

The physiological significance of pulsatile LHRH secretion in man: gonadotrophin responses to physiological doses of pulsatile versus continuous LHRH administration

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Summary

This study tested whether pulsatile LHRH stimulation of the pituitary is required for normal gonadotrophin secretion in man. Four men with idiopathic hypogonadotrophic hypogonadism (IHH) and presumed endogenous LHRH deficiency were taken off all hormonal replacement for 5–6 weeks, then 5 µg LHRH was administered every 2 h for 1 week in order to prime pituitary gonadotrophin responsiveness. A physiological dose of LHRH (10 µg every 2 h) was then administered in both pulsatile and continuous regimens, in varying order, to each man. Pulsatile LHRH was capable of stimulating LH (as measured by bioassay) and FSH secretion, while continuous administration of LHRH was not. Serum LH, measured by RIA and bioassay, and FSH and free α -subunit levels, measured by RIA, increased significantly ($P < 0.05$) over pretreatment levels during pulsatile LHRH administration. In contrast, bioactive LH and immunoactive FSH did not change significantly compared to pretreatment values during continuous infusion of the same total LHRH dose, although immunoactive LH and free α -subunit levels did increase significantly ($P < 0.05$). The ratio of LH bioactivity to immunoactivity was significantly lower during the continuous compared to pulsatile LHRH regimen ($P < 0.001$). Similar serum LHRH levels were achieved during pulsatile and continuous infusions. Serum testosterone and oestradiol levels did not increase significantly from pretreatment levels during either regimen of LHRH administration. It is concluded that a pulsatile LHRH signal pattern is essential for normal pituitary gonadotrophin secretion in men with IHH. Continuous infusion of a physiological dose of LHRH, which produced serum LHRH levels which were indistinguishable from those found during pulsatile administration, failed to stimulate FSH or bioactive LH secretion.

Keywords: FSH, gonadotrophins, hypogonadotrophic hypogonadism, LH, LHRH.

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Introduction

Transmission of hormonal signals to target organs is determined by the molecular structure of the hormone, its mean level and bioavailability in blood, and binding of the hormone to specific receptors and activation of post-receptor mechanisms within target cells. As most hormones are secreted in an episodic rather than a continuous fashion (Krieger, 1979), the endocrine signal presented to target organs is not constant, but is variable over time.

Episodic LHRH release from the hypothalamus stimulates pulsatile gonadotrophin secretion from the anterior pituitary gland. LHRH pulse generation requires a neural timing mechanism which is regulated by central neuromodulatory systems and the sex steroid milieu. The importance of the LHRH-stimulus pattern to the pituitary gland response was demonstrated in monkeys by Belchetz *et al.* (1978). In ovariectomized monkeys with lesions of the arcuate nucleus resulting in LHRH and gonadotrophin deficiency, administration of LHRH in a pulsatile fashion restored and maintained LH and FSH secretion, while continuous infusion of LHRH was incapable of sustaining gonadotrophin secretion.

In humans with idiopathic hypogonadotrophic hypogonadism (IHH) and presumed LHRH deficiency, low-dose pulsatile LHRH administration stimulates pituitary LH and FSH secretion and gonadal function (Hoffman & Crowley, 1982). Continuous administration of markedly supraphysiological doses of LHRH (McNeil *et al.*, 1979; Rabin & McNeil, 1980) or administration of very potent LHRH agonists (Labrie *et al.*, 1986) initially stimulates and then suppresses gonadotrophin secretion. However, it is not known whether, in man, continuous administration of LHRH in physiological dosages (i.e. in dosages known to be effective in restoring gonadal function when given chronically in a pulsatile fashion) can stimulate normal gonadotrophin secretion. Furthermore, studies demonstrating that mean blood LHRH levels are similar during continuous compared to pulsatile LHRH administration have not been performed in any species.

In the present study, we assessed the physiological significance of pulsatile LHRH stimulation on anterior pituitary gland response by comparing the gonadotrophin responses to a matched physiological dosage of LHRH (resulting in similar LHRH levels) administered in a pulsatile versus continuous pattern to men with IHH and presumed deficiency of endogenous LHRH.

Patients and methods

Patients

Four men, aged 19–32 years, with well documented IHH and presumed endogenous LHRH deficiency were recruited from the Endocrinology Clinics at the Seattle VA Medical Center and Pacific Medical Center and volunteered to participate in this study. The diagnosis of IHH was established by the absence of spontaneous puberty, low serum levels of testosterone and gonadotrophin, otherwise normal pituitary function, and a normal cranial computerized axial tomographic scan.

Prior to the study, IHH was treated with testosterone enanthate in three men and with low-dose pulsatile LHRH in one man. All hormonal replacement therapy

was stopped 5–6 weeks prior to beginning the experimental protocol. The subjects were on no other medication.

Experimental protocol

Five to six weeks after discontinuation of hormonal replacement therapy, all subjects were admitted to the Clinical Research Center (CRC) at the University of Washington and pretreatment blood sampling was performed every 20 min for 4 h in order to assess basal hormone production prior to LHRH treatment. Immediately following pretreatment blood sampling, each man began receiving 5 µg LHRH in a bolus subcutaneous (s.c.) injection every 2 h for 1 week in order to standardize initial LHRH exposure and to prime pituitary gonadotrophin responsiveness. This priming dose of LHRH increased LH and FSH levels into the normal adult male range in all subjects within 1 week. LHRH was delivered by a portable programmable infusion pump (Auto Syringe AS 2BH, Travenol Laboratories, Hooksett, U.S.A.) with needles being inserted antiseptically into the abdominal subcutaneous tissue.

After this period of LHRH priming, subjects were treated with the same physiological dose of LHRH given in both a pulsatile and continuous fashion. Each man received 10 µg LHRH as a bolus s.c. injection every 2 h for 1 week and 10 µg LHRH as a continuous s.c. infusion over 2 h for 1 week, in varying order without an intervening washout period. Two men received a pulsatile followed by a continuous infusion of LHRH, while the other two subjects received continuous before pulsatile LHRH treatment.

At the end of each week of LHRH treatment, subjects returned to the CRC for 4 h of blood sampling. All blood sampling was performed through an indwelling catheter inserted into an arm vein. Blood was drawn every 5 min during the initial 40 min and then every 20 min for the remaining 200 min of the sampling period. During the pulsatile regimen, blood sampling was started immediately prior to an LHRH bolus. Serum LHRH levels were measured by radioimmunoassay (RIA) in each blood sample. Serum LH and FSH levels were determined in blood samples drawn every 20 min. As serum LHRH levels peaked 20 min after an LHRH bolus, mean LHRH levels were calculated using values drawn at 20 min intervals. LH bioactivity and free α -subunit levels were measured in a pooled sample composed of equal aliquots of serum from samples drawn every 20 min. Serum total testosterone and oestradiol levels were measured in the first sample from each sampling period.

The study protocol was reviewed and approved by the Human Subjects Review Committee of the University of Washington. Informed consent was obtained from all subjects prior to participation in the study.

Hormone assays

LH and FSH RIA. The reagents and methods used for the LH and FSH RIA have been described previously (Bremner *et al.*, 1981). With the exception of the tracer used in the LH RIA, which was purified hCG supplied by Dr C. Alvin Paulsen (Department of Medicine, University of Washington, Seattle, U.S.A.), the reagents used in both assays were supplied by the National Hormone and Pituitary

Program of the NIDDK (directed by Dr Salvatore Raiti, University of Maryland School of Medicine, Baltimore, U.S.A.). The reference standard used was LER 907. Tracers were radioiodinated with ^{125}I using chloramine-T (Greenwood *et al.*, 1963). Assay results were calculated using the computer program of Burger *et al.* (1972). The lower limit of detection was 6 ng ml^{-1} for LH and 25 ng ml^{-1} for FSH. The intra- and inter-assay coefficients of variation were 5.5% and 8.4%, respectively, for the LH assay and 7.3% and 9.7%, respectively, for the FSH assay. At 50% displacement the cross-reactivity of α -subunit in the LH and FSH assays was 10% and $<1\%$, respectively.

Testosterone and oestradiol RIA. The reagents and methods used for the steroid assays have been described previously (Matsumoto *et al.*, 1983). The reagents were supplied by the WHO Matched-Reagent Programme. Both testosterone and oestradiol were extracted from serum with ether. Dextran-coated charcoal was used in both assays to separate bound from free hormone. The lower limit of detection was 0.1 ng ml^{-1} for testosterone and 8 pg ml^{-1} for oestradiol. The intra- and inter-assay coefficients of variation were 5.1% and 9.8%, respectively, for the testosterone assay and 8.2% and 8.8%, respectively, for the oestradiol assay.

Free α -subunit RIA. Serum levels of free α -subunit were determined by RIA at the Nichols Institute (Los Angeles, U.S.A.) using a monoclonal antibody to the α -subunit and ^{125}I -labelled α -subunit. The sensitivity of this assay was 0.2 ng ml^{-1} . Cross-reactivity of this assay, as determined by the Nichols Institute, was 4.5% with LH, 3.4% with FSH, 2.0% with TSH, and 1.4% with hCG.

LH bioactivity. Measurement of bioactive serum LH used modifications of procedures described by Van Damme *et al.* (1974) and Dufau *et al.* (1977) and have been described previously (Tenover *et al.*, 1987). This assay is based on the measurement of testosterone production by dispersed Leydig cells from immature Swiss Webster mice. Serial dilutions of serum samples containing high LH levels were shown to be parallel to the standards. The lower limit of detection of LH in this assay was 110 ng ml^{-1} .

LHRH RIA. This used modifications of procedures described previously (Nett *et al.*, 1973; Matsumoto *et al.*, 1986). The LHRH antiserum (N540) was supplied by Dr Robert A. Steiner (Departments of Obstetrics and Gynecology, Physiology and Biophysics, and Zoology, University of Washington School of Medicine, Seattle, U.S.A.) The tracer, [^{125}I]LHRH, was purchased from New England Nuclear (Boston, U.S.A.) and the standard preparation, synthetic LHRH, was obtained from Peninsula Laboratories (San Carlos, U.S.A.). The lower limit of detection of LHRH in this assay was 4 pg ml^{-1} .

All samples from each individual subject were analysed in the same assay.

Statistical analysis

For each subject, average LH, FSH, and LHRH levels were calculated from the 4 h blood sampling values during the pretreatment period and continuous and pulsatile LHRH treatment (using blood samples drawn every 20 min). These levels were then used for statistical analysis and to generate mean hormone levels during each study period. The area under LHRH curves generated from the 4 h blood sampling

studies at the end of the continuous and pulsatile infusions were also determined (using blood samples drawn every 5 min for the initial 40 min and then every 20 min for the remaining 200 min). Hormone levels during the pretreatment period and during continuous and pulsatile LHRH treatment were compared using repeated measures analysis of variance or Student's paired *t*-test when only continuous and pulsatile infusions were compared. For both determination of mean levels and statistical analysis, the limit of detection for the particular assay was used when results were below assay sensitivity.

Results

Mean serum levels of immunoreactive LH at 20-min intervals during each study period are shown in Fig. 1. Before starting LHRH, immunoreactive LH levels were at or below the normal adult male range ($8\text{--}50\text{ ng ml}^{-1}$) and no spontaneous LH pulses were detectable in any of the IHH men. After 1 week of continuous LHRH administration ($10\text{ }\mu\text{g}$ every 2 h), immunoreactive LH was higher than pretreatment levels. The secretory pattern of LH was nearly constant over time (i.e. without LH pulses), reflecting continuous pituitary stimulation by nearly constant LHRH levels. At the end of 1 week of pulsatile LHRH treatment at the same dose, immunoreactive LH levels were higher than during continuous infusion. Discrete LH pulses following each $10\text{ }\mu\text{g}$ dose of LHRH were evident in all subjects, reflecting episodic pituitary stimulation by pulsatile LHRH.

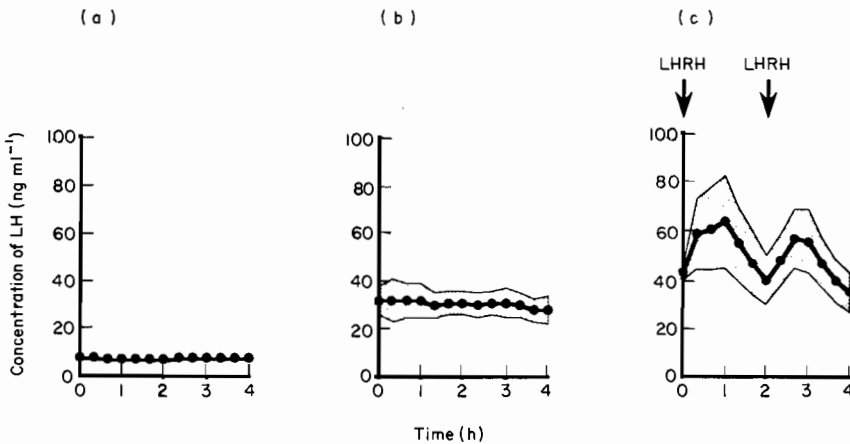


Fig. 1. Mean (\pm SEM, shaded area) serum levels of immunoreactive LH during frequent blood sampling (every 20 min for 4 h) in four men with IHH, before (a) and at the end of 1 week each of continuous (b) and pulsatile (c) LHRH infusions ($10\text{ }\mu\text{g}$ every 2 h). Prior to LHRH treatment, immunoreactive LH levels were low and nearly constant. During continuous LHRH infusion, immunoreactive LH levels were increased significantly over pretreatment values, but were constant (i.e. without detectable LH pulses). During pulsatile LHRH treatment, immunoreactive LH levels were higher than during continuous infusions and there were consistent pulses of LH following each $10\text{ }\mu\text{g}$ pulse of LHRH (\blacktriangle).

Mean serum levels of immunoreactive and bioactive LH during each period of the study are shown in Fig. 2a. During continuous LHRH infusions, immunoreactive LH levels increased significantly from 8 ± 1 (mean \pm SEM) before LHRH treatment to $31 \pm 5\text{ ng ml}^{-1}$ ($P < 0.05$). Bioactive LH was undetectable ($< 110\text{ ng ml}^{-1}$

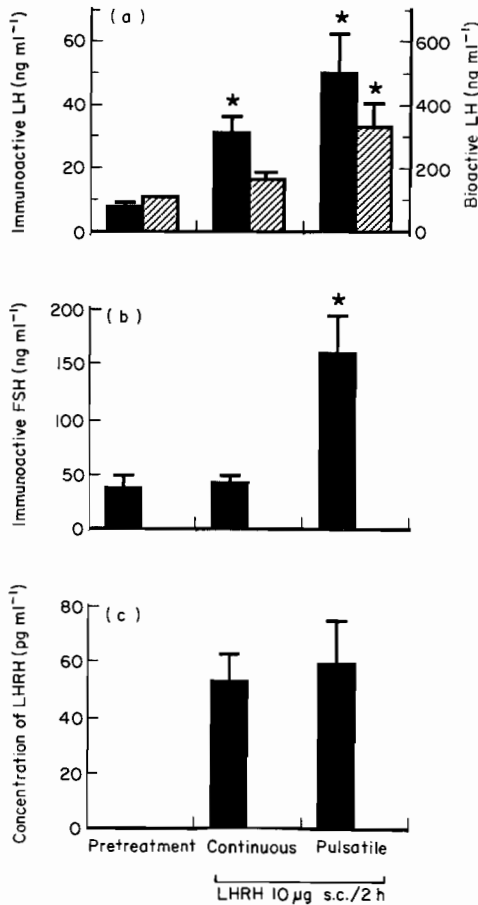


Fig. 2. Mean (\pm SEM) serum levels of immunoreactive (■) and bioactive (▨) LH and the levels of immunoreactive FSH and LHRH in four men with IHH, before (pretreatment) and at the end of 1 week each of continuous and pulsatile LHRH infusions ($10 \mu\text{g}$ every 2 h). Compared to pretreatment values, immunoreactive LH levels (a) increased significantly during both continuous and pulsatile LHRH treatment. Compared to pretreatment levels, bioactive LH (a) and immunoreactive FSH (b) were not significantly different during the continuous regimen, but did increase significantly during pulsatile LHRH administration. LHRH concentrations (c) were similar during the continuous and pulsatile regimens. * $P < 0.05$, compared to pretreatment values.

during the pretreatment period and increased only slightly to $165 \pm 24 \text{ ng ml}^{-1}$ during the continuous LHRH treatment; this increase was not statistically significant.

In contrast, LH levels determined by both RIA and bioassay increased significantly to $50 \pm 12 \text{ ng ml}^{-1}$ and $330 \pm 72 \text{ ng ml}^{-1}$, respectively, during pulsatile LHRH infusions compared to pretreatment values (both $P < 0.05$). Bioactive LH during pulsatile LHRH treatment was also significantly different from that during continuous infusion ($P < 0.05$). While immunoreactive LH levels during pulsatile LHRH treatment were also higher than those during continuous LHRH infusion, this difference did not achieve statistical significance. The ratio of bioactive to immunoreactive LH (LH B/I ratio) was significantly greater during pulsatile (6.9 ± 0.9) compared to continuous (5.7 ± 0.9) LHRH infusions ($P < 0.001$).

Serum free α -subunit levels were undetectable ($< 0.2 \text{ ng ml}^{-1}$) during the pretreatment control period in all subjects, and increased significantly during both the continuous ($0.8 \pm 0.2 \text{ ng ml}^{-1}$) and pulsatile ($0.9 \pm 0.2 \text{ ng ml}^{-1}$) LHRH regimens (both $P < 0.05$), compared to pretreatment values. Free α -subunit levels during continuous and pulsatile infusions were not significantly different.

Serum LHRH concentrations (Fig. 2c) during the 4 h blood sampling studies were similar during the continuous ($53 \pm 10 \text{ pg ml}^{-1}$) and pulsatile ($59 \pm 16 \text{ pg ml}^{-1}$) LHRH infusions ($P > 0.05$). The areas under the LHRH response curves were also similar during the continuous ($6346 \pm 1264 \text{ pg ml}^{-1} \text{ min}^{-1}$) and pulsatile ($7761 \pm 2280 \text{ pg ml}^{-1} \text{ min}^{-1}$) regimens ($P > 0.05$).

Serum levels of testosterone and oestradiol during continuous LHRH infusion ($1.7 \pm 1.3 \text{ ng ml}^{-1}$ and $21 \pm 7 \text{ pg ml}^{-1}$, respectively) were not significantly different compared to those during the pulsatile regimen ($2.2 \pm 1.4 \text{ ng ml}^{-1}$ and $26 \pm 6 \text{ pg ml}^{-1}$, respectively) or the pretreatment period ($0.4 \pm 0.1 \text{ ng ml}^{-1}$ and $19 \pm 6 \text{ pg ml}^{-1}$, respectively; both $P > 0.05$).

Discussion

In men with IHH and presumed LHRH deficiency, we found that pulsatile administration of a physiological LHRH dose stimulated normal pituitary LH and FSH secretion. In contrast, continuous infusion of the same physiological dose of LHRH increased immunoactive LH and free α -subunit levels, but did not consistently stimulate bioactive LH levels compared to pretreatment levels. Furthermore, FSH secretion was not increased significantly above pretreatment levels by continuous LHRH treatment.

Levels of immunoactive and bioactive LH and immunoactive FSH were significantly greater, while α -subunit levels were similar, during the pulsatile compared to continuous LHRH infusion. As mean serum LHRH, testosterone and oestradiol concentrations during the pulsatile and continuous infusions were comparable, the differences in gonadotrophin secretion were most likely attributable to differences in LHRH signal patterns. Together, these findings demonstrate that a pulsatile LHRH signal pattern is necessary for normal pituitary gonadotrophin secretion in men with IHH. Recently, we have also found that pulsatile administration of physiological LHRH doses is necessary to stimulate normal gonadotrophin secretion and induce ovulation in women with hypothalamic amenorrhea (Southworth *et al.*, 1991); continuous LHRH infusion was also ineffective in that clinical setting.

We have previously reported that the frequency of pulsatile LHRH administration in men with IHH can differentially regulate FSH and LH secretion, with a rapid LHRH pulse frequency favouring LH over FSH secretion (Gross *et al.*, 1987). The fact that continuous (i.e. and infinitely rapid) LHRH administration failed to stimulate FSH secretion is consistent with our previous findings and suggests that LHRH pulse frequency is an important regulator of pituitary FSH secretion.

The physiological significance of pulsatile hormone stimulation of a target gland is unclear. Recent evidence suggests that an episodic pattern of hormonal stimulation may be an important component of the endocrine signal to which target organs respond. Greater effectiveness of pulsatile versus continuous administration of glucagon (Weigle *et al.*, 1984), insulin (Matthews *et al.*, 1983), growth hormone (Clark *et al.*, 1985; Maiter *et al.*, 1988), thyrotrophin-releasing hormone (Hartnell *et al.*, 1987), adrenocorticotrophic hormone (Seely *et al.*, 1989), and LH (Peluso *et al.*, 1984) have been reported.

The importance of pulsatile versus continuous LHRH secretion in the regulation of gonadotrophin secretion in humans has been inferred from previous studies. These studies demonstrated that pulsatile LHRH treatment stimulated normal gonadotrophin secretion in men and women with IHH (Hoffman & Crowley, 1982; Santoro *et al.*, 1986). Other studies showed that high-dose continuous LHRH or potent LHRH agonist administration initially stimulated, but eventually suppressed, gonadotrophin secretion in normal men (Labrie *et al.*, 1986). However, the effect of low-dose continuous (versus pulsatile) LHRH administration on gonadotrophin secretion in IHH has remained unclear. The present study provides a direct comparison of the gonadotrophin responses to the same physiological dose of LHRH administered in a pulsatile and continuous fashion.

Low-dose continuous LHRH infusion resulted in a significant increase in serum LH and free α -subunit levels measured by RIA, but little increase in bioactive LH and a reduced LH B/I ratio in serum. We found similar results in women with hypothalamic amenorrhea treated with continuous LHRH infusions (Southworth *et al.*, 1990), and similar findings have been reported in men receiving a potent LHRH agonist (Evans *et al.*, 1984; Labrie *et al.*, 1986). The pituitary gland secretes free α -subunit, which is devoid of LH bioactivity, and multiple isoforms of gonadotrophins presumably due to variations in glycosylation; these isoforms have variable bioactivity (Strollo *et al.*, 1981; Dahl *et al.*, 1988). In the present study in men with IHH and in women with hypothalamic amenorrhea (Southworth *et al.*, 1990), we found that continuous LHRH infusion increased serum levels of free α -subunit which is not biologically active, but cross-reacts in our LH RIA. Thus, it is possible that increases in free α -subunit concentrations may be responsible for some of the decrease in LH B/I ratio during continuous LHRH infusions. It is also possible that continuous LHRH stimulates secretion of immunoactive LH isoform(s) with reduced LH biological activity, e.g. as a result of altered glycosylation.

The increase in immunoactive LH levels in our study is somewhat in contrast to earlier studies in monkeys in which LH levels measured by RIA were not stimulated by continuous LHRH infusions (Belchetz *et al.*, 1978). However, because the LH RIAs differed and the correlation of the monkey LH RIA with an LH bioassay was not known, the results of these studies are not directly comparable with ours. For example, if the LH RIA in the monkey studies reflected the LH bioassay more accurately, the results of the previous studies would be the same as in the present study.

In summary, we have found that pulsatile administration of physiological doses of LHRH is more effective than the same dose given continuously in stimulating normal gonadotrophin secretion in men with IHH. Our earlier studies, in women with hypothalamic amenorrhea (Southworth *et al.*, 1990) yielded similar results. Therefore, we conclude that pulsatile LHRH secretion is necessary for normal pituitary gonadotrophin secretion in humans and that the pattern of hormone stimulation is a critical component of endocrine signal transmission in man.

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