# Rapid Publication

## A Luteinizing Hormone-releasing Hormone Agonist Decreases Biological Activity and Modifies Chromatographic Behavior of Luteinizing Hormone in Man

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**Abstract.** The effect of the luteinizing hormone-releasing hormone (LHRH) agonist, [D-Trp$^6$,Pro$^9$-NEth]LHRH ($LHRH_A$), on luteinizing hormone (LH) bioactivity was assessed with a rat interstitial cell assay in four men during a 14-d treatment period. Biologic/immunologic (B/I) ratios were unchanged initially with treatment but by day 12 had fallen to levels lower than basal values. Frequent sampling on day 12 revealed blunted gonadotropin responsiveness to $LHRH_A$ and absence of spontaneous LH pulsations. Despite continued administration of $LHRH_A$, human chorionic gonadotropin administration resulted in elevated B/I ratios and testosterone levels. Further characterization of the serum immunoreactive LH by Sephadex chromatography revealed a later elution profile during treatment with $LHRH_A$. Thus, $LHRH_A$ appears to act, in part, by modification of the bioactivity of LH in man.

### Introduction

Synthetic agonist analogs of luteinizing hormone-releasing hormone (LHRH)[1] decrease gonadal steroidogenesis. In animals, both pituitary and gonadal effects have been observed, but, in man, a predominant pituitary site of action is favored (1, 2).

Previous studies from this laboratory during chronic treatment of men with the agonist analog, [D-Trp$^6$,Pro$^9$-NEth]LHRH ($LHRH_A$), indicate that mean 12-h gonadotropin levels are generally unchanged from pretreatment values (1). However, the acute response to $LHRH_A$ administration and the normal pulsatile pattern of luteinizing hormone (LH) release are markedly diminished during treatment. Despite low serum testosterone (T) values, the Leydig cell responds normally to infusions of exogenous human LH. These observations suggest that $LHRH_A$ effects a qualitative change in pituitary gonadotropin secretion, resulting in the observed effects on testicular steroidogenesis. Alterations in LH bioactivity (bioLH) are known to occur under several physiologic circumstances in man. The LH molecule consists of alpha and beta subunits and the unassociated individual subunits do not have biologic activity. This study examines sequential changes in bioLH, as measured by a rat interstitial cell assay, during treatment with $LHRH_A$. Further characterization of the immunoactive LH (iLH) present during treatment was performed with Sephadex G-100 chromatography.

### Methods

Four healthy adult male subjects attended the Vanderbilt University Clinical Research Center daily for 18 d. (*a*) A heparinized venous catheter was placed daily and three blood samples were obtained at 20-min intervals, pooled, and immunoassayed for LH, follicle-stimulating hormone (FSH), and T. Immediately after sampling on day 1 and for the next 13 d, each subject received 500 $\mu$g $LHRH_A$ subcutaneously. (*b*) On the twelfth day of treatment, the protocol was modified to allow a more detailed evaluation of gonadotropin and T responses. Samples were obtained at 20-min intervals for 1 h before and 3 h after injection of $LHRH_A$. LH and FSH levels were determined on serum from each of these samples, and T was determined on hourly samples. All subjects then received a single intramuscular injection of 4,000 units human chorionic gonadotropin (hCG) (A. P. L., Ayerst Laboratories, New York). (*c*) On days 13 and 14, the procedures outlined in (*a*) were followed

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1. *Abbreviations used in this paper:* B/I, biologic/immunologic; bioLH, bioactivity of LH; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; iLH, immunoactive LH; $K_{av}$, partition coefficient; LH, luteinizing hormone; LH-$\beta$, beta subunit of LH; LHRH, luteinizing hormone-releasing hormone; $LHRH_A$, luteinizing hormone-releasing hormone agonist, [d-Trp$^6$,Pro$^9$-NEth]LHRH; T, testosterone.

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and on days 15–18, the sampling continued as described, but omitting the $LHRH_A$ injection.

BioLH was assessed on all samples with a rat interstitial cell assay, as modified from Dufau et al. (3); LER 907 (National Pituitary Agency, Baltimore, MD) served as standard. All samples from a single subject were measured in duplicate in the same assay. LH and FSH immunoassays were double-antibody techniques, utilizing rabbit anti-LH and anti-FSH (National Pituitary Agency) and goat anti-rabbit γ-globulin (Calbiochem-Behring Corp., San Diego, CA). T was measured as described by Coyotupa et al. (4).

Chromatography was performed on serum obtained on day 12 of treatment from all subjects and on serum samples taken from subjects 1 and 2 after they had been off treatment for 6 wk. A Sephadex G-100 column (Pharmacia Inc., Piscataway, NJ) measuring 5 cm × 90 cm, employing 0.15 M $NH_4CO_3$ with 0.02% bovine serum albumin (BSA) as buffer was utilized. To each column, 10 ml of the subjects' serum and $^{125}$I-LH (Radioassay Systems) were added and 150 fractions were collected at 4°C. Every other fraction was lyophilized and reconstituted with 2 ml phosphate-buffered saline (PBS) and radioimmunoassayed for LH. The elution volumes of $^{125}$I-LH and beta subunit of LH-β were determined by running individual columns with each of these markers. The elution volume of unlabeled LH (LER 960) and LH were determined by radioimmunoassay and migrated with the same partition coefficient ($K_{av}$) as the labeled components. When columns were run with marker LH and LH-β, the added radioactivity was selected such that it would not contribute significantly (2–5%) to the tracer employed for the LH radioimmunoassay.

LH-β for iodination was obtained from the National Pituitary Agency. Iodination of the subunit was performed with the chloramine T method (5). 25 μg of the subunit, 1 mCi (10 μl) $^{125}$I, and 25 μg (10 μl) of chloramine T were mixed with 0.1 M K-phosphate (pH 7.2) at 25°C and reacted for 20 s. The reaction was terminated by adding 75 μg (30 μl) of sodium metabisulfite and 1.8 mg (0.9 ml) of KI. Purification of the iodinated peptide was accomplished on Sephadex G-75 chromatography (Pharmacia Inc.). Statistical analyses were performed with the two tailed *t* test.

This experiment was approved by the Vanderbilt University Committee for the Protection of Human Subjects, and all subjects gave informed consent.

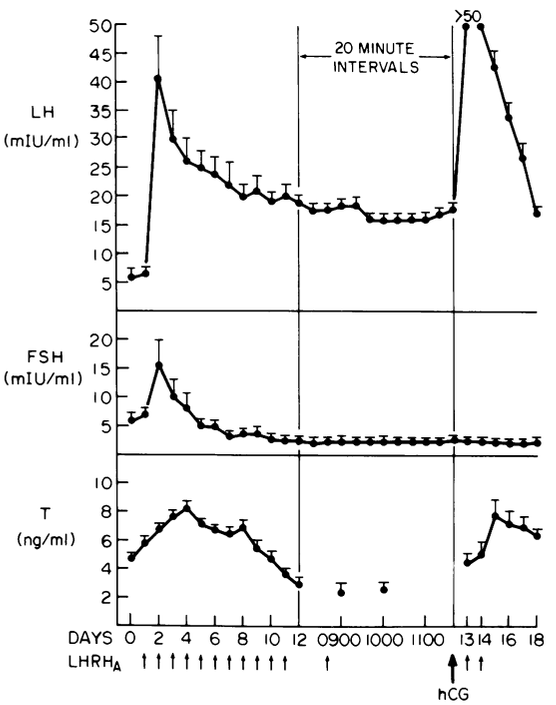


*Figure 1.* Mean (±SE) LH, FSH, and T levels in four individuals during a 14-d treatment period with 500 μg $LHRH_A$ daily. On day 12, samples were obtained at 20-min intervals. Arrows indicate injections of $LHRH_A$. All subjects receive a single injection of 4,000 U hCG i.m. on day 12.

## Results

*Immunoactive serum gonadotropin and T levels.* Mean gonadotropin and T levels initially increased but subsequently declined from peak levels on days 2 and 4, respectively, to a nadir on day 12 (Fig. 1). Mean iLH levels remained higher than basal values at day 12 ($P < 0.001$); mean FSH and T levels were lower ($P < 0.005$ and $P < 0.001$, respectively). Sampling at 20-min intervals on day 12 revealed no spontaneous pulsations and a blunted response to scheduled injection of $LHRH_A$. Administration of hCG increased both serum iLH and T concentrations.

*LH bioassay.* Basal biologic/immunologic (B/I) ratio ranged from 5.6 to 14 (Table I). Following an initial rise, bioLH levels declined. By day 12, three of the four subjects exhibited bioLH levels lower than basal values; the fourth had returned to basal levels. The corresponding LH B/I ratio fell in each subject to values lower than the pretreatment determinations. hCG administration elevated both bioLH and B/I ratios in all subjects, reflecting the cross-reactivity of this gonadotropin in the bioassay and immunoassay systems employed.

*Sephadex G-100 chromatography.* The cross-reactivity of the LH-β in our radioimmunoassay is about 4% in units of mass (insert, Fig. 2) and even lower in molar terms. The iLH obtained from all subjects during treatment eluted well after marker $^{125}$I-LH and near $^{125}$I-LH-β. Off treatment, the iLH eluted with the marker. Fig. 2 shows data on subjects 1 and 2. Results on the other two subjects revealed iLH eluted with a $K_{av}$ of 0.33 and 0.34, respectively.

## Discussion

The application of gonadotropin bioassays to the evaluation of pituitary-gonadal physiology has suggested that both gonadal steroids and LHRH may modulate gonadotropin bioactivity. The presence of estrogen has been associated with lower serum LH B/I ratios (6, 7) in humans. Conversely, the presence of T increases serum bioLH in the male rat. The mechanism is uncertain. However, Peckham and associates (8, 9) have demonstrated estrogen effects on chromatographic behavior of FSH

*Table I. Sequential Determinations of BioLH and iLH Levels, B/I, and T during Daily Treatment with 500 μg $LHRH_A$.*

| Subject | | Day 0 | 2 | 4 | 6 | 8 | 10 | 12 hCG | 14 | 16 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | bioLH | 207 | 740 | 540 | 450 | 345 | 208 | 218 | 500 | 500 | 490 |
| | iLH | 37 | 137 | 119 | 110 | 91 | 91 | 84 | 219 | 141 | 78 |
| | B/I | 5.6 | 5.4 | 4.5 | 4.1 | 3.8 | 2.3 | 2.6 | 2.3 | 3.5 | 6.3 |
| | T | 5.5 | 7.2 | 7.8 | 5.7 | 5.2 | 3.7 | 2.7 | 6.2 | 5.6 | 6.2 |
| 2 | bioLH | 285 | 740 | 680 | 93 | 111 | 95 | 63 | — | 130 | 230 |
| | iLH | 48 | 146 | 146 | 106 | 105 | 91 | 91 | — | 187 | 100 |
| | B/I | 5.9 | 5.1 | 4.7 | 0.85 | 1.1 | 1.0 | 0.7 | — | 0.69 | 2.3 |
| | T | 4.3 | 7.5 | 9.5 | 7.6 | 8 | 4.8 | 1.5 | 3.2 | 9 | 6.2 |
| 3 | bioLH | 212 | — | 660 | 540 | 560 | 420 | 101 | 500 | 500 | 500 |
| | iLH | 14.6 | — | 73 | 73 | 68 | 64 | 65 | 228 | 132 | 64 |
| | B/I | 14 | — | 9.0 | 7.4 | 8.2 | 6.6 | 1.6 | 2.2 | 3.8 | 7.8 |
| | T | 5.3 | 7 | 8.3 | 7.2 | 9.3 | 6.3 | 4 | 6.5 | 8.1 | 5.9 |
| 4 | bioLH | 182 | 1225 | 815 | 420 | 275 | 195 | 88 | 2400 | 835 | 310 |
| | iLH | 32 | 196 | 123 | 119 | 96 | 91 | 87 | 270 | 160 | 73 |
| | B/I | 5.8 | 6.2 | 6.6 | 3.5 | 2.9 | 2.1 | 1 | 8.9 | 5.2 | 4.2 |
| | T | 4 | 5.1 | 7.9 | 6.8 | 5.4 | 4.4 | 3.9 | 4.4 | 5.7 | 6.9 |

bioLH, nanograms of LER 907 per milliliter; iLH, nanograms of LER 907 per milliliter; T, nanograms per milliliter.

in Rhesus monkeys and suggested that differences in sialic acid content of gonadotropins may be involved.

The effect of the acute administration of native sequence LHRH on bioLH has also been examined. Dufau et al. (10) observed an increase in LH B/I ratio after LHRH in both pre- and postmenopausal women but not in normal men. A biphasic increase in LH B/I ratio was noted in normal and postmenopausal women by Sawyer-Steffan et al. (11). These findings, in conjunction with the known heterogeneity of gonadotropins (12), suggest the presence in the pituitary of distinct forms of LH with biochemical or immunochemical differences.

This study examines the effect of an LHRH agonist analog on LH secretion in man with the use of an LH bioassay. Although the LH B/I ratios in this study were higher than unity before treatment, this phenomenon has been observed previously (10, 11). $LHRH_A$-induced LH release initially stimulates T production, but, by the twelfth day of treatment, bioLH is diminished despite clearly elevated iLH levels, an observation consistent with recent findings (13). Frequent sampling discloses a markedly altered pattern of LH release; no spontaneous pulsations occur, and the response to injection of $LHRH_A$ is blunted. Exogenous gonadotropin stimulates T production despite continued administration of $LHRH_A$. These findings, coupled with a demonstrable change in the elution profile of iLH on Sephadex G-100 chromatography, strongly suggest that $LHRH_A$ induces the pituitary to release an immunoreactive LH with diminished bioactivity.

We believe that the effects on bioactivity and the chromatographic changes observed are a direct effect of treatment with $LHRH_A$ rather than an effect of androgen depletion. Subject 4 had a low B/I ratio on day 12 despite essentially normal T concentration. In addition, similar elution profiles have been observed in two other men who were chronically treated with 500 μg $LHRH_A$ daily and 100 mg i.m. testosterone enanthate every 2 wk, at a time that their serum T concentrations were 5.6 and 11 ng/ml, respectively (data not shown).

Possible alterations of LH that could account for these findings include (*a*) changes in carbohydrate content (14, 15), (*b*) secretion of biologically inactive fragments or subunits (16), and (*c*) changes in amino acid sequence (17). Our results indicate that the iLH identified on Sephadex G-100 chromatography is smaller than $^{125}$I-LH but elutes before $^{125}$I-LH-β. The cross-reactivity of the latter is about 4% in our LH radioimmunoassay. In light of the similarities to deglycosylated gonadotropins, alterations in carbohydrate content seem most likely, although alternative explanations are possible.

*Conclusion.* $LHRH_A$ profoundly affects the secretion of LH. After a period of initial stimulation, subsequent responses

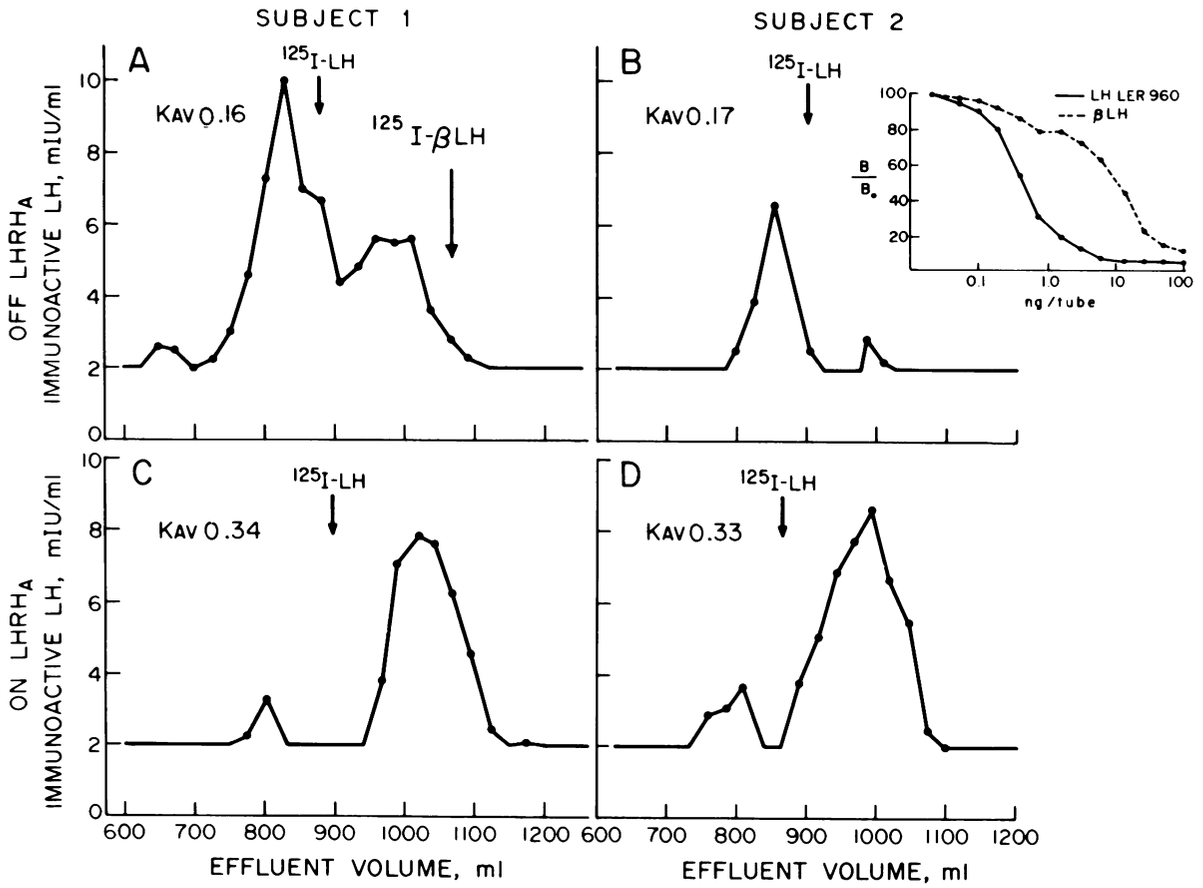


*Figure 2.* Elution profiles of immunoreactive serum LH on Sephadex G-100 chromatography. Subject 1 off of therapy (*A*) and at 12 days of 500 μg LHRH$_A$ daily (*C*) and subject 2 off therapy (*B*) and at 12 days of LHRH$_A$ (*D*) are depicted. ($K_{av}$ = $(V_e - V_0)/(V_t - V_0)$, where $V_e$ is the peak elution volume of iLH, $V_0$ is the peak elution volume of bovine thyroglobulin, and $V_t$ is the peak elution volume of $^{125}$I) Insert shows displacement of $^{125}$I-LH from anti-LH antibody by LH (LER 960) and LH-β (NPA Lot No. AFP-2444B). Results are expressed as percentage of tracer bound to that observed in the absence of added unlabeled hormone.

are blunted and spontaneous pulsations abolished. During treatment, LH B/I ratios fall dramatically, and the elution profile of iLH differs from the basal state. Thus, LHRH$_A$ appears to modify gonadal steroidogenesis in man, in part, by primary alteration of bioLH.

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