

Mode of Secretion of Bioactive Luteinizing Hormone in Man*

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ABSTRACT. The episodic nature of gonadotropin secretion was originally defined by RIA of circulating LH concentrations. We analyzed the pulsatile release of biologically active LH by measuring plasma LH concentrations in the rat interstitial cell testosterone bioassay. A computer algorithm to discriminate true biological signals (LH pulses) from background variation was applied to serially sampled LH data from seven men and seven postmenopausal women. Our results indicate the following. 1) In all subjects, mean bioactive LH values were considerably higher than immunoactive levels, (41.4 ± 15.1 and 450 ± 243 mIU/ml vs. 10.2 ± 2.3 and 83 ± 35 for men and postmenopausal women, respectively). There was a corresponding 4-fold increase in the total area under the bioactive LH secretion profile compared with that defined for the immunoactive hormone. 2) The absolute amplitude of the bioactive LH peaks was 0.5-

11-fold higher than the immunoactive values. 3) Although the majority of the LH peaks were coincident by bioassay and RIA, significant dissociation occurred in 20% and 28% of the total LH peaks (rat interstitial cell testosterone bioassay and RIA) in men and postmenopausal women, respectively. 4) Significant increases in the bioactive to immunoactive ratio over interpulse bioactive to immunoactive levels occurred in 98% of the pulses in the men and 83% of those in postmenopausal women. Also, in two men, peaks of LH bioactivity exceeding 100 mIU were followed by major increases in serum testosterone concentrations.

These findings demonstrate the value of bioactive LH determinations and indicate that LH is secreted in pulses of high biological activity. The *in vitro* LH bioassay provides a sensitive and appropriate estimate of functionally active LH in the circulation. (*J Clin Endocrinol Metab* 57: 993, 1983)

THE STIMULATORY and trophic actions of LH are responsible for maintaining the differentiated function of the steroidogenic cells in the testis and ovary. The trophic actions of LH include the regulation of LH and PRL receptors and the modulation of steroidogenic enzymes and cholesterol metabolism in gonadal target cells (1). Not only the concentration but also the quality of the circulating LH molecule are major determinants of hormonal activation of gonadal target cells. Leydig cell function and testicular androgen secretion are dependent upon stimulation by circulating LH, which is released from the pituitary gland in a pulsatile manner.

Several studies have clearly demonstrated this form of secretion using serial measurements of plasma LH by RIA (2-4). In the present study, the mode of biologically active LH secretion was analyzed and compared with the immunoactive profiles of plasma LH in young men and postmenopausal women. These two groups of subjects were selected because both secrete biologically active LH with high bioactive to immunoactive (bio:immuno) ratios that exceed those observed in spontaneously cycling women (5-7). In addition, these two groups can be studied in the absence of cyclic changes in sex steroid concentrations.

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Materials and Methods

These studies were conducted in seven healthy normal men (age range, 24-28 yr) and seven postmenopausal women (age range, 48-64 yr; from 1-10 yr postmenopausal). Each subject provided informed consent before participation in the study. Blood samples were collected at 10 (men)- to 20-min (postmenopausal women) intervals for up to 360 min. Plasma samples

were assayed for immunoreactive LH by a double antibody RIA (5), 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 rat interstitial cell testosterone assay (RICT) (5, 8), with a sensitivity of 0.4 mIU/ml or 3 pg pure LH (LER 1533). The reproducibilities of the method, expressed as the coefficient of variation for pools of plasma from normal men and postmenopausal women, were $\pm 8.2\%$ and $\pm 8.8\%$ for intraassay and $\pm 13.8\%$ and $\pm 13.5\%$ for interassay error, respectively. All samples were measured at three different dose levels in duplicate. Samples of individual subjects were assayed within a single assay. The potencies of the purified preparation in terms of the hMG standard were similar when measured by bioassay or RIA, viz. 13,500 (confidence limits, 11,200–15,200) and 13,700 (confidence limits, 11,100–14,900) IU/mg, respectively. Thus, the bio:immuno ratios of plasma samples calculated for LH are similar whether measured in terms of hMG or pure LH.

The LH plasma secretion profile were analyzed by the computerized cycle detection method of Clifton and Steiner (9) and Steiner *et al.* (10). This method performs iterative data scans for significant fluctuations (pulses) that exceed an adaptive threshold, the initial value of which is estimated from the within-assay variance. Iteration with threshold adjustment is continued until the probability of obtaining a false pulse equals the probability of missing a true pulse. Pulses are defined as successive increases above threshold separated by a decrease which is also greater than threshold (9, 10). The program determines the frequency and incremental amplitude (peak to nadir) of cycles in the presence of random measurement errors (noise) (10). An estimate of the pulse signal to noise ratio is also given. 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 area under the LH concentration *vs.* time curve and the percent amplitude of significant pulses designated by the method of Steiner *et al.* (10) were calculated by the method of Santen and Bardin (11).

Results

Analysis of LH profiles in normal men

The patterns of LH secretion measured by bioassay and RIA in four normal men are shown in Fig. 1. Measurements of LH bioactivity by RICT assay magnified the RIA patterns in all subjects. Bioactive LH levels in normal men were much higher than the immunoreactive levels, with bio:immuno ratios of 2–5. There were significant increases in the area of bioactive LH secretion and absolute amplitude of bioactive LH fluctuations, which were 0.5- to 11-fold higher than the corresponding RIA values. In addition, the bio:immuno ratio peaks for the most part were coincident with LH pulses. Thus, similar bioactive and immunoreactive periodicities were observed.

The mean concentration of bioactive LH was 41.4 ± 15.1 mIU/ml (mean \pm SD) compared to RIA values of 10.2 ± 2.3 mIU/ml ($P < 0.002$; Table 1). The integrated bioactive LH area expressed as (mIU/ml) min was also significantly higher than the RIA area (Tables 1 and 2). The incremental amplitude of LH pulses, defined as the difference between the signal value at the peak of the

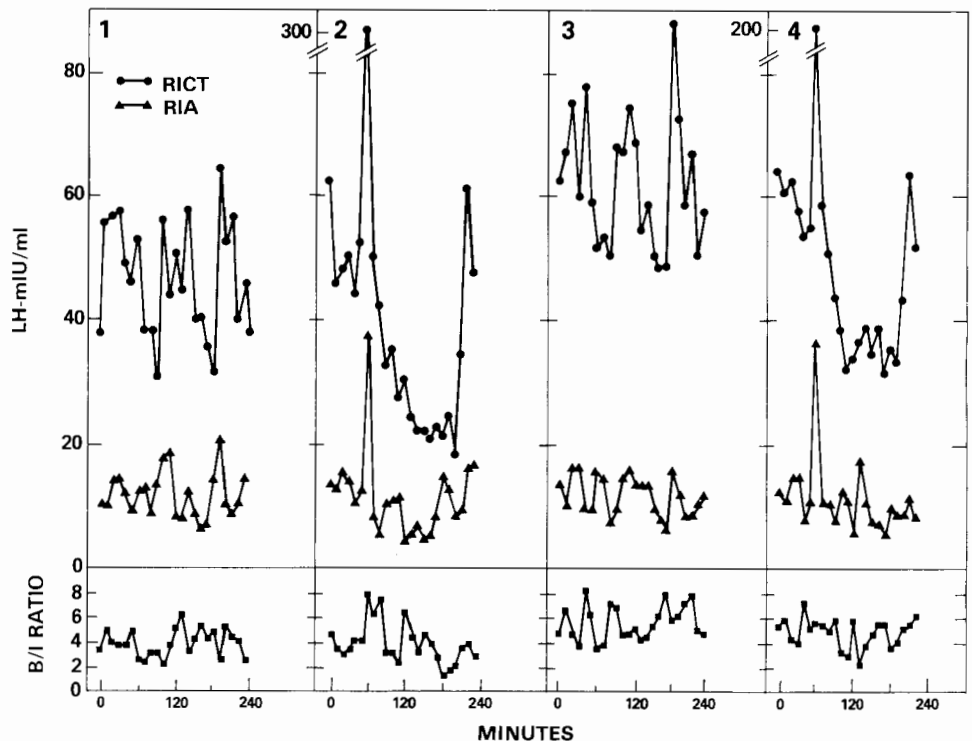


FIG. 1. Upper panel, Immunoreactive (\blacktriangle - \blacktriangle) and bioactive (\bullet - \bullet) patterns of serum LH in four men. LH values are expressed in terms of the Second International Reference Preparation (hMG). Lower panel, Bio:immuno ratio (B/I) profiles derived from the data shown in the upper panel. The horizontal axis gives the time of sampling.

TABLE 1. Bioactive LH in plasma of male subjects

Subject no.	Mean conc. (mIU/ml)	Periodicity (min)	Amplitude (%)	Amplitude (mIU/ml) ^a	LH area [(IU/ml)/min]	Bio:immuno ratio	Signal:noise ratio
1	44.9 ± 10.4	48 (5)	59 ± 42.5	18.3 ± 5.6	10.7	3.96 ± 1.17	1.96
2	47.6 ± 55.4	120 (2)	383 (552, 213)	150 (259, 42)	11.4	3.96 ± 0.94	9.52
3	62.2 ± 10.6	60 (4)	41.5 ± 24	26.5 ± 0.92	15.6	5.59 ± 1.2	1.37
4	53.2 ± 34.8	115 (2)	162 (240, 84)	109 (143, 70)	12.6	4.81 ± 1.2	6.09
5	30 ± 12.4	103 (3)	240 ± 219	21.4 ± 16	8.7	3.27 ± 1.82	4.00
6	33.8 ± 11.5	50 (7)	128 ± 118	19.4 ± 7.0	10.6	2.77 ± 0.82	3.3
7	17.8 ± 8.4	40 (9)	115 ± 60	11.3 ± 5.7	4.9	3.34 ± 1.47	4.42
Mean ± SD of means	41.4 ± 15.1	75.6 ± 35	161 ± 117	11.3–150 ^b	10.7 ± 3	3.95 ± 0.97	4.38 ± 2.55

The number of pulsations on which periodicity is based is shown in parentheses.

^aΔ increment, mean LH levels above baseline.

^bRange of means.

TABLE 2. Immunoactive and bioactive LH in plasma of male subjects

Assay	Mean conc. (mIU/ml)	Periodicity (min)	Amplitude (%)	Amplitude (mIU/ml)	LH area [(IU/ml)/min]	Signal:noise ratio
RIA	10.2 ± 2.3	63 ± 26 (37)	104 ± 36	8.10 ± 2.7	2.6 ± 0.64	2.6 ± 1.0
RICT	41.4 ± 15.1 ^a	76 ± 35 (32)	161 ± 117	19.4 ± 5.5 ^a	10.7 ± 3 ^a	4.4 ± 2.6 ^b
Bio:immuno ratio	4.0 ± 1.0					3.5 ± 0.9

The number of pulsations on which periodicity is based is shown in parentheses. Values are the mean ± SD.

^a*P* < 0.002.

^b*P* < 0.04.

cycle and the lowest value of the signal in that cycle, was also increased for bioactive LH. Signal to noise ratios were estimated as a reflection of the strength of the signal for bioassay, RIA, and bio:immuno peaks. The signal to noise ratios exceeded 1.5, except for subject 3 whose ratio was 1.37 for the bioactive LH profile. These signal to noise ratios were in the range of 1.5 or greater, which are nominally suggested for this computer method. The signal to noise ratios were significantly higher for LH measurements by RICT (mean ratio, 4.4) than by RIA (mean ratio, 2.6). Also, a high signal to noise ratio of 3.5 was observed when serial bio:immuno ratio peaks were analyzed (Tables 1 and 2).

A detailed analysis of LH data for subject 5 is shown in Fig. 2. From the LH profile (Fig. 2, upper panel), three peaks of bioactivity (indicated by a continuous line) and four significant RIA peaks (indicated by asterisks) were detected. It is evident that the RIA peaks were concordant only with certain of the bioactive hormone peaks. This concordance was not always absolute, since in some subjects, RIA peaks corresponded with only a fraction of the bioactive pulses (Fig. 2, upper panel concordance indicated by arrows). Also, pulses of immunoactivity with no concurrent pulses of bioactive LH were found. The bio:immuno ratio profile showed three peaks, each of

which was concurrent with pulses of bioactivity (Fig. 2, lower panel). In another man (subject 7) who had a high peak frequency, nine significant bioactive pulses were identified (Fig. 3, upper panel). Six significant RIA pulses that resulted from analysis of the RIA profile (Fig. 3, lower panel) concurred with six of the nine bioactive pulses, and three bioactive peaks (indicated by arrows in Fig. 3, upper panel) were dissociated.

Among the men, two individuals with low bioactive LH pulse frequency (subjects 2 and 4; Table 1) had bioactive LH peaks that reached levels of 300 and 200 mIU/ml, respectively. In both, the two bioactive LH peaks were concurrent with RIA LH peaks, while a number of nonconcurrent LH RIA peaks (Fig. 4, indicated by arrows) occurred. In addition, testosterone levels in these two subjects showed a marked peak 25–40 and 90 min after the major LH peaks. In contrast, in other men, there was a less easily definable correspondence between testosterone increases and bioactive pulses. In only one man (subject 1) was a significant positive correlation between bioactive pulses/bio:immuno peaks and testosterone (Pearson *r* = 0.31; *P* < 0.05) observed. No significant correlation of LH pulses with simultaneous or subsequent testosterone peaks was observed in the other subjects.

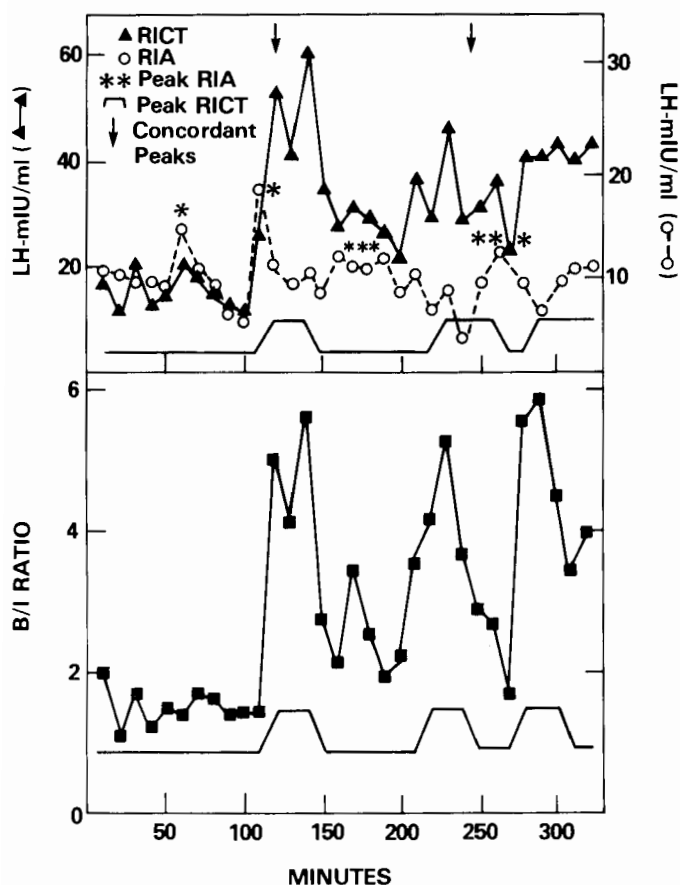


FIG. 2. Detailed analysis of LH data from subject 5. The upper panel shows the bioactive LH profile (RICT; \blacktriangle — \blacktriangle) and RIA profile (\circ — \circ). The computer analysis is given below by a continuous line, and the significant RIA peaks are indicated by asterisks. The arrows indicate concurrency of bioactive and immunoactive LH peaks. The lower panel depicts bio:immuno (B:I) ratios over time.

Overall, of a total of 69 LH pulses (bioassay plus RIA), 80% were concordant. Among the discordant peaks, 14% were RIA pulses, and 6% were bioactive LH pulses. Moreover, 87% of the bioactive LH peaks concurred with RIA peaks, and 73% of the RIA peaks were concordant with bioactive LH peaks. In this study, 98% of the bioactive pulses showed peaks of increased bio:immuno ratio.

Analysis of LH profiles in postmenopausal women

The patterns of LH secretion measured by bioassay and RIA in four postmenopausal women are shown in Fig. 5. As in the case of measurements in males, determinations of LH bioactivity in postmenopausal women amplified the RIA pattern. In contrast to results in men, analysis in postmenopausal women revealed lower frequencies of bioassay and RIA peaks. In these women, for the most part, LH peaks were less defined than in the males, but the signal to noise ratio was equal or greater than 1.5.

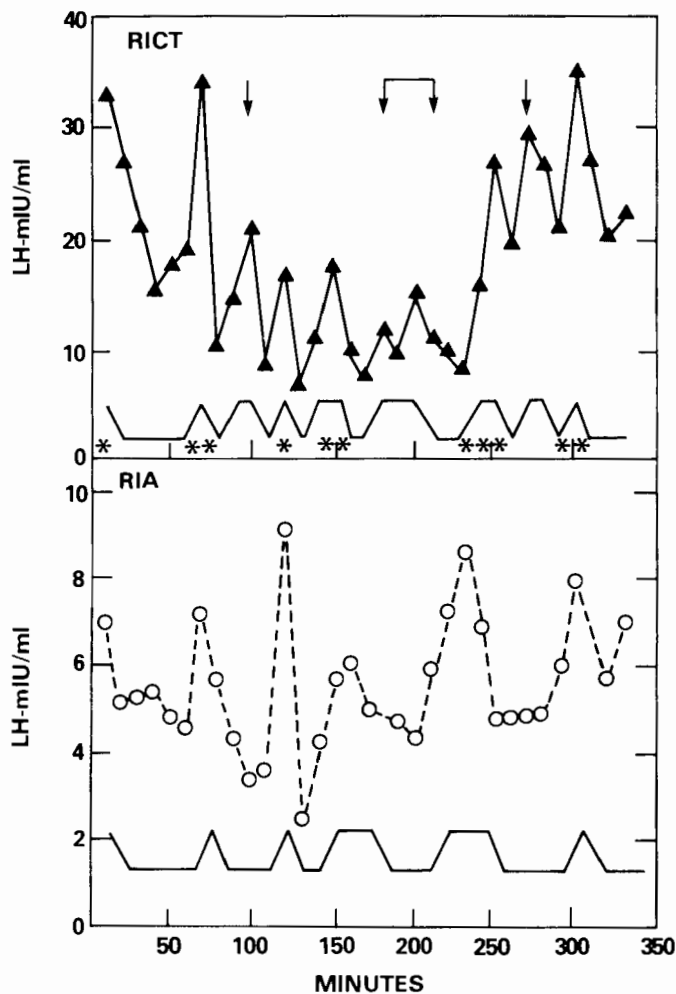


FIG. 3. Detailed analysis of serum LH from subject 7. The RICT profile and the computer analysis of the LH bioassay data designating significant biopulses are shown in the upper panel. Asterisks denote significant RIA pulses (RIA pattern shown below). Bioactive LH peaks, indicated by arrows (above), were dissociated.

The numerical parameters for the LH data in postmenopausal subjects are shown in Tables 3 and 4. Bioactive LH concentrations were significantly increased over immunoactive LH levels. This was also the case for the area of LH secretion and the absolute amplitude of LH peaks. However, no significant differences were observed in periodicity, percent amplitude, or signal to noise ratio between bioactive and immunoactive LH (Table 4). The bio:immuno ratios ranged from 3.6–7.7, which were significantly higher than those in the men. In subjects 1–5, all bioactive peaks showed increased bio:immuno ratios, while in subjects 6 and 7, such increases were only observed in 33% and 60% of the pulses, respectively.

From a total of 22 RIA LH peaks, 55% were concordant with bioactive LH peaks, and 45% showed no increase in bioactivity. From a total of 25 bioactive LH pulses, 88% were concordant, and in 12%, no immunoactivity was detected.

FIG. 4. The bioactive serum LH profile and testosterone levels (*upper panel*) and serum LH determined by RIA (*lower panel*) of two subjects [no. 2 (*left panel*) and no. 4 (*right panel*); see Table 1]. The significant pulses of bioactive and immunoactive LH designated by the computer analysis are shown by the *continuous lines* in the *upper and lower panels*, respectively. Nonconcurrent LH RIA peaks are indicated by *arrows*.

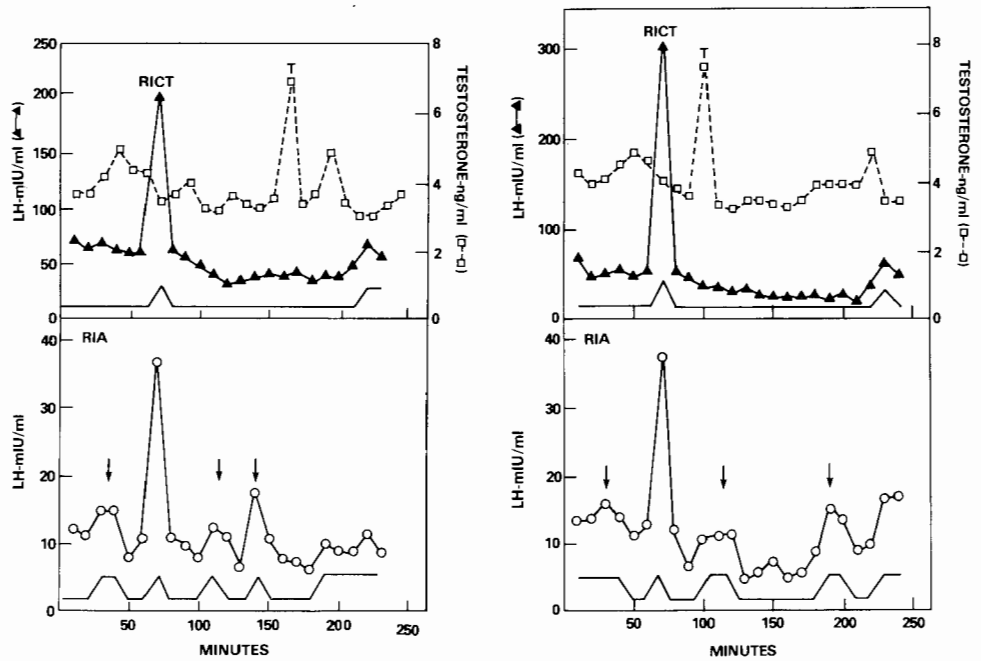
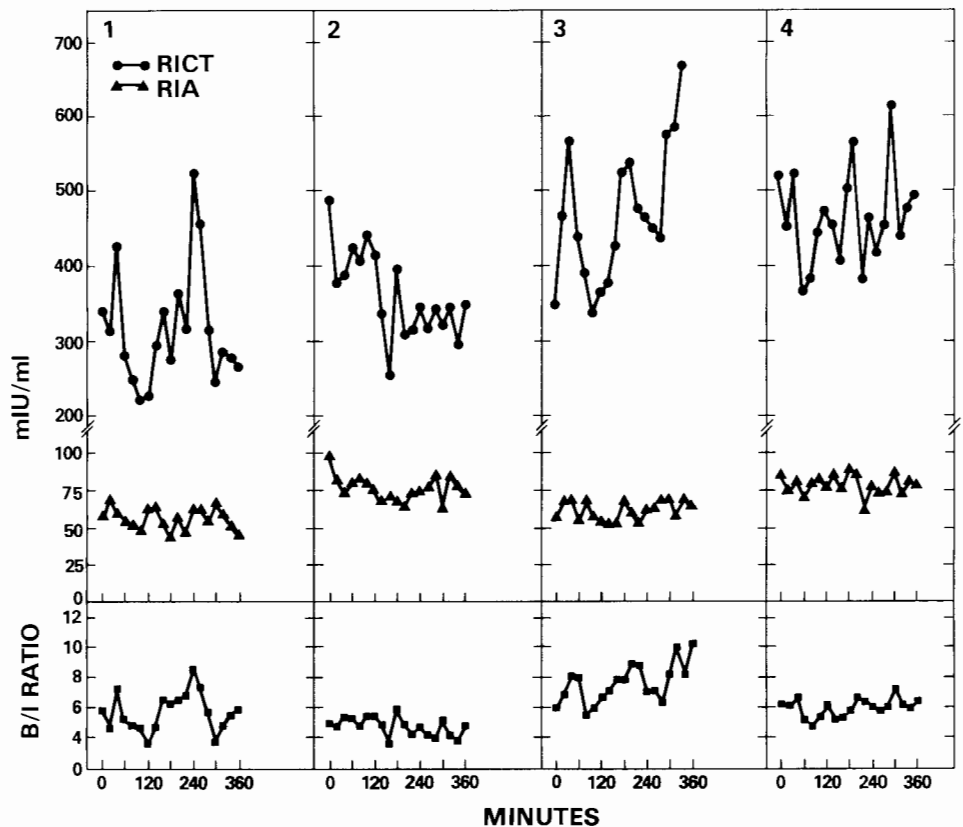


FIG. 5. Immunoactive and bioactive patterns of serum LH in four postmenopausal women are shown in the *upper panel*, and the corresponding bio: immuno (B:I) ratio profiles are given in the *lower panel*.



Discussion

In this study, we have shown that bioactive LH values were higher than immunoreactive LH levels in healthy young men and postmenopausal women using a urinary and pituitary standard. The relative or fractional excursions in LH pulses above baseline were quite similar for RIA or RICT pulses, but the absolute amplitude (mIU/

ml increase or peak) of bioactive LH pulses was considerably greater than that for RIA pulses in men and women. The majority of bioactive LH peaks were concurrent with immunoactive peaks. However, a small proportion of bioactive LH peaks were not detected by RIA. These exclusively bioactive LH pulses represented only 13% of the bioactive LH peaks. A larger proportion of

TABLE 3. Bioactive LH in plasma of postmenopausal subjects

Subject no.	Mean conc. (mIU/ml)	Periodicity (min)	Amplitude (%)	Amplitude (mIU/ml) ^a	LH area [(IU/ml)/min]	Bio:immuno ratio
1	316 ± 80	180 (2)	70 (61, 79)	182	99	5.7 ± 1.3
2	361 ± 58	— (1)	37	100	121	4.7 ± 0.6
3	472 ± 92	120 (3)	40 ± 16	113	151	7.7 ± 1.3
4	465 ± 65	72 (5)	36 ± 14	148 ± 55	136	5.9 ± 0.6
5	966 ± 224	90 (4)	47 ± 31	380	350	6.1 ± 1.4
6	221 ± 30	60 (6)	44 ± 25	54 ± 13	83	3.6 ± 1.0
7	357 ± 89	90 (4)	32 ± 10	97 ± 33	136	4.4 ± 2.5
Mean ± SD of means	450 ± 243	139 ± 105 (60–360)	44 ± 13	153 ± 107 (54–380)	153 ± 90	5.4 ± 1.3

The number of pulsations on which periodicity is based is shown in parentheses.

^a Δ increment, mean LH levels above baseline.

TABLE 4. Immunoactive and bioactive LH in plasma of postmenopausal women

Assay	Mean conc. (mIU/ml)	Periodicity (min)	Amplitude (%)	Amplitude (mIU/ml)	LH area [(IU/ml)/min]	Signal:noise ratio
RIA	83 ± 35	162 ± 135 (22)	39 ± 26	22 ± 13	30 ± 12.6	2.47 ± 1.38
RICT	450 ± 243 ^a	139 ± 105 (25)	44 ± 13	153 ± 107 ^b	153 ± 90 ^c	3.74 ± 2.03
Bio:immuno ratio	5.4 ± 1.3					2.47 ± 1.71

The number of pulsations on which periodicity is based is shown in parentheses.

^a *P* < 0.004.

^b *P* < 0.05.

^c *P* < 0.01.

immunoactive LH peaks were devoid of corresponding increases in biological activity. Such dissociation was more marked in postmenopausal women than in men. In women, 43% of the immunoactive peaks were dissociated, while in men, only 23% of the RIA peaks showed no corresponding increase in biological activity. The signal to noise ratio for bioactive and radioimmunoactive LH and bio:immuno peaks was well above the ratio required for analysis of data by this algorithm, and this ratio was significantly higher for measurements by RICT than for those by RIA in men. The higher signal to noise ratio and the prominent graphic presentation of RICT-defined LH pulses probably reflect the salient zenith and nadir of bioactive LH pulses associated with the large excursion and high absolute amplitude of these peaks. The mean levels of bioactive LH in this series were very similar to those which we have previously reported in random serum samples obtained from a large number of subjects. These values were 37 ± 19 in men and 263 ± 111 mIU/ml (±SD) in postmenopausal women. The high sensitivity of the bioassay for detection and analysis of serum LH pulses has permitted detection of LH peaks not measurable by RIA and has defined radioimmunoactive pulses devoid of biological activity.

In the male, it is evident that more than one distinct pattern of bioactive LH pulses was observed. One pattern

was of low frequency LH release (in two subjects), in which LH secretion occurred in a surge-like fashion, with bioactive pulses that reached levels up to 300 mIU/ml. Such high levels have also been recognized during LRH stimulation (6, 7). Also, such LH peaks appeared to be short-lived (single component), with a half-life of about 10 min for the down slope of the pulse, as derived recently from a series of four patients with frequent (every 4 min) sampling (Dufau, M. L., Veldhuis, J. D., and Fraioli, F., unpublished observations). In contrast, in all of the remaining male subjects, bioactive LH peaks were more frequent and reached levels up to 60–90 mIU/ml. A significant reverse correlation between secretory spike height and apparent LH half-life measured by RIA was previously demonstrated (11). In these early studies, a wide range of half-lives (from 32–96 min) was reported; however, no short-lived peaks, as detected by the present measurements of bioactive LH, were observed (11).

The patterns of release of biologically active LH in healthy men compared with those in postmenopausal women revealed certain striking differences. In particular, despite the very high concentrations of bioactive LH attained in postmenopausal women, the periodicity of LH release was considerably longer (190 ± 122 vs. 76 ± 35 min. in the men). In addition, the absolute incremental amplitude (mIU/ml) was approximately 7-fold higher

in postmenopausal women, while the fractional amplitude, expressed as a percentage of baseline, was 3.5-fold lower. These observations indicate that pulses of biologically active LH occurred less frequently in postmenopausal subjects, and that although substantial amounts of hormone were released (as reflected in the absolute increment of LH concentrations), the fractional increase over baseline was relatively small. The latter observation could reflect an increase in interpulse concentrations of bioactive LH. Thus, one mechanism for increased mean concentrations of bioactive LH appears to be increased absolute amplitudes of biologically active LH pulses (and, presumably, the mass of LH released in each pulse), associated with a substantial rise in the interpulse baseline of circulating LH concentrations, despite a significant decline in pulse frequency *per se*.

Two men had conspicuous pulses representing a major release of biologically active LH. In these individuals, a significant increase in plasma testosterone concentrations occurred within 90 min. This type of temporal association between biologically active LH release and subsequent testosterone secretion has been inferred from patterns of immunoreactive LH release in several species, including the human, ram, bull, and rat (12–17). Our data suggest a similar pattern in the human for bioactive LH release. However, the relatively frequent release of lesser quantities of bioactive LH in most of our male subjects precluded an easily definable correspondence between bioactive LH pulses and testosterone increases. Investigations in which the periodicity and amplitude of biologically active LH secretion (or administration) can be explicitly controlled will be required to explore the exact influence of bioactive LH pulses on minute to minute variations in testosterone secretion in man.

The LH secreted in the majority of pulses is presumably of significantly higher biological activity than that of basal secretion, as indicated by increased bio:immuno ratios of the pulses. Such increases could be derived from GnRH pulses and reflect the release of a compartmentalized pool of highly biologically active LH rather than increased secretion rate of the trophic hormone (6, 7). However, such a functional pool is difficult to explore and was not evident in studies using either a single bolus dose or continuous infusion with low doses of GnRH (6, 7). Demonstration of such compartment could be attained by exogenous GnRH administered in pulses, or alternatively by endogenously generated GnRH pulses that will mimic the physiological mode of secretion. It is conceivable that this form of secretion in pulses of high biological activity would prevent end organ desensitization and would maintain Leydig cells receptor numbers,

coupling functions, and steroidogenic enzymes with optimal functional activity (1, 18).

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