

Modulation of the Pulsatile Release of Biologically Active Luteinizing Hormone by Endogenous Opiates^a

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INTRODUCTION

The preferential release of pools of luteinizing hormone (LH) enriched in biologically activity has been difficult to demonstrate in humans when single bolus or continuous low dose infusions of exogenous GnRH have been used.^{1,2} As an alternative experimental approach to characterize physiological release of immunoreactive and bioactive LH in humans, we chose to enhance endogenous GnRH pulses by administering an opiate-receptor antagonist (Naltrexone, Endo Labs, Inc.)

MATERIALS AND METHODS

Blood was drawn at 20-minute intervals for eight hours in eight healthy men, after oral ingestion of placebo or Naltrexone (1 mg/kg). The samples were assayed for testosterone and immunoactive LH by RIA^{1,2} and for bioactive LH using the rat interstitial cell testosterone assay (RICT).^{1,2} Pulsatile LH secretion profiles were analyzed as described earlier.³ A chi-square table was constructed to analyze the

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TABLE I. Pulsatile Secretion of Bioactive Luteinizing Hormone in Humans

Subject	Treatment	Mean LH ^a	Area ^b	Pulses/ 8 hours	Incremental ^c	Peak ^d	Fractional (%) ^e	Mean Periodicity ^f
A	Placebo	13.71 ± 4.96	6623	2	14.0	25.9	80	220
	Naltrexone	14.16 ± 3.07	6850	5	7.3	18.9	65	100
B	Placebo	24.86 ± 7.02	11992	3	12.1	30.3	87	180
	Naltrexone	29.21 ± 7.59	14101	4	24.2	42.2	72	125
C	Placebo	10.50 ± 3.43	5032	2.5	9.8	17.2	149	200
	Naltrexone	19.64 ± 7.08	9644	6	13.4	27.4	92	63
D	Placebo	38.77 ± 6.27	18682	3	15.0	43.8	55	180
	Naltrexone	47.38 ± 7.84	22968	4.5	17.2	57.2	65	115
E	Placebo	33.90 ± 6.57	17041	2	19.6	46.3	39	220
	Naltrexone	46.64 ± 11.86	22588	4	16.9	59.8	69	125
F	Placebo	30.86 ± 3.47	14820	1	5.9	39.6	38	—
	Naltrexone	36.40 ± 7.41	17564	3	23.6	47.0	63	180
G	Placebo	40.62 ± 17.4	19657	3	28.2	60.1	79	180
	Naltrexone	42.92 ± 15.6	20479	5.5	29.3	61.4	110	100
H	Placebo	16.21 ± 5.43	7877	2	12.1	29.3	57	220
	Naltrexone	28.23 ± 8.06	13550	3	24.	41.4	70	180
Means ± SD	Placebo	26.18 ± 10.90	12175	2.31	14.6	38.6	73 ± 34	200 ± 19
	Naltrexone ^g	33.07 ± 11.5	± 5327	± 0.66	± 6.34	± 13.2	76 ± 16	124 ± 37
	Placebo		15954	± 1.02	± 6.64	± 44.4		
	Naltrexone ^g		± 5583					
<i>P</i> value	(Placebo vs Naltrexone)	<0.001	<0.002	<0.001	NS	<0.01	NS	<0.002

^a mIU/ml, mean ± SD (*N* = 25 samples).^b Area in mIU/ml × min (over eight hours of sampling).^c mIU/ml increment from nadir to peak.^d Maximal absolute LH value achieved in the pulse (mIU/ml).^e Percent increase above nadir.^f Minutes.^g Naltrexone 50 µg/l was devoid of effect in the RICT bioassay *in vitro*.

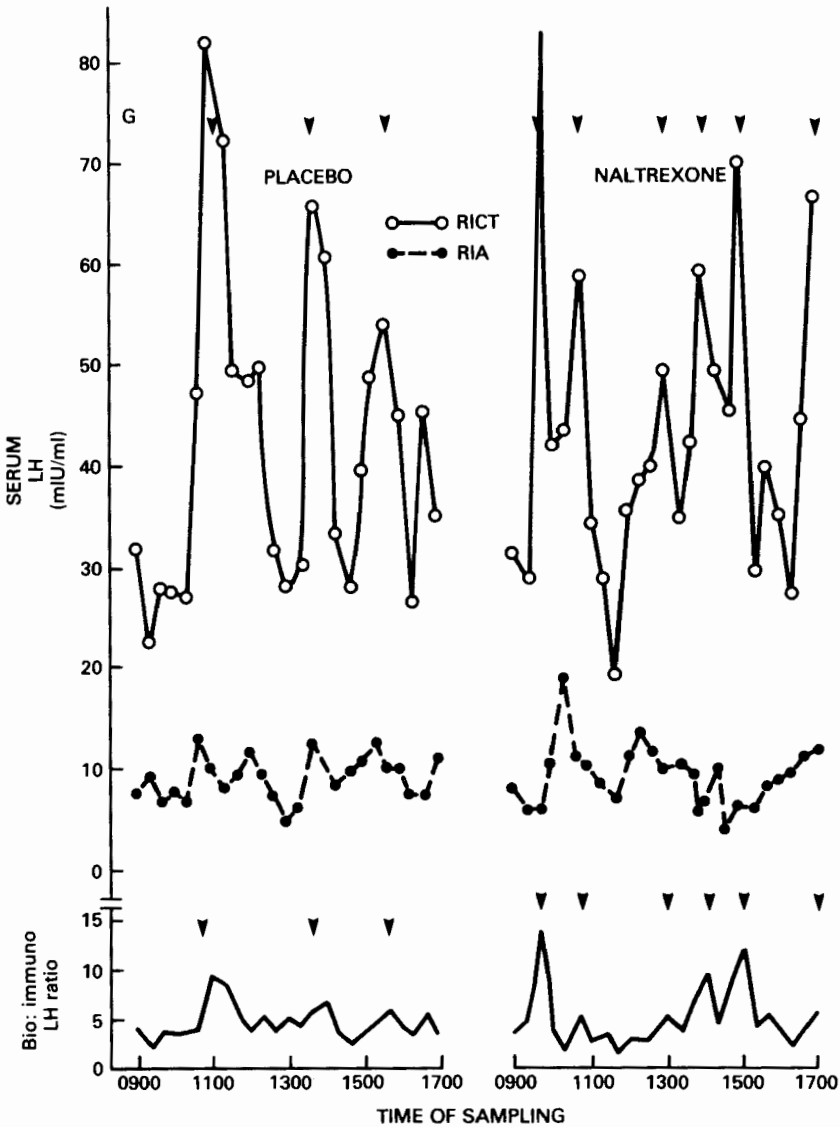


FIGURE 1. Pulsatile secretion of bioactive and immunoactive LH in one normal man following the administration of placebo or Naltrexone (1 mg/kg) at 0800. As shown on the horizontal axis, blood samples were drawn at 20-minute intervals beginning at 0900. Blood was assayed for immunoactive (RIA) and bioactive (RICT) LH, from which the corresponding profiles of LH release were constructed and the associated bioactive and immunoactive LH ratios (bio : immuno) computed. Significant bioactive LH pulses are designated by the arrows (three pulses after placebo and four pulses after Naltrexone). Note the tendency of increased bio : immuno LH ratios (lowermost curve) to accompany LH pulses after either placebo or Naltrexone ingestion.

expected versus observed distribution of increased bioactive and immunoactive LH ratios within LH pulses.³

RESULTS AND DISCUSSION

We have been able 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. Under these conditions, in which no discernible opiate agonist action can be demonstrated, there was a significant increase in mean and integrated serum concentrations of bioactive LH, associated with a striking accentuation in the pulsatile pattern of LH release ($p < 0.01$) (TABLE 1). Since the pituitary gland is devoid of intrinsic periodicity for LH release, the enhancement in bioactive LH pulse frequency must reflect an amplification of the endogenous GnRH pulse signal. In association with this presumptive augmentation of the endogenous GnRH signal, bioactive LH pulses demonstrated preferential enrichment in bioactivity, reflected by significantly increased bioactive and immunoactive LH ratios compared with interpulse baseline ratios ($p < 0.001$). We infer that modulation of the frequency of the endogenous GnRH stimulus provides one hypothalamic mechanism by which to control net pituitary release of LH molecules that retain high biological activity. This inference was supported by our demonstration of acutely increased mean or integrated serum testosterone concentrations in these men after Naltrexone administration (mean testosterone levels rose from 570 ± 151 to 645 ± 120 ng/dl, $p < 0.05$). Thus, the apparent increase in bioactive LH quantitated by the RICT assay *in vitro* correctly reflects an increase in circulating concentrations of biologically effective LH with consequent significant effects upon target cells in the gonad *in vivo*.

Our studies do not permit us to ascertain whether increased testosterone secretion represents a response to the increase in LH pulse frequency, the increase in LH pulse amplitude, or both. However, further investigations using the *in vitro* RICT bioassay to quantitate effective circulating LH concentrations in subjects in whom the frequency and amplitude of LH pulses can be experimentally controlled will be able to clarify which attributes of the pulsatile LH signal are most important in stimulating Leydig cell steroidogenesis. Use of the RICT bioassay of LH (rather than RIA alone) provides an important investigative tool, because RIA estimates of LH pulses were 29% discordant with bioactive LH pulses, while 17% of bioactive LH pulses occurred without significant immunoactive pulses. Thus, some bioactive LH pulses could not be detected from analysis of immunoactive LH data alone.

We conclude that neuroendocrine mechanisms, such as the endogenous opiate system studied here, can control the pulsatile mode of LH release and thereby significantly regulate the secretion of LH species that are enriched in bioactivity. Studies with the RICT bioassay should help 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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