

Preferential Release of Bioactive Luteinizing Hormone in Response to Endogenous and Low Dose Exogenous Gonadotropin-Releasing Hormone Pulses in Man*

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ABSTRACT. We used the rat interstitial cell testosterone (RICT) bioassay to assess biological LH activity secreted in response to endogenous and low dose exogenous GnRH pulses in normal men. The absence of nonspecific plasma effects in the LH bioassay was demonstrated by the finding of undetectable levels of LH bioactivity despite low but measurable immunoreactivity in 10 hypogonadotropic men. Moreover, bolus injections of human LH in 6 hypogonadotropic men defined a curvilinear relationship between plasma bioactive and immunoreactive LH concentrations, in which the extrapolated concentration of plasma bioactive LH at a zero dose of immunoreactive LH was indistinguishable from zero. Zero bioactive LH intercepts were also found when physiological bio- and immunoreactive LH concentrations derived from 7 intensively sampled normal men were subjected to linear regression using 2-dimensional error fitting. In these men, exogenous low dose (10 μ g) iv GnRH administration resulted in preferential release of bioactive LH, with a consequent significant increase in the median plasma bio- to immunoreactive (bio:immuno) LH ratio. This pattern mimicked

that of endogenous LH pulsatility, in which median intrapulse bio:immuno LH ratios were significantly higher than median interpulse ratios in the same individuals ($P = 0.006$). Increases in spontaneous plasma bio:immuno LH ratios were not attributable to spurious rises in bioactive LH concentrations associated with decreases in serum immunoreactive LH levels. Rather, sample cross-correlation analyses demonstrated positive correlations between bio- and immunoreactive LH at lags of 0–40 min, indicating that both hormones increased or decreased concomitantly.

These results demonstrate that LH is secreted physiologically in pulses of increased biological activity, presumably reflecting the release of a functionally compartmentalized LH pool relatively enriched in biologically active hormone. Accordingly, evaluation of the plasma bio:immuno LH ratio can provide a useful and sensitive index of qualitative changes in the LH molecule in response to endogenous (spontaneous) and exogenous GnRH stimulation. (*J Clin Endocrinol Metab* 64: 1275, 1987)

THE *IN vitro* bioassay of LH can provide a sensitive measurement of LH concentrations in man under diverse pathophysiological circumstances (1–3). In addition to estimating the amount of intrinsic biological activity, bioassay when combined with RIA permits evaluation of the biological to immunological (bio:immuno) LH ratio. The latter measurement might offer a useful sensitive index of qualitative changes in the LH molecule secreted when hypothalamic-pituitary-gonadal function is altered. Biochemically, changes in the quality of se-

creted LH are believed to reflect varying degrees of posttranslational glycosylation and possibly sulfation (4).

The validity of the gonadotropin bioassay and the interpretability of the bio:immuno LH ratio depend upon the absence of nonspecific bioactivity. In this regard, a recent study of GnRH-deficient men given exogenous GnRH concluded that no significant variation occurred in the plasma bio:immuno LH ratio during exogenous GnRH stimulation of LH release, and inferred the presence of nonspecific serum effects in their assay (5). On the other hand, earlier studies in normal men indicated that hypopituitary plasma was devoid of LH bioactivity and that spontaneous LH secretion occurred in pulses of increased intrinsic biological activity (6, 7). The latter observations suggest that the endogenous GnRH signal initiates the release of LH that is relatively enriched in bioactivity. Episodic bioactive LH secretion might min-

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imize gonadal desensitization due to sustained secretion of highly biologically active hormone (8). Nonetheless, preferential release of bioactive LH could not be demonstrated when exogenous GnRH was administered as either a single bolus dose or a continuous infusion (9, 10).

The foregoing observations raise two key issues: 1) nonspecific plasma effects would challenge the validity of extensive prior work using the LH bioassay; and 2) invariant plasma LH bioactivity in response to GnRH would question the inference that LH is released physiologically in pulses of high biological activity. To clarify these issues, we determined plasma LH bioactivity in 10 hypopituitary men basally and/or after the injection of human LH. Moreover, we sampled blood at 10-min intervals for 14 h in 7 normal men to assess the biological quality of LH secreted in response to endogenous and exogenous pulses of GnRH.

Materials and Methods

Study subjects

Sampling procedures. Seven normal men (aged 21–28 yr) and 10 hypogonadotropic men (aged 21–50 yr; median, 24.5) provided written informed consent for participation in this study, which was approved by the Human Investigation Committee of the University of Virginia School of Medicine. The healthy men had normal hepatic, renal, and hematological function; were euthyroid; and had normal plasma concentrations of TSH, LH, FSH, PRL, free T_4 , and free testosterone and normal semen analyses. After admission to the Clinical Research Center and insertion of an indwelling needle, blood was withdrawn at 10-min intervals for 14 h. The first 12 h were used to assess basal (spontaneous) endogenous LH release. Then 10 μ g GnRH were injected iv, and blood sampling was continued for an additional 120 min. The specific diagnoses in the hypogonadotropic men are listed in Table 1. These individuals received injections of human LH (see below).

TABLE 1. Assays of bioactive and immunoactive LH in hypogonadotropic men

Diagnosis	Serum FSH (mIU/mL)	Bioactive LH (mIU/mL)	Immunoactive LH (mIU/mL)
1) Chromophobe adenoma	<2	<0.4	<2
2) Panhypopituitarism	7.0	<0.4	3.0
3) Panhypopituitarism	<2	<0.4	8.9
4) IIHH ^a	11	<0.4	4.0
5) Kallmann's syndrome	<2	<0.4	<2.0
6) Kallmann's syndrome	<2	<0.4	<2.0
7) Kallmann's syndrome	<2	<0.4	2.9
8) Kallmann's syndrome	<2	<0.4	2.3
9) Kallmann's syndrome	<2	<0.4	3.8
10) Kallmann's syndrome	<2	<0.4	3.8

^a Incomplete isolated hypogonadotropic hypogonadism.

Assays of LH. Serum LH concentrations were assayed in duplicate by double antibody RIA. The assay sensitivity was 2 mIU/mL in terms of the Second International Reference Preparation of human menopausal gonadotropin. Plasma bioactive LH was assayed in four dilutions in triplicate by the rat interstitial cell testosterone assay (RICT; sensitivity 0.4 mIU/mL) (6). All samples from any one man were analyzed in the same run to eliminate the effect of interassay variance.

The RICT within-assay coefficient of variation averaged 8.5%, with a range from 8.2% in normal men to 9.2% in children and postmenopausal women. For the RIA, the coefficients of variation were 8.4% (at 2 mIU/mL), 6.8% (at 11.5 mIU/mL), and 4.6% (at 47 mIU/mL), using a LH antiserum that has less than 15% cross-reactivity with α -subunit (6).

LH injection studies. We used highly purified immunoassay grade human LH (NIADDK hLH I-2) prepared by Dr. A. F. Parlow. Its LH potency was 9,500 IU/mg LH by bioassay (ovarian ascorbic acid depletion) and 13,117 IU/mg by RIA (WHO International Standard urinary FSH/LH 70). It contained 8 IU/mg FSH by RIA and 0.03 IU/mg TSH by RIA (WHO human TSH 68/38). The potency of this preparation was 22,000 IU/mg in the RICT and 9,330 IU/mg by RIA (Second International Reference Preparation of human menopausal gonadotropin standard).

For the bolus injection studies, blood samples were withdrawn in the basal state at 15-min intervals for 1 h, after which 35 μ g highly purified LH were administered iv by bolus injection, as described previously in the clearance studies (11). Immediately after injection, blood samples were withdrawn every 5 min for 30 min, every 10 min for 40 min, every 15 min for 60 min, and then every 30 min for 180 min. These studies were done in 1983 in six hypogonadotropic men, and the plasma was frozen at -70°C (11).

Analysis and statistics. The curvilinear regressions of bioactive LH on immunoactive LH were performed using two-dimensional error fitting, since measurement error is present in both the y-axis (plasma bioactive LH) and the x-axis (serum immunoactive LH) (12). In contrast, conventional linear regression assumes that there is zero error in the x-axis values (13). The application of two-dimensional error analysis to the curvilinear regression was accomplished, as described previously, using the relationship $y = ax^2 + bx + c$, where $a = 0$ for a simple linear descriptor (12). For each of the coefficients (a , b , and c), the 67% confidence limits for the precision of fit were estimated about a mean value. This procedure assumes asymmetric, highly correlated variance spaces (12) and permits one to obtain parameter estimates while simultaneously recognizing intraassay variances in both the RIA and the bioassay.

Since plasma bio:immuno LH ratios were not normally distributed, the nonparametric Wilcoxon signed rank test was used to evaluate changes in median bio:immuno ratios (14).

Sample cross-correlation and autocorrelation matrices for plasma bioactive and immunoactive LH concentrations were determined as described previously (15). Auto- and cross-correlations were evaluated at various lag values (number of unit time intervals separating the serially correlated values), so as to assess serial correlations within the individual LH series (autocorrelations) or significant lagged (temporally separated)

correlations between the two types of LH series (cross-correlations between RIA and bioactive LH measurements).

Results

Assessment of bioactive LH measurements

Bioassay of 25, 50, or 100 μL plasma from 10 hypogonadotropic men revealed bioactive LH concentrations not distinguishable from the zero dose (*i.e.* within 3 SD of the zero dose). A range of low normal immunoactive LH concentrations was found in the same men (Table 1). In another group of 6 hypogonadotropic men, plasma bioactive and immunoactive LH concentrations were measured after LH injection. As shown in Fig. 1, plasma bioactive and immunoactive LH concentrations in these 6 men were related in a curvilinear fashion when the values in all samples collected from 5–180 min after the LH injection were considered. The curvilinear relationship was more prominent at higher bioactive and immunoactive LH concentrations, with a simple linear relationship between bioactive and immunoactive LH concentrations at lower (physiological) LH concentrations (see below). This phenomenon presumably reflects different saturabilities of *in vivo* LH clearance mechanisms for immunoactive and bioactive LH (11). The y-intercept for the 2-dimensional plot had a mean value of -21 mIU/mL (67% confidence interval, -31 to -11 mIU/mL). In the relationship, $y = ax^2 + bx + c$, the mean

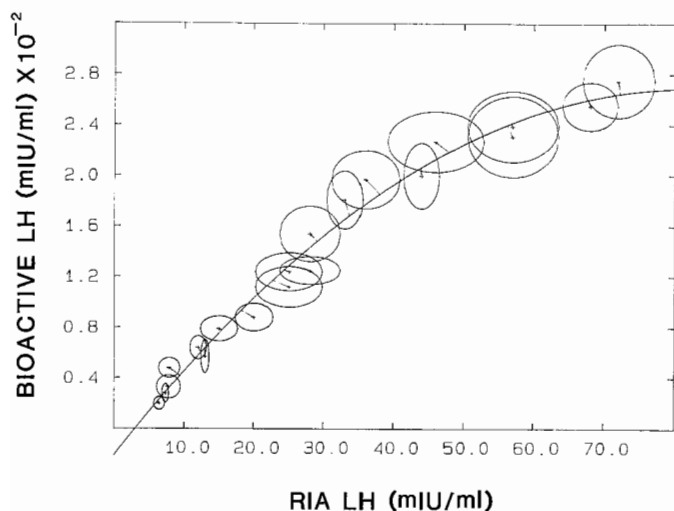


FIG. 1. Relationship between plasma bioactive and immunoactive (RIA) LH in six hypogonadotropic men injected with human LH for metabolic clearance calculations (11). The subsequently determined concentrations of plasma bio- and immunoactive LH were subjected to Gauss-Newton iterative least squares nonlinear curve fitting (see *Materials and Methods*). Each data point is the mean \pm the two-dimensional SD for the bioactive and immunoactive LH measurements, so that the corresponding ellipse depicts the asymmetric variance space associated with both the bioassay and immunoassay. The curvilinear plot denotes the best fit of the regression designed to minimize the distance from each ellipse center to its tangent on the fitted curve.

estimated values of the a and b coefficients were -0.043 (-0.059 to -0.028) and 7.1 (6.0 – 8.2), respectively. When only the physiological range of LH concentration was considered (bioactive LH, 0.4 – 160 mIU/mL; immunoactive LH, 2 – 30 mIU/mL), the slope of the linear regression was 5.01 ± 0.35 , and the y-intercept was -4.1 ± 6.4 ($r = 0.976$; $P < 0.001$). These y-intercept values indicate the absence of any nonspecific bioactivity measured at zero dose of immunoactivity.

Evaluation of the relationship between plasma bio- and immunoactive LH in normal men

In 7 normal men sampled at 10-min intervals for 12 h, the subsequent bio- and immunoactive LH concentrations were assessed by curvilinear regression. Since both the immuno- and bioassays contain inherent measurement error, we again employed curve-fitting with 2-dimensional error analysis to account for variance in both the x - and y -axes. As illustrated in Fig. 2, the relationships between plasma bio- and immunoactive LH were linear (*i.e.* the values of the a coefficients were not distinguishable from zero). The mean slope of the 7 linear regressions was 3.3 (range, 2.0 – 6.1 ; Table 2). Such linearity was observed by regression over all data points, both within and between LH peaks. The individual y -axis intercept values were not statistically different from zero in any of these 7 men, when the precision of the estimate derived from 73 individual data points was considered. These results indicate that in normal men as well as in hypogonadotropic men given exogenous human LH there is no nonspecific bioactivity estimated in plasma at a zero concentration of immunoactive LH. In contrast, there was a small degree of residual LH immunoreactivity (3.25 mIU/mL in Fig. 1 and 2.77 mIU/mL in Table 2) at zero dose of bioactive LH, *i.e.* x -intercept. This point is discussed further below.

Bio- and immunoactive LH release in response to endogenous and low dose exogenous GnRH pulses in normal men

As shown in Fig. 3, exogenous GnRH (10 μg , iv) elicited a prompt and significant increase in bioactive and immunoactive LH concentrations (Fig. 3A, *upper and middle panels*; $P < 0.001$). In these normal men, the mean plasma bioactive LH concentration rose from 33 ± 5.7 (\pm SEM) to 202 ± 41 mIU/mL, a 6-fold increase. In the same individuals, the serum immunoactive LH concentration rose from 13.2 ± 1.8 to 47 ± 2.8 mIU/mL, a 3.6-fold increase. Accordingly, as shown in the *bottom panel* of Fig. 3A, the corresponding plasma bio:immuno LH ratio rose significantly. In particular, before GnRH injection the median basal plasma bio:immuno LH ratio was 2.65 . This increased to a peak value of 4.65 approx-

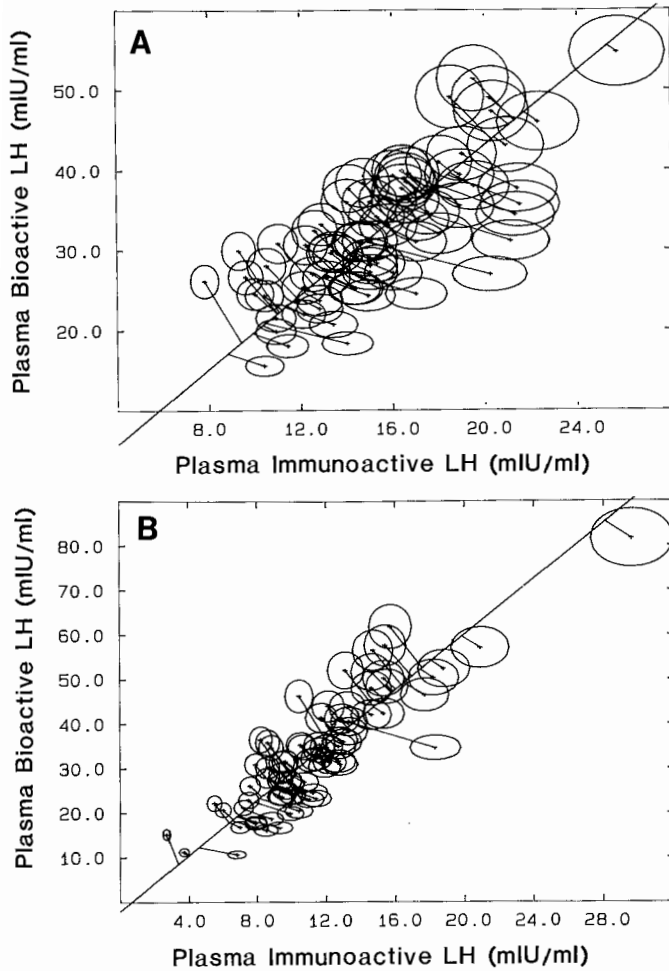


FIG. 2. Illustrative relationships between plasma bioactive and serum immunoactive LH concentrations in two of the seven normal men who underwent blood sampling at 10-min intervals for 12 h. Data are presented in the manner summarized in Fig. 1. The line represents the best fit through the two-dimensional elliptical variance spaces representing measurement errors inherent in both the bio- and immunoassays. The regression coefficients for all seven normal men studied in this fashion are summarized in Table 2. Note the break in the axes near the origin (A) to accommodate the full range of LH values observed.

imately 40 min after GnRH injection, when plasma bioactive and immunoactive LH concentrations were maximal.

The above pattern of response to exogenous GnRH was qualitatively similar to that in relation to presumptive endogenous GnRH stimulation in normal men, who had a median bioactive LH pulse frequency of 5.5 pulses/12 h and a median incremental pulse amplitude of 28 mIU/mL. Thus, when 23 endogenous LH peaks defined by cluster analysis constrained to a 1% false positive rate (16) were analyzed as a composite (mean) bioactive or immunoactive LH pulse, bioactive LH concentrations increased from a mean basal value of 23.5 ± 3.9 mIU/mL to a peak value of 57.6 ± 7.0 mIU/mL, with an associated increase in immunoactive LH concentrations

TABLE 2. Relationship between bioactive and immunoactive LH in the plasma of seven normal men

Subject no.	Slope	y-Axis intercept
1	4.3 (2.6–7.9)	-10.3 (-34 to 1.2)
2	2.3 (1.7–3.2)	-3.5 (-15 to 4.8)
3	2.0 (1.0–2.8)	-14 (-25 to -2.8)
4	6.1 (4.1–10)	-32 (-67 to -16)
5	3.1 (2.5–3.8)	-2.1 (-8.1 to 2.9)
6	2.9 (1.9–4.1)	-3.5 (-2.4 to 9.9)
7	2.5 (1.4–3.7)	-1.4 (-8.7 to 3.1)

Data are mean estimates with 67% confidence limits for the precision of the two-dimensional error fit of the relationship y (bioactive LH) = ax (immunoactive LH) + b , where a is the slope and b is the intercept. Each fit comprised 73 data points representing blood sampled at 10-min intervals for 12 h in a normal man.

from 11.8 ± 1.4 to 14.9 ± 1.9 mIU/mL. The corresponding median bio:immuno ratio increased from 2.3 to 3.3 ($P < 0.01$; Fig. 3B).

The changes in plasma bio:immuno LH ratios within endogenous LH peaks were also assessed by comparing median bio:immuno LH ratios contained in interpulse intervals with those within bioactive LH pulses (intrapulse values). In these seven men sampled at 10-min intervals for 12 h, the bio:immuno ratios within endogenous LH pulses (intrapulse values) were significantly higher than those in the same men during the interpulse (basal) intervals ($P < 0.006$; Fig. 4).

The possible effect of residual LH immunoreactivity at zero dose LH bioactivity (*i.e.* the x-intercept value) was assessed by subtracting such nonspecific LH immunoreactivity from the measured baseline and peak RIA values. The resultant revised mean bio:immuno LH ratios also increased within peaks, *e.g.* from 3.3 to 4.6 in exogenous GnRH-stimulated LH peaks and from 2.7 to 4.9 in endogenous (spontaneous) LH peaks. Consequently, residual LH immunoreactivity at zero dose LH bioactivity would not alter our overall conclusion.

Cross-correlation analyses of plasma bioactive and immunoactive LH concentrations in normal men

The 12-h bioactive and immunoactive LH series in each of the seven men were subjected to autocorrelation and cross-correlation analyses. As summarized in Table 3, only positive autocorrelations and cross-correlations were found in these normal individuals. In particular, bioactive LH exhibited significantly positive autocorrelation at various lags ranging from 10–40 min, which would be predicted from the relatively slow metabolic clearance of LH. A similar pattern was found for immunoactive LH. In addition, there were significant positive cross-correlations between the immunoactive and bioactive LH measurements at lags of 0–40 min. These positive cross-correlation coefficients indicate that when bioactive LH concentrations increase, immunoactive

FIG. 3. A, Profiles of mean (\pm SEM) plasma bioactive (upper panel) and serum immunoactive (middle panel) LH concentrations and median B:I ratios in 7 normal men before and after exogenous GnRH injection. The arrow designates the time of injection of the exogenous GnRH pulse. B, Similar data from 23 endogenous LH peaks from 7 men synchronized in relation to their maximal value (given as time zero).

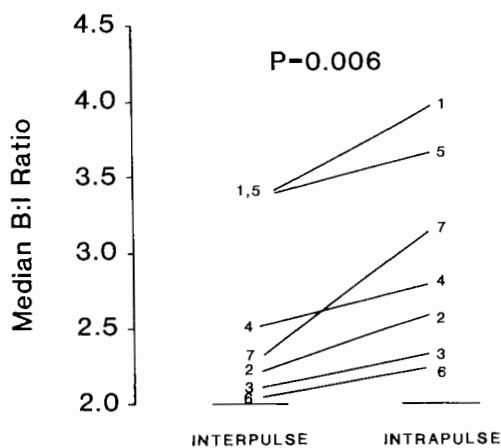
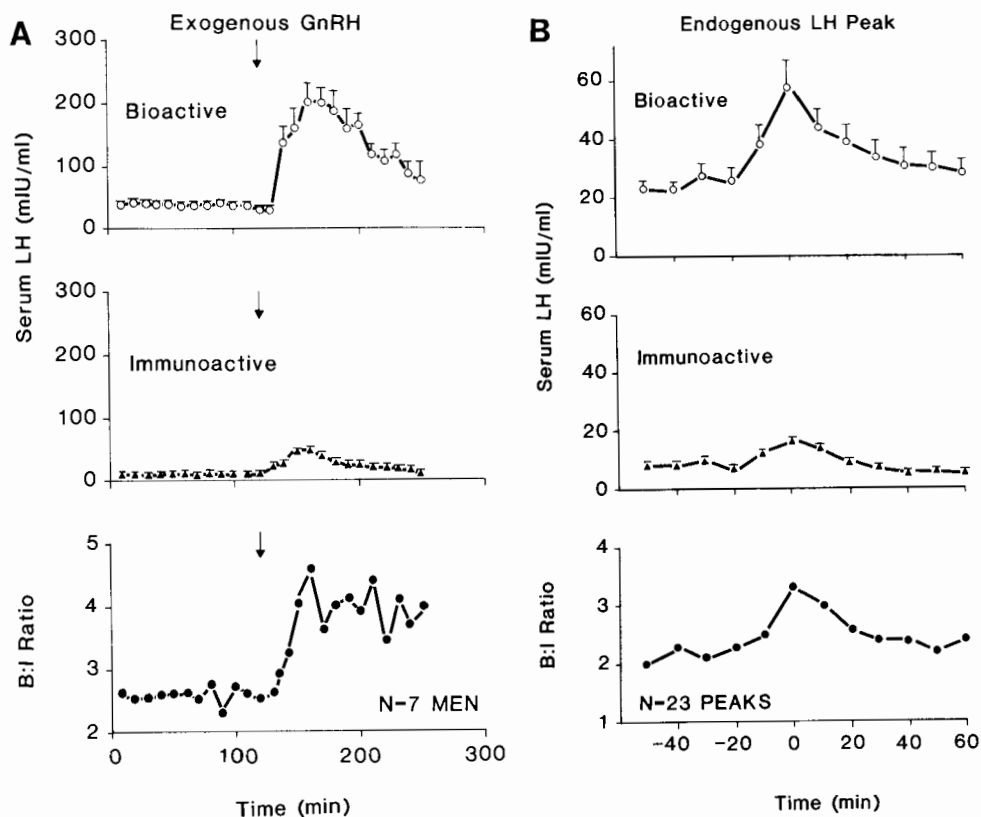


FIG. 4. Plasma bio:immuno (B:I) LH ratios between endogenous LH pulses (interpulse values) or within endogenous LH peaks (intrapulse values) under basal conditions in normal men. Data are median bio:immuno LH ratios in seven men (identified by individual numerals), each of whom underwent blood sampling at 10-min intervals for 12 h. The *P* value denotes the significant difference between median plasma bio:immuno LH ratios in the interpulse *vs.* the intrapulse intervals.

concentrations also tend to rise. Conversely, when bioactive LH concentrations decline, immunoactive concentrations also fall. Therefore, increases in the bio:immuno LH ratio in normal men must reflect preferential enhancement of bioactivity over immunoactivity, rather than an increase in bioactivity associated with a concomitant decrease or no change in immunoactivity. Patterns of auto- and cross-correlations for bioactive and immunoactive LH are illustrated for two men in Fig. 5.

TABLE 3. Median sample cross-correlation matrices of plasma bioactive and immunoactive LH concentrations in normal men

LAG (min) ^a	Correlated parameters at individual lags			
	Bioactive autocorrelations	Immunoactive autocorrelations	RIA LH leads Bio LH	Bio LH leads RIA LH
0 min	1.00 (7)	1.00 (7)	0.67 (7)	0.67 (7)
10 min	0.60 (7)	0.55 (7)	0.39 (7)	0.39 (5)
20 min	0.51 (7)	0.45 (7)	0.39 (5)	0.49 (5)
30 min	0.35 (6)	0.38 (4)	0.41 (2)	0.33 (4)
40 min	0.26 (5)	0.26 (3)	0.31 (4)	

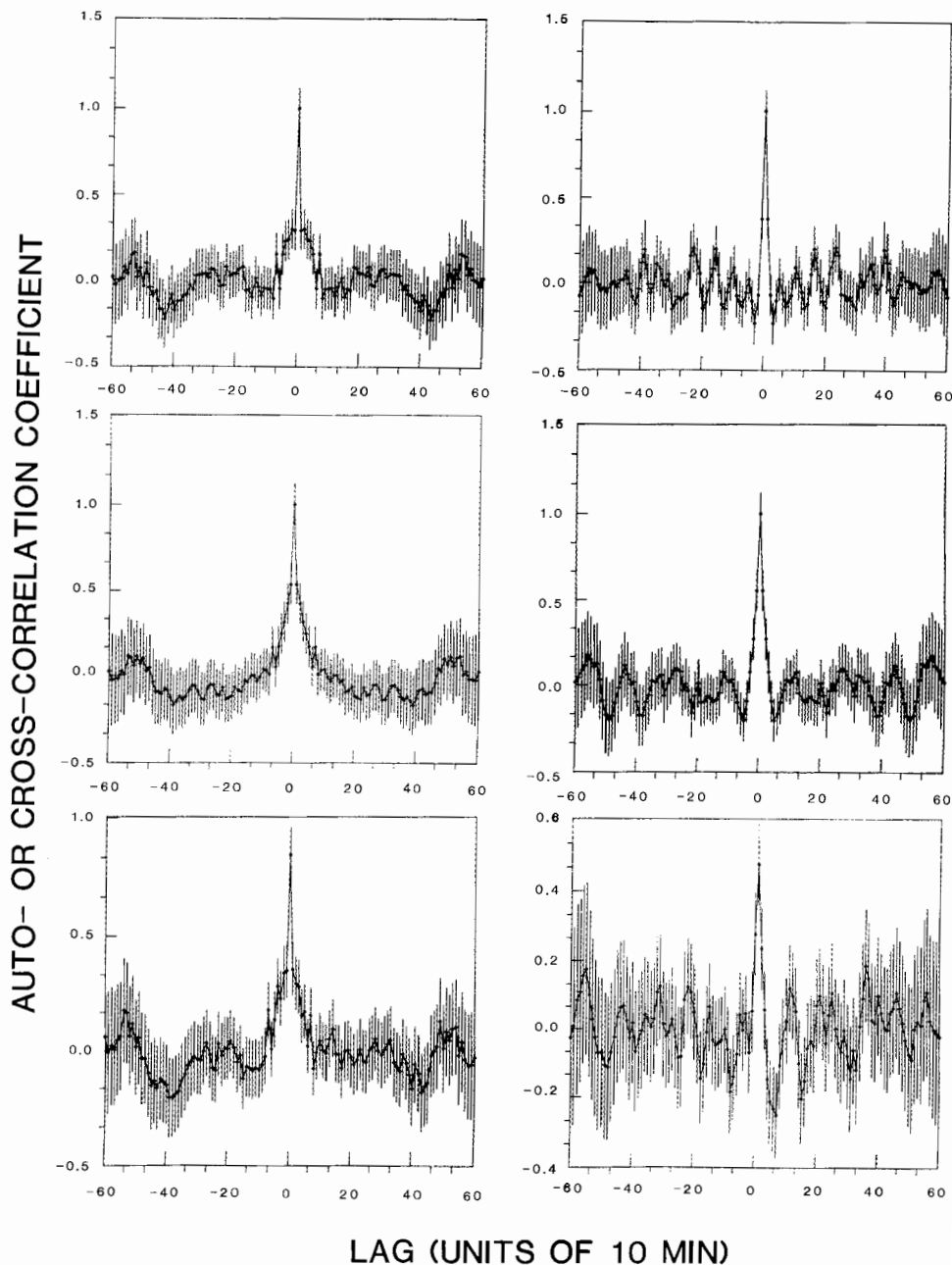
Values are median auto- or cross-correlation coefficients that are significantly nonzero. Auto- and cross-correlation analyses were performed on bioactive (Bio) and immunoactive (RIA) LH series, comprising blood sampled at 10-min intervals for 12 h in seven normal men. Values in parentheses denote the number of men whose series exhibited significant correlation coefficients at that lag.

^aThe lag denotes the number of minutes separating the serially correlated values (see *Materials and Methods*). The values in the bioactive and immunoactive LH series were permitted to lag the same series (autocorrelations columns 1 and 2) or each other (cross-correlation), with either series leading the other (columns 3 and 4).

Discussion

These results demonstrate that the RICT can detect an enhancement in the biological activity of LH secreted in response to either endogenous or exogenous pulses of GnRH in normal men. This conclusion is dependent upon our documentation that hypogonadotropic men have a bio:immuno LH ratio not distinguishable from

FIG. 5. Auto- and cross-correlation analyses of plasma bioactive and serum immunoactive LH concentrations in two normal men. Blood samples were withdrawn at 10-min intervals for 12 h for subsequent assay of bio- and immunoactive LH. The two time series (serial bio- and immunoactive LH concentrations) were then subjected to autocorrelation and cross-correlation analyses. The *upper panel* shows the autocorrelation coefficients for bioactive LH, the *middle panel* the autocorrelation coefficients for immunoactive LH, and the *bottom panel* the cross-correlation coefficients for bio- and immunoactive LH over time. The *horizontal axis* gives the lag value, k , which is the number of sampling time units (each of 10-min duration) by which the correlated samples are separated. The *vertical marks* represent the SE of the correlation coefficients at each particular lag. The results from all seven men are summarized in Table 3.



zero. In addition, our further studies in LH-deficient men given a bolus LH injection revealed a linear relationship between plasma bio- and immunoactive LH at physiological plasma hormone concentrations, with zero (or slightly negative) extrapolated bioactivity when immunoactive LH concentrations fell to zero. These results disagree with the earlier conclusion that significant LH bioactivity exists in the plasma of GnRH-deficient men when immunoactivity is extrapolated to zero and with the corresponding suggestion that bioactive LH values and consequently bio:immuno LH ratios are spuriously influenced by plasma effects (5). The occurrence of putative plasma effects in the latter study may reflect

properties of the particular RICT bioassay system, the LH-deficient patient population studied, and/or the failure to model two-dimensional error propagation in regression analysis (*i.e.* variance present in both the bio- and immunoassays).

We analyzed the relationships between plasma bio- and immunoactive LH concentrations in plasma collected at 10-min intervals for 12 h in normal men using a linear regression model designed to estimate best fit in relation to the variance in both the immuno- and bioassays (two-dimensional error propagation). In all seven men, the y-intercept of the bio:immuno LH plot was not significantly different from zero. These results in normal

men differ from those reported recently in GnRH-deficient men (see above), in which Spratt *et al.* (5) allowed for measurement variance in the y-axis (bioactive LH), but not in the x-axis (immunoactive LH). Moreover, LH secretion was maintained in the GnRH-deficient men by sc injection of GnRH at fixed 2-h intervals and fixed doses before testing. This schedule of GnRH administration may not adequately mimic the physiological pattern of endogenous GnRH secretion, the physiological (iv) route of GnRH delivery, and/or the demonstrated non-uniformity of serial LH interpulse intervals and LH pulse amplitudes observed in normal men (17, 18). In contrast, our results clearly indicate that nonspecific LH bioactivity is not present in plasma obtained from hypogonadotropic or normal men. Rather, we infer that a small degree of nonspecific immunoreactivity is present.

We previously reported the complete absence of LH bioactivity in undiluted plasma from hypogonadotropic men (19), with similar results in the hypophysectomized rat (20), rhesus monkey (21), and dark infertile mink (22). Accordingly, we used the same RICT assay conditions to determine the bioactive LH responses to endogenous and low dose exogenous GnRH pulses in normal men. GnRH administration resulted in a preferential increase in circulating bioactive compared to immunoreactive LH concentrations. There was a consequent significant increase in the plasma bio:immuno LH ratio. A qualitatively similar profile was found for spontaneous (endogenous GnRH-driven) LH peaks, wherein maximal plasma bioactive LH concentrations within LH pulses coincided with significant increases in the bio:immuno LH ratio.

The preferential increase in plasma bioactive LH concentrations in response to exogenous or endogenous GnRH with an increase in the bio:immuno LH ratio might reflect in part the somewhat smaller initial distribution volume of bioactive compared to immunoactive LH (as assessed by the injection of purified human LH in hypopituitary men) (11) and in part the somewhat slower MCR of bioactive, compared to immunoactive, LH after bolus injection or under steady state conditions (11). In addition, increased bio:immuno LH ratios within endogenously and exogenously stimulated LH peaks may result from preferential release of LH molecules enriched in bioactivity. The latter inference is further supported by deconvolution analysis of spontaneous and GnRH-stimulated LH peaks in normal men (23). An alternative consideration that plasma bio:immuno LH ratios increase because serum immunoactive LH levels decrease was excluded by the significantly positive cross-correlation coefficients between bio- and immunoactive LH at 0- to 40-min lags in these normal men. Moreover, accounting for the small amount of nonspecific immunoreactivity did not alter our conclusion that bio:immuno

LH ratios increase within LH peaks. Thus, LH is secreted in pulses of increased biological activity. Such a pattern may reflect the release of a compartmentalized pool of LH relatively enriched in bioactivity.

In previous studies, increases in plasma bio:immuno LH ratios were not found in men or postmenopausal women given synthetic GnRH either as a single sc bolus dose (100 μ g) or by continuous infusion (2 μ g/min, iv) (9, 10). Such observations could be explained 1) in the former case, by increased secretion from all pituitary pools stimulated by a single pharmacological dose of GnRH, thus yielding an integrated bio:immuno LH value rather than a selective increase from a highly bioactive LH pool; and 2) in the latter case, by the inability of the continuous GnRH infusion to stimulate LH release from a pool of presumably high bioactivity and/or by mixing such a pool with a pool of reduced bioactivity. We were able to demonstrate the release of a compartment of highly biologically active LH by evaluating responses to the endogenous GnRH pulse signal (assessing spontaneous LH pulses) and by mimicking the endogenous GnRH pulse signal with a low iv bolus dose of exogenous GnRH.

In conclusion, our studies of bioactive and immunoactive LH release in response to endogenous and low dose exogenous GnRH pulses in normal men indicate that both the quantity and biological quality of secreted LH molecules are modulated by the GnRH pulse signal. Thus, we suggest that the physiological mode of episodic bioactive LH secretion is such as to avoid gonadal desensitization that might occur if similar concentrations of highly bioactive hormone were secreted in a nearly constant manner.

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