were measured at 10-min intervals for 20–24 h during the early, mid-, and late follicular phases and the early, mid-, and late luteal phases. LH data series were analyzed using 2 different computer-assisted algorithms for pulse detection.

The LH interpulse interval decreased during the follicular phase (FP) from 94 ± 4 (±SEM) min in the early FP (EFP) to 71 ± 4 min by the late FP (LFP; $P < 0.001$). The estimation of LH pulse frequency in the EFP was significantly affected by slowing of episodic LH secretion during sleep. In the luteal phase (LP), the LH interpulse interval progressively increased from 103 ± 8 min in the early LP (ELP) to 216 ± 39 min by the late LP (LLP; $P < 0.001$). Sleep-associated slowing of episodic LH secretion also occurred in the ELP.

The mean LH pulse amplitude in the EFP (6.5 ± 0.4 mIU/ml) decreased significantly by the midfollicular phase (MFP; 5.1 ± 0.8 mIU/ml; $P < 0.05$) and increased once again by the LFP (7.2 ± 1.2 mIU/ml). LH pulse amplitude was highest in the ELP (14.9 ± 1.7 mIU/ml), decreased by the midluteal phase (MLP) to 12.2 ± 2.0 mIU/ml, and declined further by the LLP to 7.6 ± 1.1 mIU/ml ($P < 0.001$ *vs.* ELP).

FSH secretion was significantly ($P < 0.05$) correlated with LH secretion at time lags of 0–10 min in 82% of the studies.

These results indicate the following. 1) In the EFP and ELP, the frequency of gonadotropin pulsations is reduced at night in association with sleep. 2) The frequency of LH secretion increases from the EFP to MFP and LFP. 3) LH pulse amplitude decreases in the MFP, suggesting enhanced negative feedback of estrogen on the hypothalamic-pituitary axis and/or a decrease in GnRH secretion at this stage. 4) A progressive reduction of LH pulse frequency and amplitude occurs during the LP which is correlated with the duration of exposure of the hypothalamic-pituitary axis to progesterone. 5) A close relationship exists between secretion of LH and FSH, suggesting a common stimulatory factor for both gonadotropins. (*J Clin Endocrinol Metab* **62:** 1136, 1986)

THE NEUROENDOCRINE control of episodic gonadotropin secretion is a critical component of every aspect of the menstrual cycle. In the rhesus monkey, ablation of the GnRH-producing areas of the hypothalamus eliminates gonadotropin secretion and induces amenorrhea, whereas restoration of episodic gonadotropin secretion with exogenous GnRH is capable of reinstituting ovulatory cycles in these animals (1). In the human, subtle derangements of episodic gonadotropin secretion, presumably reflecting altered hypothalamic GnRH release, are associated with menstrual disturbances and amenorrhea (2, 3). Exogenous administration of low dose, pulsatile GnRH to these women is an effective method of restoring their pituitary and ovarian function, as well as fertility, to normal (4).

Since direct assessment of GnRH secretion is not possible in the human, the study of gonadotropin secretory patterns presently is the best tool with which to assess hypothalamic function. This approach relies upon the assumption that a close correspondence exists between secretory episodes of GnRH from the hypothalamus and subsequent LH release from the anterior pituitary. Such an assumption has been validated in rhesus monkeys (5), in whom direct hypothalamic measurement demonstrated that each episode of LH secretion was preceded by an antecedent burst of GnRH release from the hypothalamus. In addition, a recent report demonstrating close correspondence between hypothalamic neuronal activity and episodic LH release in the rhesus

Received October 7, 1985.

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* This work was supported by NIH Grants HD-15080 and RR-1066, and the Vincent Research Fund.

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monkey (6) further substantiated the validity of this approach.

A firm understanding of the normal pattern of episodic gonadotropin secretion in the cycling woman is important for both a comparison of presumed abnormalities of GnRH secretion and construction of a physiologically based replacement program of exogenous GnRH. However, previous studies of episodic gonadotropin release in normal cycling women were based on small numbers of subjects (3, 7–10) or did not examine all stages of the normal cycle (11, 12). Therefore, to characterize episodic gonadotropin release more precisely and to gain further insight into the normal pattern of hypothalamic-pituitary-gonadal function in the human menstrual cycle, we examined the pattern of episodic gonadotropin release during the course of 51 ovulatory cycles.

Materials and Methods

Patient population

The study population consisted of normal women aged 18–35 yr [mean age, 27.1 ± 3.5 (±SD)] who had had regular menstrual cycles of 27- to 32-day duration in the previous 2 yr. Each had a normal physical examination, with no acne, hirsutism, or galactorrhea, and a body weight within ±15% of normal according to the Sargent scale (13). No woman ran more than 20 miles/week or exercised more than 1 h/day, and none of the subjects had taken any hormonal medication in the 3 months preceding the study.

Plasma PRL levels were less than 15 ng/ml and plasma TSH, T_3, and T_4 were all within normal limits in each subject. In the month immediately preceding the study, each woman recorded her basal body temperature and had a midluteal phase (MLP) blood sample obtained for the estimation of plasma progesterone (P). Only women with a biphasic BBT, a luteal phase (LP) duration of at least 12 days, and a MLP plasma P level greater than 6 ng/ml were studied. A total of 48 women meeting each of these criteria completed the study. Some participated in the protocol more than once; consequently, a total of 68 complete cycles were evaluated. Despite the relatively stringent criteria, several study cycles were abnormal in terms of the duration of the LP (*i.e.* 10 days or less) or the maximal level of P achieved (*i.e.* <6 ng/ml), lacked critical blood samples rendering the day of ovulation indeterminate, or had incomplete frequent sampling studies. These series were omitted from the present analysis; thus, a total of 51 cycles in 36 women are reported herein.

Protocol

During a single complete menstrual cycle, blood samples were obtained daily for the estimation of plasma LH, FSH, 17-β-estradiol (E_2), and P. On a predetermined day of the same cycle, each woman was admitted to the Clinical Research Center of the Massachusetts General Hospital for a 20- to 24-h period of frequent blood sampling. Only one such frequent sampling study was performed in each menstrual cycle. Blood samples (3 ml) were obtained at 10-min intervals throughout the admission. All samples were drawn through a heparin lock (10 U heparin/ml) during the daytime, while at night, a longer iv line was inserted to permit phlebotomy while the subject slept. During the nighttime sampling procedure, a record of sleep was kept by a trained observer, although electroencephalograph recording was not performed. Additional blood samples were drawn at 6-h intervals during the period of frequent sampling for the determination of sex steroids. All women received oral iron supplementation during and immediately after the study month.

The data were analyzed in relation to the day of the midcycle surge, as defined by: 1) the day of or the day after the E_2 midcycle peak, 2) the day of the LH peak, 3) the day of the FSH peak, 4) the day on which the P levels doubled from baseline and reached 0.6 ng/ml or more (14). Day 0 of the study cycle was identified by the concomitant occurrence of at least three of these four changes on the same day. In a standard 28-day cycle with a 14-day follicular phase (FP) and a 14-day LP, the cycle stages were defined as follows in relation to day 0: early FP (EFP), days −13 to −9; mid-FP (MFP), days −8 to −5; late FP (LFP), days −4 to 0; early LP (ELP, days +1 to +4; mid-LP (MLP), days +5 to +9; and late LP (LLP), days +10 to +14. When FP or LP of differing durations occurred, the stages were defined proportionally, keeping day 0 as the reference point. Eight or 9 studies were analyzed at each stage of the cycle, for a total of 24 in the FP and 25 in the LP. One woman was studied during the midcycle gonadotropin surge, and another 1 had the onset of her menstrual flow during her 24-h admission. These 2 women's results are described, but they were omitted from the general data analysis.

Hormone assays

All plasma samples from an individual woman (daily blood sampling and 24-h studies) were processed for determination of LH and FSH in a single RIA, as previously described (15). Before assay of the samples from an entire 24-h sampling period, a pool comprised of equal aliquots of plasma from each time point was constituted, and its value determined. When this pool value was obtained, a plasma volume was selected for subsequent RIA of each time point such that this mean value fell near the center [*i.e.* (B/Bo), 50%] of the standard curve. This maneuver assured that the samples were assayed near the point of maximum assay precision.

Daily samples and the additional samples obtained at 6-h intervals during the frequent sampling studies were also analyzed by RIA for E_2 and P after extraction as previously reported (15). Therefore, the estimation of gonadal steroid levels on the day of the 24-h study is the average of five individual determinations. The LH and FSH antisera were purchased from Biodata/Serono Diagnostics Division (Milan, Italy). The gonadotropin values were expressed in milliinternational units per ml as equivalents of the Second International Reference Preparation/human menopausal gonadotropin. The sensitivity (95% confidence limits) of the LH and FSH assays varied between 0.8 and 1.6 mIU/ml plasma. The precision of the gonadotropin assays was measured by analyzing 10–20 replicates in each assay at each of 3 levels of displacement

(20%, 50%, and 80% B/Bo) using quality control samples interspersed among the unknown samples. The intraassay coefficients of variation (CVs) for the assays were 6.7% (20%), 6.4% (50%), and 8.6% (80%) for LH and 7.4% (20%), 7.5% (50%), and 11.2% (80%) for FSH. The interassay CVs were 9.3% (20%), 9.9% (50%), and 14.2% (80%) for LH and 9.9% (20%), 10.3% (50%), and 17.1% (80%) for FSH. The interassay CVs were 9.9% for E_2 and 15.9% for P determinations, respectively.

Data analysis

The analysis of pulsatile LH secretion was carried out with two different computerized algorithms for the identification of pulsatile hormone secretion (11, 16) implemented on a VAX 11/750 computer (Digital Equipment Corp., Marlborough, MA). As previously reported (15), our slightly modified version of the Santen and Bardin program (11), method A, defines a gonadotropin pulse as an increment of plasma levels of more than 1 mIU/ml which exceeds the previous nadir by 20% or more. In the assays described, a 20% increment corresponded to approximately 3–4 times the intraassay CV. The LH interpulse interval was obtained by dividing the total duration of the study by the number of pulses identified. The amplitude of a pulse was defined as the difference between the highest value in the pulse and the preceding nadir. The data were analyzed with a second independently validated computer algorithm, Pulsar, method B (16). The Pulsar computer program scores secretory peaks by height and duration of the hormone fluctuation from a smoothed baseline, using the assay SD as a scale factor. The cutoff thresholds, G (1) to G (5), used for the Pulsar program in this study were 3.5, 2.4, 1.6, 1.2, and 1.0, respectively.

The dynamics of FSH secretion in relation to plasma LH levels were assessed using cross-correlation analysis (17) of the pulsation study data series. Due to the kinetics of their clearance and the pulsatile nature of these hormones, both LH and FSH yield highly autoregressive time series (*i.e.* series that would correlate with themselves at various time-shifted intervals). This property could yield positive or negative estimates of cross-correlation even if the LH and FSH series were not correlated. The possibility of the autoregressive nature of the series leading to a spurious cross-correlation was examined, and some contribution to the correlation value was present for all time lags studied; however, its magnitude was negligible. Therefore, statistical significance was considered present for $P < 0.05$. Finally, the Mann-Whitney U test and Pearson's correlation coefficient were used to determine the significance of differences between groups and of correlations. All values in this paper are expressed as the mean ± SE.

Results

General characteristics of the study cycles

The total duration of the menstrual cycles was 28.4 ± 0.3 (±SEM) days. The FP lasted 14.9 ± 0.4 days, while the duration of the LP was 13.5 ± 0.2 days. The durations of the FP and LP in each cycle were inversely correlated ($r = -0.37$; $P < 0.01$). All cycles had a clear-cut midcycle LH and FSH surge lasting 1–2 days. The mean peak preovulatory plasma E_2 level in the daily samples was 350 ± 12 pg/ml, while the mean maximum plasma P level in the LP was 25.3 ± 1.2 ng/ml.

Hormone concentrations during the pulsation study

The mean plasma LH levels increased during the follicular phase from 8.0 ± 0.4 mIU/ml in the EFP to 15.7 ± 2.5 mIU/ml by the LFP ($P < 0.001$). With the exception of a single woman (who was excluded from the general analysis), none of the women in the LFP were studied during the midcycle gonadotropin surge. The highest mean LH levels were reached in the ELP (21.4 ± 2.2 mIU/ml). Thereafter, mean LH concentrations decreased and reached a nadir by the LLP (5.8 ± 0.7 mIU/ml; $P < 0.001$ between ELP and LLP; $P < 0.01$ between LLP and EFP).

The mean plasma FSH levels in the FP demonstrated a trend opposite that of LH, as they decreased progressively from a maximum of 10.0 ± 0.3 mIU/ml in the EFP to a LFP nadir of 7.2 ± 0.5 mIU/ml ($P < 0.001$). FSH levels then increased in the ELP (10.3 ± 1.5 mIU/ml), after which they progressively declined during the LP to a nadir of 4.7 ± 0.7 mIU/ml before menstruation ($P < 0.001$ between ELP and LLP; $P < 0.001$ between LLP and EFP).

The mean plasma E_2 levels increased progressively during the FP from 45 ± 4 pg/ml in the EFP to 61 ± 7 pg/ml in the MFP ($P < 0.05$) and 145 ± 17 pg/ml in the LFP ($P < 0.001$ between MFP and LFP, and between EFP and LFP). This progressive rise was positively correlated with the mean LH levels ($r = 0.58$; $P < 0.01$) and inversely correlated with the mean FSH levels ($r = -0.48$; $P < 0.05$). In the LP, a maximum E_2 concentration of 139 ± 8 pg/ml was achieved in the MLP. In the LP, no correlation existed between mean E_2 and mean LH or FSH levels. However, when data from all stages of the cycle were considered, an inverse correlation was found between mean E_2 and FSH levels ($r = -0.37$; $P < 0.05$), while no such correlation existed between mean E_2 and LH concentrations.

The mean P levels peaked in the MLP at a level of 17.2 ± 2.3 ng/ml ($P < 0.001$ between ELP and MLP and $P < 0.01$ between MLP and LLP) in a fashion similar to and strongly correlated with those of E_2 ($r = 0.74$; $P < 0.001$). Mean P levels were always below 0.6 ng/ml in the women studied in the FP, with the exception of the single woman studied during the midcycle LH surge who had a P level of 1.0 ± 0.1 ng/ml (not included in the general analysis). No relationship between mean FSH and P levels in the LP was apparent, whereas mean LH levels

were inversely correlated to P levels ($r = -0.46$; $P < 0.05$) during the LP.

Characteristics of pulsatile LH secretion

The results of the analysis of pulsatile LH secretion performed with two analytical approaches are shown in Table 1. Although the absolute values of LH pulse frequency and amplitude varied slightly, the patterns of change were similar, and the trends were statistically significant with both analytical methods. This degree of agreement suggests that the results are very unlikely to be artifactual. Hereafter, we report the results obtained with method A, indicating when a qualitative disagreement existed among the pulse analysis results obtained with the different methods.

Pulse frequency (Fig. 1). The mean LH interpulse interval was shorter in the entire FP (77 ± 3 min) than in the entire LP (189 ± 27 min; $P < 0.001$). The LH interpulse interval in the FP decreased from 94 ± 4 min in the EFP to 67 ± 3 min by the MFP ($P < 0.001$) and 71 ± 4 min in the LFP (P NS between MFP and LFP; $P < 0.001$ between EFP and LFP). This decrement in interpulse interval from the EFP to the MFP was not significant with method B, possibly due to the fact that this method is relatively strict and thus insensitive to the low amplitude pulses in the MFP. The LH interpulse interval was inversely correlated with the day of the FP ($r = -0.53$; $P < 0.01$; Fig. 2), but was not correlated with the mean E_2 levels on the day of the 24-h study.

When individual studies of the EFP are viewed (Figs. 2 and 3), an important characteristic becomes apparent.

TABLE 1. The frequency and amplitude of LH pulses across 49 menstrual cycles

Stage	n	LH interpulse interval		LH pulse amplitude (mIU/ml)	
		Method A	Method B	Method A	Method B
EFP	8	94 ± 4	92 ± 7	6.5 ± 0.4	6.7 ± 0.6
MFP	8	67 ± 3[a]	79 ± 6	5.1 ± 0.8[b]	4.6 ± 0.6[b]
LFP	8	71 ± 4	88 ± 6	7.2 ± 1.2	6.8 ± 1.2
ELP	8	103 ± 8[c]	119 ± 10[d]	14.9 ± 1.7[e]	15.4 ± 1.5[e]
MLP	9	206 ± 51	184 ± 31	12.2 ± 2.0[f]	12.2 ± 1.7[g]
LLP	8	216 ± 39	188 ± 17	7.6 ± 1.1	7.6 ± 1.4

The data were analyzed with two different computer algorithms and different threshold values for LH pulse amplitude were used (see *Materials and Methods* for more details). Values are expressed as the mean ± SE.

[a] $P < 0.001$ *vs.* EFP.
[b] $P < 0.05$ *vs.* EFP.
[c] $P < 0.01$ *vs.* LFP and MLP; $P < 0.001$ *vs.* LLP.
[d] $P < 0.05$ *vs.* LFP and MLP; $P < 0.01$ *vs.* LLP.
[e] $P < 0.001$ *vs.* LFP and LLP.
[f] $P < 0.05$ *vs.* LLP.
[g] $P < 0.05$ *vs.* ELP.

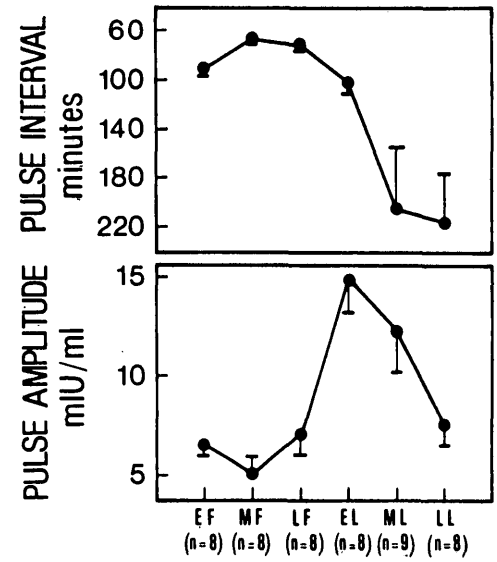

FIG. 1. The LH interpulse interval and amplitude in the different stages of the menstrual cycle. The data shown were obtained by pulse analysis method A (see text) and are expressed as the mean ± SE.

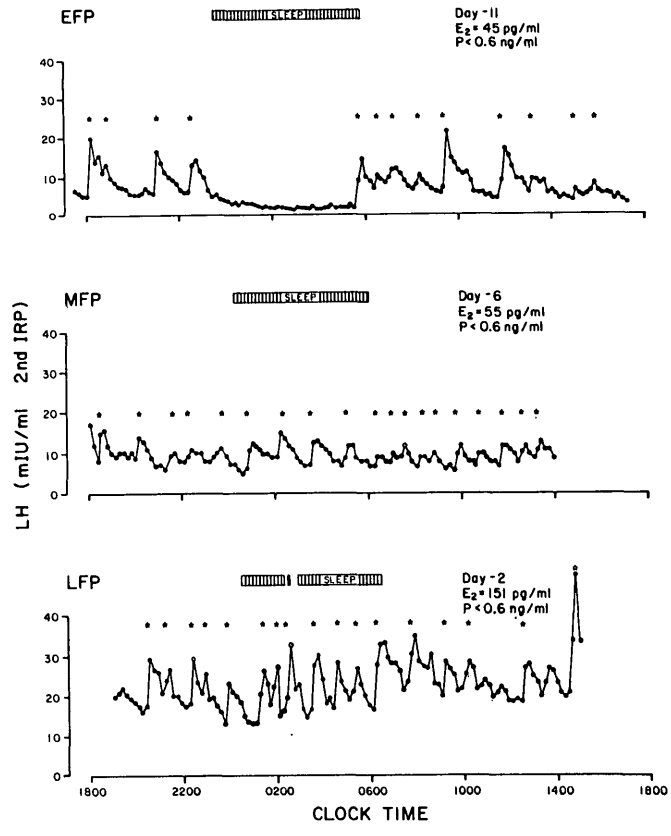

FIG. 2. Patterns of episodic LH secretion throughout the FP of the menstrual cycle. Representative examples of EFP (*top*), MFP (*middle*), and LFP (*bottom*) series are shown. The stage of the FP is indicated from day 0 (see text). LH pulsations, as identified in *Materials and Methods*, are indicated by *asterisks*. Levels of E_2 and P represent the mean of samples obtained at 6-h intervals. Sleep is indicated by the *hatched bars*.

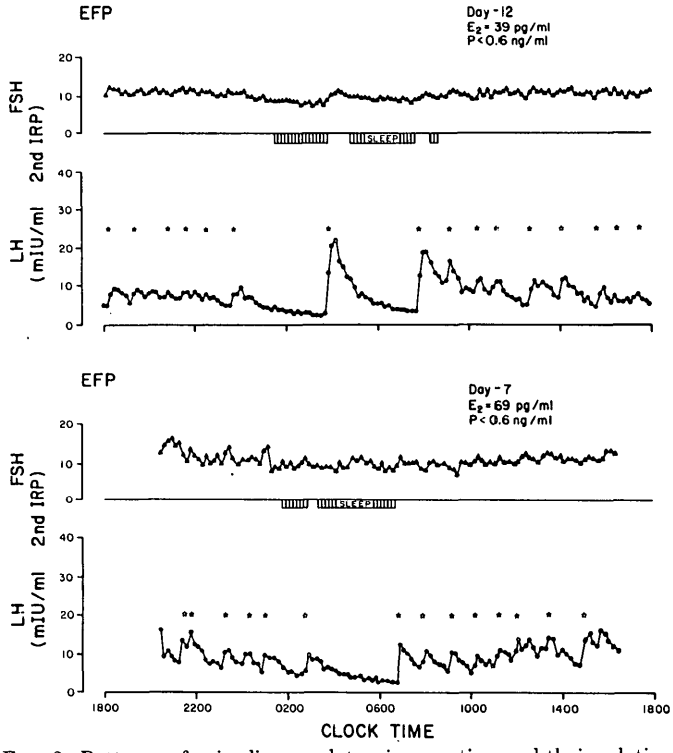

FIG. 3. Patterns of episodic gonadotropin secretion and their relation to sleep in the EFP of the normal menstrual cycle. *Hatched bars* indicate sleep, as visually monitored by trained observers; *asterisks* indicate significant LH pulsations. Note the sleep-related suspension of episodic gonadotropin secretion in both subjects studied in the EFP of the cycle and its reappearance with awakening during the night.

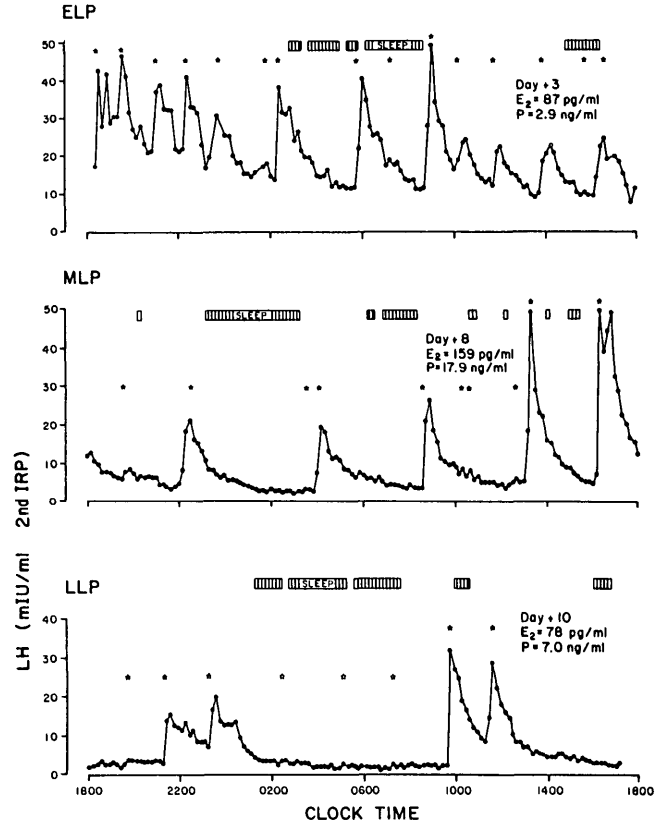

FIG. 4. Patterns of episodic LH secretion throughout the LP of the menstrual cycle. Representative examples of ELP (*top*), MLP (*middle*), and LLP (*bottom*) series are shown. The stage of the luteal phase is indicated as post-day 0 (see text).

In most women studied during this stage of the cycle (six of eight), LH pulses slowed remarkably during the night, such that almost no episodic LH secretion occurred during the sleep period. As the occurrence of sleep was not electroencephalographically recorded, the correspondence between sleep stages and the absence of episodic LH secretion could not be precisely ascertained. Therefore, to characterize this phenomenon, we estimated the LH interpulse interval from midnight to 0600 h (sleep period) and contrasted it with that during the remaining hours of the pulsation study (wake period). The mean LH interpulse interval was significantly longer during the sleep period than during the wake period in the EFP (173 ± 29 *vs.* 82 ± 4 min; $P < 0.001$). This sleep-wake difference was also evident in the ELP (161 ± 32 *vs.* 93 ± 7 min; $P < 0.05$). No other significant sleep-wake differences could be detected during any other phase of the cycle. The differences in the frequency of LH secretion during the FP were less evident when the effect of nighttime slowing was removed (by assessing pulse frequency only during the wake periods). However, an increase in pulse frequency from the EFP to the MFP was present (82 ± 4 *vs.* 67 ± 4 min; $P < 0.05$) even when only wake periods were examined.

In the LP (Fig. 4), the LH interpulse interval increased progressively from 103 ± 8 min in the ELP to 206 ± 51 min in the MLP ($P < 0.01$) and 216 ± 39 min in the LLP ($P < 0.001$ *vs.* the ELP). This change in LH pulse frequency correlated with the day of the LP ($r = 0.39$; $P < 0.05$), but not to the mean E_2 or P level on the day of the 24-h study.

Pulse amplitude (Fig. 1). The LH pulse amplitude was significantly smaller in the FP (6.3 ± 0.5 mIU/ml) than in the LP (11.6 ± 1.2 mIU/ml; $P < 0.01$). The amplitude of LH pulses in the FP decreased from 6.5 ± 0.4 mIU/ml in the EFP to 5.1 ± 0.8 mIU/ml by the MFP ($P < 0.05$). LH pulse amplitude increased slightly but not significantly during the LFP to 7.2 ± 1.2 mIU/ml. The amplitude of LH pulsations was not correlated with the mean E_2 levels during the 24-h study in the follicular phase. However, when data from the EFP and MFP only were considered, a highly significant negative correlation ($r = -0.72$; $P = 0.001$) existed between LH pulse amplitude and E_2 levels.

The largest mean LH pulse amplitude was achieved during the ELP (14.9 ± 1.7 mIU/ml), and thereafter, amplitude decreased progressively to 12.2 ± 2.0 in the MLP and 7.6 ± 1.1 mIU/ml in the LLP ($P < 0.001$ between ELP and LLP; $P < 0.05$ between MLP and LLP). LH pulse amplitude was not correlated with the

mean E_2 or P levels on the day of the 24-h study during the LP; however, a significant negative correlation ($r = -0.54$; $P < 0.01$) existed between LH pulse amplitude and the day (post-day 0) of the LP during which the pulsation study was performed.

Episodic FSH secretion

The exact pattern of episodic FSH secretion during the cycle could not be estimated reliably with these or other methods of pulse analysis due to the low amplitude of the fluctuations of this hormone. However, the use of cross-correlation analysis demonstrated that FSH secretion was significantly ($P < 0.05$) correlated with episodic LH secretion at a time lag of 0 min in 71% of the pulsation studies. In 10% of the studies there was an LH-FSH cross-correlation at a time lag of 10 min, and in 4% at time lags of more than 10 min, while in 14% no cross-correlation was found. The pulsation studies with no cross-correlation or correlation at time lags of more than 10 min were not clustered at any specific stage of the menstrual cycle. Therefore, the vast majority (71%) of FSH pulsation studies were cross-correlated to those of LH at 0 min even during those stages of the cycle when the mean LH and FSH levels were going in opposite directions, *e.g.* during the EFP. In one woman studied on day 0 (Fig. 5A), high levels of LH associated with the midcycle surge occurred along with clearly discernible FSH pulsations.

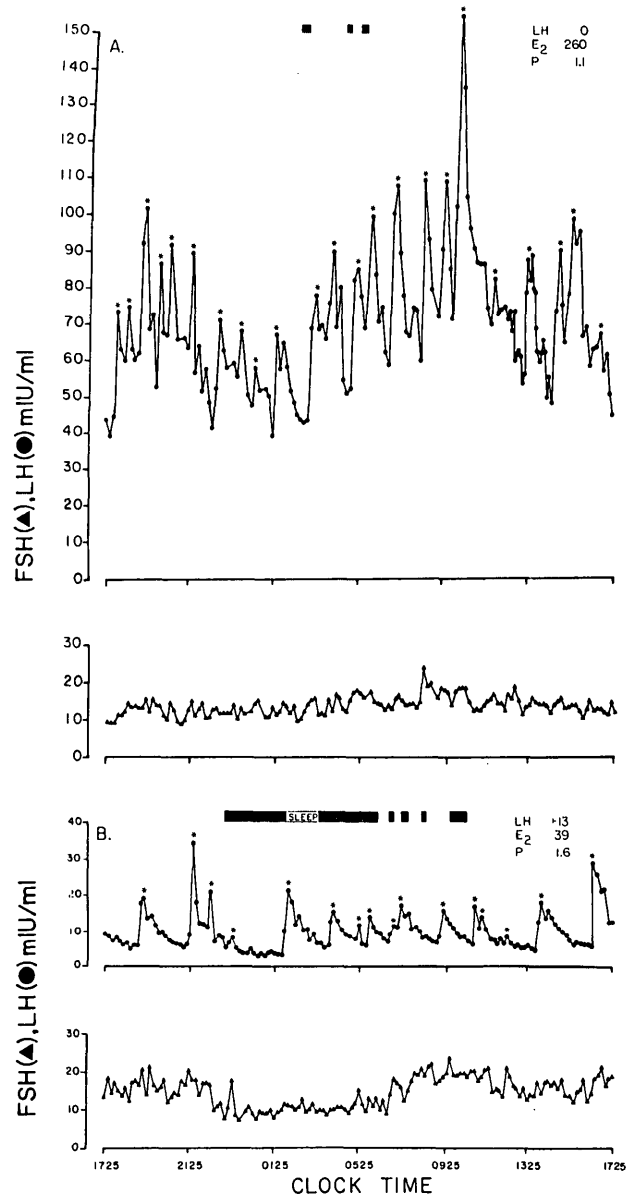

FIG. 5. Transitional patterns of episodic gonadotropin secretion. A, Episodic LH and FSH secretion in one woman during the day of her midcycle surge (day 0; see text). Note the high amplitude and frequency of LH pulsations and the emergence of discernible FSH pulsatility. Sleep is indicated by the *blackened bars*. B, Episodic LH and FSH secretion in one woman who began menstruating during the 24-h admission for frequent blood sampling.

Discussion

Since the episodic secretion of LH reflects hypothalamic GnRH release (5), a detailed study of the pattern of episodic LH secretion can provide useful information regarding changes in neurosecretory input to the pituitary. The present study used a cross-sectional approach and a frequent sampling procedure to investigate the largest series of normal women (n = 36) and cycles (n = 51) reported to date. At least eight women were studied at each of the six stages of the cycle. This cross-sectional design permitted the study to encounter a wider range of between-subject variability and ultimately provide observations that can set corresponding confidence limits for the range of normalcy.

It is now evident that a less than optimal intensity of sampling frequency may result in significant underestimations of LH pulse frequency and also affect the assessment of pulse amplitude (18, 19). However, increasing the number of samples obtained per unit of time can increase the occurrence of method- or assay-related artifacts (20) and, therefore, can result in equally undesirable overestimations of pulse frequency. For our study we chose a 10-min blood-sampling interval, a frequency that appeared to maximize the precision of gonadotropin pulse identification with a total blood requirement compatible with the execution of full 24-h studies.

The pulsatile secretion of FSH is not easily detectable in normal premenopausal women because of the low amplitude of FSH pulsations due to its relatively long half-life compared to that of LH (21, 22). In postmenopausal women, where the ratio between the amplitude and mean levels of gonadotropin is substantially greater, FSH pulsations become readily evident (10). Thus, rather than using standard techniques of pulse analysis

for the FSH series, we used cross-correlation analysis (17) to determine if FSH fluctuations were superimposable on the larger and easily identifiable LH pulses. In the vast majority of pulsation studies, such a correspondence existed at a time lag of 0–10 min. This finding suggests that despite pituitary modulation by gonadal steroids which may influence pulse amplitude or mean hormone levels, GnRH concomitantly stimulated the secretion of both gonadotropins.

The parameters of pulsatile LH secretion varied considerably throughout the menstrual cycle. A previous report by Reame *et al.* (9) suggested that LH pulse amplitude or frequency may not differ in the two phases of the cycle. However, in accord with others (10, 11), we found that the LH interpulse interval was shorter in the FP than in the LP ($P < 0.001$) and that the LH pulse amplitude was smaller in the FP than in the LP ($P < 0.01$).

In the FP, the LH interpulse interval decreased from the EFP to the LFP, a finding that confirms some (7–9) but not all (3, 12) previous studies, and was closely associated with a progressive rise in E_2. This increase in LH pulse frequency during the FP was largely, but not completely, attributable to a selective night slowing of LH pulsatility in the EFP. A relationship of LH pulse suspension to sleep is suggested by the finding that whenever our subjects awakened during the night, a clear episode of GnRH secretion occurred. Of the six women who had sleep-related slowing in the EFP, there were six episodes of awakening recorded by nursing personnel. Five of these were associated with a concomitant LH pulse. No other LH pulses were detected during recorded sleep hours in these six women. When the 6-h sleep period (2400–0600 h) was excluded from pulse analysis in all studies of the FP, a significant increase in LH pulse frequency was still detectable from the EFP to the MFP.

A sleep-related decrement in LH pulsatility was first identified by Kapen *et al.* (23) in 1973 in five normal women studied in the EFP, while normal men did not exhibit such a day-night difference. A similar study (24) failed to recognize any sleep-related change in LH secretion, although the exact stage of the menstrual cycle at which these studies were performed is not indicated. A more recent report (25) described significant day-night differences in LH pulse frequency throughout the FP but not in the LP. A similar phenomenon also was reported in women recovering from hyperprolactinemia (26). In the present study, the LH pulse slowing at night was limited to the EFP and ELP.

The mean amplitude of LH pulsations decreased from the EFP to the MFP. This decrement in LH pulse amplitude in the MFP, along with the lack of correlation of E_2 levels with LH amplitude in the FP, may be a manifestation of the biphasic nature of estrogen's action on the pituitary. Constant levels of E_2 induce an initial suppression of gonadotropin secretion followed by an amplification of pituitary responsiveness to GnRH (27). In fact, if the LFP data were not considered (because of the positive feedback induced by estrogens at this point in the cycle), a highly significant negative correlation existed between LH pulse amplitude and plasma E_2 levels. This finding suggests that the decrement of LH pulse amplitude from the EFP to the MFP may be related to this initial negative feedback effect of rising E_2 levels on the pituitary. Alternatively, since administration of a GnRH antagonist can induce menstrual bleeding in the MFP but not in other stages of the FP (28), indicating that the MFP is particularly susceptible to pituitary GnRH receptor blockade, decreased hypothalamic GnRH secretion may occur at this stage of follicular development.

The amplitude of LH pulsations was significantly larger in the LP than in the FP. Exogenous GnRH administration at different stages of the normal menstrual cycle revealed comparable pituitary sensitivity in the LFP and MLP (29), suggesting that the high amplitude endogenous LH pulsations in the LP are the consequence of greater hypothalamic GnRH secretion per pulse or, alternatively, are related to the slowing of endogenous GnRH release. Both the amplitude and frequency of LH pulses progressively and significantly decreased during the LP. Neither the frequency nor the amplitude of endogenous LH pulses was immediately related to the ambient E_2 or P levels on the day of the study. However, the day of the LP when the study was performed was positively correlated with LH interpulse interval and negatively correlated with LH pulse amplitude. It would appear that the modulatory action of sex steroids is not immediate and that their modification of GnRH secretion requires several days of exposure of the hypothalamic-pituitary axis to elevated levels of P and/or E_2. Although P activity is probably mediated through inhibition of the frequency of hypothalamic GnRH secretion (30), the progressive reduction of mean LH levels and pulse amplitude in the LP suggests an additional effect of P on GnRH pulse amplitude and/or pituitary sensitivity.

Two women were studied during key transition points in the menstrual cycle, the midcycle gonadotropin surge and the luteal-follicular transition. However, no firm conclusions can be drawn as to the characteristics of GnRH and gonadotropin secretion at these unique moments of the cycle. In one woman studied on day 0, the LH interpulse interval (69 min) was superimposable on the LFP estimate (71 ± 4 min), while the LH pulse amplitude was manyfold larger (34.9 *vs.* 7.2 ± 1.2 mIU/ml). These data agree with other recent reports concern-

ing gonadotropin dynamics at midcycle (31, 32). Recent evidence in the ewe and rhesus monkey indicates that GnRH secretion may be increased during estrogen-induced gonadotropin surges (33, 34). A second woman experienced the onset of her menstrual flow at the end of the 24-h sampling study. In this subject, features of both LLP and EFP patterns were observed, as LH pulse frequency was rapid (90 min) and mean FSH levels were already elevated to levels (15.1 mIU/ml) characteristic of the EFP, while LH pulse amplitude (11.3 mIU/ml) was still comparable to that of the LLP. Only 36 h before the initiation of menses, gonadotropin secretion in our volunteers was characterized by the infrequent LH pulses and low FSH levels typical of the LLP. On the other hand, by day 1 of the menstrual cycle, the EFP parameters of gonadotropin secretion appeared to be already established.

In conclusion, episodic gonadotropin secretion during the human menstrual cycle follows a complex and precisely timed sequence. The parameters derived from this study can be employed to characterize several reproductive disorders and to construct a physiologically based frequency regimen of exogenous pulsatile GnRH administration.

Acknowledgments

We wish to thank Ms. Franca Errani and Ms. Nancy Delaney-Perry for their expert assistance in preparing the manuscript; the nursing staff of the GCRC of the Massachusetts General Hospital for their meticulous care of these patients; and Prof. Paolo Palmieri, Dr. Guerra, and Dr. James Butler for their expert assistance in data analysis.

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