

Comments

Follicle-Stimulating Hormone Is Required for Quantitatively Normal Inhibin Secretion in Men*

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ABSTRACT. Inhibin is a glycoprotein hormone produced by the testis and ovary which is postulated to be an important regulator of pituitary FSH secretion. Animal data indicate that inhibin is produced by the Sertoli cells of the testis under the influence of FSH. To determine the role of FSH withdrawal and replacement in the control of inhibin secretion in man, we measured serum inhibin concentrations in men in whom isolated FSH deficiency had been produced by chronic hCG administration; this was followed by FSH replacement. After a 3-month control period, four normal men received hCG for 7 months, resulting in suppression of serum FSH to undetectable levels and urinary FSH excretion to prepubertal levels. Their mean serum inhibin levels fell to 70% of control values during hCG administration [362 ± 60 (\pm SE) *vs.* 518 ± 56 U/L; $P < 0.01$]. While continuing hCG, testosterone enanthate was administered for a further 6 months. Serum FSH and inhibin levels remained

suppressed to a similar degree. Testosterone administration then was ceased, and hCG continued for a further 2–4 months. Then, while continuing hCG administration, FSH was replaced as either highly purified human FSH ($n = 2$) or human menopausal gonadotropin ($n = 2$) for a period of 4–10 months. Serum FSH levels increased to the mid- and upper normal male ranges, respectively. FSH replacement restored serum inhibin levels to 522 ± 56 U/L ($P = \text{NS vs. control}$). In summary, prolonged selective FSH deficiency induced by chronic hCG administration suppressed inhibin secretion. Replacement of FSH activity restored inhibin secretion to control values. We conclude that 1) FSH is not absolutely required for inhibin secretion in men; and 2) the maintenance of quantitatively normal inhibin secretion requires the combined action of both gonadotropins. (*J Clin Endocrinol Metab* 67: 1305, 1988)

INHIBIN is a glycoprotein hormone produced by the testis and ovary that is believed to have an important role in the physiological regulation of pituitary FSH secretion (1). Animal data indicate that the Sertoli cells of the testis are the primary source of inhibin (2–5) and that FSH stimulates inhibin secretion both *in vivo* (6) and in isolated Sertoli cell cultures (5, 7–10). The control of inhibin secretion in men has not been studied. To investigate the effect of FSH on inhibin secretion in men, we measured serum inhibin concentrations during prolonged FSH deficiency and FSH replacement produced in normal men by chronic hCG administration (11) and then during combined hCG and FSH treatment (12).

Materials and Methods

Subjects

The 4 normal men, aged 30–39 yr, studied were among the 10 participants in a study of the hormonal control of spermatogenesis, the results of which were reported previously (12). These men had 6 normal seminal fluid analyses and normal basal and GnRH-stimulated serum LH, FSH and testosterone (T) levels. The study was reviewed, approved, and performed in 1982–83.

Experimental protocol

Control period. All men underwent a 3-month control period during which no hormones were given.

First hCG alone period. The men then received hCG (Profasi, Serono Laboratories, Inc., Braintree, MA; 5000 IU, im, twice weekly) for 7 months.

hCG plus T period. While continuing the same dosage of hCG, T enanthate (Delatestryl, E. R. Squibb and Sons, Princeton, NJ; 200 mg, im) was given weekly for 6 months.

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Second hCG alone period. After the period of combined hCG and T administration, the men continued to receive 5000 IU hCG, im, twice weekly alone for 2–4 months.

hCG plus FSH period. FSH then was coadministered with hCG for 4–10 months. Two men (subjects 1 and 2) received 100 IU human (h) FSH, sc, daily. This highly purified preparation (LER 1577; lot 4) was kindly provided by the National Pituitary Agency (Baltimore, MD) and contained less than 1% contamination with LH bioactivity. Due to the limited availability of this hFSH material, the remaining men (subjects 3 and 4) received human menopausal gonadotropin (hMG; Pergonal, Serono Laboratories; 75 IU, sc, daily).

Third hCG alone period. After the hCG plus FSH, two men (subjects 1 and 4) continued to receive hCG alone for an additional 3 months.

Recovery period. The men then were followed for 3–6 months until three successive sperm counts returned to the each men's own control range. The blood samples analyzed for this study were obtained monthly immediately before hormone injections, and the serum was stored at -20 C .

Analytic methods

Serum inhibin was measured using a heterologous RIA described previously, which uses an antiserum (As 1989) raised to 31K bovine follicular fluid inhibin (13). The As 1989 RIA was specific for intact inhibin; the cross-reactivity of transforming growth factor- β , bovine activin-A, and free inhibin subunits after reduction and alkylation of 31-K bovine inhibin was less than 1%. A partially purified human follicular fluid inhibin preparation was prepared for use as the RIA standard (14). The sensitivity (ED_{10}) of the assay was 100 U/L, and the ED_{50} was 550 U/L. The interassay coefficient of variation was 11% ($n = 5$ assays). The intraassay coefficients of variation in the low, mid- and high ranges of the standard curve were 12.0%, 3.3%, and 4.8%, respectively, for five assays. Inhibin immunoactivity has been found to be stable during repeated freezing and thawing (14). The control serum inhibin levels in the study subjects were approximately 25% lower than those in a recently studied group of normal young men, suggesting that there had been some loss of immunoactivity during the prolonged storage of the samples used in this study. However, all of the samples had been stored for approximately the same length of time (5 yr) so that any loss of immunoactivity during storage could not account for the differences between the various hormonal manipulations during the study. All samples from an individual man were analyzed in duplicate at the same time.

The results of measurements of serum and urinary FSH, serum T, and estradiol (E_2) and sperm concentrations in these men were included in the report describing the effects of this study protocol on spermatogenesis in 10 normal men (12). The results in these 4 men are included in this report so that the effects of the various exogenous hormone regimens on serum inhibin levels can be interpreted with knowledge of the changes in gonadotropin, sex steroid, and sperm concentrations that occurred.

Statistical analysis

The monthly serum inhibin levels during each phase of hormone administration were averaged for each man. To assure that each hormonal manipulation had sufficient time to be effective and to have been adequately washed out before the next study, only results obtained during the following time intervals were included in these analyses: 1) control period, all values until the day of commencement of hCG; 2) hCG alone periods, all values obtained at least 1 month after commencement of hCG (period 1), at least 1 month after cessation of T (period 2), and at least 2 weeks after the last FSH injection (period 3); 3) hCG plus T, at least 1 month after initiation of T and within 2 weeks of the last T injection; 4) hCG plus FSH, at least 2 weeks after initiation of hFSH/hMG treatment until the day of its cessation; 5) recovery period, all values obtained at least 1 month after cessation of hCG, starting when the first of three consecutive sperm counts were within the man's own control range. The mean monthly serum FSH, T, and E_2 levels and the mean sperm concentrations for each period of study were calculated for each man in a similar way. Sperm concentrations were log transformed before statistical analysis. The existence of a significant difference across the treatments was assessed using analysis of variance with repeated measures. Differences between individual treatments were assessed using Student's paired t test with correction for repeated comparisons using the Bonferroni method. $P < 0.05$ was considered significant. The results are expressed as the mean \pm SE.

Results

The mean serum inhibin levels in the four men during the various study periods are shown in Fig. 1. During the first period of hCG-induced FSH suppression, serum inhibin levels fell to 70% of control values (362 ± 60 vs. 518 ± 56 U/L; $P < 0.01$). During the second hCG alone period, the mean inhibin level was 392 ± 84 U/L, slightly but not significantly lower than the control level. During the third hCG alone period (subjects 1 and 4 only), serum inhibin levels were 262 and 286 U/L, respectively. During the hCG plus T period, the mean serum inhibin level remained low (312 ± 30 U/L; $P = \text{NS}$ vs. hCG alone). Replacement of FSH activity increased serum inhibin to 522 ± 56 U/L ($P = \text{NS}$ vs. control). Compared with the serum inhibin level during hCG alone, inhibin levels tended to be higher in subjects 1 and 4 given hFSH (89% and 50% increases, respectively) than in subjects 3 and 4 given hMG (19% and 43% increases, respectively). During the recovery period, the mean inhibin level remained within the control range (442 ± 40 vs. 518 ± 56 U/L; $P = \text{NS}$).

Serum FSH levels fell to undetectable levels (126 ± 14 to <25 $\mu\text{g/L}$) during the three hCG alone periods and the hCG plus T period. Similarly, urinary FSH fell from the normal adult range (398 ± 173 mIU/h) into the prepubertal range (34 ± 5 mIU/h). During hCG plus hFSH treatment (subjects 1 and 2) serum FSH increased

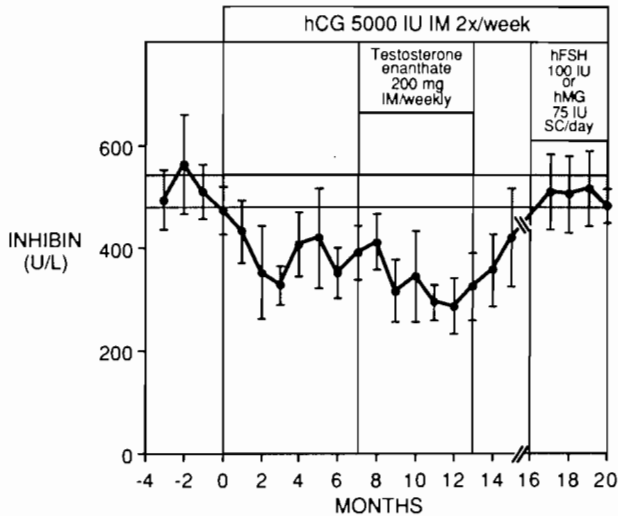


FIG. 1. Mean (\pm SE) serum inhibin levels in the four men during the control, hCG alone, hCG plus T, and hCG plus hFSH periods. The horizontal shaded area indicates the mean \pm SE control value. The break in the time axis between 15 and 17 months is due to the fact that two men received hCG alone for an additional 2 months before FSH administration. Note that the mean serum inhibin level decreased to the same extent during the hCG alone and hCG plus T periods and increased into the control range during combined hCG plus hFSH administration.

to 373 and 297 μ g/L, respectively, approximately 3-fold higher than during the control period. On the other hand, serum FSH levels increased to levels similar to those during the control period in the two men (subjects 3 and 4) who received hMG replacement (95 and 86 μ g/L, respectively).

Serum T levels were 18 ± 0.7 , 35 ± 7.3 , and 55.2 ± 10.8 nmol/L during control, the first hCG alone, and the hCG plus T periods, respectively. The corresponding E_2 values were 139 ± 51 , 338 ± 114 , and 488 ± 110 pmol/L, respectively.

Sperm concentrations during the control period were $125 \pm 39 \times 10^6$ /mL, fell after hCG treatment to $23 \pm 10 \times 10^6$ /mL, and remained suppressed to a similar degree during the hCG plus T ($29 \pm 13 \times 10^6$ /mL) and the second hCG alone periods ($34 \pm 13 \times 10^6$ /mL). During the FSH plus hCG period, sperm concentrations increased significantly in all four men, reaching a mean of $103 \pm 30 \times 10^6$ /mL during the last 2 months of hCG plus FSH administration ($P = NS$ vs. control).

Discussion

These results demonstrate that FSH is required for maintenance of normal inhibin secretion. Selective suppression of FSH by hCG administration resulted in decreased serum inhibin levels, and they returned to normal during the selective replacement of physiological amounts of FSH. The experimental approach was based

on the suppression of endogenous gonadotropins by elevated T and E_2 levels produced by high dose hCG administration (11). In these men, chronic hCG administration produced markedly elevated levels of LH-like bioactivity, as evidenced by a 2- to 3-fold rise in serum T and E_2 levels. The resultant hormonal setting was, therefore, one of normal to high intratesticular T levels and selective FSH deficiency. The latter was confirmed by the finding of undetectable serum FSH levels and suppression of urinary FSH excretion into the prepubertal range. As a result of the selective FSH deficiency, serum inhibin levels declined to 70% of control values. The further elevation of serum T during hCG plus T treatment did not further lower the already suppressed FSH or decreased inhibin levels.

Replacement of FSH activity, as either highly purified hFSH or hMG, increased serum FSH levels to control or higher levels and restored serum inhibin to control levels in all four men. Previously, the stimulatory effect of FSH on testicular inhibin secretion has been examined only in short term *in vivo* studies in rats (6) and in Sertoli cell cultures (7-10). Our study, on the other hand, describes the effect of long term FSH replacement in men during an experimental regimen designed to induced chronic isolated FSH deficiency. We attribute the stimulation of inhibin secretion by hMG to its FSH component. Although hMG does contain LH, this additional LH bioactivity would be trivial compared to the supra-physiological levels resulting from the concomitant hCG administration.

The fact that mean serum inhibin levels did not fall below 70% of the control value during hCG-induced FSH suppression suggests that factors in addition to FSH stimulate inhibin secretion. A role for both gonadotropins in the control of inhibin secretion seems likely, based on our recent study in which serum inhibin levels fell to approximately 40% of control values after suppression of LH and FSH by exogenous T administration in normal men, and serum inhibin levels were partially restored to 64% and 55% of the control value when either FSH or LH, respectively, were coadministered with T and to 63% of the control value when hCG was given in a dosage similar to that used in this report (15). The mechanism of LH stimulation of inhibin secretion is unknown. The very small amount of FSH-like bioactivity intrinsic to the hCG molecule [$\sim 0.1\%$ the amount of LH-like bioactivity (16)] was unlikely to be important in stimulating inhibin secretion. We conclude that the LH bioactivity administered in the form of hCG acted to maintain inhibin levels above those found during suppression of both LH and FSH during T administration (15).

Sperm production changed in the same way as serum inhibin in response to gonadotropin withdrawal and re-

placement (12, 17–20). Sperm counts were markedly reduced during gonadotropin withdrawal, were partially restored by either LH (17, 19, 20) or FSH (18) replacement, and were restored to normal only by combined hCG and FSH treatment (12). The covariation of inhibin secretion and sperm concentrations during the study suggests that inhibin is a marker for spermatogenesis and is consistent with its proposed role in the regulation of FSH secretion.

In summary, prolonged selective FSH deficiency, induced by chronic hCG administration, results in reduced inhibin secretion in normal men. FSH replacement restored inhibin secretion to normal. We conclude that FSH is not absolutely required for inhibin secretion, but is necessary for the maintenance of normal inhibin secretion.

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