

Stimulation of Serum Inhibin Concentrations by Gonadotropin-Releasing Hormone in Men With Idiopathic Hypogonadotropic Hypogonadism*

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ABSTRACT. Inhibin is a gonadal hormone thought to be important in FSH regulation. We investigated the effects of the hypogonadotropic state and subsequent GnRH-induced increases in gonadotropin levels on inhibin secretion. Serum levels of inhibin, LH, FSH, and testosterone (T) as well as sperm concentrations were measured in 5 men with idiopathic hypogonadotropic hypogonadism (IHH) before (baseline) and during 8 weeks of GnRH therapy (5 µg, sc, every 2 h). Baseline and peak inhibin levels were compared to those in a group of 19 normal men. Before GnRH administration, the mean serum inhibin level was significantly lower in the IHH men than in the normal men [166 ± 56 (±SE) vs. 588 ± 30 U/L; $P < 0.001$]. Serum inhibin levels rose after 1 week of GnRH therapy ($P <$

0.05) and remained higher than the baseline level thereafter. The mean peak inhibin level during GnRH administration was lower than the mean value in normal men (485 ± 166 vs. 588 ± 30 U/L; $P < 0.05$). Serum LH and FSH levels rose promptly to the midnormal range or slightly above it. Serum T levels did not significantly increase until 4–5 weeks of GnRH administration and remained in the low normal range. All IHH men were azoospermic throughout the study. These data are consistent with the hypothesis that inhibin is produced by the testis under gonadotropin control. They also suggest the possibility of defective Sertoli and Leydig cell function in men with IHH, since the men's serum inhibin and T levels did not rise to the same extent as did their normalized serum gonadotropin levels during GnRH administration. (*J Clin Endocrinol Metab* 67: 1221, 1988)

INHIBIN is a glycoprotein product of Sertoli cells and is thought to be important in the regulation of FSH secretion (1). In male animals inhibin production is stimulated by FSH *in vitro* (2) and *in vivo* (3), and inhibin levels increase in the testis during development (4, 5). In prepubertal boys, serum inhibin levels are low compared to those in men and rise concomitantly with serum gonadotropins and testosterone (T) during puberty (6). These studies suggest that gonadotropins stimulate testicular inhibin production and that inhibin may serve as a marker for the function and maturity of Sertoli cells.

The recent purification of inhibin from bovine ovarian follicular fluid allowed the development of specific het-

erologous RIA systems applicable to human serum (7–10). Idiopathic hypogonadotropic hypogonadism (IHH), characterized by low serum T and low or low normal gonadotropin levels, is thought to be due to absent or diminished secretion of GnRH from the hypothalamus and results in failure to initiate or complete pubertal maturation (11). As secondary sex characteristics and fertility can be achieved in these men by pulsatile GnRH replacement (12), IHH provides a useful clinical model for the study of the physiology of human puberty. In this study we compared serum inhibin levels in men with IHH before and during pulsatile GnRH administration to those in normal men.

Subjects and Methods

Five men with IHH, aged 21–33 yr, were studied. The diagnostic criteria for IHH included: failure to undergo spontaneous puberty before age 18 yr, subnormal serum T levels [<2.8 ng/mL (<9.7 nmol/L)], low or low normal serum gonadotropin levels (normal adult male range, 8–50 µg/L for LH; 30–230 µg/

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L for FSH), absence of other pituitary or hypothalamic pathology based on endocrine testing (serum T_4 , T_3 resin uptake, PRL, and basal and either ACTH-stimulated or insulin-induced hypoglycemia-stimulated cortisol values), and computed tomography of the sella turcica. None of the men had growth retardation. Two of the five men had been anosmic since birth, all had had scrotal testes since birth, and their mean testicular volumes ranged from 3–12 mL. Four of the men had previously received T injections, and three of these four hCG treatment from 1–5 yr before entering this study. None had received FSH in the past. The fifth man had no previous therapy. Baseline hormone measurements were made at least 8 weeks after discontinuation of any therapy.

The men gave informed consent to the study in accordance with procedures established by the University of Washington Human Subjects Review Committee. Therapy consisted of GnRH (5 μ g every 2 h) delivered sc by automatic infusion pump (Zyklomat, Ferring Laboratories Inc., Suffern, NY). In our experience, this dose and schedule for GnRH resulted in normal circulating gonadotropin levels. The men were treated for a 7- to 8-week period, sufficient for maximal or near-maximal increases in serum LH, FSH, and T levels to be induced by GnRH therapy (13). Single peripheral venous blood samples were obtained between 0800 and 1500 h (at the convenience of the men and without reference to the preceding GnRH injection) before (baseline) and after 1, 2–3, 4–5, and 7–8 weeks of GnRH administration. Serum was collected and stored at -20 C before measurements of LH, FSH, T, and inhibin. Serum LH, FSH, and T levels were measured as the samples were obtained, whereas serum inhibin levels were measured in a single assay at the completion of the study. Seminal fluid was collected every 2 weeks in men who were able to ejaculate. Two of the five men were able to provide semen specimens from the time of pump initiation, while three men did not produce an ejaculate until 3–8 weeks after starting therapy. The samples were collected by masturbation after at least 48 h of abstinence from ejaculation.

We also measured serum inhibin levels in 19 normal young men, aged 22–35 yr, taking no medications. A single peripheral venous sample was obtained from each subject between 0800 and 1000 h.

Hormone assays and seminal fluid analysis

Inhibin RIA. Serum inhibin was measured in duplicate in a heterologous RIA (9) using an inhibin antiserum (As 1989) raised against bovine follicular fluid inhibin and used [125 I]31K bovine follicular fluid inhibin as tracer. The RIA was specific for intact inhibin, with less than 1% cross-reactivity with transforming growth factor- β , bovine activin-A, and free inhibin subunits obtained after reduction and alkylation of 31K bovine inhibin, and serum from castrated men contained no detectable inhibin. A partially purified human follicular fluid inhibin preparation calibrated in terms of its *in vitro* inhibin bioactivity was used as the RIA standard (10). The sensitivity (ED_{10}) of the assay was 100 U/L, and the ED_{50} was 550 U/L. The interassay coefficient of variation (CV) was 11% ($n = 5$ assays), and the intraassay CVs in the upper, mid, and lower portions of the standard curve were 12.0%, 3.3%, and 4.8%,

respectively, for five assays.

LH, FSH, and T RIAs. Serum LH and FSH were measured by double antibody RIA, as previously described (14), using reagents distributed by the National Pituitary Agency, with reference preparation LER 907. The sensitivity of the LH assay was 6 μ g/L; the intra- and interassay CVs were 5.5% and 8.4%, respectively. The sensitivity of the FSH assay was 25 μ g/L; the intra- and interassay CVs were 7.3% and 9.7%, respectively. Serum T was measured by RIA, as previously described (15), using reagents provided by WHO Matched Reagents Program. The assay sensitivity was 0.1 ng/mL (0.35 nmol/L), with intra- and interassay CVs of 5.1% and 9.8%, respectively.

Seminal fluid analysis. Sperm concentrations in seminal fluid samples were determined using a Coulter counter (Coulter Electronics, Hialeah, FL). Concentrations less than 15 million/mL were confirmed by direct determination using a hemocytometer. The methodology for these measurements was described previously (16).

Statistics

Analysis of variance with repeated measures was used to detect differences in serum inhibin, LH, FSH, and T levels before and during treatment. Paired *t* tests were used to compare the times at which differences had occurred, and the *P* values were adjusted for multiple comparisons by the Bonferroni method (17). Baseline serum inhibin levels were averaged, as were the peak inhibin levels achieved by each man during GnRH administration, and these mean values were compared with that in the normal young men using the Wilcoxon rank sum test. $P < 0.05$ was considered significant.

Results

Before GnRH treatment, the mean serum inhibin level in the IHH men was markedly lower than that in normal young men [166 ± 56 (\pm SE) *vs.* 588 ± 30 U/L; $P < 0.001$; Fig. 1]. Serum inhibin levels increased after 1 week of GnRH administration ($P < 0.05$) and remained significantly higher than the baseline value throughout the course of treatment. At no time during treatment was the mean inhibin level in the IHH men as high as that in the normal men. The mean of the peak serum inhibin values attained in each man with IHH during treatment was 488 U/L, significantly ($P < 0.05$) less than the mean value in the normal men (588 ± 30 U/L).

Serum LH and FSH levels rose in a manner similar to the rise in inhibin, increasing significantly ($P < 0.001$) to within the normal range after 1 week and remaining within that range or slightly above it throughout treatment. Serum T levels also rose to become significantly ($P < 0.001$) higher than the baseline level after 4–5 weeks of GnRH treatment and remained in the low normal range thereafter. Testicular volumes increased in all men, ranging from 3–12 mL before and 6–20 mL after 8 weeks of GnRH administration. All men had azoospermia throughout the study period.

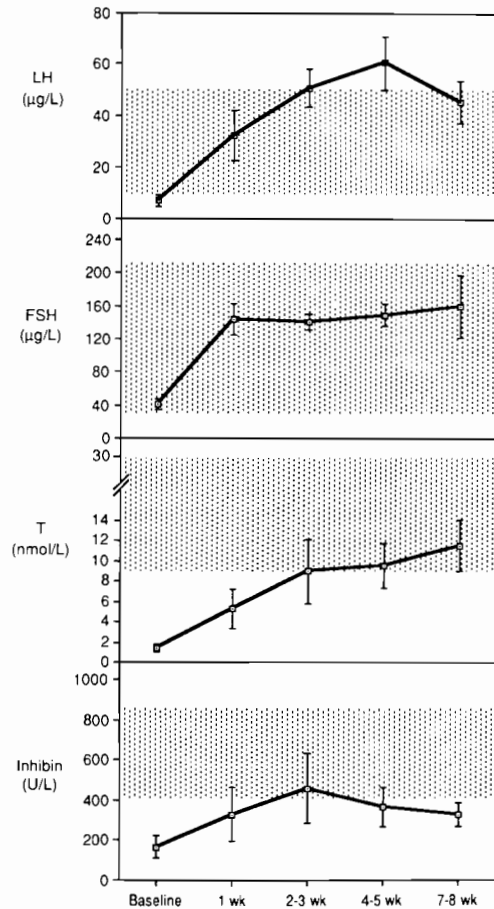


FIG. 1. Mean (\pm SE) serum inhibin (units per L), T (nanomoles per L), FSH (micrograms per L), and LH (micrograms per L) in 5 men with IHH before and during 8 weeks of treatment with GnRH (5 μ g every 2 h). The stippled areas denote the normal ranges for T, FSH, and LH, and are the means and 95% confidence limits derived from 90 normal men. For inhibin, the stippled area denotes the absolute range derived from 19 normal men.

Discussion

These results demonstrate that men with IHH have markedly decreased serum inhibin levels compared with those in normal men. Stimulation of pituitary gonadotropin secretion by pulsatile GnRH administration led to prompt (within 1 week) and persistent increases in serum inhibin levels. However, after 8 weeks of therapy, serum inhibin levels did not reach the levels in normal men. These results are consistent with the hypothesis that inhibin is produced by the testes under gonadotropic control and that gonadotropin deficiency causes inhibin deficiency.

Burger *et al.* (6) recently reported that serum inhibin levels in normal boys rose by 214%, from 161 U/L at stage I to 506 U/L in adulthood, and that serum inhibin levels correlated positively with serum LH, FSH, and T levels in these boys. Compared with normal boys, the IHH men who were treated with GnRH had a similar

pattern of inhibin stimulation, but the changes occurred with greater rapidity and were of lesser magnitude. From a baseline of 166 ± 56 U/L, serum inhibin levels rose significantly after 1 week of GnRH therapy, reaching a maximum value of 458 U/L (a 176% increase) after 2-3 weeks.

We recently demonstrated that LH as well as FSH exerts a stimulatory effect on inhibin levels (18). Using the experimental paradigm of suppressing both LH and FSH secretion in normal men by exogenous T administration, followed by selective LH or FSH replacement, we found either gonadotropin to be capable of increasing serum inhibin levels. Both LH and FSH levels were increased by GnRH therapy in this study; therefore, it is not possible to determine the relative roles of each in the increase in serum inhibin levels.

Since inhibin is synthesized and secreted by Sertoli cells, measurement of this substance may serve as an index of the function of these cells (19). Since Sertoli cell function in IHH was stimulated within 1 week by the rise in gonadotropin levels induced by GnRH, long before a spermatogenic response can be measured (20), it is evident that inhibin secretion is not dependent on or closely linked to complete spermatogenesis.

Serum inhibin and T levels both increased only into the low normal range in these men during GnRH treatment, despite the fact that serum FSH and LH levels increased to mean levels well within or slightly above their respective normal ranges. It is possible that the 8-week period of stimulation, the GnRH dose, and/or the pattern of LH and FSH pulses in response to the GnRH pulses were not sufficient for full maturation of either Sertoli or Leydig cells. Since the blood samples were obtained randomly during GnRH administration, there is no reason to believe that the serum LH and FSH values are overestimates or that the serum inhibin and T values are underestimates, particularly since the clearance of the latter two hormones is slow relative to the stimulation pattern.

Alternately, the diminished responses of these hormones could signify permanent testicular dysfunction as a result of chronic gonadotropin deficiency. Marked proliferation of Sertoli cells occurs in the first 3 months of human life (21), a process which, at least in perinatal rats, has been demonstrated to be under the control of FSH (22, 23). It is, therefore, possible that the gonadotropin-dependent postnatal growth phase fails to occur in men with IHH. If this is the case, the resulting reduction in Sertoli cell numbers may not allow quantitatively normal inhibin levels to be produced, despite optimal GnRH therapy. In addition, as Sertoli cell depletion has been shown to be associated with approximately equal depletion of spermatids (24), sperm production may also be diminished in quantity. This may

account for the finding that some men with IHH are unable to achieve complete spermatogenesis during gonadotropin or GnRH therapy, while sperm concentrations in those who respond are often lower than those in normal men (13, 25).

The possibility that defective Leydig cell function is present in men with IHH also is suggested by the finding that serum T levels do not always normalize in men with IHH during either prolonged hCG (26) or pulsatile GnRH therapy (27). It has been proposed that this failure of testicular responsiveness is not a general occurrence in IHH, but is limited to a subgroup of men with bilateral cryptorchidism (28). However, two of five noncryptorchid men with IHH who normalized their serum LH and FSH levels after 1 week of GnRH administration continued to have subnormal serum T levels after 8 weeks of therapy. Coupled with their somewhat subnormal serum inhibin levels, the low to low normal serum T levels achieved during GnRH therapy support the possibility of combined Leydig and Sertoli cell dysfunction in some men with IHH.

In conclusion, serum inhibin levels in men with IHH are low and increase in response to a GnRH-induced rise in serum gonadotropins. However, after 8 weeks of GnRH treatment neither serum T nor inhibin achieved normal levels, suggesting the possibility of defective Leydig and Sertoli cell function, respectively.

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