

Serum Inhibin B Levels Reflect Sertoli Cell Function in Normal Men and Men with Testicular Dysfunction*

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ABSTRACT

We used a recently developed ELISA format to test the hypothesis that inhibin B is the physiologically active form of inhibin in men. We measured and compared inhibin A, inhibin B, and pro- α -C-related immunoreactive peptides (pro- α -C-RI) in normal men before and after perturbations of their gonadotropin levels and baseline values in normal men and men with various disturbances of the hypothalamic-pituitary-testicular axis including men with idiopathic hypogonadotropic hypogonadism, infertile men with elevated FSH, men with Klinefelter's syndrome, and orchidectomized men.

Mean serum inhibin concentrations were significantly higher in normal men than untreated men with idiopathic hypogonadotropic hypogonadism, infertile men with elevated FSH, untreated men with Klinefelter's syndrome, and orchidectomized men (187 ± 28 vs. 45 ± 11 , 37 ± 6 , 11 ± 3 , and ≤ 10 pg/mL, respectively; $P < 0.05$). Inhibin B levels were below the limit of detection in all of the orchidectomized men. Pro- α -C-RI levels were detectable in all men studied including the orchidectomized men, and no significant differences in the pro- α -C-RI levels were noted between the normal men and men with

various testicular diseases were noted except that orchidectomized men had significantly lower pro- α -C-RI levels than all other groups ($P < 0.05$). Inhibin A was undetectable in all men tested in this study.

Six normal men who were administered exogenous levonorgestrel and testosterone had significantly lower serum gonadotropin, inhibin B, and pro- α -C-RI levels during the treatment period than the control and recovery periods ($P < 0.05$). Ten normal men who were administered human recombinant FSH had significantly higher peak serum FSH (21.85 ± 3.23 IU/L vs. 3.01 ± 0.51 IU/L), inhibin B (311 ± 88 pg/mL vs. 151 ± 23 pg/mL) and pro- α -C-RI (646 ± 69 vs. 402 ± 38 pg/mL) levels during the treatment period than the baseline values ($P < 0.05$).

We conclude that inhibin B is a unique testicular product that is not detectable in the sera of orchidectomized men, is responsive to FSH stimulation, and has a reciprocal relationship with serum FSH levels in men with various forms of testicular disease. Therefore, inhibin B is likely to be the physiologically important form of inhibin in men. (*J Clin Endocrinol Metab* 81: 3341–3345, 1996)

FOR MANY YEARS investigators have postulated that inhibin is secreted by the Sertoli cells of the testes and the granulosa and luteal cells of the ovaries and inhibits pituitary secretion of FSH in a classic feedback loop (1). However, the importance of inhibin in the regulation of gonadotropin secretion in men has remained unclear because the specific measurement of bioactive inhibin has been difficult. Most studies of blood levels of inhibin have used a

heterologous assay that does not distinguish between bioactive heterodimers (α and β subunits) and inactive forms of inhibin such as free α subunit and pro- α -C-related immunoreactive peptides (2). Studies using a heterologous assay have shown that circulating inhibin forms in healthy men are suppressed by GnRH antagonists and testosterone, and that these inhibin forms are increased by stimulation with gonadotropins (3–7). However, the heterologous inhibin assays have not been useful in the assessment of the seminiferous tubule (Sertoli cell) function in infertile men. Although it had been hoped that inhibin assays might distinguish between obstructive azoospermia (when inhibin levels would be predicted to be normal) and seminiferous tubule failure (when inhibin levels would be expected to be decreased), heterologous inhibin assays demonstrated normal or even elevated circulating inhibin levels in infertile men whether FSH levels were normal or elevated (8). These results have raised questions about whether inhibin has a significant role in negative feedback regulation of pituitary FSH secretion in men.

Recently, a new ELISA assay format has been developed and adapted for the specific measurement of different cir-

Received January 12, 1996. Revision received April 10, 1996. Accepted April 18, 1996.

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* This work was supported by Environmental Protection Agency Grant DW-36931233-01-1, NIH Grant P50-HD-12629, CONRAD Grant CSA-92-102, Andrew W. Mellon Foundation, Serono Laboratories, Inc., and Department of Veterans Medical Research Funds. Portions of this study were conducted in the Clinical Research Center at the University of Washington, which is supported by NIH Grant RR-37.

† Supported by a grant from the British Columbia Health Research Foundation.

culating inhibin forms (9). This assay format has been adapted to specifically measure the two bioactive inhibin dimers, inhibin A and inhibin B (10, 11). Inhibin A and B share the same α subunit but have different β subunits. Inhibin A has been found to be generally undetectable in men (12, 13). A third inhibin ELISA has been developed that measures the concentration of the inhibin α subunit precursor pro- α -C and other inhibin forms containing the precursor pro- α -sequence; this assay that measures pro- α -C-related immunoreactive peptides (pro- α -C-RI) has recently been shown to detect similar inhibin forms as the heterologous inhibin assays (13, 14). In a recent study, Illingworth *et al.* (13) showed that there is an inverse relationship between circulating FSH and inhibin B levels in normal men, men with infertility of unknown cause, and a heterogeneous group of men with elevated FSH.

We used these new assays to further test the hypothesis that inhibin B is the physiologically active form of inhibin in men. We sought to determine whether inhibin B levels are predictably altered by perturbations of circulating gonadotropin levels in normal men and in men with various disturbances of the hypothalamic-pituitary-testicular axis including men with idiopathic hypogonadotropic hypogonadism (IHH), infertile men with elevated FSH, men with Klinefelter's syndrome, and orchidectomized men.

Subjects and Methods

Healthy subjects

Sixteen normal men (ages 19–45 yr) were studied. All had a normal medical history, physical examination, hematological and blood chemistry studies, serum cholesterol and triglyceride levels, urinalysis, basal serum levels of gonadotropins and testosterone, and sperm counts.

Healthy men administered levonorgestrel and testosterone

Six of these 16 normal men were enrolled in a male contraceptive study using the progestogen levonorgestrel and im testosterone injections to significantly suppress gonadotropin secretion and spermatogenesis (15). After a control period of 3 months, these 6 volunteers were given levonorgestrel (Wyeth-Ayerst, Philadelphia, PA), 500 μ g po, daily and testosterone enanthate, 100 mg im, weekly for 6 months. They were then followed for a 4- to 6-month recovery period. Serum inhibin levels were determined from a sample during the last month of control and treatment periods and the second month of the recovery period.

Healthy men administered recombinant human FSH (r-hFSH)

Ten of the 16 normal men were enrolled in a study of the pharmacokinetics of sc administered r-hFSH. Venous samples for serum inhibin and gonadotropin levels were taken immediately before the administration of r-hFSH (Gonal F, Serono Laboratories, Inc., Norwell, MA) and at intervals of 2, 4, and 12 h and 1, 2, 3, 5, 7, and 10 days after the administration of r-hFSH.

Men with IHH

Seven men with IHH (age 18–38 yr) were studied. These were patients consecutively referred for pulsatile GnRH therapy and met the following criteria for IHH: men with failure to undergo spontaneous puberty by age 18 yr, with low serum testosterone levels and low or low-normal serum FSH and LH levels, azoospermia, no evidence of other pituitary dysfunction (normal serum T_4 , T_3 resin uptake, TSH, PRL, and cortisol response to ACTH), and normal imaging of the hypothalamus and pituitary. All of the men with IHH were studied after

a 2-month washout period off all androgen therapy including exogenous testosterone, gonadotropins, or GnRH.

Infertile men with elevated FSH

Twenty-nine infertile men (age 28–45 yr) with primary testicular disease of varying causes and severity and elevated basal FSH levels were studied. The types of testicular disease included cryptorchidism, previous chemotherapy, idiopathic oligo- or azoospermia, and testicular trauma. These men had no other known medical diseases. This group of men is the same group of subjects previously reported (13), and they are included in this study for comparison.

Men with Klinefelter's syndrome

A group of nine men (age 22–28 yr) with Klinefelter's syndrome (with XXY karyotype) was studied. None of these nine men had other medical diseases, and none had received any androgen therapy for at least 8 weeks. All nine men with Klinefelter's syndrome were azoospermic.

Men with bilateral orchidectomy

A group of 10 men (age 58–83 yr) who had a therapeutic bilateral orchidectomy for prostate cancer were also studied. Three of these men were on flutamide at the time the venous blood samples were drawn.

All serum samples were obtained from subjects and patients at the Puget Sound Veterans Administration Health Care System and University of Washington except for clinical serum samples from the patients with Klinefelter's syndrome, which were obtained from the University of Colorado at Denver. All serum samples were stored at -4°C for up to 4 yr before measurement of the various hormones. Informed consent was obtained from all subjects, and the University of Washington Human Subjects Review Committee approved all study protocols performed at the Puget Sound Veterans Administration Health Care System.

Hormone assays

The inhibin A and B assays were carried out as previously described (10, 11). The inhibin A and B assays are two-site ELISAs based on the use of multi-well plates coated with specific capture antibodies to the inhibin β A and β B subunits. The Fab fraction of a mouse monoclonal antibody (R1) to the N-terminal portion of the 20 kDa α inhibin subunit conjugated to alkaline phosphatase is used for detection in both assays. Recombinant inhibin A and B were used as standards (Genentech, San Francisco, CA), respectively. Coefficients of variation were $<5\%$ within plate and $<7\%$ between plates for both assays. Activin A, activin B, and follistatin and purified human pro- α -C all had $<0.1\%$ cross-reaction in both assays. Inhibin B had $<0.1\%$ cross-reaction in the inhibin A assay, whereas inhibin A had $<0.5\%$ cross-reaction in the inhibin B assay. The assay detection limit was 4 pg/mL for the inhibin A assay and 10 pg/mL for the inhibin B assay.

The pro- α -C-RI ELISA assay has been described in detail (14). In this assay we used a capture monoclonal antibody raised against the inhibin pro- α subunit sequence. The detection antibody was the same R1 monoclonal antibody used in the inhibin A and B assays. A partially purified ($>75\%$ purity) pro- α -C preparation was used as a standard. Recombinant forms of activin A, activin B, inhibin A, inhibin B, and follistatin all had $<0.02\%$ cross-reaction. The sensitivity of this assay was 3 pg/mL. Immunoblotting experiments with purified preparations of the larger precursor inhibin forms have indicated a potential for cross-reaction with larger precursor forms of dimeric inhibin containing the α -subunit sequence (14).

Statistical analysis

Between-group comparison of normal men and men with various testicular disorders was done using an ANOVA analysis followed by Dunnett's test. Comparison of baseline values with treatment values of the group of normal men receiving exogenous testosterone and levonorgestrel and comparison of baseline values with treatment values of the group receiving r-hFSH were done by repeated measures followed by a Bonferroni analysis. All data are expressed as mean values, and $P < 0.05$ was considered significant.

Results

Inhibin A levels in normal men

Inhibin A levels were undetectable ($<4\text{ pg/mL}$) in all of the men studied.

Baseline inhibin B and pro- α -C-R1 levels: in normal men and men with various disturbances of the hypothalamic-pituitary-testicular axis

The mean serum inhibin B concentration was significantly higher in normal men ($187 \pm 28\text{ pg/mL}$) than any of the groups of men with testicular dysfunction including untreated men with IHH (45 ± 11), infertile men with elevated FSH (37 ± 6), untreated men with Klinefelter's syndrome (11 ± 3), and orchidectomized men (<10) ($P < 0.05$) (Fig. 1). The inhibin B concentration was below the limit of detection in all of the orchidectomized men. Men with Klinefelter's syndrome had lower inhibin B levels than untreated men with IHH and infertile men with elevated FSH, but these trends did not attain statistical significance.

Pro- α -C-R1 was detectable in all men studied including orchidectomized men. No significant differences were noted in pro- α -C-R1 levels, except for the orchidectomized men who had significantly lower pro- α -C-R1 levels than the other groups studied ($P < 0.05$) (Fig. 1).

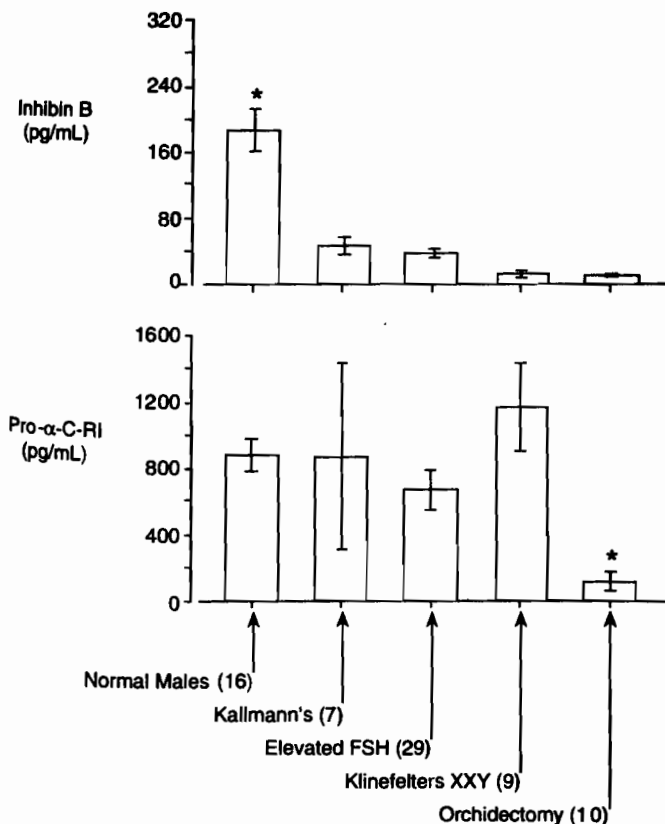


FIG. 1. Inhibin B and pro- α -C-R1 levels in normal males and various patient groups. Values are mean levels $\pm$ SEM; number of each group is shown in parentheses. *, $P < 0.05$ compared with all other groups.

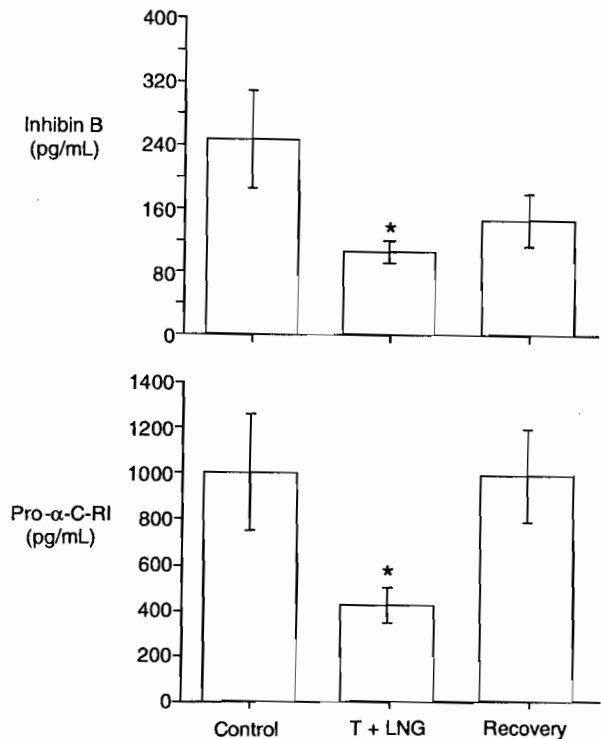


FIG. 2. Inhibin B and pro- α -C-R1 levels in normal males before, during, and after circulating serum gonadotropins were suppressed with exogenous testosterone and levonorgestrel. Values are from last month of control period and treatment period and second month of recovery period. Values are mean $\pm$ SEM, $n = 7$. *, $P < 0.05$ compared with control.

Inhibin B and pro- α -C-R1 and gonadotropin levels during gonadotropin suppression and administration

Normal men who were administered levonorgestrel and testosterone. Inhibin B and pro- α -C-R1 levels were significantly lower during gonadotropin suppression with testosterone and levonorgestrel treatment than during the control and recovery periods ($P < 0.05$) (Fig. 2). As previously reported (15), the mean serum FSH levels were 2.65 ± 0.30 , 0.10 ± 0.05 , and $2.94 \pm 0.60\text{ IU/L}$, and the mean serum LH levels were 3.26 ± 0.32 , 0.019 ± 0.003 , and $3.12 \pm 0.41\text{ IU/L}$ during the control, treatment, and recovery periods, respectively; both FSH and LH levels were significantly lower during treatment than control and recovery periods ($P < 0.05$).

Normal men who were administered r-hFSH. Inhibin B and pro- α -C-R1 levels were unchanged during the first 12 h after r-hFSH was administered, but they were significantly higher than baseline levels 24 h after r-hFSH administration ($P < 0.05$) (Fig. 3). Inhibin B and pro- α -C-R1 levels peaked at 72 h and remained significantly elevated for 7 days after the r-hFSH was administered ($P < 0.05$). Mean serum FSH levels were significantly higher 2 h after r-hFSH administration than baseline (7.59 ± 1.79 vs. $3.01 \pm 0.51\text{ IU/L}$; $P < 0.05$), and they peaked at $21.85 \pm 3.23\text{ IU/L}$ 12 h after administration of r-hFSH and remained significantly elevated above baseline 7 days after r-hFSH was administered ($P < 0.05$) (Fig. 3).

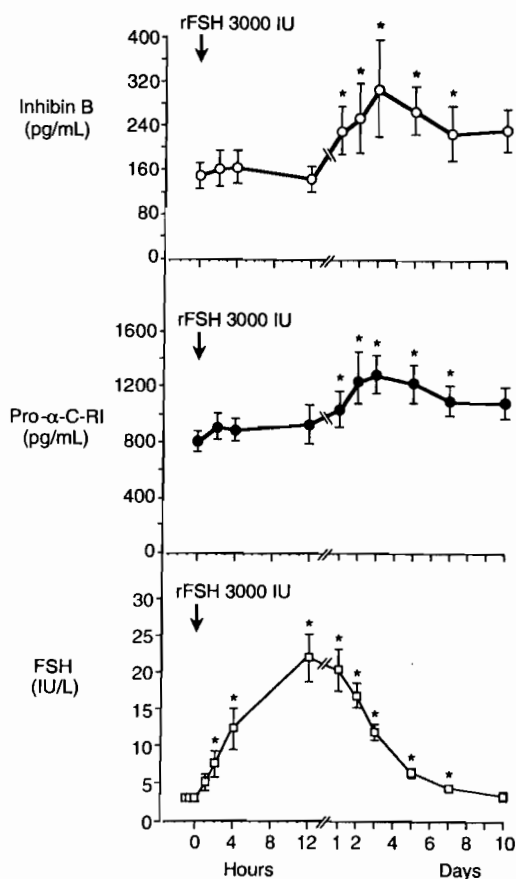


FIG. 3. FSH, inhibin B, and pro- α -C-RI levels in normal males before and after r-hFSH was administered. At 0 h 3000 IU of r-hFSH was administered. Values are mean $\pm$ SEM, $n = 10$. *, $P < 0.05$ compared with time 0.

Discussion

We demonstrated that circulating inhibin B levels are testes-dependent; serum levels of inhibin B were easily measurable in normal men and were undetectable in orchidectomized men. Pro- α -C-RI levels in serum were also easily detectable in the serum of normal men and were decreased markedly by orchidectomy, although not to undetectable levels. Presumably, therefore, pro- α -C-related substances are produced in small amounts by tissues other than the testis. Inhibin A levels were undetectable in all the men studied implying very low or absent production of this substance by any tissue in men. The absence of detectable inhibin A in men contrasts to secretion of inhibin A from the mature preovulatory follicle, the corpus luteum, and the trophoblast in women (10, 16–17).

In men with primary testicular disease caused by Klinefelter's syndrome or to various other etiologies (termed elevated FSH here), inhibin B levels were very low (Fig. 1). These results are consistent with the hypothesis that inhibin B is a Sertoli cell product and is produced in decreased amounts in clinical conditions that impair Sertoli cell function. It is likely, therefore, that inhibin B will prove to be a useful clinical measurement for assessing Sertoli cell function. In contrast, pro- α -C-RI levels did not demonstrate a clear relationship to varying degrees of primary testicular

failure (Fig. 1). These findings are similar to those reported earlier for the heterologous assay (8). Known cross-reactivity of the heterologous assay with pro- α -C-RI is likely to be at least part of the explanation for this similarity in results between the pro- α -C-RI and heterologous assays.

Gonadotropin deficiency, occurring either spontaneously (Kallmann's syndrome) or produced in normal men by administration of testosterone and levonorgestrel (Fig. 2), led to decreased levels of inhibin B. The administration of r-hFSH to normal men led to increased levels of inhibin B (Fig. 3). These results demonstrate the gonadotropin dependence of inhibin B levels. These results, together with the reciprocal relationship between inhibin B and FSH levels in men with various clinical conditions, are consistent with the hypothesis that inhibin B is the physiologically important form of the inhibin family in the pituitary-testicular hormonal control system. A further confirmation of this hypothesis would be the demonstration that the administration of recombinant inhibin B suppresses FSH production in men.

Pro- α -C-RI levels were also suppressed by gonadotropin inhibition in the setting of testosterone and levonorgestrel administration. Pro- α -C-RI was also stimulated by r-hFSH administration. However, the lack of suppression of pro- α -C-RI in primary testicular disease and the lack of inverse relationship between pro- α -C-RI and FSH suggests in men with various clinical conditions that pro- α -C-RI is a much less useful marker of Sertoli cell function than inhibin B.

Inhibin B and pro- α -C-RI levels rose 1–2 days after the initial rapid increment in serum FSH produced by the administration of r-hFSH to men. The reason for this delayed response of inhibin B and pro- α -C-RI to FSH stimulation is unknown. A similar delay in response using the heterologous inhibin assay was also found (unpublished observations).

In conclusion, inhibin B is likely to be the physiologically important form of the inhibin family in regulating pituitary FSH production in men and will also likely be a useful measure of Sertoli function. The future availability of recombinant inhibin B for experimental studies will greatly increase our knowledge of the physiological roles of this important hormone.

Acknowledgments

We are grateful to Arthur Robinson, M.D., Department of Pediatrics, National Jewish Hospital, Denver, for the supply of serum samples of patients with Klinefelter's syndrome. We are also grateful to Sero Laboratories, for the supply of r-hFSH and to Wyeth-Ayerst for the supply of levonorgestrel. We thank Liza Noonan for help with statistical analysis. We thank Jennifer Bullock and Elaine Rost for assistance in preparing the document and coordinating the project and Ian Swanson, Vivian Grant, and Dorothy McGuinness for performing the hormone assays.

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