Serum Bioactive and Immunorective Follicle Stimulating Hormone During Chronic Treatment with Gonadotropin Releasing Hormone Agonist in Elderly Men

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Summary

Chronic administration of GnRH agonists "down regulates" the pituitary and decreases LH and FSH serum levels. Changes in the bioactivity of FSH have not been adequately assessed under such treatment, for lack of a proper test. We examined serum changes under GnRH agonist treatment among 12 healthy elderly men suffering only from benign prostatic hypertrophy, for up to one year, using a modification of a granulosa cell bioassay for the determination of FSH bioactivity. While radioimmunoassay-FSH decreased, we noticed a significant increase in the bioactivity of this hormone. The clinical importance of this increase is discussed.

Key words

FSH–GnRH–FSH-Bioactivity

Introduction

Gonadotropin-releasing hormone (GnRH) is secreted in a pulsatile manner by the hypothalamus, activating the pituitary gland and maintaining gonadotropin synthesis and release. The gonadotropins, in turn, regulate gametogenesis and gonadal steroidogenesis. Chronic administration of GnRH agonists reduces gonadal function through a complex mechanism of pituitary desensitization known as "down-regulation" (*Tolis, Faure* and *Koutsilieris* 1983; *Koutsilieris* and *Tolis* 1983). In men, pituitary luteinizing hormone (LH) is responsible for the synthesis and release of testicular testosterone (T), while follicle-stimulating hormone (FSH) is essential for the maintenance of Sertoli cell function and spermatogenesis. Studies of GnRH agonist administration in men, have focused mainly on the quantitative and qualitative changes in the LH, because serum immunoreactive LH decreases less than T levels do (*Evans, Doelle, Lindner, Bradely* and *Rabin* 1984; *Bhasin, Robinson, Peterson, Stein, Handelsmann, Raijfer, Heber* and *Swerdloff* 1984). It was found that serum bioactive LH levels are drastically decreased. Indeed, serum T levels may serve as an in-vivo LH bioassay. Changes in serum bio-FSH levels are difficult to assess because of the complexity of Sertoli cell function and the long period (> 3 months) involved in estimating altered sperm production. Variable changes in serum immunoreactive FSH levels have been reported during GnRH agonist treatment (*Bhasin* et al. 1984; *Faure, Labrie, Lemay, Belanger, Gourdeau, Laroche* and *Robert* 1982; *Wenderoth* and *Jacobi* 1983; *Ahmed, Brooman, Shalet, Howell, Blacklock* and *Rickards* 1983; *Labrie, Dupont, Belanger, St. Arnaud, Giquer, Lacourciere, Ginard* and *Monfette* 1986), but information on changes in serum bioactivity is scarce and comes exclusively from one laboratory (*Pavlou, Dahl, Wakefield, Rivier, Hsueh* and *Lindner* 1988; *Huhtaniemi, Dahl, Rannikko* and *Hsueh* 1988). The recent development of a specific sensitive in vitro FSH bioassay (*Jia* and *Hsueh* 1986; *Jia, Kessel, Yen, Tucker* and *Hsueh* 1986; *Tenover, Dahl, Hsueh, Lim, Matsumoto* and *Bremner* 1987) facilitates the study of the effect of chronic GnRH agonistic agonist administration on bio-FSH. We have had the opportunity to treat a healthy group of elderly men with a monthly control-released GnRH agonist injection for prolonged periods of time. Using a modification of the above-mentioned bioassay, we report the changes in bio/immuno (B/I) ratio of FSH in this homogenous group.

Material and Methods

Patients

Twelve caucasian men aged 60–78 years, all night sleepers and nonabusers of alcohol were included in this study. All were evaluated and found to have a normal hypophyseal-gonadal axis (routine hormonal evaluation). Patients were not on any medications that might have interfered with this axis, namely hormones and drugs such as cimetidine or spironolactone. The patients formed part of a larger group of patients treated for benign prostatic hypertrophy (BPH) with a long-acting, control-released GnRH agonist (Decapeptyl 3.2 mg CR [Triptorelin-D-Trp^6-LHRH microencapsulated in biodegradable (poly DL-lactide-glycolide) material]. The study was approved by the ethical committee of our Medical Center. The injection was given intramuscularly at our clinic, every 30 days and several urological parameters were evaluated at each visit. Hormonal work-up was performed on days 0, 30, 90 and 360.

Blood sampling

Prior to the administration of the monthly injection, all blood samples were drawn between 08.00–10.00 h to avoid any diurnal variation. The pretreatment hormone levels were measured twice for each patient, and blood samples were obtained during treat-

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ment at days 30, 90 and 360. The serum was separated by centrifugation and stored at − 20 °C until analyzed. The serum for bio-FSH determination was thawed once, at most, in order to minimize the reduction in biopotency known to occur after repeated thawing.

Hormone level determination

Testosterone, estradiol (E2), prolactin and LH were all measured by previously published radioimmunologic methods using commercially available kits (*Wide, Nillus, Gremzell* and *Roos* 1973; *Zacur* 1983).

Serum immunoreactive FSH was measured in duplicate, using RIA Amerlex kit (Amerlex FSH, Amersham, International Place, Bucks., United Kingdom). The results were expressed in terms of the second International Reference Preparation 78/549.

Bio-FSH determination

Bioassay Reagents

The reference standard was the human pituitary preparation LER-907 (FSH biopotency 20 IU/mg, LH biopotency 60 IU/mg, using the Second International Preparation of human menopausal gonadotropin standard). LER-907 was a generous gift from the National Hormone and Pituitary Program, NIDDK, Bethesda, MD, USA. Human chorionic gonadotropin (hCG) was obtained from Ikapharm (Ramat Gan, Israel) (300 IU/mg). Porcine insulin (26.8 U/mg) was obtained from the Novo Company (Rotterdam, Holland). L-glutamine, fetal bovine serum (FBS), McCoy 5-A medium (without serum) and a penicillin-streptomycin solution were obtained from Biolab (Jerusalem, Israel). 1-Methyl-3-isobutylxanthine (MIX), diethylstilbestrol (DES), androstenedione and polyethyleneglycol (PEG) (mol. weight 8000) were obtained from Sigma Chemical Co. (St. louis, MO, USA). ^{125}I-progesterone was obtained from Dupont Company (Boston, Mass., USA). Monoclonal antiprogesterone antibodies were the gift of Dr. F. Cohen of the Department of Hormone Research, The Weizman Institute of Science, Rehovot, Israel. Microwells used were the Nunclon type 16 mm, 24 wells per plate (Nunc, Vyborg, Denmark).

Granulosa cell assay

Serum bioactive FSH was measured in quadruplicate according to the method of *Jia* and *Hsueh* (1986) and *Jia* et al. (1986) with the following modifications: Granulosa cells were obtained from immature, 25-day old, DES treated female rats. 100,000 cells were cultured in each well containing 0.5 ml supplemented McCoy 5-A medium. Supplementation included: 2 mmol/l L-glutamine, 100 mg/l penicillin + 100 mg/l streptomycin, 10^{-7}M DES, 0.125 mmol/l MIX, Androstenedione 10^{-4} mmol/l [Androgens have been shown to augment FSH-induced progesterone secretion by cultured rat granulosa cells and was thus added in our short incubating assay to enhance progesterone biosynthesis (*Armstrong* and *Darington* 1976; *Hsueh, Adashi, Jones* and *Welsh* 1984; *Leung, Goff* and *Armstrong* 1979; *Welsh, Jones, Ruiz de Galarreta, Fanjul* and *Hsueh* 1982)], 1 mg/l insulin and 30 ug/l hCG.

Serum samples were pretreated with 12% polyethylene glycol and a constant serum volume of 20 ul/culture was added. Standard plates were prepared by the addition of 20 µl/culture of increasing concentrations of LER-907 (from 0−4 mIU/well). A constant volume of 20 ul pretreated FBS was added to all standard cultures (Fig. 1). (We proved that FBS has no FSH activity and no inhibitory effect on the FSH stimulated granulosa cells.) Plates were allowed to incubate in a humidified 95% air 5% CO_2 incubator for 44 h only (this is a modification of the method described by *Hsueh* and *Jia* using 50000 cells per culture and incubating plates for 72 h). This short incubation period was used in order to avoid the effect of the LH receptors being induced on the granulosa cells by FSH after 48 h (*Rani, Salhanick* and *Armstrong* 1981). As both LER-907 and human serum contain LH this might cause severe interference. The progesterone produced by the granulosa cells was measured by an established RIA method (*Kohen, Bauminger* and *Lindner* 1975). Since granulosa cells produce progesterone from endogenous substrates, while estrogen synthesis requires the addition of androgens in-vitro (androstenedione), we preferred to measure progesterone as the assay endpoint. Note also that in this granulosa cell culture system, FSH-stimulated progesterone accumulation if maximal at about 48 h, compared with 3 days for oestradiol (*Hsueh* et al. 1984). The sensitivity of the assay was 0.03 mIU/culture and the intra-assay variation was 10%. All serum samples of all patients were analysed in the same assay in order to avoid interassay variations.

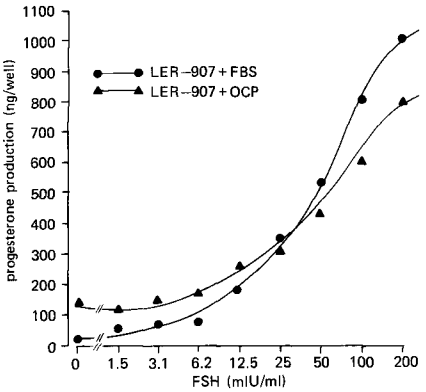

Fig. 1 Dose-response curve for the stimulation of granulosa cell progesterone production by increasing doses of FSH LER-907. Two different sera were added to the cultures, FBS and OCP (woman on oral contraceptive). Note that sensitivity was improved by using FBS. ● — ● − LER-907 standard + fetal bovine serum; ▲ — ▲ − LER-907 standard + serum from woman on oral contraceptive.

Statistical analysis

Immunoreactive FSH data and the progesterone produced by the granulosa cells (RIA) were performed using a program utilizing a weighted logit-log regression analysis. Calculation of FSH biopotency in samples was performed using a standard curve fitted with a second-degree polynomial. Comparison of mean immuno- und bio-FSH, B/I ratio, T and E_2 levels before and during treatment were done by Student's unpaired t-test, or by one-way analysis of variance (ANOVA) where appropriate.

Results

The mean pretretment serum immunoreactive FSH level was 7.00 ± 2.4 IU/l (± SEM), somewhat above the upper limit for normal men, using the Amerlex kit (0.9−7.8 IU/l). The bioactive FSH level was 14.6 ± 1.7 IU/l. Changes with time of the RIA-FSH and Bio-FSH are depicted on Fig. 2, and other details concerning the treatment outcome are given in Table 1. After one month, the FSH immunoactivity decreased significantly compared to pretreatment level ($P < 0.05$) and was maintained for up to one year, although the decrease was of a lower magnitude (53%) than the decrease in LH and T levels (66% and 90%), respectively. Contrary-wise, bio-FSH levels rose consistently and significantly ($P < 0.01$) after treatment from 14.6 ± 1.7 to 18.2 ± 2.8, 32.4 ± 11.1 and 43 ± 6 IU/l at days 30, 90 and 360, respectively. The discordant changes in serum bio- and immunoactivities are highlighted by the bio/immuno ratio that increased

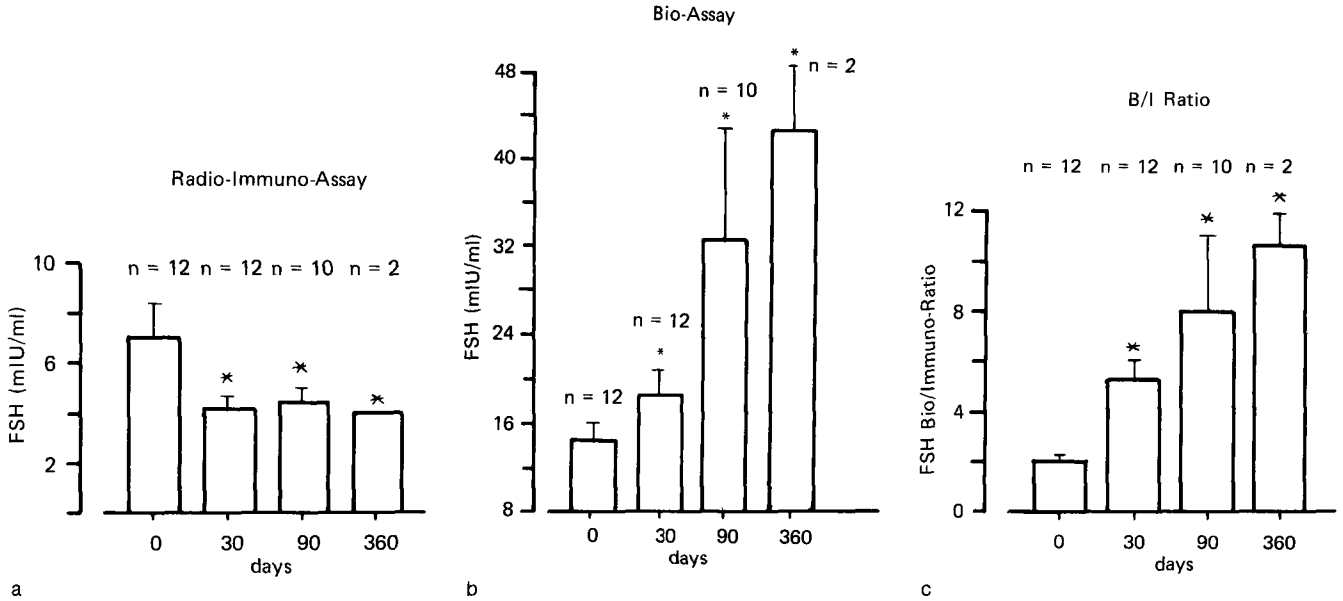

Fig. 2 a) Radioimmunoassay results of FSH; b) Bioassay results of FSH; c) Bioacting to immunoreacting FSH ratios.
[a]Results in mean ± SEM
[b]*P < 0.01 compared to pretreatment

Table 1 Pre- and post-treatment hormonal profiles among the subjects studied.

	Pre-treatment	at 30 days of treatment	at 90 days of treatment	at 360 days of treatment	Significance values (P)[a]
Testosterone (nmol/l)	19.14 ± 1.89	1.96 ± 0.17	1.75 ± 0.07	1.75 ± 0.0	P < 0.0001
LH (IU/l)	8.1 ± 2.1	2.9 ± 0.5	1.5 ± 0.4	2.0 ± 0.0	P < 0.05
Estradiol (pmol/l)	118.0 ± 13.2	39.0 ± 12.0	35.2 ± 15.7	31.2 ± 16.4	P < 0.05
Prolactin (µg/l)	7.6 ± 0.9	9.8 ± 0.7	7.4 ± 0.9	7.5 ± 1.0	P > 0.05
Testosterone/ estradiol ratio	0.16	0.05	0.05	0.06	P < 0.05

[a]comparing pre- to post-treatment measurements
[b]Results in mean ± SEM

significantly ($P < 0.01$) from 2.0 ± 0.2 to 5.2 ± 1.0, 8.0 ± 3.0 and 10.7 ± 1.5 at days 30, 90 and 360 respectively. bio/immuno ratio at day 90 was significantly higher ($P < 0.05$) than that of day 30, and the same applies to the bio/immuno ratio of day 360 compared to day 90, although one should note that only two cases reached 360 days of treatment.

Discussion

GnRH agonists have been used in men for the treatment of prostate carcinoma (*Tolis, Faure* and *Koutsilieris* 1983; *Koutsilieris* and *Tolis* 1983; *Wenderoth* and *Jacobi* 1983; *Ahmed* et al. 1983; *Labrie* et al. 1986) and lately have also been tried as a conservative modality in BPH (*Gabrilove, Levine, Kirschenbaum* and *Droller* 1987; *Peters* and *Walsh* 1987; *Keane, Tomoney, Kiely* and *Williams* 1988). The GnRH agonists with high agonist potency have also been tested in the expectation that they might be useful as contraceptive agents (*Pelletier, Cusan, Auclair, Kelly, Desy* and *Labrie* 1978; *Tcholokian, De La Cruz, Chowdhury, Steinberger, Coy* and *Schally* 1978), but they did not completely inhibit spermatogenesis. The reasons for this are not clear.

The recent introduction of the granulosa cell bioassay for the assessment of the bioactivity of FSH might cast some light on the issue, as it enables us to understand some of the mechanisms involved in the pituitary response to chronic GnRH agonist administration.

Contrary to a previous publication (*Huhtaniemi* et al. 1988), the men in our study group were physically active and healthy, and had no major co-existing disease. Therefore our results are not confounded by the problems of interacting illnesses.

In contrast to serum immunoreactive FSH levels that remained suppressed during the entire follow-up period, serum bioactive FSH levels increased consistently, compared to pretreatment values. Therefore, the B/I FSH ratio was significantly higher during treatment compared to pretreatment. This is in contrast to what is already known for the B/I ratio of LH under similar conditions (*Evans* et al. 1984; *Bhasin* et al. 1984). Bio/immuno LH ratio significantly decreases under GnRH treatment, which actually substantiates the idea that both hypophyseal hormones have different regulatory mechanisms and respond differently to chronic GnRH agonist stimulation.

Chappel, Aguirre and *Cutifaris* (1983) recently demonstrated that exposure of the pituitary gland to GnRH increases the relative proportions of the more biologically active forms of FSH in the gland. LHRH may activate pituitary enzymes necessary to produce the more biologically active FSH forms. The molecular basis for this phenomenon appears to be carbohydrate incorporation.

Interference of the long-acting GnRH agonist in our assay cannot account for the different B/I ratios obtained. This is evidenced by the mere fact that bio-FSH consistently increased after treatment, although serum was always obtained 28 days after the last injection. Furthermore, as has already been evidenced by *Hsueh, Adashi, Tucker, Valk* and *Ling* (1983), the addition of various GnRH agonists to the FSH treated granulosa cells, should cause a decrease in the steroid production. This inhibitory effect was seen also by other investigators (*Pavlou* et al. 1988; *Huhtaniemi* et al. 1988; *Dahl, Pavlou, Kovacs* and *Hsueh* 1986).

Only two other reports on the influence of GnRH agonist on Bio-FSH have been published (*Pavlou* et al. 1988; *Huhtaniemi* et al. 1988). Interestingly, in one the B/I ratios remained basically unchanged and in the second, increased B/I ratios were noted after treatment. In our opinion, this discrepancy adds support to the assumption that the agonist used, its dose, length of administration period, age and medical status are all important variables that may affect the FSH bioactivity after GnRH agonist administration.

Our findings may provide some answers to the enigmatic lack of spermatogenic suppression noted earlier under GnRH agonist treatment. It is possible that the increase of bioactive FSH enables the continuation of spermatogenesis even under the very low but still existing levels of serum T. The fact that the increase in B/I ratio was not transitory and in fact even increased with time, may suggest some kind of a pituitary "compensatory qualitative mechanism" which by improving biological activity, antagonises and decreases the clinical efficacy of GnRH agonistic treatment.

Our study clearly demonstrates that circulating bio-FSH levels can be preferentially stimulated in men after GnRH agonist treatment. The study of heterogenous populations of hormones will undoubtedly change our understanding of endocrine control mechanisms, and in vivo endocrine manipulations. In any hormonal treatment, both in men and women, one needs an assay such as the one presented here, to measure serum FSH more discriminately than by the RIA.

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