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Serum Inhibin Concentrations before and during Gonadotropin Treatment in Men with Hypogonadotropic Hypogonadism: Physiological and Clinical Implications*

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ABSTRACT. We measured by RIA the inhibin concentrations in the sera of 20 men with hypogonadotropic hypogonadism before and during treatment with gonadotropins in order to determine the role of gonadotropins in the control of inhibin secretion and the utility of the serum inhibin concentration in assessing the spermatogenic response to gonadotropin treatment in these patients.

Before treatment the mean serum inhibin concentration in the 20 hypogonadotropic men as a group (391 ± 49 U/L) was significantly lower ($P < 0.001$) than that in 27 normal men (741 ± 52 U/L). In the 7 men whose hypogonadism was of postpubertal onset, the mean serum inhibin concentration (559 ± 69 U/L) was not significantly lower than that in normal men. In the 13 men whose hypogonadism was of prepubertal onset, the serum inhibin level was significantly lower [381 ± 74 U/L ($P < 0.01$) in the 7 without a history of cryptorchidism and 207 ± 46 U/L ($P < 0.01$) in the 6 with a history of cryptorchidism]. All 20 patients were azoospermic or severely oligospermic and had distinctly subnormal serum testosterone concentrations, even those whose serum inhibin values were normal. In the 7 patients with postpubertal hypogonadism, treatment with hCG alone for 6 months increased the serum testosterone concentration and maximum sperm count to normal, even though the previously normal inhibin concentration was not increased further. In the 13 patients with prepubertal hypogonadism, treatment with hCG alone increased the serum inhibin concentration, and combined treatment with hCG and human menopausal gonadotropin (hMG) increased inhibin further, to well within the normal range (742 ± 143 U/L) in the patients without a history of cryptorchidism and to just within the normal range (487 ± 96 U/L) in those with such a history. In the 7 patients with prepubertal hypogonadism but no history of cryptorchidism, treatment with hCG and hMG increased the maximum sperm count to normal in 5. In the 6 patients with prepubertal hypogonadism who did have a history of cryptorchidism, hCG and hMG treatment produced a normal sperm count in only 1. Of 12 patients whose serum inhibin level was more than 300 U/L before treatment, 11 developed a normal maximum sperm count in response to treatment, but of 8 patients whose inhibin concentration was less than 300 U/L before treatment, only 2 developed a normal sperm count in response to treatment ($P < 0.01$).

We conclude that 1) both FSH and LH independently stimulate inhibin secretion; 2) normal Sertoli cell function, as judged by the serum inhibin concentration, appears to be necessary, but not sufficient, for spermatogenesis; and 3) the pretreatment serum inhibin concentration predicts the spermatogenic response to gonadotropin treatment. (*J Clin Endocrinol Metab* **70:** 1414–1419, 1990)

DEFICIENT spermatogenesis as a consequence of gonadotropin deficiency should, in theory, be readily treatable by the administration of hCG, which acts similarly to LH, and human menopausal gonadotropin (hMG), which contains FSH. Alternatively, if the defect is hypothalamic, administration of GnRH should stimulate endogenous LH and FSH secretion. In practice, however, such treatment is often prolonged and may be unsuccessful (1–5). A few recent studies of gonadotropin treatment of men with hypogonadotropic hypogonadism were designed to ascertain some of the factors associated with a response to treatment (1, 2, 4). In one of these studies (1), we found that men whose hypogonadism was acquired postpubertally developed sperm counts within the normal range in response to treatment with hCG alone, but that men whose hypogonadism was of prepubertal onset usually developed sperm counts within the normal range only when treated with a combination of hCG and hMG, unless the hypogonadism was associated with a history of cryptorchidism (usually unilateral), in which case the patients usually remained azoospermic even when treated with both hCG and hMG. During that

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study the adequacy of the hCG dose and the role of the Leydig cell were assessed by measuring the serum testosterone concentration. The adequacy of the hMG (FSH) dose and the role of the Sertoli cell, however, could not be ascertained, except indirectly by the number of ejaculated sperm.

The subsequent purification of inhibin (6), the heterodimeric glycoprotein that is produced and secreted principally by the Sertoli cell (7–9), and the development of an immunoassay for its measurement in human serum (10) permitted us to measure the inhibin concentrations in the stored sera from that study (1). By measuring the serum inhibin concentration before and during the course of treatment with hCG alone and the combination of hCG and hMG, we evaluated the roles of FSH and LH in controlling inhibin secretion and the usefulness of serum inhibin concentration in predicting the spermatogenic response to gonadotropin treatment.

Materials and Methods

Subjects

Twenty men with hypogonadotropic hypogonadism, 20–59 yr old (median, 31 yr), participated in a study of the effects of gonadotropin treatment on spermatogenesis. The study was approved by the University of Pennsylvania Committee on Studies Involving Humans, and all participants gave written informed consent. Gonadotropin administration was performed in 1978–1988, and the clinical data, serum FSH and testosterone concentrations, urinary FSH excretion, and sperm counts before and in response to gonadotropin treatment in 18 of these men were reported previously (1). The diagnosis of hypogonadotropic hypogonadism was based on the finding of a subnormal serum testosterone concentration (<3 ng/mL; 10.4 nmol/L) and serum LH and FSH concentrations within or below the normal adult male range (FSH, 3–14 IU/L; LH, 3–18 IU/L). Seven of the 20 men had evidence supporting a postpubertal onset of hypogonadism, including a history of normal puberty and the finding of normal virilization and testicular volumes at initial diagnosis. Three of the 7 had fathered children previously. Hypogonadism was attributed to pituitary adenoma in 4, craniopharyngioma in 2, and an unknown cause in 1. The remaining 13 had evidence supporting a prepubertal onset of hypogonadism, including a history of subnormal pubertal development and a physical examination showing incomplete virilization, small testes, and eunuchoid body proportions. Six of the men had a history of cryptorchidism (unilateral in 5, bilateral in 1), which had been corrected by orchiopexy in childhood. The 15 patients who had been taking testosterone enanthate discontinued it at least 2 months before entry into the study.

For comparison, serum was obtained from 27 normal men, 25–40 yr old, 9 who were enrolled in this original study and 18 who participated in four contemporaneous studies (11–14).

Experimental protocol

The study involved three phases: a control period, a period of hCG administration alone, and a period of hCG in combination with hMG (1). During the control period two serum samples were collected on each of 3 days, and two semen samples were obtained by masturbation after 24–48 h of abstinence. The initial hCG treatment was 2000 U, im, every Monday, Wednesday, and Friday. The dose was increased or decreased in the three men whose serum testosterone concentrations were not within the normal range within 2 months. If the sperm count did not enter the lower limit of the normal range (>43 million/ejaculate) after the serum testosterone concentration had been within the normal range for 6 months, hCG was continued, and hMG was added (75 IU, im, every Monday, Wednesday, and Friday for 4 months and 150 IU for another 4 months). Serum and semen samples were obtained every 2 weeks throughout the study period.

Hormone assays and sperm counts

Serum inhibin was measured with a heterologous double antibody RIA, using an antiserum to purified 31K bovine follicular fluid inhibin and the same antigen labeled with ^{125}I as tracer (10). The assay standard was a serum pool obtained from women undergoing ovulation induction with hMG for *in vitro* fertilization. This pool was calibrated against a partially purified human follicular fluid inhibin standard preparation of defined bioactivity (15). Serial dilutions of serum gave dose-response lines parallel to that of the standard. Bovine activin-A, Mullerian inhibitory substance, and porcine and human transforming growth factor-β had less than 1% cross-reactivity in this assay, as did free α- and β-subunits of inhibin obtained by reduction and alkylation of 31K inhibin. Recently, an α-subunit-derived dimeric protein (termed pro-α-C) has been isolated from bovine follicular fluid (16). This substance cross-reacts in this inhibin RIA, but shows no inhibin-like bioactivity. Whether pro-α-C or other cross-reacting substances occur in humans, and if so, whether they circulate in blood in significant quantity are unknown. Studies of the ratio of bioactivity to immunoactivity in women (10) and rats (17) suggest that any interference of nonbioactive substances in the present RIA is small. The sensitivity (ED_{10}) of the assay was 100 U/L, and the ED_{50} was 660 U/L. The within-assay coefficients of variation (CVs), based on repeated measurements of multiple dilutions of serum samples covering the bound to free ratio range 40–90%, were 5.8%, 3.4%, and 1.8% in the upper, middle, and lower portions of the working range. The between assay CV was 10.2% from 14 assays.

Serum LH and FSH were newly measured for the current study by RIAs described previously (18). The LH reference standard (LER 907) and antibody (antihuman LH, batch 2) were supplied by the National Hormone and Pituitary Program. The tracer was purified hCG radioiodinated with ^{125}I using chloramine-T. The sensitivity of the assay was 2.0 μg/L, and the intra- and interassay CVs were 5.5% and 8.4%, respectively. FSH reference standard (LER 907) and antibody (antihuman FSH, batch 5) were also supplied by the National Hormone and Pituitary Program. The tracer was FSH HS-1 radioiodinated with ^{125}I using chloramine-T. The sensitivity of the assay

was 15 μg/L, and the intra- and interassay CVs were 7.3% and 9.7%, respectively. All samples from an individual man were analyzed in duplicate in the same LH and FSH assays. The results of both assays were calculated using the computer program of Burger *et al.* (19).

Serum testosterone was determined by a RIA described previously (1), using ether extracts of sera. The cross-reactivity of dihydrotestosterone was 50%. The sensitivity of the assay was 20 pg/tube, and the intra- and interassay CVs were 8.9% and 13.3%, respectively.

Sperm density was determined by hemocytometer and was multiplied by the ejaculated volume to give sperm count per ejaculate.

Statistical analyses

For each subject, all data points obtained during each treatment phase were averaged, except for the sperm counts, where the three highest values during each treatment phase were averaged and called the maximum sperm count. These individual mean values were used to calculate the group mean ± SE. The existence of a hormone change between treatments was assessed by repeated measures analysis of variance as well as Fisher's protected least significant difference multiple comparison procedure. The mean hormone difference between patient groups was assessed using one-way analysis of variance and Duncan's multiple comparisons procedure. Simple multiple regression and contingency table analysis were used to evaluate the associations among measurements. $P < 0.05$ was considered significant.

Results

Pretreatment period

Pretreatment inhibin concentrations and other reproductive endocrine values in the three groups of hypogonadotropic men are shown in Table 1. The 20 hypogonadotropic patients as a group had a significantly ($P < 0.001$) lower serum inhibin concentration (391 ± 49 U/L) than the 27 normal men (741 ± 52 U/L). The 3 subgroups of patients, however, showed strikingly different degrees of inhibin deficiency. The patients who had a postpubertal onset of hypogonadism had a mean value (559 ± 69 U/L) in the lower part of the normal range (486–1201 U/L), whereas the patients who had a prepubertal onset but no history of cryptorchidism had a significantly ($P < 0.01$) lower value (381 ± 74 U/L) than normal, and the patients who had a prepubertal onset and also had a history of cryptorchidism had an even lower value (207 ± 46 U/L; $P < 0.01$).

Pretreatment serum concentrations of FSH, LH, and testosterone and pretreatment testicular size also tended to be lower in the prepubertal than in the postpubertal patients and lower in those with a history of cryptorchidism than in those without (Table 1). Pretreatment sperm counts were 0 in 17 of the 20 patients and less than 5×10^6/ejaculate in the other 3.

Simple regression analysis of the pretreatment inhibin concentration and testicular length showed a significant correlation ($r = 0.79$; $P < 0.01$), as shown in Fig. 1.

hCG treatment

hCG treatment increased the mean serum inhibin concentration significantly ($P < 0.01$) from 300 ± 50 to 427 ± 56 U/L in the 13 men with a prepubertal onset of hypogonadism, and in the group without a history of cryptorchidism the mean value increased to just within the normal range (534 ± 75 U/L; Fig. 2, *top panel*). In contrast, the mean inhibin concentration in the men with a postpubertal onset, whose values were within the normal range before treatment, did not increase significantly in response to treatment.

hCG treatment, as previously reported (1), increased the maximum total sperm count to within the normal range in all seven patients with postpubertal onset of hypogonadism, but in only one of the seven patients with a prepubertal onset without a history of cryptorchidism and in none of the six patients with a prepubertal onset and with a history of cryptorchidism (Fig. 2, *bottom panel*). hCG treatment increased the serum testosterone concentrations into the normal range in all subjects, as previously reported (1).

Combined hCG and hMG treatment

The 12 prepubertal hypogonadotropic patients who did not achieve a maximum sperm count within the

TABLE 1. Pretreatment values of 20 men with hypogonadotropic hypogonadism

	Serum LH (μg/L)	Serum FSH (μg/L)	Serum inhibin (U/L)	Serum testosterone (nmol/L)	Testicular length (cm)	Sperm count (10^6/ejaculate)
Normal men (n = 27)	25.6 ± 3.0	108.9 ± 9.1	741 ± 52	[a]	[a]	[a]
Hypogonadotropic hypogonadism						
Postpubertal onset (n = 7)	13.0 ± 1.9	77.3 ± 17.8	559 ± 69	4.3 ± 0.9	4.8 ± 0.4	1 ± 1
Prepubertal onset, noncryporchid (n = 7)	9.3 ± 1.9	45.0 ± 11.0	381 ± 74	4.1 ± 1.2	2.9 ± 0.5	1 ± 0
Prepubertal onset, cryptorchid (n = 6)	5.5 ± 0.6	17.8 ± 1.4	207 ± 46	3.1 ± 0.7	1.8 ± 0.2	0 ± 0

Data presented are the mean ± SE. The mean serum inhibin concentrations in both the prepubertal noncryptorchid and the prepubertal cryptorchid groups were significantly less ($P < 0.01$) than that in the normal men.

[a] Data not available for these men or not measured in same assay system. Normal ranges: serum testosterone. 10–33 nmol/L: total sperm count, more than 43×10^6 ejaculate; testicular length, 3.5–6.0 cm.

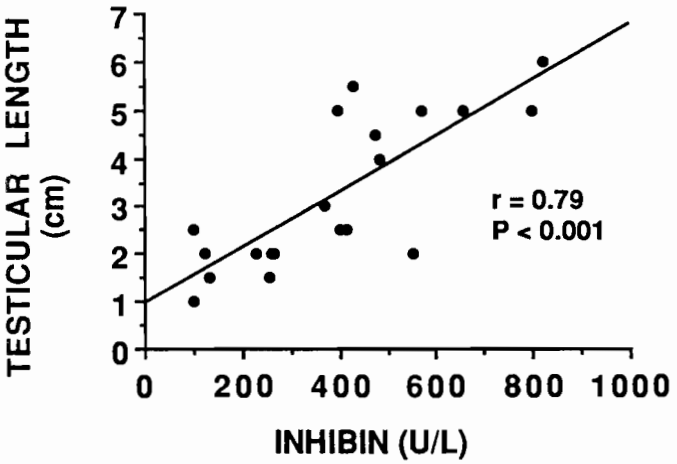

FIG. 1. Correlation of pretreatment testicular length with pretreatment serum inhibin concentration in 20 men with hypogonadotropic hypogonadism.

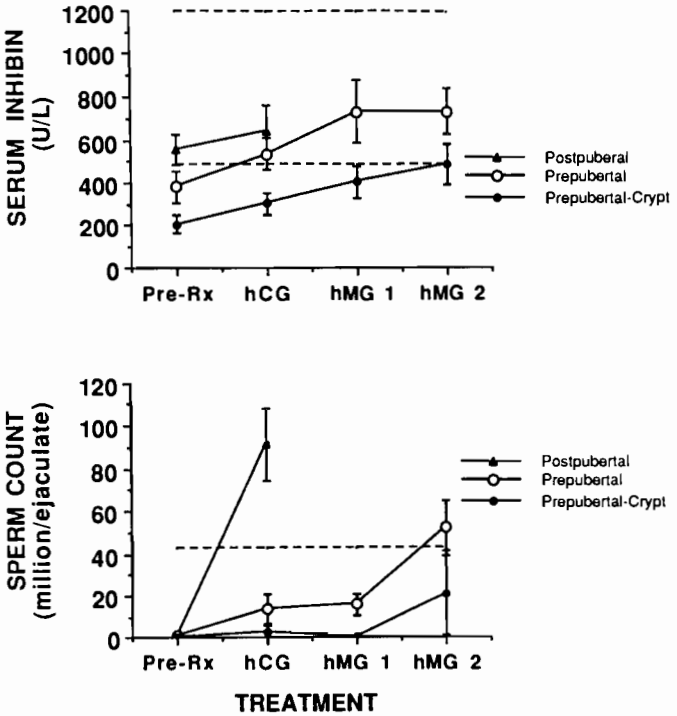

FIG. 2. The serum inhibin concentrations and maximum sperm counts in response to gonadotropin treatment in 20 men who had hypogonadotropic hypogonadism. Patients are grouped by the time of onset of their hypogonadism (postpubertal in 7, prepubertal in 13), and those with prepubertal onset by whether they had a history of corrected cryptorchidism (6 did, unilateral in 5). Treatment consisted of hCG until the serum testosterone level was normal for 6 months and, in those 12 patients who did not achieve a normal sperm count in that time, the combination of hCG and 75 U hMG 3 times a week for 4 months (hMG 1) and hCG and 150 U hMG 3 times a week for 4 months (hMG 2). Values are the mean ± SE. Sperm count values for 18 of the 20 patients were reported previously (1) and are shown here only for comparison to the inhibin values. *Dashed lines* indicate the normal range in the *upper panel* and the lower limit of normal in the *lower panel.*

normal range during treatment with hCG alone were then treated with the combination of hCG and hMG; the three times weekly hMG dose was 75 U for 4 months and 150 U for another 4 months. The lower dose of hMG increased the mean serum inhibin concentration significantly (568 ± 96 U/L; $P = 0.01$) in these 12 patients and to well within the normal range in those without a history of cryptorchidism (742 ± 143 U/L; Fig. 2, *top*). The higher dose of hMG increased the mean inhibin concentration to just within the normal range in the patients with a history of cryptorchidism (487 ± 96 U/L), but did not further increase the concentration in the noncryptorchid patients (732 ± 106 U/L).

Combined hMG treatment of the prepubertal hypogonadotropic patients increased the mean maximum sperm count to within the normal range in those without a history of cryptorchidism, but not in those with such a history (Fig. 2, *bottom*). In the latter group the small increase in the mean value shown was due entirely to an increase to 122 million in a single patient, whose pretreatment inhibin value was, interestingly, the highest in that group (400 U/L).

Correlations of pretreatment values with results of treatment

Contingency table analysis revealed a significant direct correlation between the pretreatment serum inhibin concentration and the likelihood of development of a maximum sperm count within the normal range in response to gonadotopin treatment (Table 2). Of the 12 men whose pretreatment serum inhibin concentrations were greater than 300 U/L, 11 developed sperm counts within the normal range in response to treatment, but of the 8 men whose pretreatment inhibin concentrations were less than 300 U/L, only 2 developed a sperm count within the normal range ($P < 0.01$).

Development of a sperm count within the normal range also correlated significantly with initial testicular size. All 12 patients whose average pretreatment testicular

TABLE 2. Correlation of the development of a maximum sperm count within the normal range in response to gonadotropin treatment with the pretreatment serum inhibin concentration in 20 men with hypogonadotropic hypogonadism

	Sperm count at end of Rx	
	Normal	Subnormal
Pre-Rx inhibin >300 U/L	11	1
Pre-Rx inhibin <300 U/L	2	6

Patients were treated as described in Fig. 2. A normal sperm count was considered to be greater than 43×10^6/ejaculate. By contingency table analysis the relationship between the pretreatment serum inhibin concentration and achieving a normal sperm count was statistically significant ($P < 0.01$).

length was greater than 2.5 cm developed a normal sperm count in response to gonadotropin treatment, but of the 8 patients whose average pretreatment testicular length was less than 2.5 cm, only 1 developed a sperm count within the normal range ($P < 0.001$).

Discussion

These results of measuring the serum inhibin concentrations in 20 men with hypogonadotropic hypogonadism before and during treatment with exogenous gonadotropins have several implications, both physiological and clinical. One physiological implication is that inhibin secretion is dependent upon gonadotropin secretion. This conclusion is based on finding that the patients who had the lowest serum FSH and LH concentrations, *i.e.* those whose hypogonadism was of prepubertal onset, especially when associated with a history of corrected cryptorchidism, had the lowest serum inhibin concentrations. In addition, treatment of the more severely hypogonadal patients, both with hCG alone and then with the combination of hCG and hMG, significantly increased their serum inhibin concentrations, eventually to normal. These results are consistent with those of our previous studies on the role of gonadotropins in inhibin secretion in normal men. Administration of testosterone enanthate in doses sufficient to suppress the serum FSH and LH concentrations of normal men produced a decrease in their mean serum inhibin concentration to 38% of the pretreatment value, but adding hCG to the treatment regimen increased the inhibin concentration to 63% of the pretreatment value (20). In another study, administration of hCG to normal men decreased their mean serum inhibin concentration to 70% of the pretreatment value, and addition of hMG to the treatment regimen increased inhibin to a level comparable to that before treatment (21). Those studies and the results reported here both suggest that FSH and LH independently stimulate inhibin secretion.

While FSH probably stimulates inhibin secretion by acting directly on the Sertoli cell, the mechanism by which LH stimulates inhibin secretion is unclear. By increasing intratesticular testosterone concentrations, LH may stimulate the secretion of the peritubular myoid cell protein (PModS), which has been shown to stimulate Sertoli cell function, including the secretion of inhibin (22). Recently, Leydig cells themselves have been shown to contain inhibin α-subunit mRNA and secrete inhibin immuno- and bioactivity *in vitro* (23). In that study hCG stimulated the secretion of inhibin immunoactivity, but not bioactivity, raising the possibility that the Leydig cells may secrete inhibin α-subunit-derived protein(s) in response to hCG. However, the failure of serum inhibin concentrations to fall after the destruction of Leydig cells by ethane dimethane sulfonate (24) suggests that the Leydig cell contribution to the peripheral serum inhibin concentration is not a major one.

Because the Sertoli cell appears to be the major source of inhibin, another physiological implication of the results presented here concerns the role of the Sertoli cell, as judged by its secretory product of inhibin, in human spermatogenesis. The finding that the men whose pretreatment serum inhibin concentrations were greater than 300 U/L were much more likely to develop sperm counts within the normal range in response to gonadotropin treatment than the men whose pretreatment inhibin concentrations were less than 300 U/L suggests that the Sertoli cell plays an important, perhaps an essential, role in spermatogenesis. However, the finding that the men whose hypogonadism was of postpubertal onset had severely impaired spermatogenesis before treatment even though their mean serum inhibin concentration was within the normal range suggests that Sertoli cell function associated with normal inhibin secretion is not sufficient for spermatogenesis. What these men appear to have been missing for spermatogenesis is sufficient intratesticular testosterone, as judged by their subnormal serum testosterone before treatment and their increases to normal of both serum testosterone and sperm count in response to hCG treatment without an increase in serum inhibin concentration.

One clinical implication of these results is that the serum inhibin concentration has predictive value with regard to whether treatment of a man with hypogonadotropic hypogonadism with exogenous gonadotropins will result in a sperm count within the normal range. This conclusion is based on the finding that most men whose pretreatment inhibin concentrations were greater than 300 U/L eventually developed a sperm count within the normal range, and most men whose pretreatment inhibin was less than 300 U/L did not. Pretreatment testicular length also was predictive of the eventual spermatogenic response to gonadotropins, a finding that agrees with the observations of Burris *et al.* (4).

We conclude from this study that 1) both FSH and LH independently stimulate inhibin secretion; 2) normal Sertoli cell function, as judged by the serum inhibin concentration, appears to be necessary, but not sufficient, for spermatogenesis; and 3) the pretreatment serum inhibin concentration and testicular size both predict the spermatogenic response to gonadotropin treatment.

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