

Biologically Active Luteinizing Hormone Is Secreted in Episodic Pulsations that Vary in Relation to Stage of the Menstrual Cycle*

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ABSTRACT. To characterize the physiological pattern(s) of bioactive LH secretion in normal women, serial blood samples were withdrawn at frequent intervals in each of six women at three different stages of the menstrual cycle. Plasma LH concentrations were quantitated in each sample by both rat interstitial cell testosterone (RICT) bioassay and immunoassay (RIA). When the resulting RICT and RIA LH profiles were systematically compared, we found that mean (and integrated) plasma concentrations of bioactive LH were approximately 2-fold higher than immunoactive LH levels at all stages of the menstrual cycle. In addition, unequivocal prominent pulsations of bioactive LH could be demonstrated in these women throughout the normal menstrual cycle. For all stages of the menstrual cycle, bioactive to immunoactive LH ratios within LH pulses were significantly ($P < 0.01$) increased over these ratios in the interpulse periods. The frequency of bioactive LH pulses increased dramatically from 0.44 ± 0.24 (\pm SD) pulses/h in the early follicular phase to 1.21 ± 0.07 pulses/h in the late follicular

phase ($P < 0.003$), and then declined to only 0.25 pulses/h in the luteal phase ($P < 0.001$). Notably, significant discordance existed between bioactive and immunoactive LH pulses, with 30% of immunoactive and 14% of bioactive LH pulses occurring alone.

In conclusion, using the RICT, we demonstrated that biologically active LH is secreted in discrete episodic pulsations in normal women. Estimates of the bioactive to immunoactive LH ratio indicate that these pulses of LH are preferentially enriched in biologically active compared to immunoactive hormone. The properties of bioactive LH pulses are under physiological control, since the amplitude and frequency of bioactive LH pulses vary significantly in relation to phases of the menstrual cycle. Since significant discordance exists between immunoactive and bioactive LH pulsations in normal women, we suggest that estimates of the circulating concentrations of biologically active LH (rather than immunoactive LH alone) are necessary to characterize fully physiological patterns of LH secretion during the menstrual cycle. (*J Clin Endocrinol Metab* 58: 1050, 1984)

MEAN serum levels of immunoactive LH vary significantly throughout the normal human menstrual cycle (1–5). These changes are believed to primarily reflect alterations in the amplitude and frequency of pulsatile LH secretion as estimated by RIA (6–11). More recently, mean plasma concentrations of biologically active LH have also been shown to vary during the normal menstrual cycle (12–15). However, to date, a pulsatile mode of bioactive LH release has not yet been demonstrated in women. Moreover, pulsatile patterns of bioactive LH release have not been characterized at various stages of the menstrual cycle. In addition, the degree to

which significant discordance occurs between immunoactive and bioactive estimates of LH pulses in cycling women remains unknown, despite the large body of information on immunoactive LH pulses in health and disease (1–11).

In an effort to delineate properties of bioactive LH secretion under physiological conditions in the normal menstrual cycle, we posed the following specific questions. 1) Is bioactive LH secreted in episodic pulses in women? 2) If bioactive LH is released in a pulsatile fashion, how do the frequency and amplitude of bioactive LH pulses vary during the menstrual cycle? 3) To what extent are immunoactive and bioactive LH pulses concordant or discordant in normally menstruating women?

To address these questions, we measured both the immunoactive and bioactive LH content of each of multiple serial blood samples drawn in normal women during three stages of the menstrual cycle. Using this approach,

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we were able to directly compare properties of bioactive LH secretion with those of immunoactive LH secretion in relation to different physiological phases of the menstrual cycle. These comparisons disclosed significant discrepancies between immunoactive and bioactive estimates of LH release in healthy women.

Materials and Methods

Studies were conducted in six normal women (mean age, 26.6 yr) with demonstrated normal menstrual cycles based upon serial estimates of basal body temperature and serum progesterone concentrations for at least 2 consecutive months. Each woman had a normal developmental history, including age of menarche, and normal serum concentrations of thyroid hormones, PRL, immunoactive LH and FSH, estradiol, and testosterone. No subject was receiving medications, had experienced weight loss, or was engaged in strenuous sports. All women provided written informed consent, and the study was approved by the Human Investigations Committee of the Massachusetts General Hospital.

Blood was collected from six women during each of three stages of the same menstrual cycle [except in one woman (subject A), in whom the first sampling session was performed in the luteal phase of one cycle, and the remaining sessions in the next cycle]. Women were sampled in the early follicular phase (day 3 or 4 after the onset of menstrual bleeding), in the late follicular phase (days 11–14 of the cycle), or in the luteal phase (2–7 days before the onset of menses). For these three stages of the menstrual cycle, the respective mean \pm SD serum concentrations of estradiol in these women were 53.3 ± 15.9 , 111 ± 32 , and 145 ± 35 pg/ml. Serum progesterone concentrations increased significantly from less than 1 ng/ml in the follicular phase to 15.9 ± 7.8 (range, 6.5–23.3) during the luteal phase on the day of study.

To obtain accurate estimates of average LH concentrations, serial blood samples were withdrawn at 10- or 15-min intervals for at least 4 h in all six women. To provide samples suitable for LH pulse analyses, four women had extended sessions of repetitive blood sampling at 15-min intervals for 8 h in the early follicular and luteal phases and every 10 min for 6 h at midcycle (late follicular phase).

Plasma samples were assayed for immunoactive LH by double antibody RIA, with a sensitivity of 1 mIU/ml in terms of the Second International Reference Preparation of human Menopausal gonadotropin (hMG), and for bioactive LH by the rat interstitial cell testosterone assay (RICT), with a sensitivity of 0.4 mIU/ml or 3 pg pure LH (LER 1533) (12, 16). The potencies of the purified preparation in terms of the hMG standard were similar when measured by bioassay or RIA, *viz.* 13,500 (11,200–15,200) and 13,700 (11,100–14,900) IU/mg respectively. Thus, the bioactive to immunoactive (bio:immuno) ratios of plasma samples calculated for LH measured in terms of hMG or pure LH are very similar. The RICT within-assay coefficient of variation averaged 8.8% (13 replicates) in the assays applicable to this study, with an absolute range in other studies from 8.2% in normal men to 9.2% in children and postmenopausal women (17). For the RIA, the intraassay coefficients of variation in this study were 8.4% (at 2 mIU/ml),

6.8% (at 11.5 mIU/ml), and 4.6% (at 47 mIU/ml). Serum estradiol and progesterone concentrations were measured by RIA (18).

The plasma LH secretion profiles were analyzed for significant episodic fluctuations by the computerized pulse detection method of Clifton and Steiner (19). This method performs iterative data scans to identify significant pulses that exceed a threshold value, which is initially estimated as 2.7 times the within-assay coefficient of variation. Iteration with threshold adjustment is continued until the probability of obtaining a false pulse equals the probability of missing a true pulse (19). The frequency (number of pulses per sampling interval) and incremental amplitude (nadir to peak increases in LH concentration, expressed in milliinternational units per ml) of pulses was estimated in the presence of random measurement errors (noise). For each analysis, an estimate of the pulse signal to noise ratio is also given, which in the present studies exceeded 2.3 (nominal value, ≥ 1.5). When a single prominent LH pulse was apparent (amplitude exceeding that of other LH pulses by $>50\%$), the data were rescanned after omission of the dominant pulse in order to obviate damping of the residual pulse signals. In addition, the program was modified to display the individual significant increases and decreases (pulses) detected, which were then enumerated. The area under the LH concentration *vs.* time curve and the fractional amplitude of significant pulses (given as percent above preceding nadir) were computed with the program of Santen and Bardin (8). This program was also modified to detect pulses exceeding 4 times the intraassay coefficient of variation. Smoothed (interpulse) baseline LH concentrations were estimated by a weighted moving average using a 12-h trend time (20, 21).

Pulse data are given as means \pm SD. Comparisons between bio- and immunoactive LH values were analyzed by within-subject comparisons using Student's paired two-tailed *t* test. Bio- and immunoactive LH pulses were considered concordant whenever the pulses overlapped in time. Analysis of variance with correction for repeated measures was used to assess changes in relation to the three stages of the menstrual cycle (22). Significance was assumed for $P < 0.05$. To test the hypothesis that increased bio:immuno LH ratios occurred preferentially within bioactive LH peaks, nonparametric analysis was applied. For each woman and for each stage of the menstrual cycle, bio:immuno LH ratios were ranked to determine the corresponding median ratio. We then enumerated how many bio:immuno ratios above the median occurred within actual bioactive LH pulses and how many bio:immuno ratios above the median occurred in the corresponding interpulse baseline. A χ^2 table was constructed to analyze the expected *vs.* observed distribution of increased bio:immuno ratios (22).

Results

Mean (and integrated) plasma concentrations of bioactive vs. immunoactive LH in relation to stage of the menstrual cycle

To determine mean plasma concentrations of bioactive (and immunoactive) LH accurately in relation to phase of the menstrual cycle, six women underwent repetitive venous sampling for at least 4 h. In the early follicular

phase, the mean plasma concentration of bioactive LH was more than twice that of immunoactive LH ($P < 0.05$; Table 1), yielding an average bio:immuno LH ratio of 2.33 ± 0.49 (median ratio, 2.20). Compared to the early follicular phase, the late follicular phase plasma concentrations of bioactive LH increased approximately 50%, while those of immunoactive LH increased 27% ($P < 0.02$), which led to a mean bio:immuno LH ratio of 2.81 ± 0.71 (median, 2.63). In the luteal phase, mean plasma levels of both bioactive LH and immunoactive LH declined significantly and to a similar degree compared with late follicular phase levels ($P < 0.05$) to give a mean bio:immuno LH ratio of 2.63 ± 0.74 (median, 2.20), which was not significantly different from that in the early follicular phase. Thus, mean bio:immuno LH ratios exceeded 2 in these normal women and tended to increase in the late follicular phase of the menstrual cycle. We could not discern a significant correlation between increased bio:immuno LH ratios and serum estradiol concentrations.

When areas under the time-concentration curves for bioactive and immunoactive LH were estimated for different stages of the menstrual cycle, integrated levels paralleled mean LH levels across the menstrual cycle and varied significantly in relation to stage of the cycle ($P < 0.02$; see Table 1). The integrated values for bioac-

tive LH were approximately 2.5-fold higher than those for immunoactive LH, in accordance with the estimates of mean bio:immuno LH ratios in these women.

Pulsatile secretion of bioactive and immunoactive LH in relation to stage of the menstrual cycle

To search for evidence of a pulsatile mode of bioactive LH secretion in normally cycling women, we undertook repetitive venous sampling for 6 or 8 h at three stages of the menstrual cycle. Sampling blood at 10- or 15-min intervals unmasked a strikingly pulsatile pattern of bioactive LH secretion. Results from a representative normal woman (volunteer C) are depicted in Fig. 1 (quantitative data are given below). The three panels in Fig. 1 illustrate LH time-concentration profiles in the early follicular phase (*upper panel*), late follicular phase (*middle panel*), and luteal phase (*lower panel*). Note changes in the *ordinate scale* to accommodate the wide range of LH concentrations observed during the cycle. The individual pulses actually enumerated in this woman are identified in the schematized *lower curve* of each panel. Thus, six discrete pulses occurred in the 8 h of sampling in the early follicular phase, seven pulses in the 6 h of sampling in the late follicular phase, and two pulses per 8 h in the luteal phase.

TABLE 1. Characteristics of pulsatile bioactive LH secretion: relation to immunoactive LH secretion and stage of the menstrual cycle

Stage of cycle	Mean LH ^a	Area	Pulse frequency (pulses/h)		Amplitude characteristics			Interpulse basal ^e
			C&S ^b	S&B ^c	Incremental ^a	%	Peak ^a	
Early follicular								
Bioactive	23.2 ± 15	1420 ± 890	0.44 ± 0.24 (177 ± 66) ^d	0.47 ± 0.23	10.6 ± 5.4	47 ± 17	31 ± 18	23 ± 14
Immunoactive	9.7 ± 4	430 ± 360	0.81 ± 0.22 (88 ± 34)	0.66 ± 0.16	4.2 ± 1.4	54 ± 18	12 ± 6	9 ± 4
<i>P</i> ^e	<0.05	<0.05	NS	NS	<0.05	NS	<0.05	<0.05
Late follicular								
Bioactive	43.2 ± 18	3030 ± 1710	1.21 ± 0.07 (57 ± 9)	1.0 ± 0.12	21.7 ± 10	69 ± 8	59 ± 27	38 ± 16
Immunoactive	15.0 ± 4	1050 ± 460	1.13 ± 0.18 (65 ± 18)	0.83 ± 0.30	7.1 ± 4	76 ± 41	19 ± 5	15 ± 4
<i>P</i>	<0.02	<0.05	NS	NS	<0.05	NS	<0.02	<0.02
Luteal								
Bioactive	27.0 ± 23	1630 ± 1420	0.25 (239 ± 5)	0.34 ± 0.05	15.4 ± 2	250 ± 180	43 ± 26	24 ± 26
Immunoactive	10.5 ± 6	630 ± 370	0.37 ± 0.09 (198 ± 28)	0.46 ± 0.06	13.1 ± 6	241 ± 237	20 ± 7	8 ± 7
<i>P</i>	NS	NS	NS	NS	NS	NS	NS	NS

^a Milliinternational units per ml (mean ± SD).

^b Clifton and Steiner program (19) (see *Materials and Methods*).

^c Santen and Bardin algorithm (8), modified to a threshold requirement of 4 times the relevant intraassay coefficient of variation.

^d Periodicity (minutes per pulse) is given in parentheses.

^e Immunoactive vs. bioactive LH measurements.

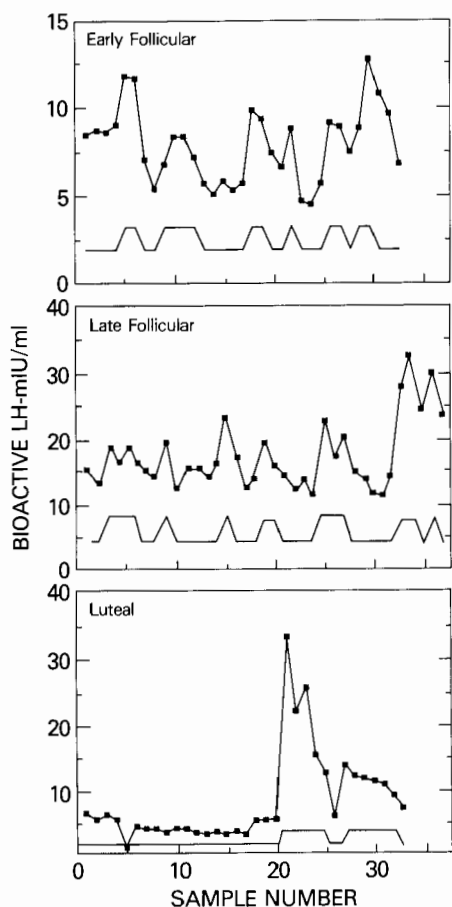


FIG. 1. Pulsatile patterns of bioactive LH release in a normal woman during different stages of the menstrual cycle. Blood was drawn serially at frequent intervals in the early follicular phase (top panel), late follicular phase (middle panel), or luteal phase (bottom panel) of subject C. The plasma concentrations of bioactive LH are denoted by the solid squares in the upper curves of each panel. The computer-detected pulses are identified in the lower schematized curves. Note that the ordinate scale in the early follicular phase is abbreviated because bioactive LH levels are lower. The abscissa gives the number of the serial plasma samples drawn every 15 min (top and bottom panels) and 10 min (middle panel). In this woman, six pulses of bioactive LH were detected during 8 h of serial sampling in the early follicular phase, seven pulses were found during 6 h in the late follicular phase, and two pulses were identified in 8 h in the luteal phase.

Quantitative analyses of bioactive LH pulse properties revealed significant changes in relation to stage of the menstrual cycle (Table 1). Most strikingly, bioactive LH pulse frequency increased significantly from 0.44 ± 0.24 pulses/h in the early follicular phase to 1.21 ± 0.07 pulses/h in the late follicular phase ($P < 0.003$), and then decreased significantly to 0.25 pulses/h ($P < 0.01$) in the luteal phase. In association with these changes in LH pulse frequency, there were corresponding changes in LH pulse periodicity (given in parentheses in Table 1). For example, one bioactive LH pulse occurred every 177 ± 66 min in the early follicular phase, every 57 ± 9 min in the late follicular phase, and every 239 ± 5 min in the luteal phase. Changes in immunoreactive LH pulse

frequency and periodicity during the menstrual cycle closely mirrored those for bioactive LH. Thus, bioactive LH was secreted in prominent pulses, which in the late follicular phase occurred very frequently (approximately once per h) and in the luteal phase very uncommonly (once every 4 h).

The amplitude of bioactive LH pulses expressed as an increment (milliinternational units per ml) above nadir was significantly greater than that for immunoreactive LH in the early and late follicular phases ($P < 0.05$). Both bioactive and immunoreactive LH pulse amplitudes increased approximately 2-fold from the early follicular to the late follicular phase. Bioactive, but not immunoreactive, LH pulse amplitude then declined in the luteal phase.

When the amplitude of bioactive LH pulses was alternatively calculated as a fractional increase (percent above nadir), pulse amplitude in the luteal phase was remarkably higher than that in the early or late follicular phase. Results for immunoreactive LH were not different from those for bioactive LH (Table 1). Comparison of the absolute (milliinternational units per ml) peak levels of bioactive LH that were attained within pulses revealed a tendency toward higher values in the late follicular phase than in the early follicular or luteal phase ($P = \text{NS}$). The trend toward higher peak bioactive LH levels in the late follicular phase (despite a similar fractional increase above nadir) was associated with a significant ($P < 0.05$) increase in mean interpulse baseline bioactive LH concentrations compared with the early follicular phase. A similar pattern was found for immunoreactive LH. Thus, in the late follicular phase, absolute peak bioactive and immunoreactive concentrations within LH pulses tended to increase in conjunction with significant increases in interpulse LH concentrations. These patterns are illustrated in Fig. 2.

The detection of bioactive LH pulses was associated with a mean signal to noise ratio of 4.1 ± 2.2 ($\pm \text{SD}$; range, 2.3–10), which was not statistically different from that for immunoreactive LH (mean signal to noise ratio, 3.67 ± 2.8 ; range, 1.7–12.0). The individual signal to noise ratios all exceeded 1.5, which satisfies criteria necessary for reliable pulse identification (19). However, as an independent analytical approach, we also assessed pulse frequency using a modification of the method of Santen and Bardin (8). For this purpose, all data were analyzed separately using the criterion that LH peaks must exhibit an increase above nadir that exceeds 4 times our intra-assay coefficient of variation (Table 1, Modified criteria). This alternative assessment provided remarkably similar estimates of bioactive LH pulse frequency, with significant effects of stage of the cycle on LH pulse frequency ($P < 0.01$). Data for individual women are given in Table 2.

Pulse frequency estimates were not significantly dif-

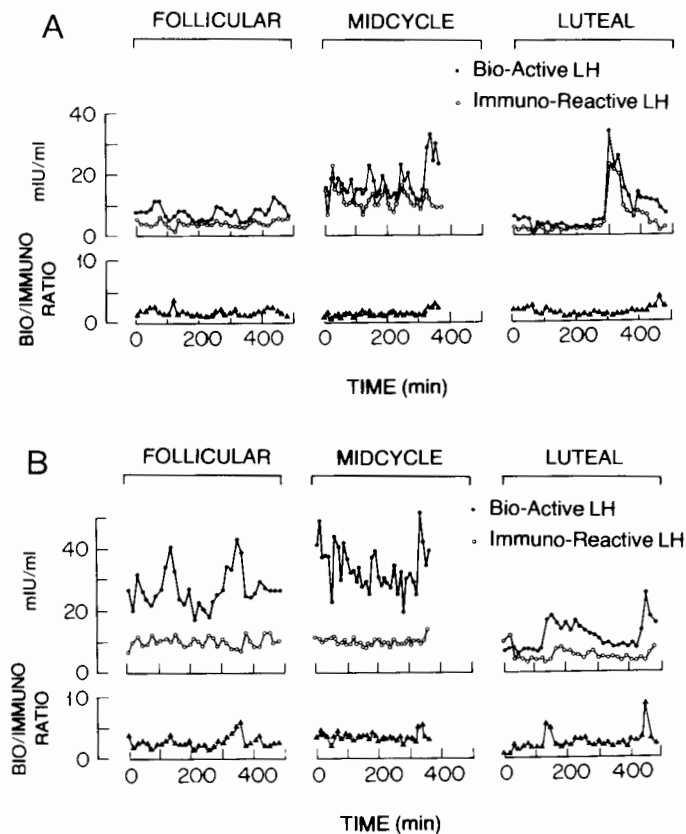


FIG. 2. Relationship between pulsatile bioactive and immunoactive LH secretion during three stages of the menstrual cycle in normal women (subjects C and B). Serial blood samples were collected for 360–480 min, as shown on the horizontal axis. Profiles are given for bioactive LH (upper solid curve), immunoactive LH (middle curve, open circles), and bio:immuno LH ratios (bottom curve, solid triangles) for each sampling session. Sampling was performed in the early follicular, late follicular (midcycle), and luteal phases, as noted.

ferent for the immunoactive and bioactive LH measurements at any stage of the menstrual cycle (Table 1). While this observation might suggest a high degree of concordance between individual bio- and immunoactive LH pulses, systematic analyses of the concordance between bio- and immunoactive LH peaks revealed that only 78% of all peaks were concordant (Table 3). Discordance was attributable predominantly to immunoactive peaks, 30% of which occurred without a corresponding bioactive LH peak. Remaining discordance was contributed by bioactive LH peaks, 14% of which occurred without a demonstrable immunoactive LH pulse. When separately analyzed, the concordance between RICT and RIA did not differ in relation to stage of the menstrual cycle *per se* (data not shown).

Bio:immuno LH ratios in individual women: relation to LH pulses

As shown in Table 4, for all stages of the menstrual cycle, bio:immuno ratios within LH pulses were significantly ($P < 0.01$) increased over bio:immuno ratios in

TABLE 2. Plasma concentrations and pulse frequency of bioactive LH release in the menstrual cycle of individual women

Subject	Mean LH ± SD ^a	No. of pulses/h	
		Modified method ^b	Clifton and Steiner ^c
Early follicular phase			
A	11.4 ± 3.1	0.12	0.25
B	27.3 ± 6.1	0.50	0.25
C	7.9 ± 2.2	0.75	0.75
D	46.2 ± 6.4	0.50	0.50
Mean ± SD	23.2 ± 15	0.47 ± 0.23 ^d	0.44 ± 0.24 ^d
Late follicular phase			
A	58.9 ± 23.7	1.17	1.17
B	33.9 ± 7.1	0.83	1.33
C	17.6 ± 5.3	1.0	1.17
D	62.3 ± 17.8	1.0	1.17
Mean ± SD	43.2 ± 18	1.00 ± 0.12 ^e	1.21 ± 0.07 ^e
Luteal phase			
A	21.4 ± 9.6	0.37	0.25
B	11.2 ± 4.6	0.25	0.25
C	8.5 ± 7.2	0.37	0.25
D	66.7 ± 11.5	0.37	0.25
Mean ± SD	27.0 ± 23	0.34 ± 0.05 ^d	0.25 ^d

Differing superscripts (^d or ^e) denote significantly different means.

^a Milliinternational units per ml in the RICT bioassay.

^b Determined by a modified method of Santen and Bardin (8), which requires a threshold increase of 4 times the intraassay coefficient of variation.

^c Method of Clifton and Steiner (19).

TABLE 3. Analysis of correlation between bioactive (BIO) and immunoactive (RIA) LH pulses in women

	LH pulses (n)	Concordant pulses (%)	Discordant pulses (%)	
			RIA	BIO
Total	116	78	17	6
RIA	65	69	30	
BIO	51	87		14

TABLE 4. Association of increased bio:immuno LH ratios with actual LH peaks

	Within-peak bio:immuno ratios	Between-peak bio:immuno ratios
No. of bio:immuno ratios > median	281	81
No. of bio:immuno ratios ≤ median	48	177

the interpulse baselines. When data were separately analyzed at individual stages of the menstrual cycle, bioactive LH pulses at each stage contained significantly higher bio:immuno ratios than those found in the inter-

pulse baseline. Thus, throughout the menstrual cycle, LH pulses were relatively enriched in bioactive LH compared to the interpulse baseline.

Discussion

The present study demonstrates that plasma concentrations of biologically active LH exhibit strikingly episodic fluctuations in normally cycling women. Moreover, the nature of such pulsations varied significantly in relation to stage of the menstrual cycle. These spontaneous pulses of bioactive LH were qualitatively similar to those previously recognized for immunoactive LH in men and women (6–11) and were akin to those recently reported by Dufau *et al.* (17) for bioactive LH in normal men or postmenopausal women. Our delineation of prominent pulses of bioactive LH in young women significantly extends available knowledge of episodic LH secretion in premenopausal women, because in previous reports, properties of pulsatile LH release were evaluated only by RIA estimates. However, the present work demonstrates that significant discrepancies occur between immunoactive and bioactive estimates of LH secretion in women during the menstrual cycle.

Our assessment of bioactive LH concentrations revealed unambiguous pulses in normal women, which we quantitated using a cycle detection method (19) and a modified program of Santen and Bardin (8) to distinguish significant biological signals from random assay and procedural errors. Using the former method, we found that bioactive LH pulse frequency increased significantly in the late follicular phase and then declined markedly in the luteal phase. The same pattern was found for immunoactive LH. The validity of these observations was confirmed by an independent estimate of LH pulse frequency using the method of Santen and Bardin (8) with a modified threshold of 4 times the intraassay coefficient of variation. In addition, although estimates of bioactive LH pulse frequency have not been previously reported, our estimates of immunoactive LH pulse frequency are similar to those reported by other workers using 6 to 24 h sampling sessions (8, 11, 23). Thus, we conclude that plasma bioactive LH concentrations in healthy women exhibit unequivocal pulsations, and that the frequency of such bioactive LH pulses changes significantly during the menstrual cycle. A similar pattern of increased bioactive LH pulse frequency in the late follicular phase has also been suggested, but not yet rigorously analyzed, in the monkey (24).

As the frequency of bioactive LH pulses increased significantly from the early to late follicular phase, there was an accompanying nearly 2-fold increase in both the incremental and the peak amplitude of LH pulses. These amplitude changes were associated with a corresponding

increase in interpulse (baseline) LH levels, so that the fractional (percent above baseline) amplitude of bioactive LH pulses did not increase. In contrast, in each woman's luteal phase, the fractional amplitude of bioactive LH pulses was considerably greater than in the early follicular phase. This distinctive pattern of high fractional amplitude for luteal phase pulses of bioactive LH is qualitatively similar to that previously recognized for immunoactive LH pulses (6–11).

The overall patterns of bioactive LH secretion are generally similar to those reported for immunoactive LH secretion (6–11). However, by analyzing each sample by both RIA and RICT to compare bio- and immunoactive LH patterns, we have unmasked significant discrepancies. In particular, mean and integrated concentrations of bioactive LH, incremental and peak amplitudes of bioactive LH pulses, and interpulse baseline concentrations of bioactive LH all significantly exceeded corresponding immunoactive LH values in the early follicular and late follicular stages. The fractional (percent) pulse amplitudes were similar for immunoactive and bioactive LH pulses, indicating that the higher absolute amplitude of bioactive LH pulses was commensurate with higher interpulse basal levels of bioactive hormone. In addition, individual peaks of bioactive and immunoactive LH were significantly discrepant. Specifically, among 116 total bioactive and immunoactive LH pulses identified in this study, only 78% were concordant. The majority of the discordance was accounted for by immunoactive LH pulses, 30% of which occurred without a significant rise in bioactivity. The exact basis for discrepant immunoactive LH peaks is not known, but these results agree closely with our earlier observations in normal men, in whom we found a similarly high prevalence of discordant immunoactive LH pulses (17). Thus, we conclude that increased quantities of LH immunoactivity in serum cannot be equated with corresponding changes in biological activity. Moreover, in both normal men and normal women, a significant minority (approximately 14%) of bioactive LH pulses occurs without a demonstrable immunoactive LH peak. Thus, direct estimates of circulating concentrations of biologically active LH are useful as a complementary means of characterizing physiological patterns of LH secretion during the menstrual cycle.

To estimate the relative content of bioactive and immunoactive LH, we used the bio:immuno LH ratio. At each of the three stages of the menstrual cycle studied, the mean bio:immuno LH ratio exceeded unity. The mean bio:immuno LH ratio in the six young women studied was 2.42, significantly lower than those which we recently defined in normal men (3.95 ± 0.97 ; $P < 0.05$ *vs.* premenopausal women) and postmenopausal women (5.4 ± 1.3 ; $P < 0.02$ *vs.* premenopausal women) (17). Thus, mean bio:immuno LH ratios characteristically ex-

ceed unity in premenopausal women, but are lower in these women than in men or postmenopausal women. These results, derived from multiple (25–29) blood samples in each woman, validate earlier observations by us and other investigators who used infrequent sampling techniques (12, 14, 15, 17, 24, 30) which yield less precise estimates because of the pulsatile nature of LH release. For example, in the present work, we found that bio:immuno LH ratios within bioactive LH pulses were significantly higher than bio:immuno ratios between LH pulses during all three stages of the menstrual cycle. In addition, the fluctuations in bio:immuno LH ratios are consistent with the hypothesis that LH can be released from functional pools preferentially enriched in bioactivity (25, 31–37). A similar relative increase in bio:immuno LH ratios in LH pulses was described by Dufau *et al.* (17) in normal men and postmenopausal women. Moreover, an increase in bio:immuno LH ratios could also be recognized in normal men when the frequency of the endogenous GnRH signal was amplified by administration of an opiate receptor antagonist (26). Thus, both spontaneous GnRH signals and amplified GnRH signals of endogenous origin can promote pulsatile LH release in a form that is preferentially enriched in biologically active hormone. In contrast, either bolus or continuous infusion of exogenous GnRH does not readily demonstrate preferential release of bioactivity (30, 32, 34). These observations suggest that the intermittency of the endogenous GnRH signal may be critical in regulating the biopotency of LH.

Although the exact endocrine factors that influence variations in the biopotency of circulating LH in women are not fully known, estrogen *per se* may significantly modify LH biopotency (24, 31, 32). In the present studies, mean and median bio:immuno LH ratios increased in the late follicular phase, when serum estradiol concentrations rose significantly. Although the correlation between estradiol and bio:immuno LH ratios was not statistically significant, the tendency to higher bio:immuno LH ratios in the late follicular phase is in accord with observations in the preovulatory stages of the menstrual/estrous cycle of the monkey and rat (27–29, 31, 33, 38). In addition, in the rat, the striking proestrus increase in bio:immuno LH ratios is abolished by ovariectomy (33), if gonadectomy precedes the acute preovulatory rise in estrogen secretion. Moreover, in the late follicular phase of the human menstrual cycle (but not at other times), bolus injection of GnRH can lead to increased bio:immuno LH ratios (14, 32, 34). The latter observation suggests that in women, relative release of bio- *vs.* immunoactive LH is dependent upon phase of the menstrual cycle and may be controlled by ovarian factors, such as sex steroids.

The physiological significance of the pulsatile nature

of bioactive LH release is suggested by a recently recognized possible correlation between immunoactive LH pulses and corresponding increases in sex steroid concentrations in normal women (11) as well as in men (38–40), monkeys (41), mice (42), and sheep (43). In addition, the pulsatile administration of LH *in vitro* to perfused rat ovaries enhanced estradiol secretion more effectively than tonic LH administration (44). Moreover, in hypogonadotropic ewes (45), monkeys (46), or women (47, 48), progressive follicular development and ultimately ovulation can be induced by pulsatile, but not by continuous, GnRH administration. Although the preceding observations applied to immunoactive LH pulses, our demonstration that bioactive LH is also released in prominent pulses further underscores the pivotal importance of the pulsatile nature of the LH signal in the female. The present studies also delineate significant changes in the patterns of bioactive LH release during different physiological stages of the menstrual cycle. Because the number of patients studied was relatively small, our data should not be taken to define an exact normal range for bioactive LH pulse characteristics that would apply to all cohorts of normal women. However, the significant relative changes in bioactive LH pulse properties should be representative of the patterns of change that occur in the normal menstrual cycle. Thus, this work provides basis for investigating the endocrine mechanisms that regulate bioactive LH pulse frequency and amplitude and relative bioactive/immunoactive LH release in women in health and disease.

In summary, the highly sensitive bioassay for LH in plasma has demonstrated that LH is secreted into the circulation as pulses of high biological activity, and that modulation of the frequency of bioactive pulses occurs during the follicular and luteal phases of the normal menstrual cycle. Such frequency modulation is an important physiological mechanism for regulating the blood concentration of bioactive LH available to the ovary prior to ovulation. This information can also provide a rational physiological basis for designing optimal schedules or pulsed GnRH administration for the induction of ovulation.

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