Abstract

**Background:** Several studies have indicated that cryptorchidism is associated with degenerative changes in both Sertoli cells and germ cells. The gonadal peptide hormone inhibin B reflects Sertoli cell function. Low inhibin B levels are found in a large portion of formerly cryptorchid men who show compromised seminiferous tubule function. It is not known if inhibin B can be used to demonstrate early damage of seminiferous tubules in prepubertal boys with cryptorchidism.

**Methods:** We investigated the relationship between serum levels of inhibin B, testosterone, FSH and LH in 62 prepubertal boys with uni- and bilateral cryptorchidism. Furthermore, we investigated the changes in serum levels of inhibin B and the corresponding changes in serum levels of FSH, LH and testosterone during a short course (3 weeks) of human chorionic gonadotropin (hCG) injections in 18 of these cryptorchid boys.

**Results:** In the 62 prepubertal boys with uni- or bilateral cryptorchidism there were no significant differences in baseline levels (median and range) of inhibin B (88 (20 – 195) pg/ml vs 78 (35 – 182) pg/ml; not significant), LH (0.08 (0.05 – 0.99) IU/l vs 0.06 (0.05 – 1.61) IU/l; not significant) and FSH (0.60 (0.08 – 3.73) IU/l vs 0.85 (0.25 – 2.55); not significant) compared with 156 healthy prepubertal boys, and there were no differences in hormonal levels between boys with uni- or bilateral cryptorchidism. There was no correlation between baseline levels of inhibin B and FSH. In boys younger than 9 years, we found no correlation between baseline levels of inhibin B and LH whereas, in boys older than 9 years, baseline levels of inhibin B were positively correlated to baseline LH (Spearman rank correlation coefficient (R_s) = 0.58, P = 0.03). Treatment with hCG (1500 IU intramuscularly twice weekly for 3 weeks) resulted in descensus of testes in 9 out of 18 patients. In all boys but one, irrespective of age, hCG induced a marked increase in testosterone into the adult range (from undetectable to 21.8 (7.0 – 35.4) nmol/l; P < 0.001) and completely suppressed FSH and LH levels. Serum levels of inhibin B increased significantly from 116 (50 – 195) pg/ml to 147 (94 – 248) pg/ml (P < 0.05), but not uniformly. The increase in serum levels of inhibin B was inversely correlated to baseline inhibin B (R_s = −0.52, P = 0.03) and baseline FSH (R_s = −0.59, P < 0.01).

**Conclusions:** We therefore suggest that, in the prepubertal testes, inhibin B is secreted from the prepubertal Sertoli cells following hCG, whereas early pubertal testes with more differentiated Sertoli cells are not able to secrete inhibin B in response to hCG stimulation, perhaps due to lack of germ cell-derived βB-subunits. We found (a) normal inhibin B levels in prepubertal boys with uni- or bilateral cryptorchidism, (b) that hCG stimulated testosterone markedly and suppressed FSH and LH levels and (c) that hCG treatment stimulated inhibin B levels in the youngest cryptorchid boys. In the oldest prepubertal boys no hCG-induced changes in inhibin B were shown.

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**Introduction**

The gonadal peptide hormone inhibin B is the principal circulating bioactive form of inhibin in men and reflects Sertoli cell function (1, 2). In prepubertal boys, stimulation of Sertoli cells by follicle-stimulating hormone (FSH) increases circulating inhibin B levels (3). In boys of 3 months of age, early activation of the pituitary–gonadal axis results in markedly increased inhibin B levels, which remain elevated up to the age of 15 months, while FSH, luteinizing hormone (LH) and testosterone at the age of 6–9 months decrease into the normal range observed later in childhood (4). The onset of male puberty is associated with increasing
serum concentrations of gonadotropins, testosterone (5) and inhibin B (6–8). By pubertal stage II, the adult serum level of inhibin B has been reached, and stage III is characterized by a negative correlation between serum levels of inhibin B and FSH, reflecting the establishment of the closed loop feedback regulation of the hypothalamic–pituitary–gonadal axis operating in adult men (7). In the prepubertal human testes, both α- and βB-subunits of inhibin are co-localized in Sertoli and interstitial cells whereas, in the adult human testes, the βB-subunits are localized to pachytene spermatocytes and round spermatids as well as to Leydig cells, and the α-subunit is localized to Sertoli cells and Leydig cells (7). This suggests that Sertoli cell maturation induces a change in inhibin subunit expression during puberty. Treatment of boys with delayed puberty with testosterone plus an aromatase inhibitor increases FSH and inhibin B levels (9) and sustains the elevations of inhibin B levels despite normal FSH and LH levels, as demonstrated in a boy with aromatase deficiency (10). Together, these findings suggest that estrogen may be involved in the pituitary–gonadal feedback in boys. Several studies have indicated that cryptorchidism is associated with degenerative changes in both Sertoli cells and germ cells (11, 12). It has been shown in monkeys that inhibin B represents an early marker of testicular damage, which is more sensitive than FSH (13). Three out of 17 patients with cryptorchidism had low inhibin levels and elevated FSH levels after orchidopexy (14). In line with these preliminary findings, low inhibin B levels are found in a large portion of formerly cryptorchid men who show compromised seminiferous tubule function (15). Preoperative testicular location (16) or testicular size at orchidopexy (17) in men with previous unilateral cryptorchidism was not a major determinant of inhibin B levels, whereas early operation appears to be beneficial. Thus, adult men with a history of cryptorchidism and who underwent orchidopexy by the age of 2 years have higher inhibin B levels compared with those who underwent surgery later in life (18). In the present study, we investigated the relationship between serum levels of inhibin B, testosterone, FSH and LH in prepubertal boys with uni- and bilateral cryptorchidism. Furthermore, we investigated the changes in serum levels of inhibin B and the corresponding changes in serum levels of FSH, LH and testosterone during a short course of human chorionic gonadotropin (hCG) injections.

Patients and methods

Patients

Sixty-two otherwise healthy prepubertal boys (testis volume, if palpable, less than 4 ml) with uni- or bilateral cryptorchidism, aged 7.7 (4.1–13.6) years, who were referred to our department for treatment, participated in the study. None of the boys had previously received hormonal treatment or had undergone operation in the inguinoscrotal region. Boys with retractile testes had been excluded. Seventeen boys presented with bilateral and 45 boys presented with unilateral cryptorchidism. The testes were palpated between the internal ring of the inguinal canal and the neck of the scrotum, or not at all. The parents were informed about potential treatment regimens (hCG and/or orchidopexy). The decision on whether or not to try medical therapy was based on clinical decision and parental request, which resulted in 23 boys (7 with bilateral and 16 with unilateral cryptorchidism) receiving hCG treatment (Pregnyl; NV Organon, Oss, The Netherlands; 1500 IU intramuscularly twice a week for 3 weeks). The remaining 39 boys were assigned for an operation (orchidopexy) without pretreatment.

Study design

Serum levels of inhibin B, FSH, LH, testosterone and sex hormone-binding globulin were determined at baseline in all 62 boys. Blood samples were taken 72 h after the last (sixth) hCG injection. Venous blood was sampled from an antecubital vein between 0900 and 1200 h at every visit, centrifuged and serum stored at −20 °C until analysis. Data from five patients were excluded from the analysis because more than 72 h had passed between the last hCG treatments, leaving paired samples from 18 boys for analysis.

Hormone analyses

Inhibin B was determined by a double-antibody enzyme immunometric assay using a monoclonal antibody raised against the inhibin βB subunit in combination with a labeled antibody raised against the α-subunit as previously described (19). The detection limit was 20 pg/ml, and intra- and interassay coefficients of variation were 15% and 18% respectively. FSH and LH were measured by time-resolved immunofluorimetric assay (DELFIA; Wallac, Turku, Finland) with detection limits of 0.06 and 0.05 U/l respectively. Intra- and interassay coefficients of variation were both below in 8% in the FSH and LH assays. Testosterone was determined by radioimmunoassay (Count-a-Count; Diagnostic Products, Los Angeles, CA, USA). The detection limit was 0.23 nmol/l, and the intra- and interassay coefficients of variation were both less than 10%. Normal ranges for inhibin B, testosterone, FSH and LH levels in normal prepubertal boys have been published previously (6).

Statistics

The results are expressed as the median (range). The changes in serum levels of inhibin B and testosterone
before and after hCG treatment were compared using the Wilcoxon matched-pairs signed-ranks test. Spearman rank correlation coefficients were calculated to determine any correlation between variables. Two-sided P values less than 0.05 were considered significant.

**Ethical considerations**

The study was approved by the local ethics committee. Informed consent was obtained from the patients, and the study was conducted according to the Helsinki II declaration.

**Results**

**Basal hormone levels in cryptorchidism**

The medians (ranges) of serum inhibin, testosterone, FSH and LH levels are summarized in Table 1. For comparison, hormone levels in a normal population of 156 prepubertal boys, which have been published previously (6), are also included in Table 1 and Fig. 1. There were no significant differences in baseline levels of inhibin B, LH and FSH compared with 156 healthy prepubertal boys, and there were no differences in hormonal levels between boys with uni- or bilateral cryptorchidism. There was no correlation between baseline levels of inhibin B and FSH. In boys younger than 9 years, we found no correlation between baseline levels of inhibin B and LH. In contrast, in boys older than 9 years (n = 14), baseline levels of inhibin B was positively correlated to baseline LH (Spearman rank correlation coefficient (Rs) = 0.58, P = 0.03). In boys with bilateral cryptorchidism and at least one non-palpable testes, basal levels of inhibin B were significantly lower (50 pg/ml (20–63)) compared with boys with bilateral cryptorchidism and palpable testes (90 pg/ml (47–195); P < 0.02). Detailed analysis of boys presenting with basal inhibin B levels outside the normal range for age-matched boys did not reveal any significant differences compared with those with normal levels of inhibin B, except in one boy presenting with bilateral non-palpable testes.

**Effect of hCG treatment in cryptorchidism**

Treatment with hCG resulted in desensus in six patients with unilateral cryptorchidism, and complete desensus of both testes in three patients with bilateral cryptorchidism. Treatment with hCG resulted in markedly increased testosterone levels in all boys (P < 0.001), irrespective of their age (Table 1 and Fig. 2). Similarly, FSH and LH levels were completely suppressed in all patients (P < 0.001). Serum levels of inhibin B increased significantly (P < 0.05), but not uniformly. Individual hormone concentrations in the 18 boys receiving hCG treatment, measured at baseline and 72 h after the last hCG injection, are shown in Fig. 2. Baseline inhibin B did not correlate with the testosterone response to hCG. The increase in serum levels of inhibin B was inversely correlated to the baseline levels of inhibin B (Rs = −0.52, P = 0.03) and FSH (Rs = −0.59, P < 0.01).

**Discussion**

We found normal levels of inhibin B, FSH and LH levels in our study of 62 boys with cryptorchidism who were all clinically prepubertal (testicular volume less than or equal to 3 ml). This is in line with the findings of Kubini and coworkers (20) who found undetectable inhibin B levels in boys with anorchia as compared with normal levels in cryptorchid boys with intra-abdominal testes. Treatment with hCG increased testosterone into the adult range and suppressed FSH and LH levels completely. Inhibin B levels increased in response to hCG in the youngest prepubertal children only, whereas no changes or even decreases were seen in the oldest prepubertal/early pubertal boys with cryptorchidism.

Treatment with hCG markedly increased testosterone levels in all boys irrespective of their age, and suppressed FSH and LH levels completely after our current regimen of twice weekly administration of hCG (1500 IU i.m. for 3 weeks; six injections). Testosterone levels increased into the normal adult range (>10 nmol/l) in all boys but one. Previous assessment of testosterone levels after hCG in cryptorchid prepubertal boys have been studied after varying hCG treatment regimens (a single dose of 5000 IU i.m. (21), a single dose of

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Table 1 Hormone values (median (range)) in 62 cryptorchid boys of whom 18 were treated with hCG, as compared with 156 healthy prepubertal boys.

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Controls (n = 156)</th>
<th>All (n = 62)</th>
<th>Before hCG (n = 18)</th>
<th>After hCG (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (IU/l)</td>
<td>0.85 (0.25–2.55)</td>
<td>0.60 (0.08–3.73)</td>
<td>0.72 (0.27–2.97)</td>
<td>0.05** (0.05–0.11)</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>0.08 (&lt;0.05–0.99)</td>
<td>0.06 (0.05–1.61)</td>
<td>0.08 (0.05–1.61)</td>
<td>0.05** (0.05–0.06)</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>&lt;0.2 (&lt;0.2–0.9)</td>
<td>0.23 (0.23–0.33)</td>
<td>0.23 (0.23–0.32)</td>
<td>21.8** (7.0–35.4)</td>
</tr>
<tr>
<td>Inhibin B (pg/ml)</td>
<td>78 (35–182)</td>
<td>88 (20–195)</td>
<td>116 (50–195)</td>
<td>147* (94–248)</td>
</tr>
</tbody>
</table>

Values at baseline in cryptorchid boys (all) did not differ from healthy boys (controls). *P < 0.05, **P < 0.001 compared with before hCG treatment.
5000 IU/m² (20), 1500, 3000 or 5000 IU once weekly depending on age for 3 weeks (22–24), but these studies uniformly describe elevated testosterone after hCG. However, it remains difficult to evaluate the results from these studies as wide discrepancies exist with respect to selection of patients, heterogeneity in pathogenesis and the use of varying hCG protocols. It is believed that the effect of hCG on undescended testes is mediated by the rise in endogeneous testosterone (25), but the optimal hCG regimen and rise in testosterone levels are unknown. In our study, testosterone levels increased to values well above those of infant boys. Prepubertal boys have a monophasic testosterone response to hGH with peak levels 4–5 days after hCG administration (26, 27). Four injections of hCG (100 IU/kg at 5-day intervals) produced testosterone levels within the normal adult male range, and similar success rates compared with seven injections of 1500 IU every other day, with no correlation to testosterone levels (28). Thus, we speculate that very high testosterone levels should be avoided, and future hCG regimens should include lower hCG doses, perhaps over longer periods of time.

In our study, inhibin B levels did not indicate signs of seminiferous tubule damage in prepubertal children with cryptorchidism (aged 4–13 years) as has been described in studies of adult men with a history of cryptorchidism (15). However, in the few cases with more severe forms of cryptorchidism with bilateral cryptorchidism, and at least one with non-palpable testes, we found evidence of lowered levels of inhibin B. This discrepancy may be a result of the duration of cryptorchidism or of the selection bias in the adult studies in which the males who have been operated for cryptorchidism earlier in life may represent the more severe part of the disease spectrum. We found no correlation between baseline levels of inhibin B and LH in boys younger than 9 years, but in boys older than 9 years of age a positive correlation between baseline levels of inhibin B and LH was indicated. This is in accordance with our previous results in normal prepubertal/early pubertal boys (6). In contrast, Raivio & Dunkel found an inverse relationship between basal levels of serum FSH and inhibin B in prepubertal boys (aged 1–8 years) with cryptorchidism (22), and between inhibin B and gonadotropin-releasing hormone (GnRH)-stimulated FSH levels in boys with constitutional delay of puberty (29), suggesting that the reciprocal regulation between FSH and inhibin B is operational in early childhood.

Figure 1 Serum levels of inhibin B, FSH, testosterone and LH levels in 62 prepubertal boys with uni- or bilateral cryptorchidism. Lines represent means and 95% confidence intervals in healthy prepubertal boys as previously published (6).
The present data indicated that the changes in inhibin B during hCG treatment were dependent on basal levels of inhibin B and FSH, suggesting a decreasing inhibin B response to hCG with increasing maturation of the testes. The results support our previously published hypothesis of the changing regulation of inhibin B secretion during male puberty (6, 7). In the prepubertal human testis, both α- and βB-subunits are co-localized in Sertoli and interstitial cells whereas, in the adult testis, the two subunits constituting inhibin B are expressed by different cell types: βB-subunits are localized to germ cells (from pachytene spermatocytes to the early spermatid stages) and to a lesser degree to Leydig cells, and the α-subunit is localized to Sertoli cells. This suggests that Sertoli cell maturation induces a change in inhibin subunit expression during puberty, the immature prepubertal Sertoli cell expressing both α- and βB-subunits, whereas the fully differentiated Sertoli cells express the α-subunit only (7). Thus, in adult men, inhibin B is possibly a joint product of Sertoli cells and germ cells. The stimulatory effect of gonadotropins on the secretion of inhibin in prepubertal boys has been shown previously (30). However, the immunoassay for inhibin used in this study suffered from cross-reactions with inactive monomeric precursor forms present in plasma and lack of discrimination between inhibin A and B.

The effect of hCG stimulation on the secretion of inhibin B in prepubertal boys has been investigated in two previous but smaller studies using specific immunoassays (20, 22). Raivio & Dunkel (22) analyzed paired samples from nine boys before and after hCG treatment for cryptorchidism. They found no increase in inhibin B levels during hCG treatment, but when data from the four oldest boys, aged 1.8 – 6.4 years, were analyzed, inhibin B increased slightly from 91 pg/ml to 135 pg/ml following hCG treatment (22). However, the boys included in that study were younger compared with our present study. Kubini and coworkers (20) found no influence of hCG stimulation on inhibin B levels. However, the data in this study are not comparable to our data, since the patients in the study of Kubini et al. (20) suffered from various testicular disorders, including intra-abdominally located cryptorchidism, and only a single hCG injection (5000 IU/m²) was given. Treatment with hCG altered inhibin B levels in males with hypogonadotropic hypogonadism (32), whereas no effects of hCG on inhibin B were seen in normal men (33). Conversely, inhibin α-subunit precursor (pro-αC) concentrations increased rapidly.
following hCG, suggesting that the adult Leydig as well as Sertoli cells secrete inhibin α-subunit in response to hCG, whereas no secretion of inhibin B from mature Leydig cells could be shown (33). Pulsatile GnRH treatment in patients with idiopathic hypogonadotropic hypogonadism (IHH) increased inhibin B, suggesting regulation of inhibin B by gonadotropins (34), but did not affect inhibin B levels in late-pubertal boys with varicoceles (35). Recombinant FSH increased inhibin B in normal men (34), IHH (36), oligozoospermic subjects (37) and infertile men with unilateral cryptorchidism (38). By contrast, recombinant LH did not affect serum inhibin B levels in males with hypogonadotropic hypogonadism (36). Intramuscular testosterone treatment decreased inhibin B (and sperm concentrations) in healthy men (39), altogether supporting the hypothesis of inhibin B playing a physiological role in the feedback control of FSH secretion and reflecting FSH-stimulated Sertoli cell function.

Thus, we suggest that in the prepubertal testis, inhibin B is secreted from the Sertoli cells following hCG, whereas early pubertal testes with more differentiated Sertoli cells are not able to secrete inhibin B following hCG stimulation, perhaps due to lack of germ cell-derived βB-subunits. However, we cannot exclude the possibility that duration of cryptorchidism per se might limit the Sertoli cell response to hCG.

In conclusion, we found (a) normal inhibin B levels in prepubertal boys with uni- or bilateral cryptorchidism, (b) that hCG stimulated testosterone markedly and suppressed FSH and LH levels and (c) that hCG treatment stimulated inhibin B in the youngest cryptorchid boys, whereas no hCG-induced changes in inhibin B were shown in the oldest prepubertal boys.

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