CLINICAL STUDY

Signaling between the pituitary gland and the testes: inverse relationship between serum FSH and inhibin B concentrations in boys in early puberty

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Abstract

Objectives: To study the relationship between serum inhibin B and sex steroid concentrations and pituitary FSH responsiveness to GnRH in boys in early puberty, and to examine serum inhibin B levels in prepubertal boys with different timing of the onset of gonadotropin deficiency (GD).

Design: Twenty-five boys with constitutional delay of puberty (CDP; 20 in Tanner stage G2 and 5 in G3; age range, 13.5–16.8 years) and eight prepubertal boys (G1P1) with GD (age range, 10.0–13.2 years) were clinically examined, and serum inhibin B, testosterone and estradiol concentrations were measured from sera obtained immediately before the administration of GnRH (Relefact; 3.5 μg/kg, maximum 100 μg i.v.). Thereafter, FSH levels were measured at 30 min intervals up to 90 min.

Results: In the boys with CDP, basal inhibin B and FSH levels did not correlate. However, inhibin B and GnRH-stimulated FSH concentrations ($r_S = 0.43$ to $0.45$, $n = 25$, $P < 0.05$) and the difference between basal and peak serum FSH levels were inversely related ($r_S = -0.63$, $n = 25$, $P < 0.005$). This relationship remained significant in boys at stage G2 ($r_S = -0.66$, $n = 20$, $P < 0.005$). Basal testosterone concentrations and GnRH-induced FSH levels did not correlate. Estradiol levels were too low (64% of the boys had estradiol levels below the assay sensitivity) to allow correlation analysis.

Conclusions: These results suggest that, in boys, the reciprocal regulation between inhibin B and FSH is in operation before mid-puberty. Moreover, autonomous inhibin B secretion by the prepubertal human testis is likely to reflect the number of Sertoli cells.

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Introduction

Inhibin B, a glycoprotein hormone with two dissimilar subunits (α/βB), has been suggested to be the endocrinologically most important form of inhibin in men. In adults (1) and prepubertal boys (2), inhibin B is produced by Sertoli cells in response to follicle-stimulating hormone (FSH). Inhibin B appears to reciprocally inhibit FSH secretion, since in healthy men, men with various testicular disorders and in gonadotropin-deficient men during their gonadotropin-releasing hormone (GnRH) treatment, serum inhibin B and FSH levels correlate negatively (3–6). In addition, chemotherapy given to treat hematological malignancies and testicular irradiation given to treat germ cell cancer both suppress inhibin B secretion with a concomitant increase in circulating FSH levels (7, 8). During puberty, basal inhibin B and FSH levels correlate negatively in boys at Tanner stage G3 or G4, suggesting the establishment of a feedback regulation loop at this stage of development (9, 10). However, prepubertal- and early pubertal-aged boys with primary hypogonadism have higher basal (11–13) and GnRH-induced (14) FSH concentrations than healthy boys of similar age, suggesting that the Sertoli cells regulate FSH secretion earlier.

Gonadotropin drive to the testis is undoubtedly the most important factor stimulating inhibin B secretion; serum levels increase during the postnatal activation of the hypothalamo–pituitary–testicular axis (15), subside during the juvenile hiatus of gonadotropin secretion, rise in early puberty (9, 10), and slowly decrease towards senescence (16). On the other hand, Sertoli cells appear to be capable of autonomous inhibin B production. For example, testes of men with gonadotropin deficiency (GD) produce inhibin B (5, 6), and, in boys, the
postnatal secretion of inhibin B is sustained up to 18 months of age (15). However, the factors affecting the autonomous inhibin B secretion are largely unknown.

In the present study, we examined the relationship between basal serum inhibin B concentration and GnRH-induced FSH responses in boys with constitutional delay of puberty (CDP). In addition, to investigate physiological correlates of autonomous inhibin B secretion, we examined eight prepubertal boys with different timing of the onset of GD.

Subjects and methods

Subjects

Twenty-five boys with CDP (mean age 15.3 years; range, 13.5–16.8 years) were enrolled. These boys had presented with delayed onset and progression of puberty (Tanner genital or pubic hair stage observed at an age equal to or older than the mean +2 S.D. for healthy Finnish boys (17) or a testicular volume <4 ml at or after 13.5 years of age). The boys were short for their chronological ages (range of height S.D. score after 13.5 years of age). The boys were short for their delayed puberty; instead, 68% had laboratory evidence of any disease that could have progressed in each subject.

The second group consisted of eight prepubertal boys with GD. The diagnoses were based on clinical histories, prepubertal genitalia, low basal gonadotropin and sex steroid levels, and subnormal gonadotropin responses to GnRH (Table 1). Three boys had clinical histories suggesting congenital onset of the disease (subjects 2, 5 and 8). None of the boys had received previous androgen or gonadotropin treatment. Other pituitary hormone deficiencies were adequately substituted. Clinical information, serum inhibin B (measured in a different laboratory), luteinizing hormone (LH) and FSH levels of three boys (subjects 3, 6 and 7; Table 1) have been published previously (2).

Serum inhibin B and sex steroid concentrations (testosterone and estradiol in boys with CDP, and testosterone in boys with GD) were measured from samples obtained immediately before (0 min) the administration of GnRH (Relefact, Hoechst, Frankfurt am Main, Germany; 3.5 μg/kg, maximum 100 μg i.v. as a rapid bolus); in subject 1 (Table 1), serum inhibin B and testosterone levels were measured 3 months after the GnRH test. Serum FSH levels were measured from samples obtained 0 (before), 30, 60 and 90 min after the administration of GnRH. The corresponding time points for LH measurements were 0 (before), 20, 30 and 60 min. The GnRH test was performed between 0830 and 1500 h. In two prepubertal boys with GD (subjects 1 and 2; Table 1), serum FSH and LH levels were measured 0 (before), 20 and 70 min after the administration of GnRH. All blood samples were allowed to clot, after which the serum was separated by centrifugation. The sera were stored at −20°C until required for analyses. Boys with CDP had higher GnRH-induced LH peaks (range, 2.6–30 IU/l) than those with GD (Table 1).

The study protocol was accepted by the ethics committee of the Hospital for Children and Adolescents, University of Helsinki. Informed consent was obtained from the boys and their parents.

Assays

Gonadotropins and inhibin B

Serum FSH and LH levels were measured with time-resolved ultrasensitive fluororimmunometric assays (Wallac, Turku, Finland) as described previously (11). The FSH standards were calibrated against the 2nd IRP of pituitary FSH/LH (78/549). The sensitivity of the FSH assay was 0.05 IU/l, as defined by a mean +2 S.D. of multiple (singleton) zero-sample measurements. The samples were also analyzed in singletons. For FSH, the within- and between-plate coefficients of variation (CVs) were less than 6%. The corresponding CVs for LH were less than 7%. We used a commercially available kit (Serotec, Oxford, Oxon, UK) to measure serum inhibin B levels. According to the manufacturer, sensitivity of the assay was 15.6 pg/ml. The standard provided was inhibin extracted from human follicular fluid. At a mean concentration of 115 pg/ml, calculated from the repeated measurement of the same sample, the within-assay CV was less than 5%. The between-assay CV, determined from 11 consecutive assay runs, was less than 7%.

Sex steroids

Serum testosterone levels were measured with an RIA kit (Orion Diagnostica, Espoo, Finland). According to the manufacturer, the assay has a sensitivity of 0.1 nmol/l and crossreacts minimally with other steroid hormones. The within- and between-assay CVs were less than 5 and 7% respectively. Serum estradiol concentrations were measured with an RIA kit (DiaSorin, Vercelli, Italy). The assay sensitivity, determined as a mean +2 S.D. of multiple zero samples, was 24 pmol/l. All samples were analyzed in a single assay run; the within-assay CV was 15%.
Table 1 Clinical characteristics of eight prepubertal boys with gonadotropin deficiency. Data on subjects 3, 6 and 7 have been published previously (2).

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age/bone age (years)a</th>
<th>Genital stageb</th>
<th>Testis volume (ml)</th>
<th>Etiology</th>
<th>Therapy (age in years)</th>
<th>Other pituitary hormone deficienciesc</th>
<th>Genital anomalies</th>
<th>GnRH testd</th>
<th>S-LH (IU/l)</th>
<th>S-FSH (IU/l)</th>
<th>S-inhibin B (pg/ml)</th>
<th>S-testosterone (nmol/l)</th>
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<tbody>
<tr>
<td>1</td>
<td>10.1/8.3</td>
<td>1</td>
<td>1.0</td>
<td>Craniopharyngioma</td>
<td>Surgery (5.1)</td>
<td>GH, TSH, ADH, ACTH</td>
<td>No</td>
<td>&lt;0.1/0.1*</td>
<td>&lt;0.1/0.2*</td>
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<td>&lt;0.1</td>
<td></td>
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<tr>
<td>2</td>
<td>10.0/9.5</td>
<td>1</td>
<td>0.6</td>
<td>Craniopharyngioma</td>
<td>Surgery at birth</td>
<td>GH, TSH, ADH, ACTH</td>
<td>No</td>
<td>&lt;0.1/0.1</td>
<td>&lt;0.1/0.1</td>
<td>&lt;15.6</td>
<td>&lt;0.1</td>
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<tr>
<td>3</td>
<td>13.2/12.5</td>
<td>2</td>
<td>2.5</td>
<td>Craniopharyngioma</td>
<td>Surgery (10.0)</td>
<td>GH, TSH, ADH, ACTH</td>
<td>No</td>
<td>0.1/0.8</td>
<td>0.7/1.1</td>
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<tr>
<td>4</td>
<td>10.0/9.0</td>
<td>1</td>
<td>0.8</td>
<td>Pilocytic astrocytoma</td>
<td>Radiotherapy (3.0)</td>
<td>GH, TSH</td>
<td>No</td>
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<td>0.4</td>
<td>Idiopathic</td>
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<td>GH, TSH, ACTH</td>
<td>Bilateral cryptorchidism, micropenis</td>
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<td>&lt;0.1/0.1</td>
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<td>&lt;0.1</td>
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<tr>
<td>6</td>
<td>13.2/12.3</td>
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<td>0.7</td>
<td>Idiopathic</td>
<td>–</td>
<td>GH, TSH</td>
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<td>0.1/0.5</td>
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<tr>
<td>7</td>
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<td>0.8</td>
<td>Idiopathic</td>
<td>–</td>
<td>GH, TSH</td>
<td>Unilateral cryptorchidism</td>
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<td>&lt;0.1</td>
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<tr>
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<td>10.3/10.7</td>
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<td>0.9</td>
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<td>–</td>
<td>–</td>
<td>Bilateral cryptorchidism, micropenis</td>
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<td>0.1/0.6</td>
<td>&lt;15.6</td>
<td>0.4</td>
<td></td>
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</table>

* Determined by the RUS method (19).
* Estimated by the method of Tanner.
* GH, growth hormone; TSH, thyrotropin; ADH, antidiuretic hormone; ACTH, adrenocorticotropic (the deficient hormones were adequately substituted).
* Relafact 3.5 µg/kg, maximum 100 µg i.v.
* Basal/stimulated maximum.
Statistical analyses

Spearman rank correlation was used to investigate the relationships between serum hormone levels and other parameters of puberty. Estradiol levels were not included in the analyses, because 64% of boys with CDP had concentrations below the assay sensitivity. Serum inhibin B levels within the group of boys with GD were compared using the Mann–Whitney U-test. Values are expressed as median (range). Values of P less than 0.05 were accepted as indicating statistical significance.

Results

In the 25 boys with CDP, the basal serum hormone concentrations were distributed as follows: inhibin B, median 179 pg/ml (range, 86–276 pg/ml); FSH, 2.1 IU/l (0.7–6.8 IU/l); LH, 1.8 IU/l (0.1–5.0 IU/l); testosterone, 3.8 nmol/l (0.5–17.8 nmol/l). In 16 boys, serum estradiol levels were below the assay detection limit; the median of the concentrations above the limit was 48 nmol/l (27–185 nmol/l). Serum inhibin B levels correlated with circulating testosterone concentrations (r_S = 0.42, n = 25, P < 0.05), but not with chronological or bone ages and not with basal serum gonadotropin concentrations.

In each boy with CDP, the highest FSH level was observed 60 min after the administration of GnRH. Serum inhibin B levels correlated with circulating testosterone concentrations (r_S = 0.42, n = 25, P < 0.05), but not with chronological or bone ages and not with basal serum gonadotropin concentrations.

Clinical parameters of puberty together with serum inhibin B, gonadotropin, and testosterone levels in boys with GD are presented in Table 1. Subjects 2, 5 and 8 had lower inhibin B levels than the other five boys with GD (P < 0.05); in these boys, inhibin B concentrations were below the assay sensitivity. Boys with GD had a combination of low inhibin B levels and minimal GnRH-induced FSH concentrations (data shown in Table 1). In both subgroups, serum inhibin B concentrations and testis volumes correlated positively (r_S = 0.49, n = 25, P < 0.05 in boys with CDP; r_S = 0.70, n = 8, P = 0.07 in boys with GD; Fig. 2).

Discussion

Several previous findings suggest that the testes regulate FSH secretion before mid-puberty. In boys at early puberty, for example, serum LH levels increase rapidly, whereas the corresponding rise in FSH levels is much smaller (12, 20–22). At the transition from pre- to peripuberty, FSH responses to exogenous GnRH may even show a decreasing trend (23). Direct evidence that inhibin B would mediate these changes is lacking. This is suggested, however, by the findings that inhibin B secretion increases in early puberty (9, 10) and, during short-term GnRH treatment of gonadotropin-deficient men, serum inhibin B and FSH levels correlate negatively (6). In boys in early puberty, we found an observed between circulating testosterone and GnRH-stimulated FSH concentrations. The inverse relationship between inhibin B and ΔS-FSH remained statistically significant, even though only the boys at Tanner stage G2 were included in the correlation analysis (r_S = −0.66, n = 20, P < 0.005).

Figure 1 (A) Correlations between basal serum inhibin B (S-inhibin B) levels and GnRH-induced serum FSH (S-FSH) concentrations immediately before (P = NS; upper left), and 30, 60 and 90 min after the administration of GnRH (Relefact; 3.5 μg/kg, maximum 100 μg i.v.) to 25 boys with CDP (r_S = −0.43 to −0.45, P < 0.05, Fig. 1A), and strongly with the difference between basal and peak serum FSH levels (ΔS-FSH; r_S = −0.63, n = 25, P < 0.005, Fig. 1B). ΔS-FSH ranged from 0.9 to 4.5 IU/l. No inverse relationships were observed between circulating testosterone and GnRH-stimulated FSH concentrations. The inverse relationship between inhibin B and ΔS-FSH remained statistically significant, even though only the boys at Tanner stage G2 were included in the correlation analysis (r_S = −0.66, n = 20, P < 0.005).

Figure 1 (B) Relationship between basal S-inhibin B levels and GnRH-induced ΔS-FSH in the same group of boys (r_S = −0.63, P < 0.005). Linear regression lines are shown.
Prepubertal boys with GD had low basal and GnRH-induced gonadotropin concentrations in combination with low inhibin B levels (below the reference range of our laboratory for healthy boys of similar age). The lowest inhibin B levels were observed in boys with congenital onset of the disease: two boys with GD had undervirilized genitalia suggesting GD in utero, and one boy had had a craniopharyngioma resected immediately after birth, resulting in a defective postnatal activation of the hypothalamo–pituitary–gonadal axis. These developmental disturbances probably impaired Sertoli cell proliferation, resulting in non-detectable serum inhibin B levels later in prepuberty. For example, in the rat, neonatal reduction of Sertoli cell number suppresses inhibin B production later in life (33), and men with prepubertal onset of GD have lower inhibin B levels than those with histories of endogenous pubertal maturation (5). Moreover, in prepubertal boys, inhibin B has been suggested to be produced exclusively by Sertoli cells (34), the number of which correlates with the length of seminiferous tubules (35). Subject 3 had the highest inhibin B concentration and the largest testis size, and for subject 5 the findings were opposite. On the basis of these findings, we suggest that in boys, similar to the previous suggestion for non-human primates (36), the amount of inhibin B autonomously produced by the prepubertal testis reflects the number of Sertoli cells.

In conclusion, we found that in boys in early puberty, circulating inhibin B and GnRH-induced increases in serum FSH concentrations correlated negatively, suggesting feedback regulation between these hormones before mid-puberty. Moreover, we investigated inhibin B levels in eight prepubertal boys with GD. The findings suggest that the autonomous inhibin B production by the prepubertal human testis reflects the number of Sertoli cells.

Acknowledgements
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References
4. Anderson RA, Wallace EM, Groome NP, Bellis AJ & Wu FC. Physiological relationships between inhibin B, follicle stimulating

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**Figure 2** Relationship between testis volumes and serum inhibin B (S-inhibin B) levels in 25 boys with CDP. Twenty boys were at stage G2, and five at G3. Insert: relationship between testis volumes and S-inhibin B levels in eight prepubertal boys with GD ($r = 0.70, n=8, P = 0.07$).
hormone secretion and spermatogenesis in normal men and response to gonadotropin suppression by exogenous testoster-

5 Nachtigall LB, Boepple PA, Seminara SH, Khoury RH, Sluss PM, Lecain AE et al. Inhibit B secretion in males with gonadotropin-
releasing hormone (GnRH) deficiency before and during long-


7 Wallace EM, Groome NP, Riley SC, Parker AC & Wu FC. Effects of chemotherapy-induced testicular damage on inhibin, gonado-


9 Andersson AM, Jaul A, Petersen JH, Müller J, Groome NP & Skakkebaek NE. Serum inhibit B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-
stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. Journal of Clinical Endocrinology and Metabolism 1997 82 3976–3981.


13 Dunkel L, Stimes MA & Bremner WJ. Reduced inhibit and elevated gonadotropin levels in early pubertal boys with testicular defects. Pediatric Research 1993 33 514–518.


22 Albertsson-Wikland K, Rosberg S, Lannering B, Dunkel L, Selstam G & Norjavaara E. Twenty-four-hour profiles of luteiniz-


28 Tapanainen JS, Altmann K, Jin J, Vaskivuo T & Huhtaniemi IT. Men homoygous for an inactivating mutation of the follicle-


30 Phillip M, Arbelle JE, Segev Y & Parvari R. Male hypogonadism due to a mutation in the gene for the beta-subunit of follicle-

31 Kaiser J, Simoni M, Gromoll J, Braun M, Bergmann M, Michalk D et al. Bilateral macroorchidism in a prepubescent boy with idio-


34 Andersson A, Muller J & Skakkebaek NE. Different roles of prepubertal and postpubertal germ cells and Sertoli cells in the regulation of serum inhibit B levels. Journal of Clinical Endocrinol-
yogy and Metabolism 1998 83 4451–4458.


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