Pituitary and gonadal function in prepubertal and pubertal boys with hypospadias


Departments of Urology, Obstetrics and Gynecology and Pathology, Osaka University Medical School, Osaka, Japan.

Department of Urology, Osaka Labour-Injury Hospital, Osaka, Japan.

Abstract. Luteinizing hormone releasing hormone (LRH) and human chorionic gonadotrophin (hCG) tests were performed in 18 prepubertal and 29 pubertal boys with hypospadias to examine pituitary and gonadal function. Thirty-one normal boys were also examined as controls. In the prepubertal group, the mean level of serum peak LH after LRH was significantly higher and the mean level of serum peak testosterone after hCG was significantly lower in boys with hypospadias than in normal boys, though about half of the patients had peak LH levels within ±2 SD of the normal mean. There was little or no response to hCG in 3 out of 18 boys with hypospadias. In the pubertal group, serum levels of LH, FSH and testosterone in all cases were clearly higher than those in the prepubertal group and distinct or moderate responses to LRH and hCG were found in all boys including patients examined. Although serum basal FSH and testosterone levels were similar in the two groups, the mean levels of serum basal LH, peak LH and FSH in LRH test were significantly higher and the mean level of serum peak testosterone after hCG was significantly lower in boys with hypospadias than in normal boys. However, more than half of the patients had these levels within ±2 SD of the normal means. The difference was more marked in LH than in FSH levels. An elevated mean serum FSH suggests hypofunction of not only the Leydig cells but also of the seminiferous tubules at least in some boys with hypospadias.

The development of the male external genitalia is dependent on androgens secreted by the foetal testis. This development starts early in foetal life, about the seventh week after conception, and the urethral canal of the corporal part of penis is completed about 7 weeks later. In hypospadias, this development may be arrested at any stage. Theoretically hypospadias may result from many different errors in organogenesis, including both endocrine and non-endocrine disorders. In hypospadias, both normal and hypofunction of Leydig cells obtained from estimation of basal testosterone levels and the levels after hCG administration have been reported (Walsh et al. 1976; Raboch et al. 1976; Knorr et al. 1979). Recently, androgen unresponsiveness of the target tissue has been described in boys with hypospadias (Savage et al. 1978; Svensson & Snochowski 1979). In this study, luteinizing hormone releasing hormone (LRH) and human chorionic gonadotrophin (hCG) tests were carried out in prepubertal and pubertal boys with hypospadias to study the function of the Leydig cells and seminiferous tubules.

Subjects and Methods

Clinical material
Thirty-one normal boys and 47 boys with hypospadias were investigated during the prepubertal or pubertal stage. The hypospadias were penile and penisscrotal in type, and 3 prepubertal cases were complicated by crypt-
torchidism with the testes retained at the inguinal portion, with no indication of dysosmia, obesity or chromosomal abnormalities.

The prepubertal group consisted of 11 normal boys (aged 6½ to 9½ years, averaged 7½ years) and 18 boys with hypospadias (aged 6½ to 9½ years, averaged 7½ years). In the pubertal group, 7, 6 and 7 normal boys (aged 11½ to 16½ years, averaged 14½ years) were respectively P2, P3 and P4 (Marshall & Tanner 1970) in pubertal stage. Of the boys with hypospadias (aged 11½ to 16½ years, averaged 14½ years), 11, 9 and 9 boys were P2, P3 and P4, respectively. In all subjects, 100 μg/m² body surface area of synthetic LH was administered iv under resting and fasting conditions. Blood samples were taken before administration and 30, 60, 90 and 120 min after administration to measure serum LH and FSH levels. The hCG test was carried out following the LRH test. In all subjects, 10 000 IU/m² body surface area of hCG was administered im, and blood samples were taken before administration and 4 days after administration to measure serum testosterone levels (Okuyama 1978). The blood samples were kept frozen at −20°C. When normal boys were treated with LRH and hCG, all the parents had been informed and agreed with the administration of both drugs.

**LH and FSH radioimmunoassay**

Serum levels of LH and FSH were determined by double antibody radioimmunoassay as described previously (Aono et al. 1972). Human pituitary LH and FSH, which had been obtained from Calbiochem., Jolla, Calif. USA, were labelled with 125I. Antisera, also obtained from Calbiochem., were diluted with 0.01 M phosphate buffered saline (pH 7.6) containing 1% normal rabbit serum. Two IU of hCG was added per tube to enhance the specificity in FSH assay. The separation of bound from free hormone was achieved by sheep antirabbit γ-globulin serum. The 2nd International Reference of Human Menopausal Gonadotrophin was employed as the standard material. The assay was performed in duplicate using 0.1–0.4 ml of serum per tube. The minimal detectable dose was 0.5 mIU/ml in the LH assay and 1.0 mIU/ml in the FSH assay. The intra- and interassay coefficients of variation in the normal male range obtained from 8 assays were respectively, 9.0 and 14.1% in the LH assay and 13.3 and 19.4% in the FSH assay. A specificity study of the LH assay revealed less than 1% cross-reaction by weight for synthetic human adrenocorticotrophin (Richter, βh1-39), human growth hormone (Wilhelmi) and human FSH (Calbiochem.), and less than 4% for human thyrotrophin (Condliffe). FSH assay was also specific for human FSH having less than 1% cross-reactivity by weight for human adrenocorticotropic, growth hormone, thyrotrophin, LH (Calbiochem.) and hCG (Teizo).

**Testosterone radioimmunoassay**

Serum testosterone level was measured by radioimmunoassay (Yoshimi et al. 1972) with slight modifications. Antiserum was prepared in the rabbit by immunization with testosterone-3-carboxymethylxol-mxime-bovine serum albumin. The radioactive steroid used was [1,2-3H] tes-

![Fig. 1.](image)

Serum basal and peak LH, FSH and testosterone levels in LRH and hCG tests in prepubertal boys with hypospadias. The vertical bars, ( volunteered study) and (aged study) represent mean ± sd in prepubertal normal boys and patients, respectively. Differences from "Normal" (P): **<0.01, ***<0.001 (t-test was used). Shaded areas indicate mean ± 2 sd in normal boys.

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testosterone (Daiichi Pure Chemical), 40 Ci/mmol. The separation of the free from bound testosterone was achieved by the dextran-coated charcoal method and the assay was performed in duplicate using 0.1–0.5 ml of serum per tube. For the estimation of low serum testosterone levels in prepubertal boys, a more diluted antiserum and 0.5 ml serum were used. The minimal detectable dose was 0.06 ng/ml. The intra- and interassay coefficients of variation in male serum obtained from 10 assays were 8.2 and 9.9%, respectively. The antiserum showed cross-reacts with 5α-reduced androstanes: 5α-dihydrotestosterone (65%), 5α-androstan-3α,17β-diol (17%) and 5α-androstane-3β,17β-diol (7%) but not with oestradiol-17β, progesterone, cortisol and corticosterone.

Results

**Prepubertal boys with hypospadias**

In prepubertal boys, the basal levels of LH, FSH and testosterone were low in all groups (Fig. 1), and no statistically significant differences could be demonstrated between normal boys and boys with hypospadias. Increases in serum LH and FSH levels by the LRH test were found in all normal boys and all boys with hypospadias. Although increases in serum testosterone levels by the hCG test were found in all normal boys, 3 out of 18 boys with hypospadias had slight or no responses to hCG stimulation. The mean level of serum peak LH after LRH was significantly higher and the mean level of serum peak testosterone after hCG was significantly lower in boys with hypospadias than in normal boys (Fig. 1). Although the mean level of peak LH was significantly higher in the patients, about half of the patients had peak LH levels within ±2 SD of the normal mean. However, most of the patients had peak testosterone levels lower than 2 SD below the normal mean. The data in detail of the 3 non-responders to hCG and 3 cryptorchid boys with cryptorchidism are shown in Table 1.

**Pubertal boys with hypospadias**

In most pubertal boys, serum basal and peak levels of LH, FSH and testosterone in both groups were clearly higher than those in the prepubertal group (Fig. 2). Distinct or moderate responses to LRH and hCG were found in all boys including boys with hypospadias. Although serum basal FSH and testosterone levels were similar in the two groups, the mean levels of serum basal LH, peak LH and FSH in the LRH test were significantly higher and the mean level of serum peak testosterone in the hCG test was significantly lower in boys with hypospadias than in normal boys. The difference was more marked in LH than FSH levels (Fig. 2). As groups, the patients had significantly higher or lower levels than the normal boys. However, about two thirds of the patients had the levels within ±2 SD of the normal means. There were a few patients with a gonadal hypofunction but also normal responders, the levels of LH, FSH and testosterone had increased in progress of pubertal stage in boys with hypospadias as in normal boys and the tendency in patients was more remarkable in LH than FSH levels (Table 2).
Serum basal and peak LH, FSH and testosterone levels in LRH and hCG tests in pubertal boys with hypospadias. The vertical bars, ( ) and ( ) represent mean ± sd in pubertal normal boys and patients, respectively. Differences from “Normal” ($P$): *<0.05, ***<0.01 ($t$-test was used). Shaded areas indicate mean ± 2 sd in normal boys.

Discussion

The evolution of gonadal function in boys with hypospadias in prepubertal or early pubertal ages, seems to be needed for both completion of sexual maturation and fertility in adulthood. Since serum testosterone level is known to be very low in prepubertal boys, the hCG test is required to estimate accurately the endocrine function of Leydig cells in prepubertal boys with hypospadias. It has been reported that the prepubertal boys with hypospadias had significant responses to hCG stimulation (Walsh et al. 1976). In this study, 15 out of 18 prepubertal boys with hypospadias responded clearly to the hCG stimulation but the levels were significantly lower than in normal boys. All re-

Table 2.

Serum LH and FSH levels in LRH test and serum testosterone levels in hCG test in normal boys and boys with hypospadias at different pubertal stages (mean ± sd).

<table>
<thead>
<tr>
<th>Pubertal stage</th>
<th>LH (mIU/ml)</th>
<th>FSH (mIU/ml)</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Peak</td>
<td>Basal</td>
</tr>
<tr>
<td>Normal boys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2 (n=7)</td>
<td>7.6 ± 1.7</td>
<td>21.8 ± 3.0</td>
<td>7.0 ± 1.1</td>
</tr>
<tr>
<td>P3 (n=6)</td>
<td>8.2 ± 1.4</td>
<td>21.6 ± 3.2</td>
<td>8.4 ± 1.4</td>
</tr>
<tr>
<td>P4 (n=7)</td>
<td>9.0 ± 1.2</td>
<td>23.4 ± 4.0</td>
<td>9.5 ± 1.6</td>
</tr>
<tr>
<td>Boys with hypospadias</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2 (n=11)</td>
<td>6.8 ± 1.0</td>
<td>23.2 ± 2.6</td>
<td>6.8 ± 1.8</td>
</tr>
<tr>
<td>P3 (n=9)</td>
<td>12.4 ± 2.0</td>
<td>25.8 ± 3.6</td>
<td>8.0 ± 1.8</td>
</tr>
<tr>
<td>P4 (n=9)</td>
<td>14.6 ± 1.8</td>
<td>29.4 ± 2.8</td>
<td>8.6 ± 2.0</td>
</tr>
</tbody>
</table>
responded to LRH stimulation, but the high mean level of peak LH in boys with hypospadias suggested that primary hypofunction of Leydig cells was present at least in some patients (Fig. 1). In 3 patients which showed a slight or no response to hCG, the Leydig cell hypofunction was more marked than in other boys with hypospadias. The results of the LRH test (Table 1) suggested a primary gonadal hypofunction in these 3 patients. Other investigators have also reported on prepubertal boys with hypospadias who failed to respond to hCG stimulation (Grant et al. 1976; Knorr et al. 1979), and if they are left untreated sexual maturation may not occur (Grant et al. 1976; Gendrel et al. 1977).

It has already been reported that the basal and peak serum gonadotrophin and testosterone levels in the LRH and hCG tests increase as sexual maturation progresses in normal pubertal boys (Canlorbe et al. 1974; Lee et al. 1974; Reiter et al. 1977). Our study has confirmed these findings (Table 2). We also found that the mean serum levels of LH and FSH following the LRH test were significantly higher and the mean level of serum peak testosterone in the hCG test was significantly lower in pubertal boys with hypospadias than in normal boys, though more than half of the patients had the levels within ±2 sd of the normal means. The difference was more marked in LH than in FSH levels. With the onset of puberty, such a disorder of the Leydig cells is thought to be further increased, and there is a considerable change in the endocrine dynamics of the pituitary-gonadal system. It is assumed that a negative feed-back mechanism comes into play to raise the serum LH level in pubertal boys with hypospadias at least in some patients, and that the secreting potential of the Leydig cells is then maintained by hypersecretion of LH from the pituitary gland.

Although, a considerable number of patients showed a normal response of FSH levels to LRH, the elevated mean level of serum FSH in boys with hypospadias suggests hypofunction of not only the Leydig cells but also of the seminiferous tubules in some patients (Fig. 2). The low mean levels of serum testosterone and the high mean levels of serum LH in prepubertal and pubertal boys with hypospadias which we found, suggest that an endocrine testicular insufficiency is present at least in some patients from the foetal stage, while the high mean levels of serum FSH in pubertal boys with hypospadias also implies that hypofunction in the seminiferous tubules is also present (Aafjes et al. 1977). The high doses of hCG used seemed to be appropriate because the boys with hypospadias were in a state of hypergonadotropinism. We estimated serum testosterone levels a few days after single hCG administration to assess reserve Leydig cell function as had been reported previously (Saenz & Forest 1979; Davies et al. 1979).

References


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