Endogenous opiates modulate the pulsatile secretion of biologically active luteinizing hormone in man.

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Research Article

We studied the secretion of physiological pools of immunoreactive and biologically active luteinizing hormone in response to endogenous pulses of gonadotropin-releasing hormone (GNRH) in eugonadal men. Concentrations of immunoactive and bioactive luteinizing hormone (LH) were determined in blood drawn at 20-min intervals for 8 h in eight normal men under two conditions: (a) after placebo, in order to evaluate spontaneous LH pulsations in the basal state, and (b) after administration of the opiate-receptor antagonist, naltrexone, which is believed to amplify the pulsatile release of endogenous GNRH. Spontaneous and naltrexone-stimulated secretion of LH occurred in pulses of high biological activity, as measured in the RICT (rat interstitial cell testosterone bioassay), i.e., bioactive:immunoactive LH ratios within both spontaneous and naltrexone-stimulated LH pulses were higher than corresponding interpulse ratios (P less than 0.001). Quantitative characterization of the pulsatile release of bioactive LH revealed the following specific effects of opiate-receptor blockade: increased 8-h mean and integrated serum concentrations of bioactive LH (P less than 0.002), enhanced pulse frequency of bioactive LH release (P less than 0.001), and augmented peak amplitude of bio-LH pulses (P less than 0.01). Moreover, this increase in episodic secretion of bioactive LH was associated with increased 8-h mean and integrated serum testosterone concentrations in these men (P less than 0.05). We conclude the following: (a) LH is normally released in […]

Endogenous Opiates Modulate the Pulsatile Secretion of Biologically Active Luteinizing Hormone in Man

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ABSTRACT We studied the secretion of physiological pools of immunoreactive and biologically active luteinizing hormone in response to endogenous pulses of gonadotropin-releasing hormone (GNRH) in eugonadal men. Concentrations of immunoactive and bioactive luteinizing hormone (LH) were determined in blood drawn at 20-min intervals for 8 h in eight normal men under two conditions: (*a*) after placebo, in order to evaluate spontaneous LH pulsations in the basal state, and (*b*) after administration of the opiate-receptor antagonist, naltrexone, which is believed to amplify the pulsatile release of endogenous GNRH. Spontaneous and naltrexone-stimulated secretion of LH occurred in pulses of high biological activity, as measured in the RICT (rat interstitial cell testosterone bioassay), i.e., bioactive:immunoactive LH ratios within both spontaneous and naltrexone-stimulated LH pulses were higher than corresponding interpulse ratios ($P < 0.001$). Quantitative characterization of the pulsatile release of bioactive LH revealed the following specific effects of opiate-receptor blockade: increased 8-h mean and integrated serum concentrations of bioactive LH ($P < 0.002$), enhanced pulse frequency of bioactive LH release ($P < 0.001$), and augmented peak amplitude of bio-LH pulses ($P < 0.01$). Moreover, this increase in episodic secretion of bioactive LH was associated with increased 8-h mean and integrated serum testosterone concentrations in these men ($P < 0.05$).

We conclude the following: (*a*) LH is normally released in spontaneous pulses of high biological activity in men; (*b*) when the endogenous GNRH signal is amplified by opiate-receptor blockade, the pituitary gland releases more frequent bioactive LH pulses, which are of high amplitude and contain a high bioactive:immunoactive LH ratio. This increase in pulsatile release of bioactive LH quantitated in the RICT assay in vitro is reflected by acutely increased serum testosterone concentrations in vivo. We infer that modulation of the episodic GNRH signal by endogenous opiates provides another significant mechanism by which the hypothalamus can alter the biological activity of circulating gonadotropic hormone in man. Moreover, observed alterations in the pulsatile pattern of bioactive LH release were associated in turn with significant changes in testosterone concentrations. Thus, we hypothesize that alterations in the properties of the bioactive LH pulse signal can provide an important mechanism for regulating target-cell function within the gonad in states of health or disease.

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INTRODUCTION

Luteinizing hormone (LH)[1] is normally secreted in an episodic fashion. In most studies to date, this pulsatile pattern of LH release has been characterized solely in terms of LH immunoactivity (1–9). More recently, we have demonstrated prominent high amplitude, low frequency pulses of biologically active LH in the human circulation (10). On the basis of studies in experimental animals, intermittent fluctuations in circulating (immunoactive) LH concentrations are believed to reflect pituitary stimulation by corresponding pulses of endogenous gonadotropin-releasing hormone (GNRH) (11, 12). Exogenously administered GNRH can also promote pituitary release of both immunoactive and bioactive LH (13–15). However, preferential release of pool(s) of LH enriched in biological activity has been difficult to demonstrate in man, whether single-bolus or continuous low dose infusions of GNRH have been used (14, 15). Thus, alternative experimental approaches are required to characterize the physiological release of functional pools of immunoactive and bioactive LH in man. Such approaches could include either mimicking the physiological mode of endogenous GNRH secretion by infusing exogenous GNRH in discrete pulses, or enhancing the endogenous generation of GNRH pulses.

In the present work we have used an opiate-receptor antagonist to amplify the endogenous GNRH signal for LH release. Prior investigations in experimental animals and in man indicate that opiate-receptor antagonists stimulate pulsatile secretion of immunoactive LH by disinhibiting brain mechanisms that otherwise suppress GNRH secretion (16–21). By blocking the endogenous opiate system, we have been able to characterize the release of physiological pools of immunoactive and bioactive LH in response to endogenously generated GNRH pulses in normal man, and test the impact of an altered LH signal on testosterone production.

METHODS

Studies were conducted in eight healthy normal male volunteers (age range from 24 to 35 yr), who had normal serum concentrations of free thyroxine, prolactin, immunoactive LH and follicle-stimulating hormone, free testosterone, and 17β-estradiol. Each subject provided written informed consent before participation.

Blood samples were collected at 20-min intervals for 8 h beginning 60 min after oral ingestion of placebo elixir or naltrexone (1 mg/kg) at 0800 on separate days. Plasma samples were assayed for immunoactive LH by a double-antibody radioimmunoassay (22), with a sensitivity of 1 mIU/ml in terms of the 2nd International Reference Preparation of human Menopausal Gonadotropin (hMG); and for bioactive LH, by rat interstitial cell testosterone assay (RICT) (23, 24) with a sensitivity of 0.4 mIU/ml, or 3 pg of pure LH (LER 1533). The intraassay coefficient of variation for the bioassay was 8.3% (computed from 13 replicates) and for the immunoassay, 8.8%. The potency of the purified preparation in terms of the hMG standard was very similar when measured by bioassay or by RIA, namely, 13,500 (11,200–15,200) and 13,700 (11,100–14,900) IU/mg, respectively. Thus, the bioimmunoactive ratio of plasma samples calculated for LH measured in terms of hMG or pure LH is very similar. The serum testosterone concentration was measured in each sample by radioimmunoassay (25), after diethyl ether extraction and celite chromatography. In one subject, there was insufficient blood for serum immunoactive LH and testosterone assays, so that only bioactive LH was measured.

The plasma LH secretion profiles were analyzed for significant fluctuations by the computerized pulse-detection algorithm of Clifton and Steiner (26) and Steiner et al. (27). This method performs iterative data scans to identify significant fluctuations (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 (26, 27). The frequency (number of pulses per sampling interval) and incremental amplitude (nadir-to-peak increases in LH concentration, expressed in milli International Units per milliliter) of pulses can be estimated in the presence of random measurement errors (noise). 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. For each analysis, an estimate of the pulse signal-to-noise ratio is also given, which in the present studies exceeded 2.5. Bioactive and immunoactive LH pulses were considered concordant whenever the point (or points) inscribed above base line within the pulses overlapped in time. The area under the LH concentration-vs.-time curve, and the fractional amplitude of significant pulses (given as percentage above preceding nadir) were also computed with the program of Santen and Bardin (28). Pulse data are given as means±SD, and were analyzed by within-subject comparisons using a paired, two-tailed t test (29). To test the hypothesis that increased bioimmunoactive LH ratios occurred preferentially within bioactive LH peaks, nonparametric analysis was applied. In any given subject, the number of bioimmunoactive LH ratios above the median was determined both in bioactive LH pulses and in the corresponding interpulse base line. A χ^2 table was constructed to analyze the expected vs. observed distribution of increased bioimmuno ratios (29).

RESULTS

Bioactive LH. In the basal state (after placebo administration), bioactive LH was secreted in prominent pulses having a mean amplitude of 14.6±6.34 mIU/ml above preceding nadir, with mean absolute peak levels of 38.6±13.2 mIU/ml (see Table I). The fractional increase of bioactive LH in these pulses was 73±35%

[1] *Abbreviations used in this paper:* GNRH, gonadotropin-releasing hormone; hMG, human menopausal gonadotropin; LH, luteinizing hormone; RICT, rat interstitial-cell testosterone bioassay.

TABLE I
Pulsatile Secretion of Bioactive LH in Man

Subject	Treatment	Mean LH*	Area‡	Pulses/8 h	Incremental§	Peak‖	Fractional (%)¶	Mean periodicity**
A	Placebo	13.71±4.96	6,623	2	14.0	25.9	80	220
	Naltrexone	14.16±3.07	6,850	5	7.3	18.9	65	100
B	Placebo	24.86±7.02	11,992	3	12.1	30.3	87	180
	Naltrexone	29.21±7.59	14,101	4	24.2	42.2	72	125
C	Placebo	10.50±3.43	5,032	2.5	9.8	17.2	149	200
	Naltrexone	19.64±7.08	9,644	6	13.4	27.4	92	63
D	Placebo	38.77±6.27	18,682	3	15.0	43.8	55	180
	Naltrexone	47.38±7.84	22,968	4.5	17.2	57.2	65	115
E	Placebo	33.90±6.57	17,041	2	19.6	46.3	39	220
	Naltrexone	46.64±11.86	22,588	4	16.9	59.8	69	125
F	Placebo	30.86±3.47	14,820	1	5.9	39.6	38	—
	Naltrexone	36.40±7.41	17,564	3	23.6	47.0	63	180
G	Placebo	40.62±17.4	19,657	3	28.2	60.1	79	180
	Naltrexone	42.92±15.6	20,479	5.5	29.3	61.4	110	100
H	Placebo	16.21±5.43	7,877	2	12.1	29.3	57	220
	Naltrexone	28.23±8.06	13,550	3	24.0	41.4	70	180
Means±SD	Placebo	26.18±10.90	12,175 ±5,327	2.31 ±0.66	14.6 ±6.34	38.6 ±13.2	73±34	200±19
	Naltrexone	33.07±11.5	15,954 ±5,583	4.375 ±1.02	19.5 ±6.64	44.4 ±14.4	76±16	124±37
P value (Placebo vs. Naltrexone)		<0.001	<0.002	<0.001	NS	<0.01	NS	<0.002

* mIU/ml, mean±SD (n = 25 samples).
‡ Area in mIU/ml × min (over 8 h of sampling).
§ mIU/ml increment from nadir to peak.
‖ Maximal absolute LH value achieved in the pulse (mIU/ml).
¶ Percentage increase above nadir.
** Minutes.

above preceding base line. There were 2.31±0.66 bioactive LH pulses per 8 h. The mean (and integrated) serum bioactive LH levels are very similar to those previously reported in other healthy men (10, 14, and 15).

After naltrexone administration, there was a highly significant increase in mean serum bioactive LH levels from 26.2±10.9 to 33.1±11.5 mIU/ml ($P < 0.001$), with a corresponding increase in integrated concentrations of bioactive LH ($P < 0.002$, see Table I). Treatment with this opiate-receptor antagonist also significantly enhanced the frequency of bioactive LH pulses from 2.31±0.66 to 4.38±1.0 pulses/8 h ($P < 0.001$), and augmented the absolute peak LH values attained within individual pulses from 38.6±13.2 to 44.4±14.4 mIU/ml ($P < 0.01$ treatment effect). Neither the fractional (percentage above base line) nor the incremental (milli International Units per milliliter above preceding nadir) amplitude of bioactive LH pulses was altered (Table I). In accord with the increase in LH pulse frequency, there was a corresponding significant decrease in the mean periodicity of bioactive LH pulses from 200±19 min (basal) to 124±37 min, after naltrexone ($P < 0.002$). None of these effects could be attributed to interfering properties of naltrexone in the bioassay, since naltrexone was inactive in the RICT at concentrations (10 ng/ml and 50 ng/ml) equal and exceeding those attained in plasma (10 ng/ml [30, 31]). (Table II). In addition, naltrexone did not alter the signal-to-noise ratio for the pulse detection methodology (the mean signal-to-noise ratio was 3.28±0.28 after placebo, and 3.54±0.26 after naltrexone). These signal-

TABLE II

Basal and Gonadotropin-stimulated Testosterone Production by Leydig Cells In Vitro: Lack of Effect of Naltrexone or Vehicle on the RICT Assay

Experimental	Incubation±hMG (0.625 mIU)	Mean	±SE
1. Control assay	−	8.9	0.34
	+	50.2	0.49
2. In presence of vehicle:	−	9.3	0.85
	−	9.7	0.33
	+	49.1	1.0
	+	48.9	0.79
3. In presence of naltrexone:	−	9.1	0.8
	−	8.9	0.50
	+	49.7	0.8
	+	50.6	0.69

Naltrexone concentrations used in the in vitro incubations are equivalent to circulating levels of 10 and 50 μg/liter (31). An equivalent volume of vehicle was used in separate incubations.

to-noise ratios are well within the range of accurate pulse detection for this computer algorithm (26).

Immunoactive LH. Immunoactive LH concentrations were also measured for each of the 20-min samples drawn over 8 h in seven men. The changes in serum immunoactive LH levels basally and after naltrexone were similar qualitatively to those described above for bioactive LH. Mean serum immunoactive LH concentrations rose from 8.6±2.61 mIU/ml basally to 12.2±5.6 mIU/ml after naltrexone administration ($P < 0.02$), and integrated LH concentrations increased from 4,120±1,330 basally to 5,828±2,680 mIU/ml x min after drug ($P < 0.02$). Naltrexone also significantly increased the frequency of LH pulses from 3.22±0.6 basally to 4.57±0.7 pulses/8 h ($P < 0.008$), and increased mean absolute peak immunoactive LH values from 12.8±3.5 to 16.0±5.6 mIU/ml after naltrexone administration ($P < 0.02$). There was a corresponding significant decline in pulse periodicity from 166±37 to 112±16 min/pulse ($P < 0.009$), with no significant alteration in incremental or fractional (percentage) amplitude of immunoactive LH pulses.

Representative profiles of serum bioactive and immunoactive LH are given for three men in Fig. 1. When all data were separately analyzed by the method of Santen and Bardin (5), LH pulses detected agreed well with those of the method of Clifton and Steiner (26) (82.5% concordance). The small discrepancy reflects the different cut-off criteria used (5, 26).

Comparison of bioactive and immunoactive LH. Bioactive LH pulses were concordant with immunoactive LH pulses in 83% of cases overall, i.e., 38 of 46 bioactive LH pulses were associated with coincident immunoactive LH pulses. There was a somewhat greater discordance for immunoactive pulses than for bioactive pulses, i.e., 17 of 57 or 29% of immunoactive LH peaks were not associated with a corresponding increase in bioactivity, while 8 of 46 or 17% of bioactive LH peaks did not have a coincident increase in immunoactivity. These values are similar to those we described previously (10). When analyzed further, neither concordance nor discordance of immunoactive and bioactive LH pulses was affected by naltrexone compared with placebo administration.

Bioactive:immunoactive LH ratios fluctuated over time after placebo ingestion (spontaneous) and after naltrexone administration. After placebo ingestion, spontaneous increases in bioactive:immunoactive LH ratios occurred significantly more commonly within bioactive LH peaks, than in the interpulse base line ($P < 0.001$, Table III, *A*). Similarly, after naltrexone administration, increases in bioactive:immunoactive LH ratios also occurred significantly more often within bioactive LH pulses, than in the corresponding interpulse base line ($P < 0.001$, Table III, *B*). In particular, the mean (±SD) values of the bioactive:immunoactive LH ratios within bioactive LH pulses were 5.27 (±3.60) for placebo and 4.44 (±1.88) for naltrexone, which compare with corresponding interpulse base-line ratios of 3.26 (±1.65) and 3.48 (±1.5) for placebo and naltrexone, respectively.

Compared with placebo, administration of naltrexone did not significantly influence overall bioactive:immunoactive LH ratios (3.84±1.71 for placebo, 3.72±1.66 for naltrexone). Moreover, compared with placebo, naltrexone did not curtail the preferential distribution of increased bioactive:immunoactive LH ratios within bioactive LH pulses (Table III, *C*). Thus, after naltrexone administration the same significant tendency for increased bioactive:immunoactive LH ratios to occur preferentially within bioactive LH pulses was observed.

Serum testosterone concentrations. The serum testosterone concentration was measured in each sample collected at 20-min intervals for 8 h. Mean serum testosterone increased significantly after naltrexone administration, i.e., from 570±151 ng/dl basally, to 645±120 ng/dl after drug ingestion ($P < 0.05$ within-subject treatment effect). The integrated areas under the testosterone concentration-vs.-time curves also increased significantly ($P < 0.05$). Data for individual men are given in Fig. 2.

When pulse analysis was applied to the serial testosterone values, we were unable to define any facile relationship between fluctuating testosterone levels (average of 3.5±1.0 peaks/8 h) and preceding bioactive or immunoactive LH peaks. Examination of the profiles

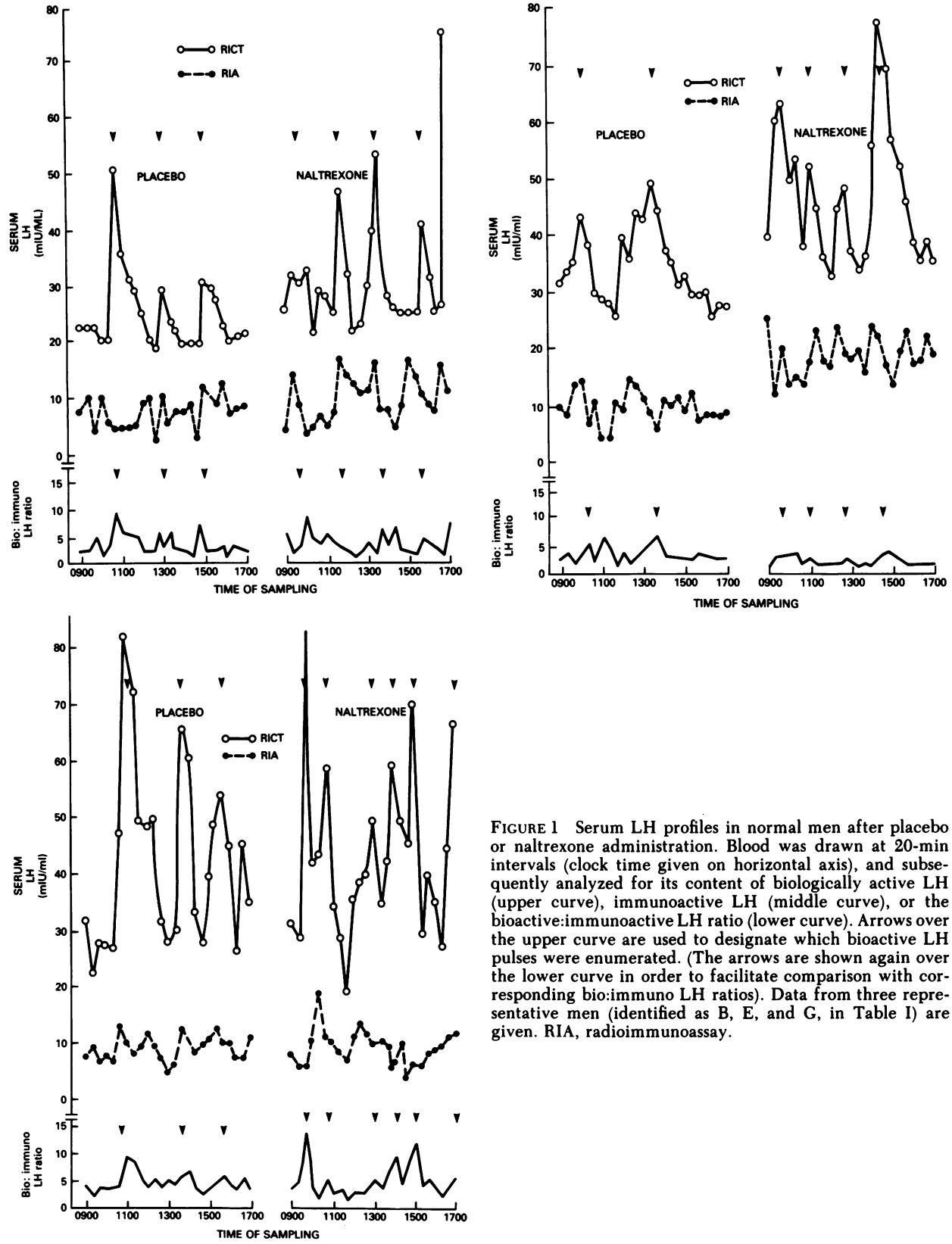

FIGURE 1 Serum LH profiles in normal men after placebo or naltrexone administration. Blood was drawn at 20-min intervals (clock time given on horizontal axis), and subsequently analyzed for its content of biologically active LH (upper curve), immunoactive LH (middle curve), or the bioactive:immunoactive LH ratio (lower curve). Arrows over the upper curve are used to designate which bioactive LH pulses were enumerated. (The arrows are shown again over the lower curve in order to facilitate comparison with corresponding bio:immuno LH ratios). Data from three representative men (identified as B, E, and G, in Table I) are given. RIA, radioimmunoassay.

TABLE III

LH Bioactive:Immunoactive Ratios in Eugonadal Men

	A. Spontaneous (placebo)		B. After naltrexone administration		C. Within bioactive LH pulses	
	Within-pulse bioactive:immunoactive ratios	Interpulse bioactive:immunoactive ratios	Within-pulse bioactive:immunoactive ratios	Interpulse bioactive:immunoactive ratios	Placebo	Naltrexone
Number of bioactive:immunoactive ratios > median	38 (24.29)	51 (64.08)	44 (27.54)	35 (51.46)	34 (35.89)	44 (42.11)
Number of bioactive:immunoactive ratios ≤ median	18 (31.08)	93 (79.92)	17 (33.46)	79 (62.53)	18 (16.11)	17 (18.89)
	$\chi^2 = 17.181$ $P < 0.001$		$\chi^2 = 27.53$ $P < 0.001$		$\chi^2 = 0.595$ $P < 0.384$	

Expected values are given in parentheses.

of serial testosterone levels and LH peaks in these men indicated that bioactive LH peaks occasionally (but not invariably) preceded increases in testosterone (see Discussion).

Serum prolactin concentrations. In five men, mean or integrated serum prolactin concentrations measured at 20-min intervals for 8 h were not influenced by naltrexone (data not shown), indicating the absence of any discernible opiate-agonist effect of this drug under these conditions.

DISCUSSION

In the present work, we have explored the mode of release of physiological pools of immunoactive and bioactive LH in response to endogenous pulses of GNRH in eugonadal men. First, we have formally presented the novel observation that LH secretion in the adult male occurs in spontaneous pulses of high biological activity, reflected in episodically increased bioactive:immunoactive LH ratios within LH pulses compared with interpulse base-line ratios (10). Secondly, the present studies permit us to characterize for the first time changes in the release of bioactive and immunoactive LH, when the endogenous GNRH signal is amplified by opiate-receptor blockade.

Blockade of opiate receptors with the potent, selective and long-acting (half-time ≃ 10.3 h) antagonist, naltrexone (30, 31), evoked a significant increase in mean

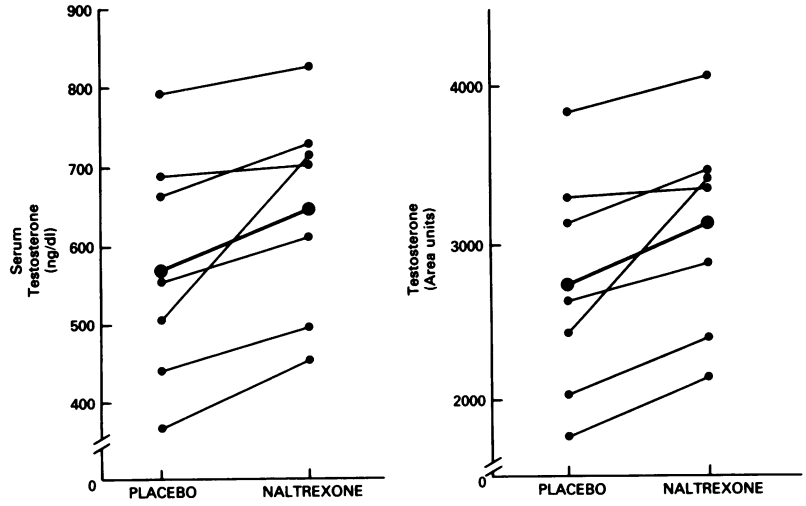

FIGURE 2 Mean (*left*) and integrated (*right*) serum concentrations of testosterone are given for seven individual men after placebo or naltrexone administration. Each mean derives from 25 samples drawn at 20-min intervals over 8 h of study. The integrated concentrations over the 8 h of sampling were determined by the method of Santen and Bardin (28).

and integrated serum concentrations of bioactive LH with continuation of the pulsatile pattern of LH secretion. Absence of any discernible opiate agonist action was confirmed by demonstrating no change in mean serum prolactin concentrations (32, and this study). The increase in bioactive LH concentrations was associated with a proportionate increase in immunoactive LH levels, so that mean bioactive:immunoactive LH ratios derived over the entire period of venous sampling did not change in response to naltrexone. Since naltrexone was devoid of effect in the RICT bioassay in vitro, and is not considered to alter hormone clearance in vivo, we believe that the major increase in serum concentrations of bioactive LH in response to naltrexone administration reflects an actual augmentation of secretion of biologically active LH molecules (discussed further below).

The higher mean and integrated serum concentrations of bioactive LH in the presence of an opiate-receptor antagonist were accompanied by a significantly increased number of bioactive LH pulses in all eight men. This increase was demonstrable by two different pulse-detection methods (5, 26), whose results agreed by 83%. Since the pituitary gland is devoid of any intrinsic periodicity of LH release (6), the observed enhancement in bioactive LH pulse frequency must reflect an amplification of the endogenous GNRH signal. A similar conclusion has been reached from analyses of immunoactive LH pulses (21). However, immunoactive and bioactive LH pulses are sometimes discordant, possibly reflecting variations in the degree of glycosylation or other potency properties of the LH molecule released or cleared in vivo (33–35). The present work significantly extends prior reports on immunoactive LH pulsations by demonstrating for the first time, in any species, that brain opiate systems that modulate endogenous GNRH mechanisms are actually coupled to the effective secretion of biologically active LH.

Analyses of the naltrexone-associated pulses of bioactive LH reveal that these pulses are rich in bioactivity, containing significantly increased bioactive:immunoactive LH ratios compared with interpulse base-line ratios. The degree of increase in bioactive:immunoactive ratios within LH pulses after naltrexone administration is quantitatively similar to that observed in spontaneous LH pulses that occur at a lower frequency. Thus, modulating the frequency of the endogenous GNRH signal (at least within this physiological range of pulse frequencies) provides one hypothalamic mechanism by which to control net pituitary release of LH molecules that retain high biological activity. Our inference that the frequency of endogenously generated GNRH pulses is an important modulator of circulating concentrations of bioactive LH is in accord with several previous studies that have documented changes in immunoactive LH pulse frequency in diverse conditions of health and disease (1–12, 27, 36–39). Moreover, our inference is congruent with the recent report that serum concentrations of bioactive LH increase strikingly in the spontaneous or induced preovulatory phase of the menstrual cycle in the rhesus monkey (40, 41) at a time when immunoactive LH pulse frequency increases (2, 5–7, 9). Similar physiological alterations in bioactive LH secretion in normally cycling women have been observed and correlated with increased pulse frequency in the late follicular phase (42).

The observed increase in pulsatile bioactive LH secretion after naltrexone administration was associated with a corresponding significant rise in absolute peak bioactive LH concentrations within pulses. Neither the fractional amplitude (percentage above interpulse nadir, reference 28) nor the incremental amplitude (milli International Units per milliliter above interpulse base line, reference 27) was increased after naltrexone. These observations are consistent with a rise in interpulse concentrations of bioactive LH in response to naltrexone. Such increases in interpulse hormone concentrations could reflect either release of larger quantities of bioactive LH within each pulse (producing the observed, higher peak LH concentration in blood), or the demonstrated occurrence of more frequent pulses, with interpulse intervals that are consequently shorter (and hence allow less metabolic clearance of bioactive LH), or both.

The increase in pulsatile secretion of bioactive LH was also associated with a significant increase in mean and integrated serum testosterone concentrations in these men. This observation provides important evidence that the apparent increase in bioactive LH, as quantitated in the RICT assay in vitro, correctly reflects an actual increase in circulating concentrations of biologically effective LH in man in vivo. These findings are also consistent with earlier studies describing diminished serum testosterone concentrations in heroin and methadone users (43), and decreased androgen levels in male rats receiving opiate agonists chronically (44, 45). Moreover, in the human, more recent studies indicate that acute heroin administration suppresses serum testosterone levels, while chronic naltrexone administration increases mean testosterone concentrations (46). In the present work, we have demonstrated that opiate-receptor blockade with naltrexone acutely increases serum testosterone levels in normal men, and that this increase in androgen concentrations occurs pari passu with augmented pulsatile release of bioactive LH.

Our data do not permit us to ascertain whether the increase in mean serum testosterone concentrations

represents a response to the increase in LH pulse frequency or pulse amplitude, or perhaps to the consequent increase in mean and interpulse concentrations of biologically active LH. However, in the ram, bull, rat, monkey, and peri-pubertal or, in occasional cases, in the adult human, episodic increases in immunoactive LH concentrations have been correlated with subsequent presumptive pulses of testosterone secretion (8, 47–58). In the present work, we found 3.5±1.0 statistically significant fluctuations in serum testosterone concentrations per 8 h. Nonetheless, we cannot make any definitive inference that these represent true "pulses" of testosterone release. In addition, we believe that the relatively high frequency of spontaneous and naltrexone-stimulated pulsations of bioactive (or immunoactive) LH in adult male subjects precludes defining a facile one-to-one correspondence between individual bioactive (or immunoactive) LH peaks and fluctuating testosterone concentrations. Further investigations using the in vitro RICT bioassay to quantitate effective circulating LH concentrations in subjects in whom the frequency and amplitude of bioactive LH pulses are manipulated selectively would be likely to clarify the exact nature of the pulsatile bioactive LH signal that is most effective in stimulating Leydig cell steroidogenesis.

In summary, we conclude that brain neuroendocrine mechanisms, such as the endogenous opiate system studied here, which are capable of controlling the pulsatile character of LH release can thereby significantly regulate the secretion of LH species enriched in bioactivity. Acute changes in circulating quantities of bioactive LH, quantitated by the sensitive and specific RICT assay in vitro, are also reflected in corresponding changes in testosterone concentrations in normal men in vivo. Thus, the in vitro bioassay of LH is likely to provide an important investigative tool to ultimately clarify the exact nature of the pulsatile bioactive LH signal that is most effective in enhancing trophic and steroidogenic functions of the gonad in health and disease.

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