Neuroendocrine-gonadal axis in men: frequent sampling of LH, FSH, and testosterone

DANIEL I. SPRATT, LOUIS ST. L. O'DEA, DAVID SCHOENFELD, JAMES BUTLER, P. NARASIMHA RAO, AND WILLIAM F. CROWLEY, JR.
Reproductive Endocrine Unit, Vincent Memorial Research Laboratories, and Departments of Medicine and Gynecology, Massachusetts General Hospital, Boston, Massachusetts 02114

SPRATT, DANIEL I., LOUIS ST. L. O'DEA, DAVID SCHOENFELD, JAMES BUTLER, P. NARASIMHA RAO, AND WILLIAM F. CROWLEY, JR. *Neuroendocrine-gonadal axis in men: frequent sampling of LH, FSH, and testosterone.* Am. J. Physiol. 254 (Endocrinol. Metab. 17): E658–E666, 1988.—Previous studies of episodic hormone secretion of the hypothalamic-pituitary-gonadal axis in normal men have produced conflicting results due to examinations of small cohorts of subjects or to limited sampling techniques. We evaluated gonadotropin and testosterone (T) secretory patterns in 20 normal men by sampling blood at 10-min intervals for luteinizing hormone (LH) and follicle-stimulating hormone (FSH). T concentrations were also analyzed at 20-min intervals in 10 subjects. A previously unappreciated spectrum of gonadotropin and T secretory patterns was observed in normal men. Both mean LH concentrations and mean LH pulse amplitudes varied fourfold between individuals. LH interpulse intervals varied from 30 to 480 min (mean 119 ± 32). Results also suggested a relative refractory period at the level of the hypothalamus or pituitary. In three subjects, a striking nighttime accentuation of LH pulsations was noted. Through use of Fourier analysis, a diurnal variation in LH was observed in the population ($P < 0.02$). Mean FSH levels showed marked variation between individual subjects, with discrete pulses rarely observed. No diurnal variation in FSH secretion was noted. Serum T concentrations determined at 6-h intervals ranged from 105 to 1,316 ng/dl between subjects. When T was measured at 20-min intervals, marked intermittent declines in the T concentrations to levels well below the normal range were observed in 3 of 10 subjects. T secretion was found to lag behind LH secretion by ~40 min ($P < 0.02$).

pituitary-gonadal axis; episodic hormone secretion; gonadotropins; normal men

EPISODIC RELEASE of luteinizing hormone (LH) in men was first described in 1971 (28). Although considerable data regarding gonadotropin and testosterone (T) secretion have been collected since then (1, 3–5, 9, 10, 12–14, 17–23, 25, 27, 29, 30–33, 36–40, 42, 44, 47), conflicting results have been a striking feature of these previous attempts to characterize the normal male hypothalamic-pituitary-gonadal axis. Most studies have demonstrated marked variations in gonadotropin and T secretion between individuals within their study populations (1, 3, 5, 9, 10, 12–14, 21, 23, 25, 27, 29–33, 37, 39, 40, 42, 44, 47). Some groups have reported gonadotropins to be released in a diurnal rhythm (13, 23, 33, 37, 40, 44), whereas others have not (1, 3, 5, 9, 10, 21, 30, 32, 39, 42). Similarly, diurnal variation in serum T concentrations has been demonstrated by most groups (9, 10, 14, 17, 31, 33, 37). However, this finding has not been uniformly reported (1, 4, 19, 20). Finally, the specifics of the temporal relationship between episodic LH release and T secretion have yet to be clearly elucidated in humans (9, 10, 12, 18, 22, 36, 38, 42). To date, no study has been reported that examines the patterns of pulsatile secretion of the pituitary-gonadal hormones, their diurnal rhythms, and the temporal interrelationships of these hormones concomitantly in a large series of normal men.

Several principles should be followed to characterize such reproductive hormone secretory patterns more accurately. For evaluation of hormones released in a pulsatile fashion, a sufficiently intensive sampling frequency (in relation to the hormone secretory rate and serum half-life) must be used (3). We have previously demonstrated that the appropriate sampling interval for evaluation of gonadotropin secretion in humans appears to be ~10 min (8, 16), and this observation has subsequently been confirmed by others (45). In addition, previous studies have suggested that serial analysis of blood samples obtained at frequent intervals from individual subjects is required to evaluate both diurnal variations in release of gonadal steroids and the temporal correlations between different hormones (11, 15). Furthermore, when evaluating a diurnal pattern of secretion, the sampling must also be of sufficient duration (i.e., 24 h) to encompass the period of that rhythm. Finally, a sufficiently large study population must be studied to encompass the anticipated variance (34).

With these considerations in mind, over 24-h periods, we have examined the patterns of pituitary-gonadal-hormonal secretion in 20 normal men at 10-min intervals for gonadotropins and in 10 of these men at 20-min intervals for T. Mean parameters for hormone secretion and diurnal variation of hormones and dynamics of pulsatile secretion have been analyzed. Several subjects also underwent repeated evaluations to assess the reproducibility of these secretory patterns over time. Temporal relationships between secretion of the hormones of the pituitary-gonadal axis were also evaluated.

METHODS

Protocol. Twenty normal men between the ages of 18 and 37 yr were selected on the basis of *1*) normal pubertal

development, sexual function, and general health; *2*) a normal physical examination with testicular volumes >15 ml and weight within 10% of the ideal predicted body weight; *3*) normal screening serum LH (3–18 mIU/ml), FSH (3–18 mIU/ml), and T (300–1,100 ng/dl) concentrations; *4*) a normal semen analysis (>30 × 10^6 sperm/ml, >60% motility, and >2.5 ml volume); and *5*) normal serum prolactin and thyroxine concentrations. Exclusion criteria were *1*) concurrent or recent major illness; *2*) weight change of >15 lb within the previous year; *3*) history of narcotic or sedative use; *4*) history of moderate or excessive alcohol use; and *5*) concurrent medication other than aspirin or acetominophen. Subjects were also interviewed with regard to daily exercise patterns and major factors of stress in their lives.

Subjects were admitted to the General Clinical Research Center of the Massachusetts General Hospital where blood was drawn at 10-min intervals for 24 h via a catheter placed in a peripheral vein. Serum gonadotropin concentrations were determined at 10-min intervals, and serum T and estradiol (E_2) concentrations were determined at 6-h intervals in all subjects. T concentrations were also determined on serum pools from each study composed of equal aliquots of each sample (n = 145) obtained during the study. In 10 subjects, T concentrations were also determined at 20-min intervals. These latter 10 subjects consisted of 5 subjects with regular LH pulsation patterns and mean LH pulse amplitudes and interpulse intervals that approximated the study mean and 5 subjects whose LH pulsation pattern showed the greatest variation from the mean (i.e., nighttime predominance of pulsations or low frequency or amplitude of pulsations). The study was initiated either during the morning hours (n = 10) or the evening hours (n = 10) to avoid possible influences of initiation of sampling on interpretation of day and night variations in gonadotropin secretion. Sampling could be performed without disturbing the subjects during sleep periods via a 6-ft polyethylene line connected to the catheter during the night. This "long line" contained 7 ml dead space and was cleared with a 10-ml discard volume that was found during preliminary studies to prevent any dilution of blood samples. Subjects were asked to go to sleep between 2300 and 2400 and were awakened at 0700 if not already awake. Apparent sleep was observed and recorded at 10- or 20-min intervals. All subjects were observed to sleep during most of this period. Only one subject took a brief 30-min nap during the day. Seven of the 20 subjects underwent repeat studies at least 2 mo apart to determine the reproducibility of gonadotropin secretory patterns over time. These seven men consisted of three subjects with regular LH pulsations, with parameters approximating the population mean, and 4 subjects with marked nighttime predominance of LH pulsations or LH pulsations of minimal amplitude or frequency for the population. Whenever possible (n = 4), the repeat studies were begun with a 12-h difference in time of initiation of the first study.

Radioimmunoassays. Serum gonadotropin concentrations were determined by previously reported double-antibody radioimmunoassays (RIA) using the second international reference protein as the reference standard (15). Serum E_2 and T concentrations were determined by previously described radioimmunoassays (15, 35). Intra-assay and interassay coefficients of variation were <10% for the gonadotropin assays and <15% for the T and E_2 assays.

Statistics: mean concentrations, diurnal variations, and pulse detection. Mean LH and FSH levels were evaluated by calculating the arithmetic mean of all LH and FSH determinations over the 24-h sampling period. All results are expressed as means ± SD. Diurnal variations of gonadotropins in the 20 men were analyzed by two methods. First, mean LH and FSH concentrations and the mean amplitude of LH pulses were determined for each subject during the periods between 0700 and 2300 (day) and between 2300 and 0700 (night). Day and night values were compared by a two-tailed, paired t test. The difference in LH pulse frequency between day and night was also calculated for each man. The Wilcoxon matched-pairs sign-rank test was used to determine whether the mean of these values was different from zero. Second, the following procedure was used to test whether the series of LH and FSH measurements exhibited diurnal variation. The sine and cosine of the Fourier coefficients for a period of 24 h were calculated for each patient and subsequently tested to determine whether they were zero using Hotelling's t square test (41).

Diurnal variation of T secretion in the population was also determined by two methods. First, T determinations obtained at 6-h intervals were examined. A single T measurement in each subject obtained in the morning between 0400 and 1000 was compared with a sample obtained in each subject in the evening between 1600 and 2200 by a one-tailed, paired t test. Second, in the 10 men undergoing frequent sampling for T, the Fourier coefficients for a period of 24 h were tested as above to determine whether they were zero. To determine whether diurnal variations in LH were related to those observed in T secretion, the correlation coefficients were calculated for day-night differences in LH and day-night differences in T. For this comparison, mean values for T and LH were calculated for each subject from all samples obtained between 0700 and 2300 (day) and all samples collected between 2300 and 0700 (night). Correlations between differences in day and night LH values and differences in day and night T values were determined by regression analysis.

The presence of gonadotropin pulses was determined by the criterion of Santen and Bardin (39), which requires a 20% increase from nadir to peak LH concentrations for pulse detection. To minimize false-positive pulse detection, each pulse was also required to contain at least 2 points and to have an amplitude of at least 2 mIU/ml (at least 2 times the SD of our assays) as previously reported (8). The use of alternative minimal-amplitude criteria of 1 or 3 mIU/ml had minimal effect on mean amplitude or frequency of LH pulses (8). The presence of pulses was also evaluated by the PULSAR computer program (26). The G values of the PULSAR program were set as follows: G1 = 3.5, G2 = 2.4, G3 = 1.6, G4 = 1.2, and G5 = 1.0. In general, there was

excellent agreement between the two methods. LH pulse amplitude was defined as the difference between nadir and peak values. LH interpulse interval was defined as the time interval (in minutes) from the beginning of one LH pulse to the beginning of a subsequent LH pulse.

Statistics: normal ranges. The normal ranges of mean LH and FSH and of LH pulse frequency were defined by the maximum and minimum values observed in our population. Establishing normal boundaries for LH pulse amplitude was more problematic: the lower boundary was defined as the limit used for pulse detection (2 mIU/ml); a wide range of amplitudes was observed. Thus we wished to establish 95% confidence limits to improve discrimination of normal from abnormal and to identify the range of LH pulse amplitudes desirable during subsequent gonadotropin-releasing hormone (GnRH) therapy in idiopathic hypogonadotropic hypogonadism men more precisely. To avoid subjects with more frequent LH pulsations exerting greater influences on the calculation of these 95% confidence limits, we chose to weight all subjects equally. Thus the estimate of the 95th percentile in the population is the average of each subject's 95th percentile of amplitude.

Statistics: comparisons and correlations. The relationship of pulse amplitude to the interpulse interval either preceding or succeeding each pulse was analyzed by deriving the correlation of amplitudes to the preceding and succeeding interpulse intervals for each subject. The results of these correlations were then compared with zero, the null hypothesis, by *t* testing.

The relationships between LH and FSH secretion in all 20 subjects and between either gonadotropin and T secretion in the 10 subjects with frequent sampling for T were evaluated by cross-correlation analysis similar to the method previously described (15). First, each series was filtered with a symmetrical linear high-pass filter to remove any diurnal variation and linear trend. The correlation coefficients of one series with the other (lagged at 0, 10, 20, 30, 40, and 60 min) were then calculated for each subject (41). A Wilcoxon matched-pairs sign-rank test was used to determine whether the mean correlation coefficient was significantly greater than zero.

RESULTS

Discrete LH pulsations were observed in all 20 men (Figs. 1–3), with marked variations in rhythms of gonadotropin and T secretion observed between subjects. Our sampling frequency of 10-min intervals permitted us to describe in detail the dynamics of pulsatile hormone secretion and the temporal relationships between the secretion of hormones of the hypothalamic-pituitary-gonadal axis. The individual parameters of gonadotropin and T secretion are listed in Table 1.

LH secretion: mean concentrations and diurnal variation. Mean LH values in individual subjects ranged from 4.7 to 18.4 mIU/ml (mean 10.6 ± 3.2). A marked diurnal variation of LH was apparent in *subjects 2, 7,* and *16* during frequent sampling studies (Fig. 3). Mean serum LH concentrations calculated during day and night periods in each subject revealed significantly greater concentrations of LH during the night (mean 9.8 ± 3.5 mIU/ml ± SE day vs. 11.5 ± 4.2 night; $P < 0.02$) (Fig. 4). Fourier analysis, which used all data points in each subject, also revealed a significant diurnal variation in serum LH concentrations ($P < 0.02$).

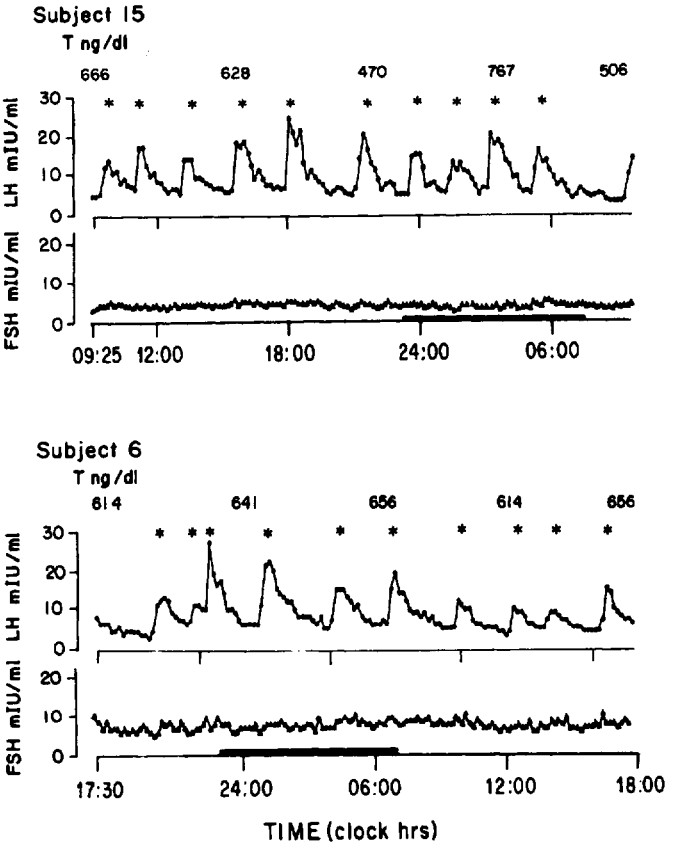

FIG. 1. Serum luteinizing hormone (LH; ●) and follicle-stimulating hormone (FSH; ▲) concentrations determined at 10-min intervals in 2 subjects showing a fairly regular episodic discharge of LH from the pituitary. No obvious pulsatile pattern of FSH release is evident. Serum testosterone (T) concentrations determined at 6-h intervals (listed at top of each study) were consistently within normal range of 300–1,100 ng/dl. Bar at *bottom* of each panel indicates nighttime hours. * LH pulsations as determined by modified Santen and Barden criteria.

Increases in pulse amplitude and frequency during the night were apparent by visual inspection of data from frequent sampling in *subject 16* (Fig. 3) and *subjects 2* and *7*. No diurnal variations, however, were demonstrable in our population during day and night periods in LH pulse amplitude (10.27 ± 3.6 day vs. 11.29 ± 3.6 mIU/ml night; NS) or frequency (0.44 ± 0.17 pulses/h day vs. 0.48 ± 0.14 pulses/h night; NS) (Fig. 4).

LH secretion: dynamics of pulsatile secretion. Figures 1–3 illustrate the marked variance in gonadotropin secretory patterns among our subjects. Figure 1 displays LH and FSH secretory patterns in two men with LH pulse amplitudes and interpulse intervals approximating the mean values observed in the population. Marked variations in LH pulse amplitude and frequency were observed (Fig. 2). As noted above, LH pulses occurred predominantly during the night in *subjects 2, 7,* and *16* (Fig. 3). *Subjects 7* and *16* subsequently underwent a second 24-study, and a similar pattern of nocturnal predominance of pulsatile LH secretion was observed (Fig. 3). The age of these three men ranged from 18 to 31 yr;

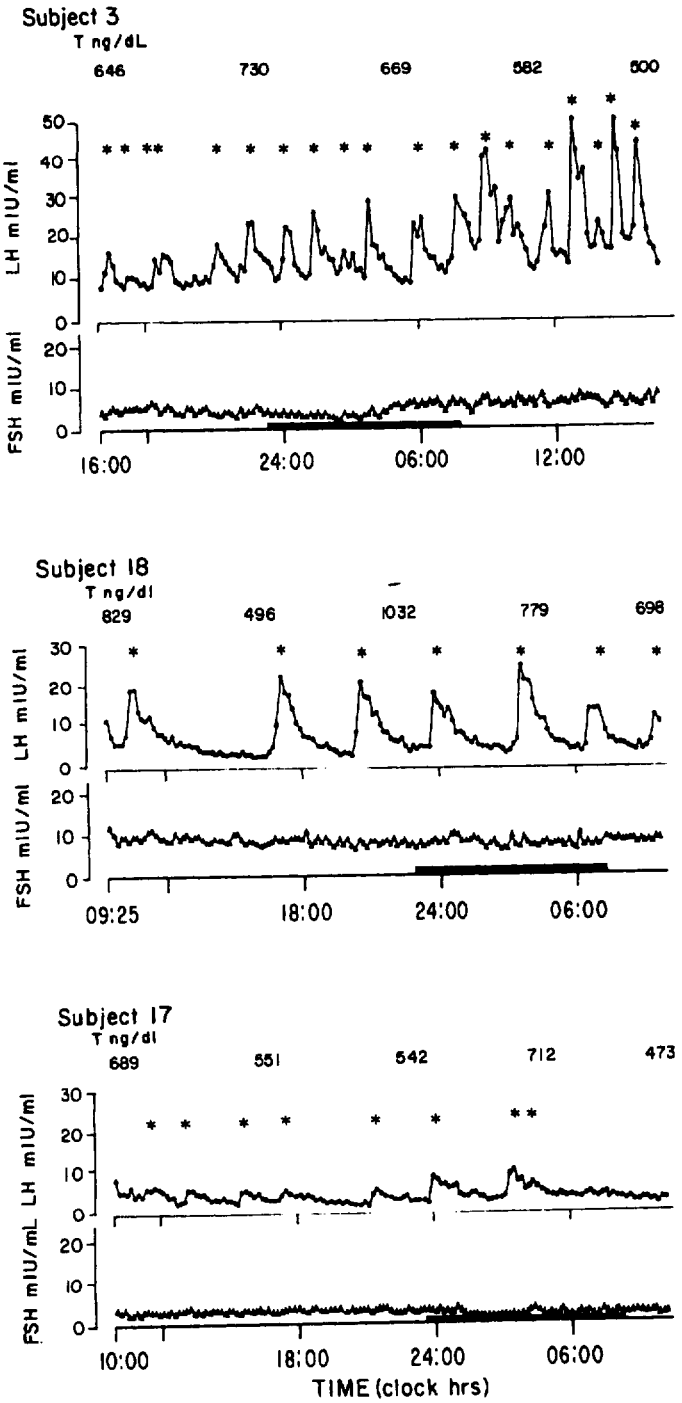

FIG. 2. Serum luteinizing hormone (LH; ●) and follicle-stimulating hormone (FSH; ▲) concentrations determined at 10-min intervals in 3 subjects demonstrating range of LH pulse amplitude and frequency observed in our normal men. *Top*: gonadotropin secretory patterns from our subject with greatest amplitude and frequency of LH pulses. *Middle*: results from our subject with slowest pulse frequency (except for those subjects with a nocturnal dominance of LH pulses). *Bottom*: subject with lowest LH pulse amplitude. Note that FSH levels vary between subjects but that FSH pulses are only rarely evident. Serum testosterone (T) concentrations were consistently within normal range in all 3 subjects. Bar at *bottom* of each panel indicates nighttime hours. * LH pulsations as determined by modified Santen and Barden criteria.

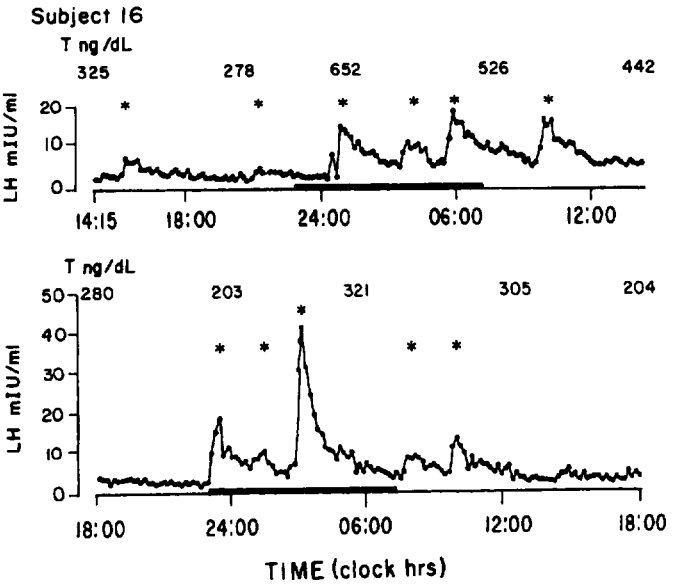

FIG. 3. Serum luteinizing hormone (LH; ●) concentrations in a subject demonstrating a nighttime predominance of LH secretion. Pattern was reproducible during a second 24-h study (*bottom*). No nocturnal increase in FSH levels occurred during either study (not shown). Note that serum testosterone (T) concentrations decreased well below normal range of 300–1,100 ng/dl during long LH interpulse intervals. Bar at *bottom* of each panel indicates nighttime hours. * LH pulsations.

thus no clear relationship of age to the presence of nighttime augmentation of LH secretion was apparent. No other historical or physical factors such as weight or amount of exercise distinguished these three subjects from the remainder of the population. *Subjects 10, 16,* and *19* reported long hours of work at careers that they enjoyed. Of these, only *subject 16* had a nocturnal pattern to his LH secretion. However, *subject 16* was studied on a second occasion at the end of a 2-wk vacation when he reported no "stress." His pattern of LH secretion showed no apparent change (Fig. 3). No other major factors of stress could be identified in any of the subjects. Visual inspection of LH pulse patterns in the other six subjects who underwent a second 24-h period of sampling also revealed similarities in the pattern of gonadotropin secretion on the two occasions.

The mean amplitude of LH pulses ranged from 4.5 ± 0.8 to 16.3 ± 2.5 mIU/ml (mean 10.6 ± 3.2). Figure 5*A* is a histogram of LH pulse amplitudes in these 20 men. The upper 95th percentile for LH pulse amplitude was 24.5 mIU/ml. The lower boundary of normal was set as the pulse-detection limit of 2 mIU/ml. The frequency of episodic LH secretion varied from 6 to 19 pulses/24 h (mean 10.9 ± 3.2) using the modified Santen and Barden criteria of pulse detection and from 7 to 18 pulses/24 h (mean 11.4 ± 3.0) using the PULSAR program. The mean LH interpulse interval was 119 ± 32 min, and the median interpulse interval was 120 min. Figure 5*B* is a histogram of LH interpulse intervals in the 20 subjects. There was a significant positive correlation between the amplitude of the LH pulse and both the preceding and succeeding interpulse interval ($r = 0.286$ and 0.338; $P < 0.003$ and 0.002, respectively).

FSH secretion. Mean FSH values varied from 3.3 to

TABLE 1. *Parameters of gonadotropin and T secretion in 20 normal men*

Subject	Age, yr	LH	FSH	LH Amplitude	Pulse Frequency	AM T	PM T	T	E
1	37	14.1	9.9	13.1	10	819	829	969	41
2	31	8.0	3.9	8.5	11	933	420	589	<20
3	19	18.4	5.7	16.3	19	582	588	590	<20
4	22	12.3	4.8	10.1	13	680	488	479	<20
5	29	6.9	3.4	6.8	10	634	762	499	30
6	26	9.4	7.8	10.3	10	656	656	552	22
7	18	8.8	7.5	8.2	10	470	239	329	<20
8	24	13.7	3.9	13.1	14	1,014	332	485	<20
9	37	15.1	15.7	15.2	10	1,184	903	829	43
10	35	11.7	12.2	10.1	11	509	625	473	39
11	29	8.8	6.5	8.5	13	1,220	1,316	820	<20
12	29	8.7	8.3	10.6	7	891	619	694	<20
13	32	13.1	14.6	14.4	10	753	855	863	41
14	26	9.2	4.8	11.5	9	823	982	855	34
15	29	9.7	4.1	10.7	11	506	470	559	35
16	31	7.4	8.6	8.4	6	525	278	467	28
17	22	4.7	3.3	4.5	7	712	551	628	26
18	24	8.5	8.6	15.3	7	698	1,032	358	45
19	34	10.1	5.2	9.1	14	828	647	765	<20
20	32	12.2	5.6	6.3	16	637	732	761	40
Mean ± SD	28±6	10.6±3.2	7.2±3.6	10.9±3.2	10.9±3.2	754±214	672±270	628±182	

Mean luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in mIU/ml calculated from all determinations in each 24-h study. LH amplitude is measured in mIU/ml. Pulse frequency, no. of pulses observed in 24 h; AM T, serum testosterone (T) concentration (ng/dl) determined between 0400 and 1000; PM T, serum T concentration (ng/dl) determined between 1600 and 2000; T, T concentration determined from serum pools from each 24-h study; E, serum estradiol determined on initial serum sample in pg/ml.

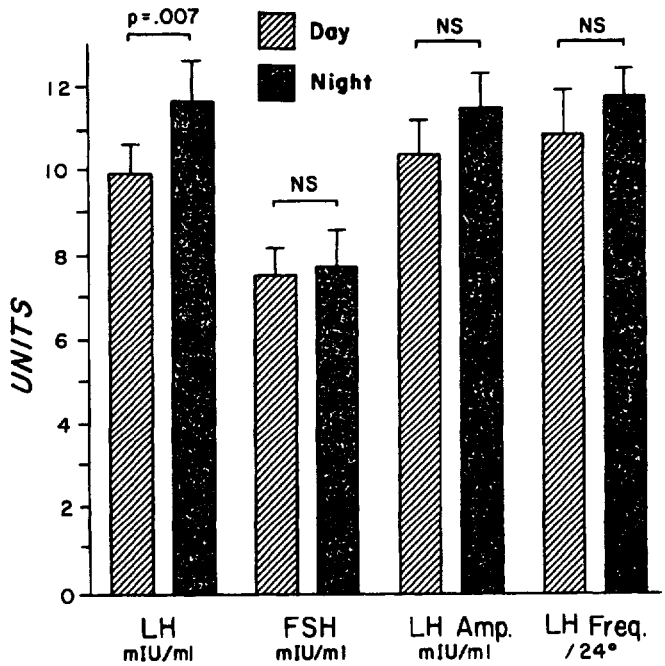

FIG. 4. Daytime and nighttime values of gonadotropin secretory parameters determined from initial 24-h frequent-sampling studies in 20 normal men. Mean serum luteinizing hormone (LH) concentrations were significantly greater during nighttime hours, although nocturnal increases in LH pulse amplitude and frequency were not significant by themselves. No diurnal variation in serum follicle-stimulating hormone (FSH) concentrations was evident.

15.7 mIU/ml (mean 7.2 ± 3.6). No significant difference was found in mean serum FSH concentrations calculated during day and night periods in each subject (7.18 ± 3.5 mIU/ml day vs. 7.28 ± 3.8 mIU/ml night; NS) (Fig. 4). Fourier analysis also revealed no diurnal variation in FSH concentrations. In most men, FSH pulses were only rarely detected. The correlation coefficient between FSH and LH measurements (with a time lag of 0 min) varied from −0.04 to 0.43 for the 20 men. A small but highly significant correlation was demonstrated with a mean correlation coefficient of 0.3, which was significantly greater than zero ($P < 0.001$). Furthermore, when one series was lagged in time, the correlation was reduced, indicating a predominance of simultaneous secretion of LH and FSH in these normal men.

Testosterone secretion. T concentrations determined on serum pools varied from 329 to 969 ng/dl (mean 628 ± 182). Serum T concentrations determined at 6-h intervals in the initial studies of the 20 men ranged from 105 to 1,316 ng/dl (mean 687 ± 232). Although some subjects displayed a marked decline in serum T levels from morning to evening (Table 1), no diurnal difference of T levels could be demonstrated statistically across the population using the T concentrations determined at 6-h intervals. There was no significant difference between the mean morning T (mean 754 ± 214 ng/dl) and mean evening T (mean 672 ± 270 ng/dl) in the population. However, Fourier analysis, using all data points in the 10 subjects sampled for T at 20-min intervals, demonstrated a significant diurnal variation in T levels across this subpopulation ($P < 0.01$). This Fourier analysis did not determine the relationship of time of day to the diurnal variation. The men with clear nighttime increases in LH secretion (*subjects 2, 7,* and *16*) appeared to have the greatest diurnal variation of T levels (Figs. 3 and 6, *bottom*; Table 1).

Frequent sampling of serum T concentrations in the 10 subjects selected on the basis of their LH pulsation patterns demonstrated several findings of interest. First,

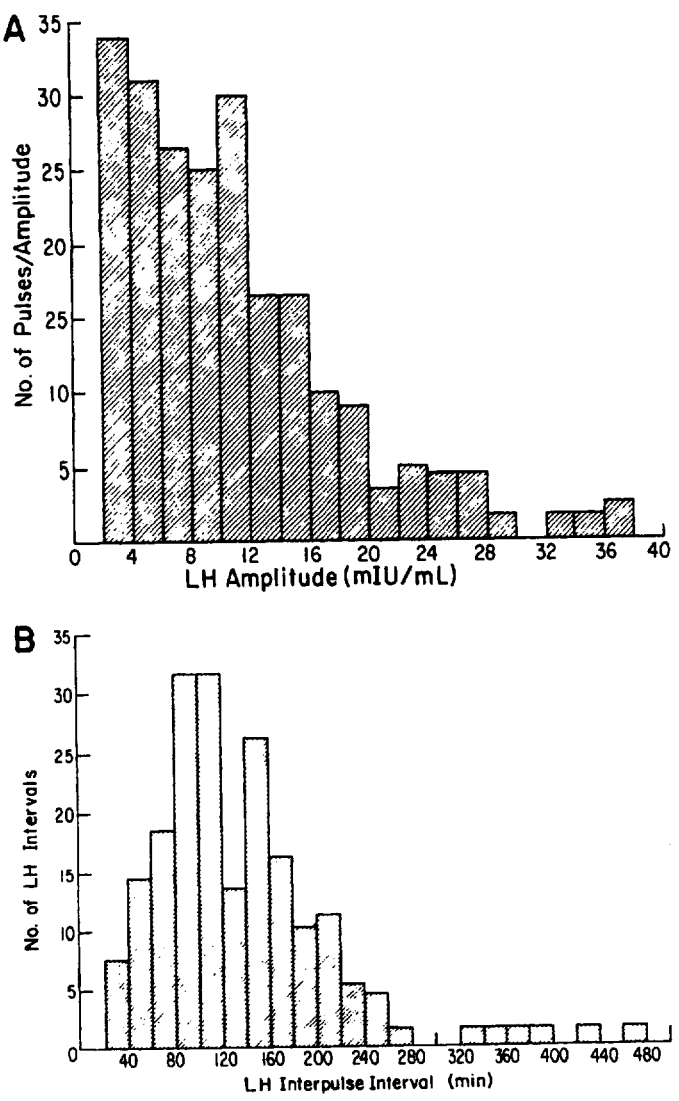

FIG. 5. *A*: histogram of luteinizing hormone (LH) pulse amplitudes (calculated to nearest 2 mIU/ml) measured in initial 24-h sampling periods of 20 normal men. LH pulses of small amplitude were more common than those of large amplitude. *B*: histogram of LH interpulse intervals (calculated to nearest 20 min) in same sampling studies as *A*. Interpulse intervals of ~120 min were most common in our men.

in five subjects, T concentrations remained consistently within the normal range (Fig. 6, *top*). In three subjects, T concentrations were occasionally observed to drop below the normal level as illustrated by *subject 10* (Fig. 6, *middle*). In the remaining two men (*subjects 7* and *16*), T concentrations decreased well below the normal range for prolonged periods (Fig. 6, *bottom*). A detailed review of symptoms of these latter three men revealed no clinical evidence of T deficiency such as decreased libido, potency, or frequency of shaving. Decreases in serum T concentrations were especially prone to occur during the longer LH interpulse intervals (Fig. 6). In addition, pulsatile T secretion was most evident to visual inspection when the frequency of LH pulses was markedly decreased (Fig. 6, *bottom*).

Estradiol. Serum E_2 concentrations determined on the initial blood sample in each subject ranged from <20 to 45 pg/ml (Table 1). Because the concentration was less

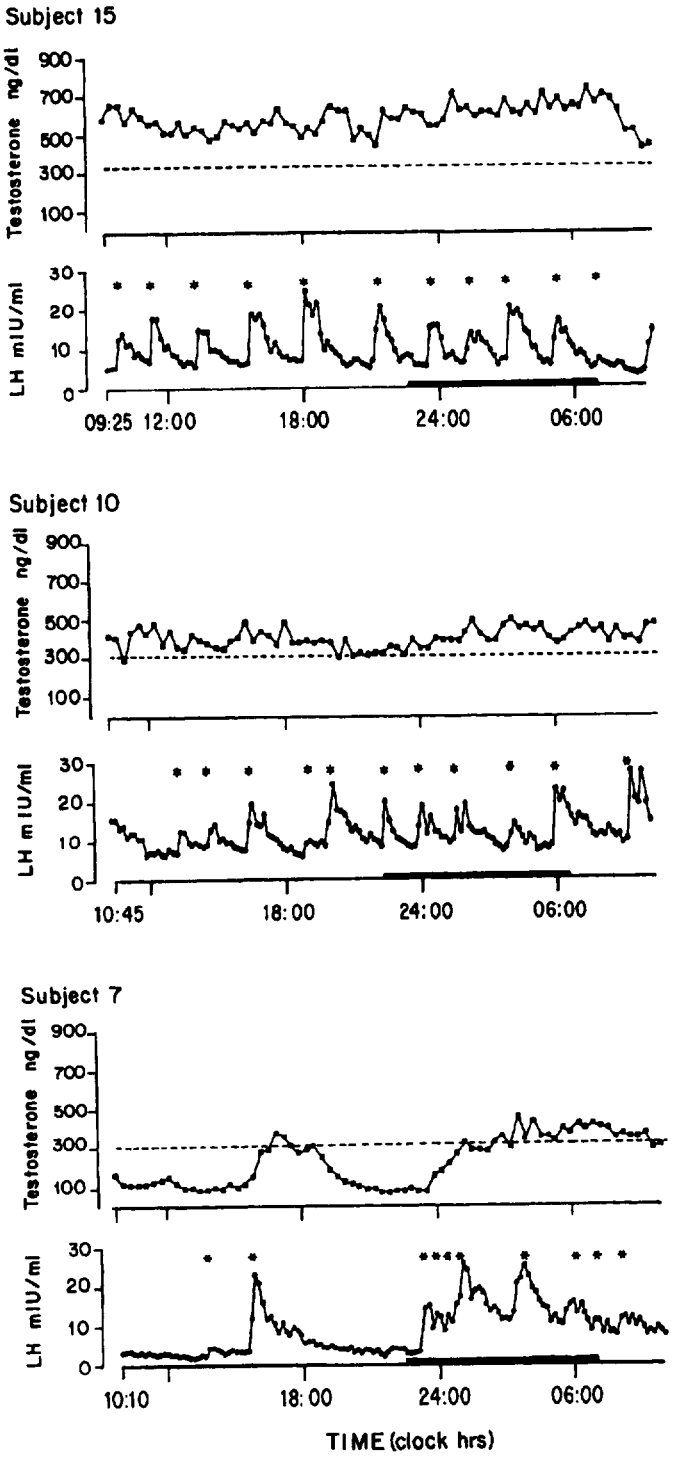

FIG. 6. Serum luteinizing hormone (LH; •) concentrations determined at 10-min intervals and testosterone (T) concentrations determined at 20-min intervals in 3 subjects. ----, Lower limit of normal range of serum T concentrations. Serum T concentrations were sometimes determined to be below normal range in these men with normal reproductive function. Note that diurnal variation of T secretion is not always evident. Bar at *bottom* of each panel indicates nighttime hours. * LH pulsations.

than the assay-detection limit in several subjects, a mean value could not be calculated. Concentrations of E_2 did not correlate with either LH pulse amplitude or frequency.

Relation of gonadotropin to T secretion. Cross-correlation analysis of LH values to T values and FSH values to T values in the 10 men who had T concentrations measured at 20-min intervals demonstrated a positive relationship between LH and T concentrations, with mean correlation coefficients for the population of −0.05, −0.05, 0.12, 0.13, 0.10, and −0.03 at 0, 20, 40, 60, 80, and 100 min, respectively. The mean correlation of T to LH was significant at a lag of 40 min ($P < 0.02$). FSH and T were also correlated at 40 min ($P < 0.05$). By use of regression analysis, the pooled serum T concentration of each subject did not correlate with mean LH or FSH concentrations ($r = 0.18$ and 0.18, respectively; NS), with LH pulse frequency ($r = 0.15$; NS), or with LH pulse amplitude ($r = 0.014$; NS).

DISCUSSION

By employing frequent blood sampling in a large cohort of normal men over 24-h sampling periods, we were able to demonstrate a previously unappreciated spectrum of patterns of gonadotropin and T secretion occurring in normal men. Striking variations occurred in both LH and T secretion in our rigorously defined normal subjects. In addition, these data permit us to deduce several principles of male hypothalamic-pituitary-gonadal physiology.

With regard to LH secretion, a marked variability in the rhythm of LH release exists both within and between individual men. Wide variations in LH pulse frequency, amplitude, and rhythm were observed across the population. The striking nighttime accentuation of LH pulsations found in some of our men has not previously been reported, except in pubertal children (6). Moreover, this presence or absence of the nocturnal accentuation of LH secretion appeared to be a reproducible characteristic of some individuals when examined over time. No factors such as age, levels of exercise, or stress could be identified to correlate with the nighttime accentuation of LH pulses in these subjects. As demonstrated in *subject 4* (Fig. 3), insertion of the intravenous catheter and initiation of the study did not appear to be related to the sparsity of LH pulses observed during the day in these subjects. Because electroencephalographic tracings were not employed, differences in stages or patterns of sleep cannot be excluded.

Some previous studies which have not used frequent sampling suggested the presence of diurnal variation of LH secretion (23, 33, 37, 40, 44), particularly in young adults (44), whereas others have not (1, 3, 9, 10, 21, 30, 32, 42). In a study employing frequent sampling in five adult men, Boyar et al. (5) observed no augmentation of LH release during sleep. The detection of men with diurnal variation of LH secretion in our study in contrast to Boyar's study may result from evaluation of a greater number of subjects and/or a more intensive sampling regimen. This reasoning is supported by a recent study that evaluated 36 normal men with frequent sampling over 8-h periods during the day and noted that some subjects (similar to our men with nighttime LH secretion) exhibited only 1 LH pulse (47). Thus our results demonstrate that although some normal men have no apparent diurnal variation of LH secretion, others have a dramatic nighttime predominance of LH secretion.

In addition to this spectrum of rhythms of LH release, variations in both the LH pulse amplitude and interpulse interval were apparent, both within each subject's study and across the population. A similar variability between subjects has been observed using shorter study periods and less frequent sampling (47).

In our subjects, the mean and median interpulse interval of 120 min was similar to that reported in other studies (3, 5, 24, 25, 27–29, 39, 45, 48).

Examination of the histogram of interpulse intervals suggests the existence of a refractory period at the level of the hypothalamus or pituitary, a previously unreported characteristic of the hypothalamic-pituitary axis. If there is no effect of length of interval on generation of the subsequent pulse, the process is characterized as "Poisson." With a Poisson process, the frequency of occurrence of intervals decreases exponentially (i.e., shorter intervals are more common and longer intervals are less common) (2). However, in our data, such an exponential decrease occurred only with interpulse intervals >120 min and was thus consistent with a relative refractory period occurring for ~120 min after an LH pulse. The number of interpulse intervals of <120 min could also be explained by the occurrence of undetectable LH pulses (amplitude <2 mIU/ml) occurring at these shorter intervals. This phenomenon would also be consistent with a relative refractory period.

Histograms of LH interpulse intervals previously reported in normal men and women (7, 45) are consistent with the notion of a refractory period. The presence of this refractory period is also implied by our previous observation that when GnRH is administered at intervals of <120 min to GnRH-deficient men, detectable pulses of LH do not consistently follow (43). This refractoriness to GnRH occurring at the level of the anterior pituitary may provide one means of regulating the frequency of gonadotropin release.

Further evidence for a refractory period at the hypothalamus or pituitary was provided by the correlations between LH pulse amplitude and both the preceding and succeeding interpulse intervals. Larger LH pulses were more likely to be followed by longer intervals, suggesting a refractory period whose duration varies with the magnitude of the amount of LH discharged within a given pulse of secretion. Furthermore, shorter interpulse intervals tended to be followed by smaller LH pulses, implying a relative refractory period during which the LH pulse amplitude varies with the time interval from the previous GnRH or LH secretory episode. Our previous report that more frequent administration of GnRH boluses to GnRH-deficient men results in a decrease in the amplitude of the ensuing LH response is also consistent with this latter notion (43). The extent to which these correlations indicate a relation between the interpulse interval of GnRH secretion from the hypothalamus and the ensuing LH pituitary response rather than a correlation directly between the preceding and succeeding intervals of hypothalamic secretion of GnRH was established by our previous study, which found no direct effect of the

length of one interpulse interval on the length of the subsequent interval (7).

With regard to FSH secretion, a variation in mean levels across our population of normal men similar to those observed with LH was present. However, no diurnal variation was observed, and pulses of FSH secretion were only rarely detected. As anticipated, LH and FSH secretion were highly concordant in their cross-correlated levels. The rarity of FSH pulses in normal men has been common to most studies with the exception of the recent findings of one group (46).

Analysis of T secretory dynamics in normal men and their relationship to gonadotropins also revealed several unexpected observations. First, a diurnal pattern of T secretion was quite evident in some subjects but not others. Most previous studies have noted a diurnal variation in T levels across their populations (9, 10, 14, 17, 31, 33, 37), although others have failed to substantiate this observation (1, 4, 19, 20). In those studies reporting such a variation, examination of data from individual subjects (when available) revealed in two studies (9, 37) that some of their subjects exhibited this apparent diurnal variation of T that others did not. In a third study (33), all 10 subjects revealed a diurnal variation of T. Therefore a decline in T levels from morning to evening most likely does not occur in all men.

A second striking observation in our study was the intermittent decrease in serum T concentrations to levels well below the normal range in some of our otherwise normal subjects. Serum T concentrations fell below the previously accepted normal range in half of the subjects undergoing sampling at 20-min intervals for T. However, three of the subjects whose T dynamics were examined in greater detail were selected on the basis of screening T levels that were intermittently low. Therefore the incidence of this finding may be somewhat overestimated in our population. Nonetheless, these findings clearly demonstrate that serum T concentrations may occasionally fall well below the normal range in men with normal reproductive function. The practical consequence of this finding is that a low serum T concentration determined on a single blood specimen may not, by itself, be abnormal.

Finally, the precise temporal relationship of LH to T secretion has been difficult to establish in normal men. It is well established that human LH stimulates Leydig cells to produce T. However, some studies have shown no correlation between LH and T levels in normal men (9, 36, 42). These studies employed sampling intervals of ≥2 h or studied fewer than five subjects and thus may not have had the statistical power or intensity of sampling to discern relationships of LH to T release with precision. Other studies that have employed more frequent sampling have demonstrated a relationship to LH to T secretion in pubertal children or young men with a lag time of 20–120 min (10, 12, 18, 22). Data from our study demonstrate a temporal correlation of LH to T secretion in normal men with a lag of ~40 min in T secretion.

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Present addresses: J. Butler, Dept. of Environmental Science and Physiology, Harvard School of Public Health, Boston, MA 02115; P. N. Rao, Southwest Foundation for Biomedical Research, San Antonio, TX.

Address for reprint requests: D. I. Spratt, Div. of Endocrinology, Maine Medical Center, Portland, ME 04102.

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