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PATHOPHYSIOLOGICAL FEATURES OF THE PULSATILE SECRETION OF BIOLOGICALLY ACTIVE LUTEINIZING HORMONE IN MAN

JOHANNES D. VELDHUIS,[1]* RANDALL J. URBAN,[1] INESE Z. BEITINS,[2] ROBERT M. BLIZZARD,[1] MICHAEL L. JOHNSON[1] and MARIA L. DUFAU[3]

[1]Division of Endocrinology and Metabolism, Departments of Internal Medicine and Pediatrics; and Sections of Biomathematics and Biophysics, Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Va, [2]Division of Pediatric Endocrinology, University of Michigan, Ann Arbor, MI 48109 and [3]Endocrinology and Reproduction Research Branch, The National Institute of Child Health, and Human Development, National Institute of Health, Bethesda, MD 20892, U.S.A.

Summary—The development of an *in vitro* bioassay of high specificity, sensitivity and precision for the measurement of low circulating concentrations of biologically active glycoprotein hormones has offered exciting new insights into the *in vivo* secretion and metabolic clearance of luteinizing hormone (LH) in various pathophysiological states. Moreover, the most recent combined application of the rat interstitial cell testosterone (RICT) bioassay and a novel multiple-parameter deconvolution model has allowed investigators to dissect plasma concentration profiles of bioactive LH into defined secretory bursts, which have numerically explicit amplitudes, locations in time, and durations, and are acted upon by determinable subject- and study-specific endogenous metabolic clearance rates. Here, we have: (i) reviewed the ability of the endogenous GnRH pulse signal to regulate the *in vivo* secretion of biologically active LH molecules as assessed in the RICT and by deconvolution mechanics; (ii) demonstrated that low-dose exogenous GnRH pulses effectively mimic spontaneous bioactive LH pulsatility; (iii) investigated the role of endogenous androgen and estrogen in modulating bioactive gonadotropin secretion in men and women; and (iv) described significant alterations in endogenous LH bioactivity in puberty and healthy aging.

INTRODUCTION

With the advent of radioimmunoassays more than a decade ago, the properties of *immunoactive* glycoprotein hormone secretion and metabolic clearance have been characterized under various pathophysiological conditions [1]. However, the development of a valid *in vitro* assay system for the quantitation of biologically active LH in plasma (the rat interstitial cell testosterone bioassay, RICT) revealed a range of discrepancies between immunoactive estimates and bioactivity in various pathological and physiological states [2]. In particular, studies of bioactive LH release in healthy young men, in young women of reproductive age evaluated throughout the normal menstrual cycle, and in postmenopausal women disclosed that significant discordance existed between immunoactive and bioactive LH pulsations, such that 14–30% of bioactive LH pulses were *un*accompanied by significant immunoactive LH pulses [2–4]. Moreover, serum bioactive LH concentrations in spontaneous gonadotropin pulses increased preferentially over immunoactive LH concentrations, resulting in a significantly enhanced bio:immuno LH ratio, which could be mimicked by the exogenous administration of low-dose pulsed GnRH but not by continuous GnRH infusion and/or pharmacological GnRH injections [5–7]. In addition to pulsatile bioactive LH release in individual subjects, mean serum bioactive LH concentrations could be shown to vary strikingly in puberty, during the normal menstrual cycle, with healthy aging, and in response to manipulations of the sex-steroid hormone milieu [1–5, 8, 9]. Moreover, in each of these circumstances, the RICT-determined bioactivity of LH can confer substantial additional information regarding physiological regulation and pathological alterations in LH secretion and/or clearance. Thus, the measurement of bioactive LH can be viewed as a clinical and experimental probe of the gonadal axis that offers distinct and complementary insights into hypothalamo–pituitary function in health and disease. Here, we have presented a systematic examination of the physiological and pathological regulation of bioactive LH secretion and clearance under selected clinical conditions of health and disease.

*Address correspondence to: Dr Veldhuis, Box 202, University of Virginia School of Medicine, Charlottesville, VA 22908, U.S.A. [*Tel.* (804) 924-9697].

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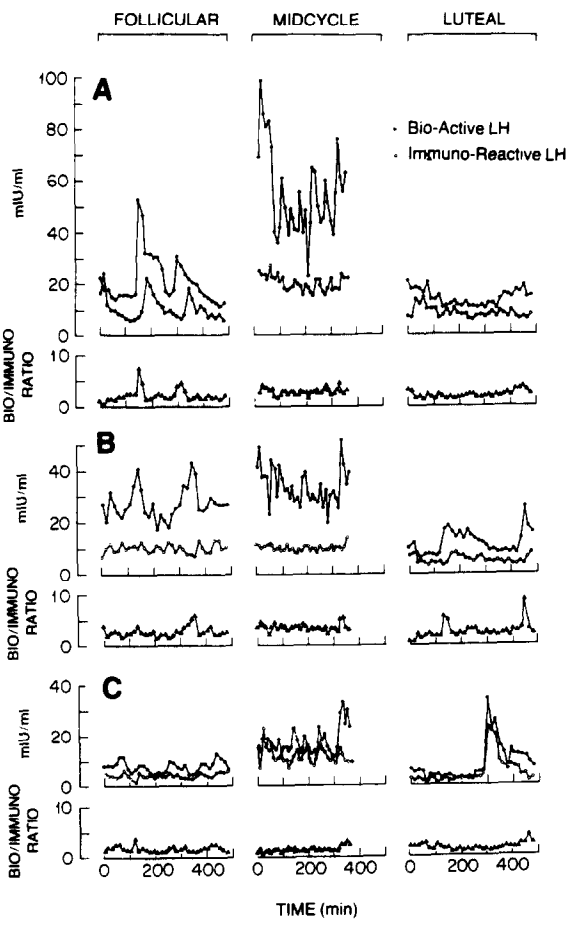

Fig. 1. Pulsatile bioactive and immunoactive LH release and resultant bio:immuno LH ratios obtained during three stages of the menstrual cycle in three normal women. Serial blood samples were collected at 10 or 15 min intervals for 360–480 min (horizontal axis) in the early follicular, late follicular (midcycle), and luteal phases. (Adapted with permission from Ref. [4].)

1. IMPACT OF THE GNRH SIGNAL ON BIOACTIVE LH SECRETION

(a) Role of the endogenous GnRH pulse signal in regulating bioactive LH release in man: Pulsatile nature of bioactive LH release

Initial studies of healthy young men and postmenopausal women during estrogen deprivation revealed a distinctly *pulsatile* mode of bioactive LH release *in vivo* [3]. In these early studies, bioactive LH pulses were detected at a median periodicity of approximately every 76 min in young men and every 90 min in estrogen-unreplaced postmenopausal individuals. Moreover, mean (±SD) serum bioactive LH concentrations were 41 ± 15 mIU/ml in young men and 450 ± 24 mIU/ml in postmenopausal women, with corresponding bio:immuno LH ratios of 4.0 ± 1.0 (men) and 5.4 ± 1.3 (postmenopausal women). The incremental amplitudes of bioactive LH pulsations were 19 ± 5.5 mIU/ml in young men and 153 ± 107 mIU/ml in postmenopausal women ($P < 0.01$). In addition, statistical analyses revealed that bio:immuno LH ratios within LH pulses significantly exceeded those in the inter-peak "valleys" [2, 3]. Collectively, these observations indicated that bioactive LH release occurs in distinct and episodic pulsations, which are associated with an increase in circulating concentrations of LH enriched in biological activity. As discussed further below, the high bioactivity of spontaneous LH pulsations could result from the preferential secretion of LH molecules of high bioactivity when the pituitary gland is stimulated by quanta of endogenous GnRH, and/or differential clearance of bioactive and immunoactive hormone with or without interconversion of various molecular species of circulating LH.

In addition to the striking *amplitude* differences in pulsatile LH release between young men and postmenopausal women (*vide supra*), young women of reproductive age exhibited both frequency and amplitude modulation of pulsatile bioactive LH release throughout the menstrual cycle [4]. This pattern is illustrated in Fig. 1. Mean bioactive LH concentrations (±SD) in repetitively sampled young women varied from 23 ± 15 mIU/ml in the early follicular phase to 43 ± 18 mIU/ml in the late follicular phase and 27 ± 23 mIU/ml in the luteal phase of the menstrual cycle [4]. These changes in mean serum bioactive LH concentrations were accompanied by significant alterations in bioactive LH pulse frequency. In particular, bioactive LH pulse frequency increased from 0.47 ± 0.23 pulses/h in the early follicular phase to 1.0 ± 0.12 pulses/h in the late follicular phase, and then decreased to 0.34 ± 0.05 pulses/h in the mid-luteal phase. The maximal amplitude of LH peaks was also modulated throughout the menstrual cycle, with respective values of 31 ± 18 mIU/ml (early follicular phase), 59 ± 27 mIU/ml (late follicular phase), and 43 ± 26 mIU/ml (mid-luteal phase). Thus, we can conclude that bioactive LH release occurs in distinct pulsations in healthy young women, and that this pulsatile mode of bioactive gonadotropin secretion exhibits *both* amplitude and frequency modulation throughout the normal menstrual cycle [4].

Since experimental studies in several animal species have demonstrated a close correspondence between LH pulsatility and episodic GnRH release in hypothalamo-pituitary-portal blood [10, 11], we can infer that the *pulsatile* release of *bioactive* LH in normal men, postmenopausal women, and young women studied throughout the menstrual cycle reflects episodic endogenous GnRH action. Moreover, although variations in bioactive LH pulse amplitude can result from changes in either the amplitude of the endogenous GnRH pulse signal and/or changes in pituitary gonadotrope-cell responsiveness, significant alterations in bioactive LH pulse *frequency* can be taken to reflect corresponding modulation of the hypothalamic GnRH pulse generator [1]. Thus, the

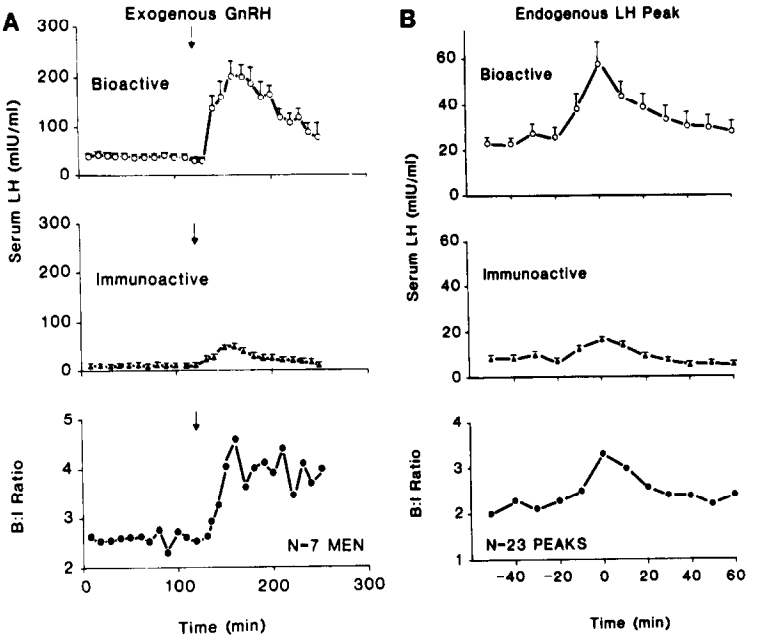

Fig. 2. *Panel A.* Profiles of mean (±SEM) plasma bioactive (upper subpanel) and immunoactive (middle subpanel) LH concentrations and median B:I ratios (lower subpanel) in 7 normal men before and after exogenous low-dose (10 mcg) pulsed GnRH injection. The arrow designates the time of injection of the exogenous GnRH pulse. *Panel B.* Similar data from 23 endogenous LH peaks from 7 men synchronized in relation to their maximal intrapulse value (given as time 0). (Adapted with permission from Ref. [5].)

impact of GnRH pulse frequency on the release of bioactive LH can be investigated under clinical or pathological conditions in which LH pulse frequency is accelerated experimentally or occurs at various frequencies physiologically. The latter circumstance prevails during the normal human and primate menstrual cycles, when spontaneous bioactive LH pulse frequency exhibits significant variation, such that increased LH pulse frequency occurs in the late follicular phase associated with enhanced circulating concentrations of estradiol and bioactive gonadotropin hormone [4, 12].

Experimental acceleration of the GnRH pulse generator can be accomplished by pharmacological blockade of the endogenous mu-opiate receptor system [13]. Administration of the long-acting selective mu opiate-receptor antagonist, naltrexone, significantly increased spontaneous bioactive LH pulse frequency, with a resultant enhancement in mean serum bioactive LH concentrations from 26 ± 11 mIU/ml to 33 ± 12 mIU/ml in healthy young men [14]. In addition, maximal bioactive LH peak amplitudes increased from 39 ± 13 to 44 ± 14 mIU/ml, as the bioactive LH interpulse interval declined from 200 ± 19 min to 124 ± 37 min ($P < 0.002$). This paradigm, in which the *endogenous* GnRH pulse signal frequency is selectively amplified, demonstrates that frequency modulation of GnRH's stimulation of the pituitary gland offers one major mechanism for regulating the amount of biologically active LH secreted *in vivo*.

The trophic role of endogenous GnRH pulses on bioactive LH release in man is further supported by the prompt and major suppression of serum bioactive LH concentrations induced by injection of a potent and long-acting selective decapeptide antagonist of GnRH action [15]. Further studies utilizing a more potent GnRH antagonist revealed that serum bioactive LH concentrations declined to a greater degree than immunoactive LH concentrations, which resulted in a gradual fall in the serum bio:immuno LH ratio [16]. The latter observation could reflect a preferential decrease in the release of bioactive over immunoactive hormone, altered interconversion between released bioactive and immunoreactive LH, and/or differences in the metabolic clearance rates of bioactive and immunoactive gonadotropin. Thereafter, "escape" occurred, with the reappearance of low-amplitude bioactive LH pulsations and stabilization of the serum bioactive LH concentration at a new but reduced level.

The role of GnRH in controlling the secretion of biologically active LH molecules *in vivo* can be examined further by injections of synthetically pure GnRH. Earlier investigations employing continuous infusions and/or pharmacologically large doses of GnRH failed to reveal any preferential release of bioactive over immunoactive LH in men or women, except during the late follicular phase of the menstrual cycle [6, 7]. However, more recent studies utilizing low-dose (10 mcg rather than 100 mcg) intravenous pulses of GnRH have demonstrated a quantitatively greater increase in bioactive than immunoactive LH concentrations with a transient

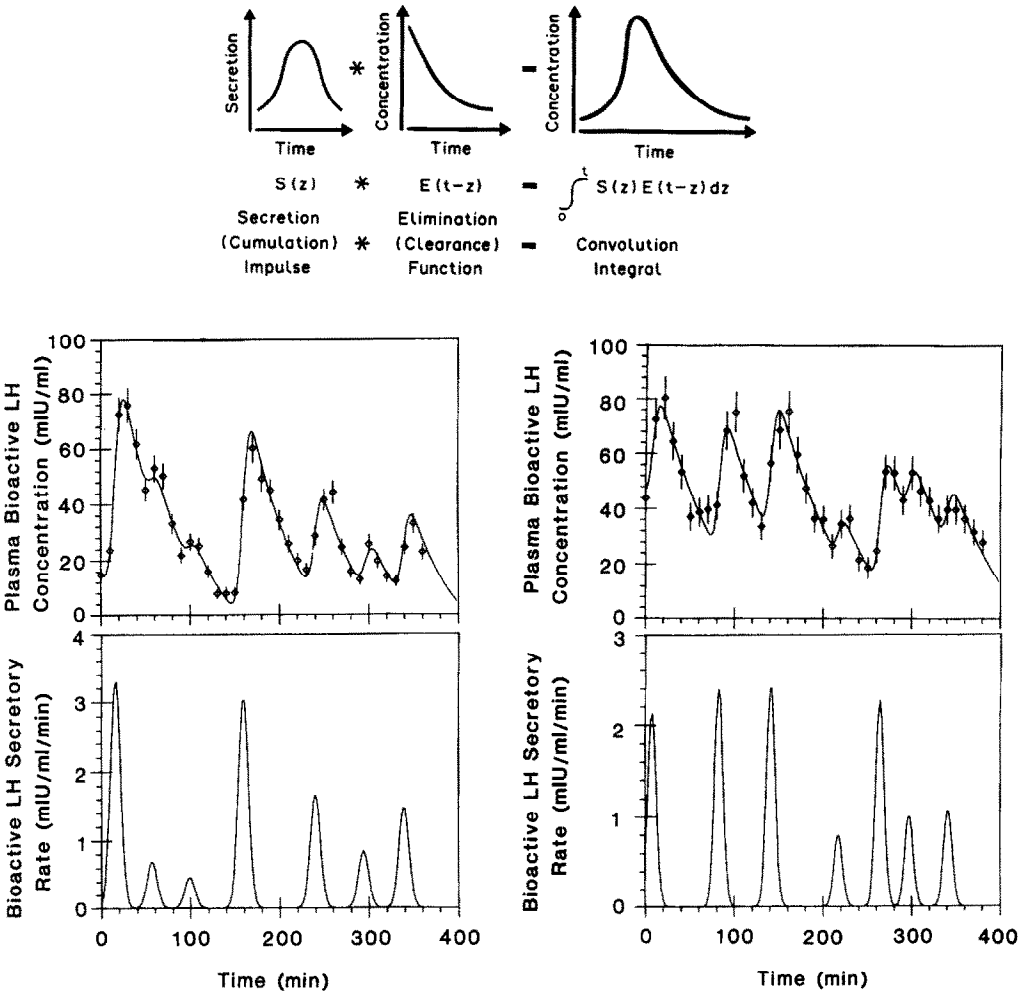

Fig. 3. *Panel A.* Model of multiple-parameter convolution integral, in which circulating hormone concentrations are controlled by discrete secretory bursts which are acted upon by endogenous metabolic clearance rates [discussed fully in Ref. 17]. *Panels B and C.* Illustrative profiles of spontaneous bioactive LH release with deconvolution-resolved secretory bursts in 2 healthy men. For each individual, the upper subpanel depicts serial serum bioactive LH concentrations measured by RICT in blood sampled at 10-min intervals for 6 h. The continuous curve through the data represents the calculated reconvolution fit derived from simultaneous non-linear, multiple parameter estimation (see [17]). The lower panel gives the underlying significant individually resolved secretory bursts, as a plot of secretion rate versus time. Note that the resolved secretory pattern consists of punctuated burst-like episodes of bioactive hormone release without any intervening "tonic" secretion. (Adapted with permission from Reference [19].)

increase in the bio:immuno ratio: Fig. 2. This pattern closely recapitulated that recognized in spontaneous LH pulsations: Fig. 2B. Such results suggest that physiological GnRH pulse signals elicit the episodic secretion of LH molecules of high biological activity.

The preferential increase in serum bioactive LH concentrations during spontaneous LH pulsations or in response to exogenous low-dose pulsed injections of GnRH could result from increased LH secretion and/or decreased clearance [17]. Using deconvolution modeling, it is possible to resolve the amplitude, temporal location, and duration of individual underlying LH *secretory* bursts and simultaneously estimate the half-time of endogenous LH disappearance from plasma [17, 18], as schematized in Fig. 3A. Accordingly, this new model of combined secretion and clearance was applied to spontaneous bioactive LH pulse profiles. In particular, as shown in Fig. 3B, episodic fluctuations in serum bioactive LH concentrations could be accounted for by underlying secretory bursts of quantitatively defined amplitude, temporal location, and duration. The resolved *secretory* bursts of bioactive LH manifested an average amplitude (maximal rate of secretion achieved within any given release episode) of 2.1 ± 0.26 mIU/ml/min [19]. The *frequency* of bioactive LH secretory bursts was 6.5 ± 0.25 pulses/6 h, which corresponded to a mean interpulse interval of 56 ± 1.3 min [19].

Table 1. Exogenous GnRH-stimulated LH release in young men assessed by deconvolution analysis

	Bioacative LH	RIA LH
Maximal secretory rate (mIU/ml/min)	6.5 ± 0.9	2.2 ± 0.70*
Mass of LH released per burst (mIU/ml)	215 ± 30	27 ± 8.7*
Half-time of endogenous LH disappearance (min)	49 ± 5	114 ± 12*

*$P < 0.01$ vs bioactive LH.

Data in Fig. 4 were subjected to multiple-parameter deconvolution analysis to resolve underlying LH *secretory* rates and half-times of endogenous LH disappearance simultaneously [17]. Secretory rates and mass are expressed per unit distribution volume, which is similar for bioactive and immunoactive LH [20]. The deconvolution model assumes that concentration peaks result from secretory bursts of finite and determinable amplitudes, durations and temporal locations, which are acted upon by endogenous clearance kinetics. The individual secretory and clearance values of interest are determined by non-linear least squares parameter estimation after solution of the relevant convolution integral (schematized in Fig. 3A, see Ref [17]).

Moreover, computer-resolved LH secretory bursts had a mean half-duration (duration at half-maximal amplitude) of 12 ± 1.5 min, which is remarkably shorter than the serum bioactive LH *concentration* peak of 56 ± 5.5 min ($P < 0.01$). The simultaneously calculated half-time of endogenous bioactive LH disappearance in normal men was 53 ± 5.4 min, which conforms closely to the independently determined half-time of exogenous LH disappearance of 65 ± 4.9 min in hypopituitary men infused with highly purified human pituitary LH (NIH I-2) [20]. Thus, deconvolution modeling of serum bioactive LH concentration series demonstrates not only that biologically active gonadotropin is secreted in short-lived, distinct and frequent bursts, but also that reasonable estimates of *endogenous* bioactive LH half-lives can be accomplished without the injection of radiolabeled or unlabeled gonadotropin [19].

To assess whether the preferential increase in serum bioactive LH concentrations following pulsed GnRH injection results from increased *secretion*, we applied deconvolution analysis to the bioactive and immunoactive LH pulses elicited by two consecutive low-dose (10 mcg) GnRH bolus infusions in a normal man. As shown in Fig. 4, and summarized numerically in Table 1, the calculated underlying secretory bursts of bioactive LH exhibited significantly greater amplitudes (maximal rate of secretion) and were associated with a significantly larger mass of hormone released per secretory burst (area under the secretory impulse) than immunoactive hormone. The calculated half-lives of endogenous bioactive and immunoactive LH clearance in this example were 49 ± 5 min and 114 ± 12 min. Thus, deconvolution analysis combined with the RICT bioassay permits us to infer that bioactive gonadotropic hormone is

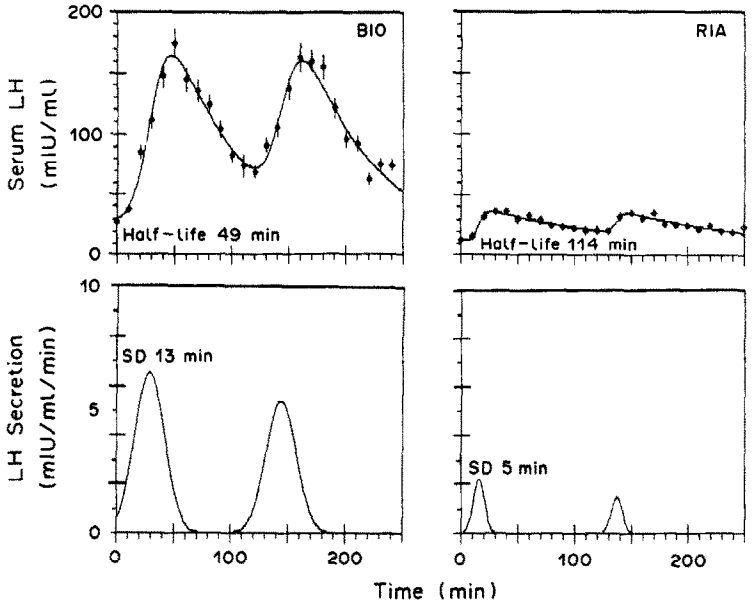

Fig. 4. Deconvolution analysis of serum bioactive (*left panel*) and immunoactive (*right panel*) LH concentrations after two consecutive two-hourly injections of low-dose pulsed GnRH (10 mcg) in a young normal man. The upper subpanels give the observed serial serum LH concentrations over time. The calculated reconvolution curve is shown through the observed data [17]. In the lower subpanels, the computer-resolved LH secretory bursts are depicted. The computer analysis assumes a multiple-parameter convolution integral, in which serum hormone concentrations are controlled by the number, amplitudes, durations, and temporal locations of all underlying secretory bursts, which are acted upon continuously by subject- and study-specific metabolic clearance rates [17]. Secretory *and* clearance (half-life) parameters are calculated simultaneously from all data points and their intrasample variances considered jointly [17]. The "SD" is the standard deviation of the resolved Gaussian secretory burst [Veldhuis J. D., Dufau M. L. and Johnson M. L., unpublished data].

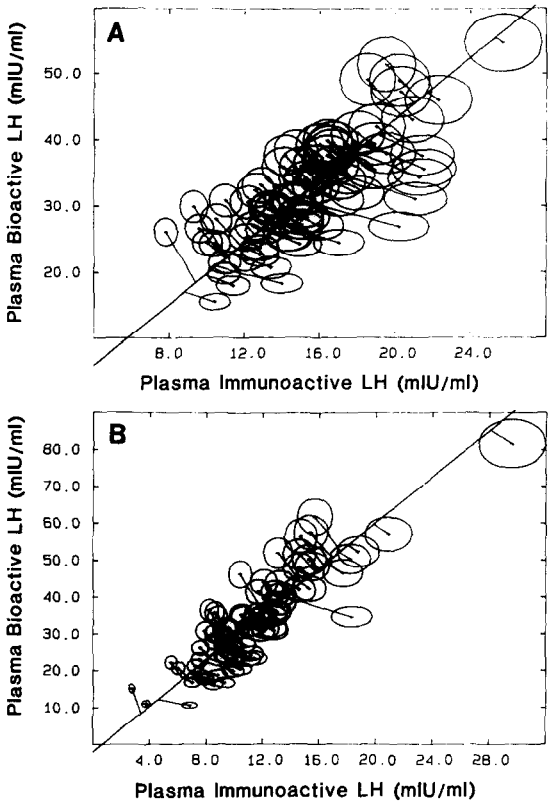

Fig. 5. Illustrative relationships between plasma bioactive and serum immunoactive LH concentrations in two of the seven normal men who underwent blood sampling at 10 min intervals for 12 h. The line represents the best fit through the 2-dimensional elliptical variance spaces representing measurement errors inherent in both the bio- and immunoassays. The y-intercepts for the regression lines for all 7 normal men studied in this fashion were zero or slightly negative. Note break in axes near origin (Panel A) to accommodate the full range of LH values observed. (Adapted with permission from Ref. 5.)

secreted at a more rapid rate and in larger amounts than immunoactive material. Moreover, bioactive LH is cleared more (rather than less) rapidly than its immunoactive counterpart in the same individual.

Summary

We conclude that bioactive LH is released in episodic pulsations that exhibit both frequency and amplitude modulation under different pathophysiological conditions. In response to endogenous or exogenous pulses of GnRH, bioactive LH secretion occurs preferentially and in a burst-like mode. Deconvolution analysis of LH concentrations measured in the RICT bioassay which is devoid of a nonspecific "blank" permits one to estimate the amplitudes, frequency, and duration of bioactive LH secretory bursts, and simultaneously calculate the half-time of endogenous bioactive LH disappearance *in vivo*.

Altered serum bio:immuno LH ratios could alternatively result if the RICT bioassay measured a certain "non-specific" or "blank" amount of bioactive LH. However, as shown in Fig. 5, the linear regressions of bioactive LH on immunoactive LH measured in serum samples collected at 10-min intervals for 12 h in normal men have y intercept values of 0 or less. Here, we used linear regression in which experimental uncertainty exists in both the dependent and independent variables (immunoactive and bioactive LH measurements) to estimate the *two-dimensional error ellipses* and the mean and statistical confidence limits for the regression coefficients and intercept [5]. Accordingly, no "blank" bioactivity is demonstrable. Rather, positive x-axis intercepts indicated the occurrence of small quantities of nonspecific immunoactivity when bioactivity fell to 0 [5]. Moreover, we demonstrated that hypopituitary serum, although it contains small quantities of immunoactive LH, is devoid of measurable LH bioactivity in our RICT [5]. Thus, we can exclude the problems observed in certain other assays of a "nonspecific" bioactivity.

2. ROLE OF ENDOGENOUS ANDROGENS AND ESTROGENS IN REGULATING BIOACTIVE LH SECRETION

(a) Role of estrogens

To probe the role of estrogens in regulating the secretion of bioactive LH, we have infused estradiol under steady-state conditions in healthy young men. The equilibrium infusion of estradiol at its daily endogenous production rate results in significant suppression of serum bioactive LH concentrations [21]: Fig. 6. Suppression of bioactive LH is more pronounced than that for immunoactive hormone, resulting in a decrease in the mean serum bio:immuno LH ratio. Conversely, when the nonsteroidal, selective antiestrogen, tamoxifen hydrochloride, is administered orally for 7 days, bioactive LH concentrations increase, and this increase is disproportionately large compared to the accompanying rise in immunoactive LH concentrations. The mechanisms subserving this stimulatory effect of antiestrogen in men include an acceleration of endogenous bioactive LH pulse frequency, which is associated with an increase in the maximal amplitude of LH concentration peaks but no major change in pituitary bioactive LH release in response to exogenous pulses of GnRH [21]. Consequently, we infer that GnRH pulse frequency can modulate bioactive LH secretion and that inhibition of endogenous estrogen's negative feedback action results in augmented bioactive LH release in the human.

The clinical importance of estrogen's negative feedback effect on bioactive LH secretion was observed in a man with hyperestrogenemia associated with a benign, surgically resectable adrenal adenoma [22]. Prior to surgical removal of the tumor, the patient's serum concentrations of bioactive LH and total and free testosterone were profoundly suppressed. After surgical cure, serum bioactive (and to a lesser degree,

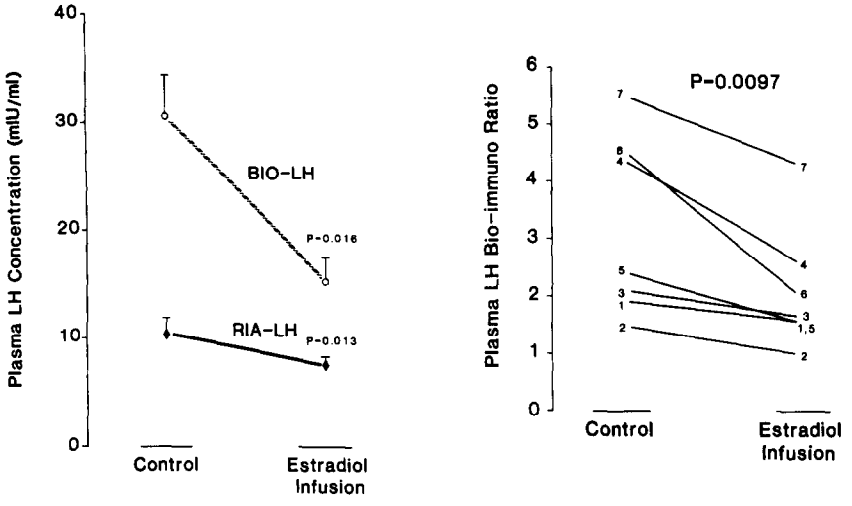

Fig. 6. Suppressive effects of steady-state estradiol infusion on mean serum LH concentrations in normal men. Seven normal men received 48 mcg of estradiol-17β per day by continuous intravenous infusion for $3\frac{1}{2}$ days. Mean serum concentrations of LH were determined by RICT bioassay (bio-LH) and immunoassay (RIA-LH) under baseline (control) conditions and during the last 8 h of the estradiol infusion. Data represent mean $\pm$ SEM (mIU/ml) serum LH concentrations obtained from blood withdrawn at 4 min intervals for 8 h. Corresponding changes in the serum LH bio:immuno ratio in response to steady-state estradiol infusion as shown in the right subpanel. The numerals in the control and estradiol-infusion columns denote individual men. (Adapted with permission from Ref. [21].)

immunoactive) LH concentrations increased significantly into the normal range in association with a recovery of sexual function and restoration of plasma androgen concentrations. Thus, we can infer that in normal men endogenous estrogen operating at physiological concentrations restrains the secretion of bioactive LH and that pathological excess of estrogen significantly suppresses bioactive LH release with resultant clinical hypogonadism.

Estrogen administration to postmenopausal women via an intravaginally-placed silastic ring impregnated with pure crystalline steroid provides another model in which to examine feedback actions of estrogen on the hypothalamo-pituitary axis [23]. We have observed that estrogen exerts a bipotential effect on LH release, with an acute suppression of LH pulse frequency and to a lesser degree LH pulse amplitude within 24 h, followed by a secondary increase in LH pulse frequency and amplitude on days 5–10 of estrogen exposure. Thereafter, sustained suppression of LH concentrations occurs [23]. The secondary increases in serum LH concentrations observed on days 5–10 of estrogen exposure in postmenopausal women reflect not only an increase in LH pulse frequency, but also an enhanced responsiveness to GnRH's stimulation of LH release [24]. In particular, when two consecutive pulses of exogenous low-dose GnRH (10 mcg) are administered at 2-hourly intervals after five or ten days of estrogen exposure, the second pulse of GnRH elicits a significantly larger increase in LH concentrations than the first. This property of enhanced responsiveness to the serial doses of GnRH is referred to as GnRH self-priming. Accordingly, we infer that estrogen exerts *time-dependent* effects on the kinetics of GnRH's self-priming action on the human pituitary gland. These results with RIA have been confirmed and extended recently by RICT bioassay. This self-priming action of GnRH on bioactive LH release is maximal after 5 and 10 days of estrogen exposure, and can be demonstrated whether it is defined as an increase in the percentage amplitude of the second peak compared to the first, or as an incremental increase of the second peak maximum over the first (Veldhuis and Dufau, unpublished). Thus, we can infer that estrogen in the human regulates the pituitary gland's responsiveness to pulses of GnRH, such that enhanced secretion of biologically active gonadotropin occurs during serial exposure to GnRH stimuli.

Our finding of GnRH self-priming induced by estradiol is of particular importance to an understanding of possible mechanisms subserving the generation of the pre-ovulatory LH surge. The progressive increase in bioactive LH concentrations observed in the late follicular phase [4] that culminates in the preovulatory LH surge [12] could result from GnRH self-priming by a relevant strength-duration effect of endogenous estradiol on anterior pituitary gonadotrope function, i.e. on the synthesis, processing, and secretion of LH molecules enriched in biological activity. Although this formulation does not necessitate concurrent increases in GnRH or LH pulse *frequency* during the preovulatory surge, possible further acceleration of LH pulse frequency during the LH surge proper (as distinguished from during the late follicular phase, where bioactive

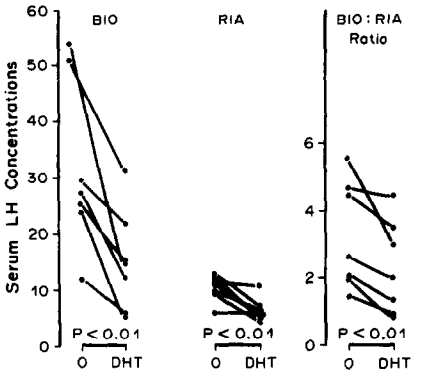

Fig. 7. Suppressive effect of equilibrium androgen (5-alpha-dihydrotestoserone) infusion on 8-h mean serum bioactive LH ("BIO") and immunoactive ("RIA") LH concentrations and the resulting bio:immuno LH ratio in normal men. Data are means ± SEM ($N = 7$ subjects). [Veldhuis J. D. and Dufau M. L., unpublished observations.]

LH pulse frequency does increase [4]) will require investigation.

(b) Role of androgens

To assess the ability of androgens to regulate bioactive LH secretion, we have infused the potent non-aromatizable androgen, 5-alpha-dihydrotestosterone, at twice the production rate of testosterone in normal men [25]. This pharmacological maneuver significantly suppresses immunoactive LH pulse frequency, which can be restored to nearly normal when an opiate-receptor antagonist is co-administered with the androgen [25]. During dihydrotestosterone infusion, bioactive LH concentrations also decline significantly, as shown in Fig. 7. In fact, there is preferential suppression of serum bioactive over immunoactive LH concentrations in this circumstance. Consequently, the mean bio:immuno LH ratio falls. The converse experiment, in which a selective nonsteroidal antiandrogen, flutamide hydrochloride, is administered to young men to block *endogenous* androgen negative-feedback action has revealed an increase in plasma bioactive LH concentrations with an accompanying rise the plasma bio:immuno LH ratio (Veldhuis and Dufau, unpublished observations in four men). Although further detailed analyses are required to explore the mechanisms and nature of androgen's regulation of bioactive LH secretion and/or clearance in the human, the preceding studies demonstrate that androgens can regulate circulating concentrations of bioactive gonadotropin in men. However, the exact extent to which such regulation occurs via frequency modulation of the GnRH pulse signal and/or by direct actions of androgen on gonadotrope cellular function has not been elucidated. In addition, further studies will need to focus on the explicit modulation by steroid hormones of distinct molecular isoforms of LH.

In women, the role of endogenous androgens in regulating bioactive LH secretion has not been defined. However, we have recently observed several patients in whom androgen-secreting tumors of the adrenal gland or ovary were surgically curable. Under these conditions of excess endogenous androgen secretion, serum bioactive LH concentrations were profoundly suppressed in a postmenopausal individual, but relatively minimally suppressed in a premenopausal amenorrheic individual. After surgical cure of the androgen-secreting tumor, bioactive LH concentrations returned to the castrate range in the postmenopausal patient, whereas they changed only slightly in the premenopausal woman despite a similarly profound fall in androgen levels. The mean serum bio:immuno LH ratio increased after androgen withdrawal in the postmenopausal patient, but did not change significantly after restoration of normal androgen concentrations in the premenopausal woman. Accordingly, further study will be required to dissect the nature and degree of regulation by endogenous androgen of bioactive LH secretion in women.

3. ALTERATIONS IN BIOACTIVE LH RELEASE IN PUBERTY AND HEALTHY AGING

(a) Puberty

Prior to puberty, bioactive LH concentrations in girls are virtually undetectable [8]. During the early stages of puberty, as breast development is initiated (Tanner Stages 3 and 4), estrogen concentrations increase and serum bioactive LH concentrations also rise. There is a corresponding increase in the serum bio:immuno LH ratio [8]. However, even prior to the onset of increased endogenous estrogen secretion, some degree of pulsatility of bioactive LH release can be observed in healthy young girls, but frequent dissociation occurs between bioactive LH pulsatility and that of alpha subunit and/or immunoactive LH. Thus, the prepubertal context may be one in which bioactive LH measurements are particularly important if the status of activation of the gonadal axis is difficult to determine clinically. Nonetheless, much further effort is required to delineate the properties of bioactive LH secretion and clearance throughout spontaneous normal pubertal stages and in various clinical conditions of arrested puberty, precocious puberty, or failure of pubertal activation.

(b) Aging

Although striking increases in serum bioactive LH concentrations occur in the postmenopausal setting, such castrate levels of gonadotropins are not observed in healthy aging men [2, 3, 9]. Population studies of bioactive LH concentrations over a wide age-span indicate that a gradual decline in the bio:immuno-LH ratio can be observed [26]. This decline may reflect development of intercurrent illness and/or debility, as well as aging *per se*. Nonetheless,

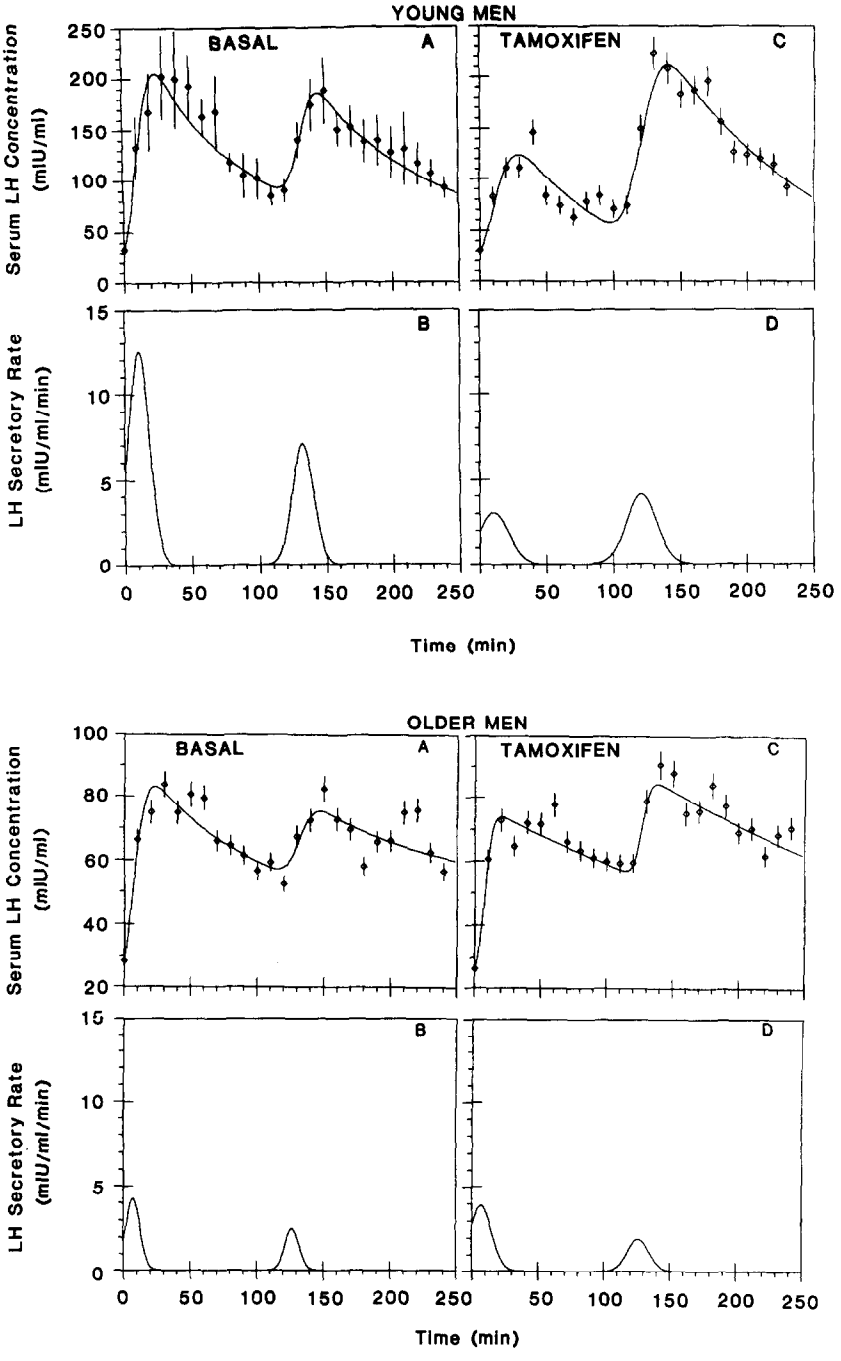

Fig. 8. Bioactive LH concentration peaks and calculated underlying secretory episodes in reponse to two consecutive intravenous pulses of exogenous GnRH (10 mcg) in six young men and six older men. Plasma bioactive LH concentrations (upper panels, A and D) are given as means ± SEM ($N = 6$ men), for which the accompanying mean calculated reconvolution curves are shown. Secretory episodes are plotted as mean regressions of secretory rates versus time (lower panels, B and C) which were estimated by multiple-parameter deconvolution [17]. Data in young and older men are presented in both the basal (control) and tamoxifen-treated state. Time zero denotes the time when the first of two consecutive intravenous injections of 10 mcg exogenous GnRH was administered. (Adapted with permission from Reference [9].)

healthy older men (ages greater than 65 and less than 80) who are in robust, active, and exemplary health have serum bioactive LH concentrations similar to those of young men (ages 25–35) and exhibit similar serum bio:immuno LH ratios [9]. Moreover, recent studies of *bioactive* LH *pulsatility* indicate that 12-h profiles of healthy aging men are quantitatively indistinguishable from those of normal young men [9].

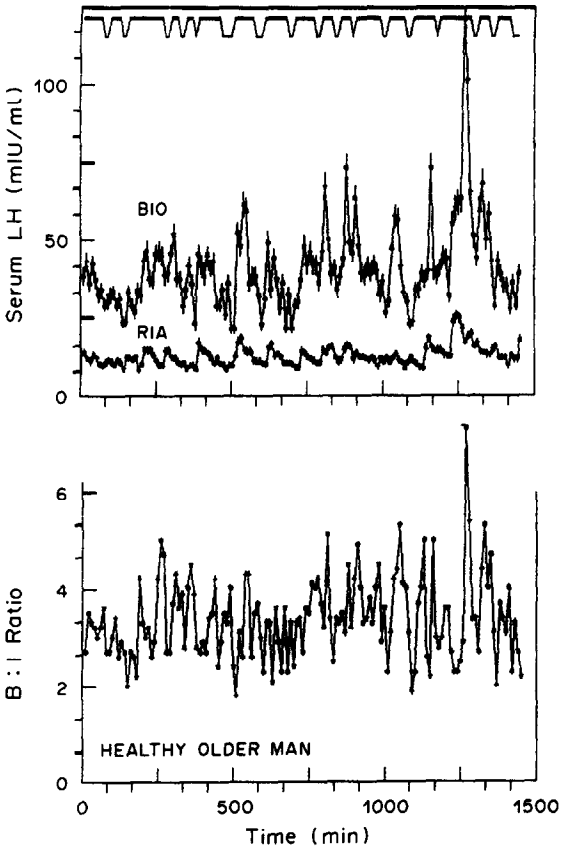

Fig. 9. Entire 24-h profile of pulsatile bioactive LH in a healthy older man (age 72). Blood was sampled at 10 min intervals for 24 h and the resultant sera subjected to RICT (bioactive LH) and RIA. The corresponding LH time series and the serial bio:immuno LH ratios are shown (Urban R. J., Veldhuis J. D., Blizzard R. M. and Dufau M. L., unpublished data).

On the other hand, provocative tests of the hypothalamo–pituitary–gonadal axis have revealed a clear diminution in bioactive LH secretory reserve with healthy aging. Thus, low-dose pulses of GnRH in healthy aging men elicit considerably less bioactive LH secretion, as assessed either by the plasma concentration profile or by deconvolution analysis: Fig. 8. Although the half-life of exogenously injected LH in older men is not known, deconvolution analysis has suggested that the half-life is not remarkably different from that in younger individuals [9]. Stimulation of the hypothalamo–pituitary–gonadal axis with the anti-estrogen, tamoxifen, also unmasked a deficit in bioactive LH secretory reserve in healthy aging men. The amplitude but not the frequency of bioactive LH pulses in tamoxifen-treated older men was significantly reduced compared to that in younger men. Thus, we can infer that healthy aging in men is associated with a decreased secretory reserve capacity of the gonadotrope for bioactive LH.

Further studies are required in aging individuals in order to elucidate the range of (patho-)physiological features present in healthy and/or debilitated populations. For example, by sampling blood at 10 min intervals for 24 h, we have observed the entire nycthemeral profiles of bioactive LH release in healthy aging individuals. As shown in Fig. 9, during certain intervals throughout the day, serum bioactive LH concentrations fall almost to undetectable, as secretory burst activity is nearly completely arrested. Although 24 h data are of limited availability in younger men, the occurrence of such intervals of markedly decreased and/or polyphasic secretory burst activity in healthy older men raises the possibility of a defect in the neuroregulation of episodic LH release in this setting.

4. SUMMARY

Significant advances have been made in the last decade in our knowledge of the secretion and metabolic clearance of biologically active LH *in vivo* in the human. Evidence is presented that pulsatile or burst-like secretory episodes characterize bioactive LH release, and that the endogenous GnRH pulse signal exerts both amplitude and frequency-dependent control over bioactive LH secretion. Enrichment in bioactivity is further modulated by sex steroid hormones and varies in puberty and healthy aging. Further studies will need to focus on the specific regulation of discrete molecular isoforms of LH in health and disease.

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