Increased Frequency of Pulsatile Luteinizing Hormone-Releasing Hormone Administration Selectively Decreases Follicle-Stimulating Hormone Levels in Men with Idiopathic Azoospermia

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Increased frequency of pulsatile luteinizing hormone-releasing hormone administration selectively decreases follicle-stimulating hormone levels in men with idiopathic azoospermia*†

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Men with idiopathic azoo-oligospermia and selective elevations of follicle-stimulating hormone (FSH) levels have evidence for decreased luteinizing hormone-releasing hormone (LH-RH) pulse frequency. We assessed whether increasing the frequency of pulsatile LH-RH stimulation can lower FSH levels in such men. We administered 5.0 μg of pulsatile LH-RH subcutaneously at intervals of 30, 60, and 120 minutes for 1 week to four men who had azoospermia, elevated FSH levels, and normal LH and testosterone (T) levels. The order of administration was varied among subjects. Frequent blood samples were obtained for 6 hours before LH-RH treatment and during the last 6 hours of each regimen. Before LH-RH treatment, the FSH levels (mean ± standard error of the mean) were 359 ± 18 ng/ml (normal range, 30 to 230 ng/ml). During LH-RH treatment, FSH levels progressively declined from 397 ± 68 ng/ml to 237 ± 70 ng/ml to 175 ± 43 ng/ml as the frequency of administration increased from every 120 to 60 to 30 minutes, respectively ($P < 0.05$). Unlike the FSH levels, which showed a progressive decline, LH, T, and estradiol levels showed no consistent relationship to LH-RH pulse frequency. We conclude that (1) in men with idiopathic azoospermia and elevated FSH levels, it is possible to decrease FSH levels by increasing the frequency of pulsatile LH-RH stimulation; (2) this decline does not appear to be a result of changes in steroid feedback or pituitary down-regulation; and (3) the frequency of pulsatile LH-RH stimulation can differentially modulate LH and FSH secretion by the pituitary gland. Fertil Steril 45:392, 1986

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Approximately 20% of men with azoo-oligospermia have elevated follicle-stimulating hormone (FSH) and normal luteinizing hormone (LH) levels.[1] Although several mechanisms have been proposed to account for the high FSH levels, such as decreased gonadal production of inhibin[2] and decreased gonadal production of sex steroids,[3] the precise explanation for the hormonal pattern in these patients is not well understood. We examined an alternative hypothesis for the generation of selective elevations of FSH levels. We explored the possibility that the frequency of pulsatile LH-releasing hormone (LH-RH) stimulation is capable of differentially modulating FSH and LH levels.

It is well-known that the secretion of LH-RH occurs in a pulsatile pattern, with an apparent frequency of approximately every 120 minutes in normal men.[4] There is a growing body of evidence that the pattern of LH-RH stimulation imparts information to the pituitary gland and that different pulse frequencies result in changes in the relative amounts of FSH and LH secreted by the pituitary. In monkeys with hypothalamic lesions and in humans with Kallmann's syndrome, it has been demonstrated that as the frequency of administered LH-RH decreases, FSH secretion is favored over LH.[5, 6] We have also found that men with selective elevations of FSH have a decreased frequency of episodic LH secretion, compared with normal men.[7] These results are consistent with the hypothesis that the frequency of spontaneous episodic discharges of LH-RH from the hypothalamus is decreased in this patient group, compared with normal men. In this study, we tested whether FSH levels could be lowered in men with selective FSH elevations by administering LH-RH at increasing frequencies.

MATERIALS AND METHODS

SUBJECTS

Four men, 25 to 36 years of age, volunteered to participate in the study. Each man presented as a member of an infertile couple and was found on semen analysis to be azoospermic. Hormonal evaluation revealed elevated serum FSH levels and normal serum LH and testosterone (T) levels in each man. Single random serum levels of these hormones are shown in Figure 1. The patients were otherwise healthy and were taking no medication. There was no history of orchitis, trauma, cryptorchidism, or exposure to radiation or known toxins. No patient had a varicocele.

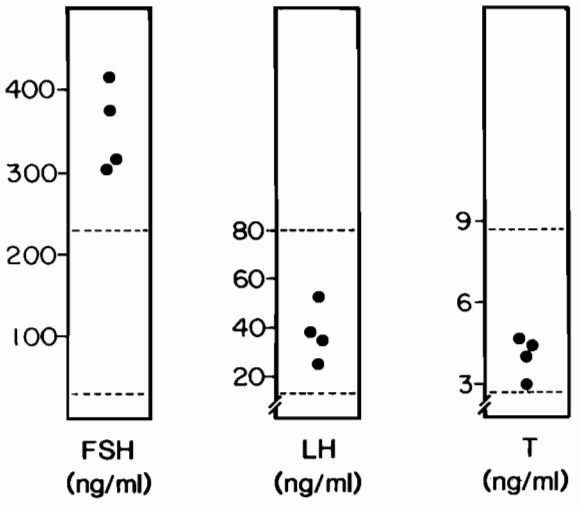

Figure 1
Single, random serum levels of FSH, LH, and T in each man before entering the study. The *broken lines* indicate the normal ranges for each hormone.

PROTOCOL

Before LH-RH administration, control blood sampling was performed every 20 minutes for 6 hours. After the control blood sampling, each patient was instructed in the use of a portable infusion pump (Autosyringe AS 6H, Auto Syringe, Inc., Hooksett, NH) for LH-RH administration and insertion of needles into the abdominal subcutaneous tissue with the use of an aseptic technique.

Each patient was treated for 3 weeks with LH-RH (Ayerst Laboratories, Inc., New York, NY) administered in frequent pulses. The amount of LH-RH administered per pulse was held constant, at 5.0 μg. Three different pulse frequencies were used, each administered for 1 week. The dosing frequencies employed were every 30 minutes, every 60 minutes, and every 120 minutes. Each patient received each frequency, but the order was varied so that each subject received the regimens in a unique order. During the last 6 hours of each week of LH-RH treatment, frequent blood sampling was performed. Samples were obtained every 10 minutes during 30- and 60-minute frequencies and every 20 minutes during the 120-minute frequency. These sampling frequencies were chosen to best assess mean hormone levels between LH-RH injections. Immediately

after each blood sampling period, the subjects were begun on the next pulse frequency in their protocol.

All blood sampling was performed through an indwelling intravenous catheter inserted in an arm vein kept patent with a heparin flush solution. LH and FSH were measured in each blood sample. T and estradiol (E_2) were determined in a pool from hourly aliquots during each sampling period. During the study, all samples obtained for a patient were analyzed in the same assay. Before taking part in the study, each patient signed an informed consent document approved by the Human Subjects Review Committee of the University of Washington.

HORMONE ASSAYS

FSH and LH Radioimmunoassay (RIA)

The RIA for serum FSH[8, 9] was with reagents distributed by the National Pituitary Agency. The reference standard was LER-907, the first antibody was antihuman FSH batch 5, and the tracer was HS-1, radioiodinated with ^{125}I with the use of chloramine T.[10] The limit of detectability of FSH in this assay was 25 ng/ml. The intraassay variability was 7.3%, and the interassay variability was 9.7%.

The RIA for LH[8, 9] had as a reference standard LER-907 and first antibody (antihuman LH batch 2) supplied by the National Pituitary Agency. The tracer was purified human chorionic gonadotropin radioiodinated with ^{125}I with the use of chloramine T.[10] The limit of detectability of this assay was 6 ng/ml, and the intraassay and interassay coefficients of variation were 5.5% and 8.4%, respectively. Assay results for both LH and FSH RIA were calculated with the use of the computer program of Burger et al.[11]

T and E_2 RIA

The RIA for serum T and E_2 was with reagents supplied by the World Health Organization Matched Reagent Programme.[12] The antisera were raised in rabbits against bovine serum albumin conjugates of T and E_2. Anti-T antiserum exhibited cross-reactivity of 14% with 5α-dihydrotestosterone, 6% with 5α-androstanediol, and < 2% with other steroids tested. Anti-E_2 antiserum exhibited 17% cross-reactivity with estrone. Both T and E_2 assays were preceded by ether extraction. Separation of bound from free hormone in these assays was accomplished with the use of dextran-coated charcoal. The assay sensitivity was 0.1 ng/ml for T and 8 pg/ml for E_2. The intraassay and interassay variabilities were 5.1% and 9.8%, respectively, for T and 8.2% and 8.8%, respectively, for E_2.

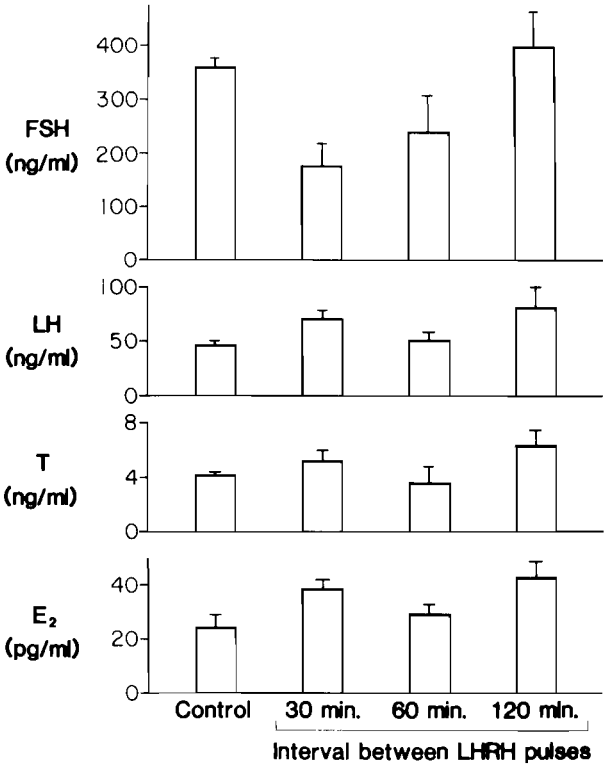

Figure 2
Mean (± standard error of the mean) serum levels of FSH, LH, T, and E_2 for all four men during 6 hours of sampling in the control period and in each of the three dose frequencies of LH-RH administration.

STATISTICAL ANALYSIS

Gonadotropin and steroid results obtained during the control period and during the different frequencies of LH-RH administration were compared with the use of a one-way analysis of variance. When a P value < 0.05 was found, Duncan's multiple range test was employed to determine the location of the difference.

RESULTS

Serum levels of FSH and LH (Fig. 2) obtained during the control periods confirmed that our patients had elevations of FSH with normal LH lev-

els (mean ± standard error of the mean, FSH = 359 ± 18 ng/ml; LH = 46 ng/ml). FSH levels decreased from control values in all patients during the 30-minute (175 ± 43 ng/ml) and 60-minute (237 ± 70 ng/ml) frequencies. In two patients, FSH levels also declined from control values during the 120-minute frequency. The FSH results demonstrated statistically significant decreases during the 30-minute frequency, compared with both the 120-minute frequency and the control values ($P < 0.05$).

LH levels demonstrated no consistent relationship to LH-RH pulse frequency. Mean LH levels were 69 ± 10 ng/ml during the 30-minute regimen, 50 ± 9 ng/ml during the 60-minute regimen, and 81 ± 19 ng/ml during the 120-minute regimen. There were no significant differences in the mean LH levels.

T and E_2 values (Fig. 2) paralleled the LH results. The mean control T level was 4.2 ± 0.3 ng/ml. As the pulse frequency varied from every 30 minutes to every 60 minutes to every 120 minutes, the mean T level was 5.3 ± 0.7, 3.6 ± 1.2, and 6.4 ± 1.1 ng/ml, respectively. The mean control E_2 level was 24 ± 5 pg/ml. As the pulse frequency varied from every 30 minutes to every 60 minutes to every 120 minutes, the mean E_2 level was 39 ± 4, 29 ± 4, and 43 ± 6 pg/ml, respectively. There were no statistically significant differences in either T or E_2 levels during the study.

DISCUSSION

To determine whether increases in the frequency of pulsatile LH-RH stimulation can decrease FSH levels, we treated four men with azoospermia and selective elevations of FSH, each with LH-RH in three different pulse intervals. As the frequency of LH-RH administration increased, FSH levels progressively decreased, whereas LH, T, and E_2 values demonstrated no significant changes. These findings support the hypothesis that the frequency of LH-RH stimulation is capable of differentially modulating FSH and LH secretion.

FSH levels consistently decreased into or toward the normal range during the 30-minute and 60-minute frequencies. This occurred despite the higher total dosage of LH-RH used with increasing frequency and provides further evidence for the capability of LH-RH pulse frequency to direct the pituitary's secretion rates of FSH and LH. The results of this study are consistent with those of Wildt et al.[5] They found that faster frequencies of LH-RH stimulation resulted in lower FSH levels in castrated monkeys with hypothalamic lesions (which abolished endogenous LH-RH production).[5] The results of this study also confirm our findings for men with Kallmann's syndrome, who had decreased FSH levels but no change in LH levels during faster frequencies of LH-RH administration.[6]

Our results suggest that steroid feedback was not the cause for the decreased FSH levels found during the study. Although steroids have been shown to be capable of selectively inhibiting FSH secretion,[13, 14] the finding of similar T and E_2 levels but decreased FSH levels makes it probable that the FSH levels decreased independently of any steroid effect. It is similarly difficult to invoke pituitary down-regulation as a cause for the decreased FSH levels. Down-regulation occurs when high doses of LH-RH are administered continuously and results in decreased LH, FSH, and gonadal steroid secretion.[15] We found no decline in LH or steroid levels at the faster LH-RH pulse frequencies, when FSH levels were markedly decreased.

The mechanism underlying the differential modulation of FSH and LH by LH-RH pulse frequency is not understood. Recent findings[16, 17] on the production of a molecule with LH-like immunoreactivity but little LH bioactivity during continuous LH-RH treatment suggest that changes in the pattern of LH-RH stimulation may affect gonadotropin secretion at a posttranslational level of hormone synthesis. It is possible that the intracellular processes responsible for FSH and LH production have different responses to changes in the temporal characteristics of LH-RH stimulation.

The findings in this study, that FSH levels bear an inverse relationship to LH-RH pulse frequency, and of our previous study, that men with high FSH levels show evidence for decreased LH-RH pulse frequency, raise the question of a causal relationship between the decreased LH-RH pulse frequency and increased FSH level in men with azoo-oligospermia. In this study, the institution of a normal LH-RH pulse frequency of every 120 minutes decreased FSH levels in some men but not in others. It is possible that 1 week of treatment at every 120 minutes is not sufficient to lower FSH levels. Wagner and Warsch[18] reported that FSH levels decrease over 3 months when

LH-RH is administered every 120 minutes to men with selective FSH elevations. It is also possible that current methods of determining LH-RH pulse frequency underestimate the true frequency, so that every 120 minutes is still subphysiologic. A third possibility for our inability to lower FSH levels during the 120-minute period is that the dose of 5.0 μg was not optimal.

We hypothesize that the decreased LH-RH pulse frequency observed in men with high FSH levels is related to, and may be responsible for, their spermatogenic defect. Most such men are classified as having infertility of unknown cause. Their histories are negative for known causes of testicular injury, and biopsy studies of their testes demonstrate no pathologic correlates of injury, such as inflammation or fibrosis.[1] We believe it possible that some men with idiopathic azoo-oligospermia have a primary hypothalamic disorder, resulting in decreased frequency of LH-RH pulsation, elevated FSH levels, and spermatogenic dysfunction as a result of the abnormal hormonal milieu. This hypothesis is testable by long-term administration of LH-RH in increased pulse frequencies and assessment of the effects of this regimen on spermatogenesis.

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