Decrease in serum sex hormone binding globulin during human chorionic gonadotrophin stimulation in prepubertal boys

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Abstract. Temporal relationships between steroidogenic and sex hormone binding globulin (SHBG) responses to hCG were studied in 27 prepubertal boys: 19 with incomplete testicular descent and 8 with hypogonadotropic hypogonadism (HH). Nine of the boys with incomplete testicular descent were given a single im injection of hCG and blood samples were taken daily for 5 days. Six of them showed a slight decrease in SHBG concentration by day 5. All the other 18 boys were given four im injections of hCG on days 0, 4, 7 and 10. Blood was taken before each injection and on day 14. In the boys with incomplete testicular descent SHBG concentration decreased by day 14 (P < 0.01). All the boys with HH had an impaired testosterone response to hCG, and SHBG levels did not decrease after hCG. In only 2 of these boys SHBG concentrations were >10% below the basal by day 14. These boys, however, also had the highest testosterone responses of their group. Thus it appears that if testosterone increases in prepubertal boys, SHBG decreases.

The serum concentration of sex hormone binding globulin (SHBG) depends on the hormonal milieu. In adults it is increased by oestrogens and thyroid hormones and decreased by androgens (Anderson 1974).

Very little is known about the regulation of the serum concentration of SHBG in children. hCG stimulation has been reported to cause a decrease in prepubertal cryptorchid boys but the time course of the decrease was evaluated in only one boy (Belgorosky & Rivarola 1982). Since this decrease has been suggested as providing a test of biological response to androgens, the purpose of the present paper was to study the temporal relationships between the steroidogenic and SHBG responses to hCG in greater detail.

Subjects and Methods

Subjects

Twenty-seven prepubertal boys were studied (Table 1). Nineteen had been referred to us because of incomplete descent of the testis; 13 had true unilateral incomplete descent and 6 retractile testis (groups A and B). Eight boys had isolated hypogonadotropic hypogonadism (HH) (group C). In the two youngest boys the diagnosis of HH was based on subnormal gonadotrophin response to GnRH (Dunkel et al. 1985a). The diagnosis of HH in the other 6 boys was confirmed by two clinical criteria during a 2.5-2.9 year follow-up after the study: 1) a clearly prepubertal testis size at bone age 13 years or older and 2) absence of pubertal penis growth at bone age 13 years or older. None of the boys with HH had received androgen therapy before the study or showed elevated basal or post-GnRH gonadotrophin levels in the follow-up.

The relative body weight of all the boys varied between 80-120% of the mean weight for boys of the same height.

Protocols

Testosterone (T), oestradiol (E2) and SHBG responses were studied with two different protocols. In protocol I 9 boys of the boys with incomplete testicular descent...
Incomplete testicular descent

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (mean range)</th>
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<tbody>
<tr>
<td>Group A (protocol I)</td>
<td>5.8 (1.7–10.5)</td>
</tr>
<tr>
<td>Group B (protocol II)</td>
<td>7.3 (2.4–12.5)</td>
</tr>
<tr>
<td>Hypogonadotrophic hypogonadism</td>
<td>11.4 (7.4–14.3)</td>
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(group A) were given a single im injection of 5000 IU hCG/1.7 m² (Pregnyl, Organon, Oss, The Netherlands). Blood samples were taken at 0 and 12 h and then daily for 5 days. In protocol II the other 10 boys with incomplete testicular descent (group B) and all 8 boys with HH (group C) were given four im injections of 5000 IU hCG/1.7 m², on days 0, 4, 7 and 10. Blood samples were taken before each injection and on day 14. The results of the T and E₂ responses for both protocol I (Tapanainen et al. 1983) and protocol II (Dunkel et al. 1985b) have been published previously.

Normal values of gonadotrophin responses to GnRH were obtained from 44 prepubertal boys with incomplete testicular descent (Dunkel et al. 1985a). They were given a single iv injection, 3.5 μg/kg, of GnRH (Relefact, Hoechst AG, Frankfurt, West-Germany). Serum for LH and FSH assays was obtained at −20, 0, 20, 30, 60, 90 and 120 min after the injection.

Methods

The sera were stored at −20°C until analyzed. T was quantified by RIA after chromatography on Lipidex 5000 (Apter et al. 1976). E₂ of protocol I by a RIA kit (Nordiclab Oy, Oulunsalo, Finland) and of protocol II by RIA after chromatography on Sephadex-LH20 (Adlercreutz et al. 1982). The coefficients of variation were for T assay between 10 and 15% (inter-assay) and 5.2 and 6.2% (intra-assay) at serum concentrations of 8.2 and 15.1 nmol/l, respectively, for E₂ assay of protocol I 14.1 and 12.2% (inter-assay) at serum concentrations of 164 and 1915 pmol/l and 10.5 and 9% (intra-assay) at serum concentrations of 128 and 1592 pmol/l, respectively, and for E₂ assay of protocol II 7.7 and 5.5% (inter-assay) at serum concentrations of 195 and 478 pmol/l, respectively. SHBG was measured by using a liquid-phase immunoradiometric assay (IRMA) with monoclonal antibody to SHBG (Hammond & Robinson 1984). Briefly, after appropriate dilution a mixture of ¹²⁵I-labelled monoclonal SHBG antibody (mouse) and anti-SHBG antiserum (rabbit) was added. The tube contents were mixed and incubated at room temperature for 1 h. A solid-phase anti-rabbit IgG antiserum (donkey) was then added, followed 15 min later by 2 ml 0.9% NaCl. After centrifugation (2000 × g for 15 min) to pellet the solid-phase matrix, the supernatants were decanted to waste. The pellets were then counted in a gamma scintillation counter. A standard series of human pregnancy serum standard was used in each assay, the SHBG content of which was determined by Scatchard analysis using tritium labelled DHT. The coefficients of variation for SHBG IRMA were 5.2 and 6.2% (inter-assay) and 4.2 and 2.3% (intra-assay) at serum concentrations of 20 and 105 nmol/l, respectively. Samples from an individual subject were analyzed at the same time.

Statistics

The data were analyzed by BMDP computer programs (Dixon 1981). The means were compared by Student's...
\textit{t}-tests for independent and dependent samples (program 3D) and by general univariate and multivariate analysis of variance (program 4V). Because of positive skewness of the distributions of T and E2, calculations of these variables were made after logarithmic transformation and the geometric mean and mean $\pm$ SEM values are given.

**Results**

In group A treated according to protocol I the serum concentration of T was clearly increased in all boys 12 h after the single injection of hCG, with peaks at 2–5 days. The mean E2 concentration did not change. The mean basal SHBG concentration was 127 nmol/l. After the second day SHBG concentration showed a tendency to decrease, and decreased finally to a level significantly ($P < 0.05$) below the basal (Fig. 1).

In groups B and C treated according to protocol II the mean T concentrations were significantly ($P < 0.001$) above the basal levels after the first dose. The remaining injections further increased the concentrations in both groups. There was no overlapping between the two groups on day 14.

The mean E2 concentrations were not significantly affected by hCG in either of the groups.

The mean basal SHBG concentrations were 103 and 89 nmol/l for groups B and C, respectively. In group C the SHBG concentration was not affected by hCG. By contrast, in group B, the concentration showed a tendency to decrease, and decreased finally to a level significantly below the basal on day 14 ($P < 0.01$) (Fig. 2).

In group A, on day 5, the SHBG concentration was 89 $\pm$ 5% (SEM, range 78–116%) of the basal level. In groups B and C, on day 14, the concentrations were 71 $\pm$ 6% (range 43–89%) and 101 $\pm$ 5% (range 83–112%) of the basal level, respectively. In groups A and B the decrease of SHBG below the basal level was significantly greater after four injections (group B, protocol II) than 5 days after a single injection (group A, protocol I) ($P < 0.05$). Three of the 9 boys of group A and 5 of the 8 boys of group C did not have a SHBG level below the basal at the end of the stimulation. The 2 boys of group C whose SHBG concentration decreased by $> 10\%$, had the highest T responses (7.3 and 8.4 nmol/l) of their group. Of the different measurements of the responses of SHBG, T and E2 on any day the best correlation was found between the relative SHBG decrease on day 14 and the post-hCG T concentration on the same day ($r = -0.61$, $P < 0.01$) (Fig. 3).

![Graph](image-url)
Discussion

The present study clearly indicates that serum concentrations of SHBG decrease during hCG stimulation in prepubertal boys with incomplete testicular descent. The decrease is probably mediated by the rise in serum concentrations of androgens as seen by the fact that there was no decrease when post-hCG T concentration remained below 7 nmol/l.

Although some of the boys with HH were older than any of the boys with incomplete testicular descent, they were all prepubertal and had basal sex steroid levels of very low prepubertal range. Therefore, they had probably not had any activation of gonadotrophin secretion which would have modulated their steroidogenic responses to hCG. Hence it was considered justified to compare these groups.

Serum SHBG concentration has previously been reported to decrease in prepubertal cryptorchid boys after five consecutive daily im injections of hCG (Belgorosky & Rivarola 1982). The overall decrease in SHBG in that study was much greater. These discrepancies could be due to methodological differences. In the previous work a DHT-binding method was used, which might give erroneous results in this situation. In prepubertal boys the increase in endogenous androgens after hCG can be 100-fold or even more. These androgen molecules may occupy the DHT-binding sites of SHBG thereby causing a greater decrease in the serum capacity for binding DHT than in the actual SHBG concentration. In the present study, an immunoradiometric SHBG assay was used, which is free from interference by endogenous steroids. The reason for Chaussain et al. (1979) not seeing any changes in the SHBG concentration in prepubertal boys after hCG administration is not clear.

Recent studies in adult men of short-term (Willemse et al. 1984) or long-term (Plymate et al. 1983) Leydig cell stimulation by hCG have not found any changes in SHBG concentration. This difference from prepubertal boys is most likely due to fundamental differences in steroidogenic response; an increase in oestrogens is known to occur in adult men after acute administration of hCG, whereas prepubertal boys (present study) have no E₂ response. The rise in E₂ in adult men probably counterbalances the rise in T, in regard to effect on SHBG levels. Furthermore, serum testosterone response to a single dose of hCG is only 2.4-fold in adult men (Saez & Forest 1979), compared with 70-fold in boys with incomplete testicular descent (present study).

A decrease in SHBG after hCG was seen in all boys who had an adequate T response. Whether this decrease could be used as a test of biological response to androgens is debatable. A stimulation of at least 2 weeks would be necessary and, due to the slightness of the decrease, there would probably be many false positive results in the diagnosis of androgen insensitivity. Thus there is still a need for a good biological test for the diagnosis of this syndrome.

References


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