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FSH Is Produced by GnRH-Deficient Men and Is Suppressed by Testosterone

STEPHEN J. WINTERS

From the Division of Endocrinology, Department of Medicine, Montefiore-University Hospital, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

ABSTRACT: Because there is an unexpected action of testosterone (T) to increase follicle-stimulating hormone (FSH) production in the absence of gonadotropin-releasing hormone (GnRH) in the rat, the effects of T treatment on circulating FSH were studied in men with GnRH deficiency. FSH immunoreactivity was identified in serum using a sensitive two-site immunoassay in each of five untreated GnRH-deficient men. Analysis by gel filtration chromatography revealed that circulating immunoreactive FSH coeluted with radiolabeled authentic FSH. T enanthate treatment suppressed serum FSH levels in each subject from (mean ± standard error of the mean [SEM]) 1.02 ± 0.94 to 0.26 ± 0.21 mIU/ml ($P = 0.061$, Wilcoxon rank sum test). Thus, FSH is produced in GnRH-deficient men, but there is no evidence for the stimulatory effect of T on FSH production in the absence of GnRH, as observed in the rat. These preliminary data provide further evidence that male contraceptive strategies using GnRH antagonists to suppress LH and FSH production in normal men will not be counteracted by T replacement therapy, although the issue deserves further attention in that study population.

Key words: Follicle-stimulating hormone, hypogonadotropic hypogonadism, androgens.

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Men with gonadotropin-releasing hormone (GnRH) deficiency are a clinical model for studying the actions of GnRH and other factors on FSH and luteinizing hormone (LH) secretion. With newer, sensitive two-site immunoassays, low levels of FSH and LH are detected in the serum in many patients with GnRH deficiency (Wu et al, 1991; Goji and Tanikaze, 1992), but there is no increase during the sleeping hours as in prepubertal boys (Goji and Tanikaze, 1992). There has been no further identification of this immunoreactivity as dimeric FSH and LH, however.

LH and FSH are products of the same cell, and their synthesis and secretion are stimulated by GnRH. But FSH pulsatile release in male rats (Culler and Negro-Vilar, 1986) and FSH synthesis and secretion by cultured rat pituitary cells (Kitahara et al, 1990) are less dependent on GnRH stimulation than is LH. The finding that GnRH antagonists produce an immediate and pronounced suppression of LH secretion in men, whereas the fall in serum FSH concentrations is delayed and incomplete (Pavlou et al, 1989), suggests that circulating FSH in men is likewise less dependent than LH on GnRH stimulation. When the rat pituitary is deprived of its GnRH support *in vivo* by GnRH antagonist treatment, or rat pituitary cells are cultured in the absence of GnRH, testosterone (T) increases FSH production and FSHβ mRNA levels while suppressing LHβ and α-subunit mRNA concentrations (Rhea et al, 1986; Bhasin et al, 1987; Wierman and Wang, 1990; Winters et al, 1992). This unexpected effect of T on FSH could be clinically important, because if FSH were stimulated by T in men, then contraceptive strategies using GnRH antagonists and T supplementation could be compromised. The present study was performed to characterize the FSH immunoreactivity in the serum of GnRH-deficient men, and to determine whether T stimulates FSH in these men.

Materials and Methods

Subjects

The clinical characteristics of the men with hypogonadotropic hypogonadism (HH) are summarized in Table 1. Three men had congenital GnRH deficiency. Each man had normal thyroid, adrenal, and growth hormone secretion. Computerized tomography (CT) or magnetic resonance (MR) scans of the hypothalamus/pituitary region were normal. Two men had acquired GnRH deficiency. One subject had a germinoma of the hypothalamus and diabetes insipidus; the second subject has had normal CT and MR scans together with otherwise normal anterior and posterior pituitary function for 4 years since presenting with GnRH deficiency. There was a remote history of head trauma. His serum iron saturation and erythrocyte sedimenta-

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Correspondence to: Dr. Stephen J. Winters, Department of Medicine, Montefiore-University Hospital, 3459 Fifth Avenue, Pittsburgh, Pennsylvania 15213.

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Table 1. *Clinical characteristics of men with hypogonadotropic hypogonadism (HH)*

Diagnosis	Age (years)	Other defects	Testis size (R/L, ml)	LH (mIU/ml)	FSH (mIU/ml)	T (ng/dl)
1. Congenital HH	19	None	3/3	<0.15	1.10	16
2. Congenital HH	21	Anosmia	2/1	<0.15	0.24	12
3. Congenital HH*	34	None	6/6	<0.15	0.51	30
4. Germinoma	26	DI†	15/15	0.35	0.65	14
5. Idiopathic HH	35	None	12/12	0.22	3.42	25
Prepubertal boys	5–9			<0.15–1.38	0.61–1.38	5.0–17.0
Adult men‡				3.44 ±0.53	6.04 ±2.11	566 ±65

* Previously treated with human chorionic gonadotropin (hCG) (6 months) and T (8 years), but no treatment for 6 years.
† Diabetes insipidus.
‡ Mean ± SEM (n = 15).

tion rate are normal. Testis volumes were estimated using a Prader orchidometer. One to three morning serum samples before and after 4–12 months of treatment with T enanthate were analyzed. Treatment schedules ranged from 150 to 200 mg T enanthate intramuscularly every 2–3 weeks. Blood samples were also obtained from 10 healthy prepubertal boys aged 5–9 years old.

Assays

FSH and LH were measured with Nichols Allegro LH and FSH two-site immunoradiometric assays. The minimal detectable dose of FSH (3SD above the zero standard) ranged from 0.2 to 0.3 mIU/ml. The within-assay coefficient of variation was 5.0% at 3.8 mIU/ml and 29% at 0.79 mIU/ml. The between-assay coefficient of variation at 20 mIU/ml was 8.0%. The minimal detectable LH dose was 0.15 mIU/ml. The within-assay coefficient of variation at 2.7 mIU/ml was 4.7%, and the between-assay coefficient was 3.4% at 18.6 mIU/ml. All samples from a given subject were analyzed in the same immunoassay.

Gel Filtration Chromatography

To examine the nature of the immunoreactive FSH in serum in GnRH deficiency, serum was chromatographed on a Sephadex G-100 column (1.6 × 96 cm) at 4°C in 0.1 M ammonium bicarbonate buffer. Rat [^{125}I]FSH was cochromatographed with the samples as an internal molecular weight marker. Fractions (2 ml) were collected, counted in a Packard gamma counter, lyophilized in a Speed Vac concentrator, and assayed for FSH.

Results

The clinical and hormonal characteristics of the men with HH are summarized in Table 1. Pretreatment serum T levels were in the range of values characteristic of prepubertal boys. LH levels were below the limits of detection in all three men with congenital GnRH deficiency. Serum LH levels in the men with acquired GnRH deficiency were measurable and within the range of values for prepubertal boys. In contrast to LH, FSH was detectable in serum in all patients with GnRH deficiency. Mean FSH levels were similar in men with GnRH deficiency (1.02 ± 0.94 mIU/ml) and in prepubertal boys (0.74 ± 0.29 mIU/ml).

To confirm the identity of immunoreactive FSH, serum from two of the subjects was studied by gel filtration chromatography, and the fractions were analyzed in the FSH assay. Figure 1 reveals that a single peak of FSH immunoreactivity was found that coeluted with rat [^{125}I]FSH.

The effect of T enanthate treatment on serum FSH levels in GnRH-deficient men is illustrated in Figure 2. Serum FSH levels declined during T enanthate treatment in each of the five subjects to 0.26 ± 0.21 mIU/ml (P = 0.061, Wilcoxon rank sum test). Mean (± standard error of the mean [SEM]) serum T levels during T enanthate treatment (463 ± 95 ng/dl) were similar to those of normal adult men.

Discussion

The results of this study indicate that dimeric FSH is produced and secreted into the circulation in men with severe GnRH deficiency. The characterization of the serum FSH immunoreactivity by gel-filtration chromatography extends recent findings with two-site immunofluorescent assays, using monoclonal antibodies to FSHβ and α-subunit, in which serum FSH levels in men with Kallmann's syndrome were measurable and similar to values in prepubertal boys (Wu et al, 1991; Goji and Tanikaze, 1992).

The factors responsible for the predominance of FSH relative to LH secretion in GnRH-deficient men are uncertain. One possibility is that activin is produced in the human pituitary, as in the rat, where it partially maintains FSH production (Corrigan et al, 1991). Although no data are available for humans, activin subunit mRNAs are present in the pituitary gland of adult male rhesus mon-

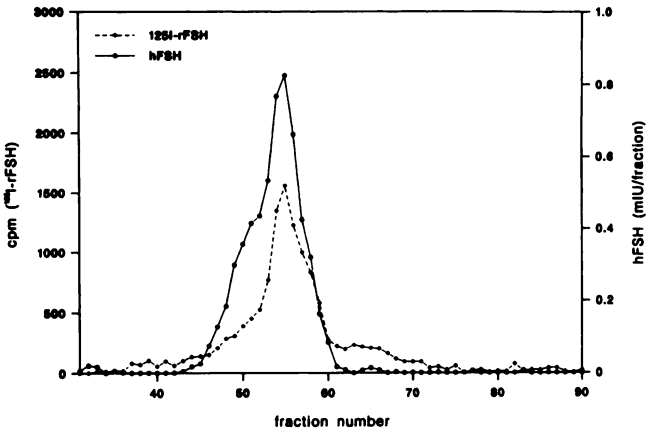

FIG. 1. Gel filtration chromatography of serum from one man with GnRH deficiency. Six ml of serum (subject 1) was centrifuged at 1,500 × *g* for 20 minutes, marker rat [^{125}I]FSH was added, and the sample was applied to the column. Fractions of 2 ml were collected and counted, lyophilized, and analyzed in the FSH immunoassay. The analysis was repeated with a sample from subject 3 with similar results.

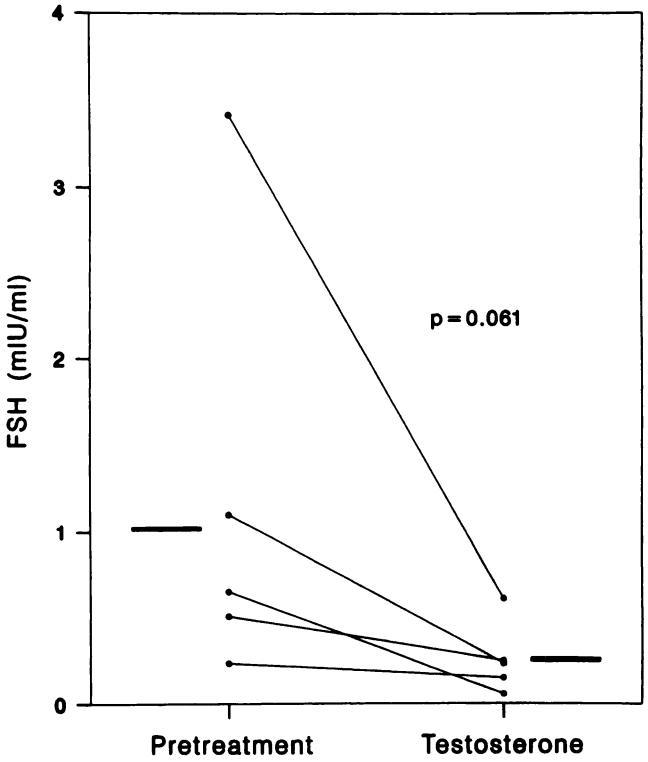

FIG. 2. Serum FSH levels in GnRH-deficient men before and during treatment with T enanthate.

keys (Attardi et al, 1992). Moreover, activin has been found to increase FSHβ mRNA levels and FSHβ and dimeric FSH secretion *in vitro* in selected human glycoprotein hormone-producing pituitary adenomas (Alexander et al, 1991; Takano et al, 1992). Although there was clinical and laboratory evidence for pronounced GnRH deficiency in the patients in this study, the possibility that small amounts of GnRH were produced and delivered via hypothalamic portal blood to gonadotrophs cannot be ruled out. In fact, initiation of pulsatile GnRH treatment of men with complete GnRH deficiency also resulted in predominant FSH secretion (Barkan et al, 1985).

Unlike the effect of T to increase FSHβ mRNA levels and stimulate FSH production in the absence of GnRH in the rat, T treatment of GnRH-deficient men suppressed circulating FSH concentrations. Similarly, T decreases serum FSH levels in normal men (Matsumoto, 1990), in normal men treated with the Nal-Glu GnRH antagonist (Bagatell et al, 1989), and in GnRH-deficient men treated with pulsatile GnRH (Finkelstein et al, 1991b). But those findings could reflect a T-mediated decrease in GnRH production (Jackson et al, 1991), a fall in GnRH receptor concentration (Giguere et al, 1981) or impeded signal transduction, or a submaximal dose of the Nal-Glu GnRH antagonist, respectively, as well as a direct effect on FSHβ gene expression. Further studies are needed to distinguish among these possibilities. Another unresolved question is whether the suppression of FSH secretion observed during T treatment was due to aromatization of the administered T to estradiol, because estradiol also decreases circulating FSH levels in normal (Sherins and Loriaux, 1973) and GnRH-treated GnRH-deficient men (Finkelstein et al, 1991a).

GnRH antagonist suppression of LH and FSH production together with T replacement therapy represents a possible approach to hormonal male contraception. Most, but not all men so treated, develop azoospermia (Pavlou et al, 1991; Tom et al, 1992), and with long-term treatment serum FSH levels also decline to near the limits of detection of sensitive immunoassays (Tom et al, 1992). The current finding that replacement therapy with T suppresses circulating FSH levels in GnRH-deficient men provides further preliminary evidence that the spermatogenic effect of T to increase FSH production in the GnRH antagonist-treated rat does not occur in men. Finally, the factors responsible for this species difference in the control of FSH synthesis and secretion remain to be established.

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