NOTE

Decreased Immunoreactive Inhibin and Increased FSH Levels in Cryptorchidism after Orchidopexy

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Abstract. The blood FSH level is often high in patients with severe testicular disorders including cryptorchids. To examine whether inhibin is involved in the increase in FSH we measured immunoreactive inhibin, FSH, LH, and testosterone in 17 patients after orchidopexy. FSH was extremely high (20 mIU/ml or above) in 3 patients. The inhibin level was significantly lower (P<0.01) in these 3 patients (6.47 ± 2.19 IU/ml; mean ± SEM) than in the other 14 patients (14.31 ± 3.96 IU/ml). All 3 high-FSH patients had azoospermia. Testosterone and LH were normal in one of them. Even considering problems involved in the inhibin assay, the high FSH levels are considered to reflect reductions in the blood inhibin level due to Sertoli cell dysfunction. These findings suggest that inhibin plays an important role in the suppression of FSH at least in some patients after orchidopexy.

Key words: Inhibin, FSH, Cryptorchidism

INHIBIN is a peptide hormone with a molecular weight of 32,000 purified in 1985. It has the biological activity of suppressing the biosynthesis and secretion of FSH in the hypophysis. Its localization has been demonstrated primarily in Sertoli cells of the testis in males [1-7]. Microassay methods based on RIA have been developed since 1985, when inhibin was purified, and various microassays have been performed on biological samples [10-12]. In 1989, de Kretser et al. of Australia measured serum FSH and inhibin in males with primary testicular insufficiency but noted no correlation between them [16]. Since that report, the role of inhibin in the negative feedback mechanism of FSH in males has come to be controversial. We also reported azoospermia and increases in FSH in 4 of 24 cryptorchids [8]. In this study, we measured serum inhibin in the same patients to examine whether inhibin is a negative regulator of FSH.

Materials and Methods

Subjects

Of the 178 patients who underwent orchidopexy at the Department of Urology, Gunma University hospital since 1961 and reached the age of 18 years or above, 24 visited the outpatient clinic for follow-up evaluation at our request. The subjects were 17 of these patients in whom the inhibin level could be measured. Cryptorchidism was unilateral in all these subjects and no retractile testis was included. The serum FSH, LH and testosterone (T) levels were examined, a general sperm count was performed and the testicular volume was measured in these 17 patients. The serum inhibin, FSH, LH and T levels were also measured in 26 healthy male volunteers. The 17 patients were aged 19 to 44 years with a mean of 27.1 years at the follow-up
evaluation, and the 26 healthy volunteers were aged 18 to 39 years with a mean of 27.3 years.

**Hormone assays**

Inhibin: Serum inhibin was measured in a heterologous double antibody RIA based on the bovine inhibin RIA system. The anti serum (TNDH-1) was raised in a castrated male rabbit to partial purified bovine follicular fluid inhibin prepared by immunoaffinity chromatography [9]. The anti serum showed no significant cross-reaction with FSH, LH, transforming growth factor-β, activin, or [Tyr30] inhibin-α-(1-30) [10]. The bovine 32-kDa inhibin was iodinated with chloramine-T [11], and labeled antigen was further purified on Affigel-10 (Bio-Rad, Richmond, CA) coupled with a monoclonal antibody to bovine 32-kDa inhibin. Its potency was defined in terms of its in vitro inhibin bioactivity and calibrated against WHO/NID inhibin standard 86/690 [13]. There was no difference between the inhibin concentration in serum and that in plasma in this RIA system. All samples were measured in triplicate. The same amount of IFS as in the samples was added to each standard to compensate for the nonspecific effects of serum in the RIA.

Samples of standards and antiserum (1:100,000 in PBS, pH 7.5, containing 50 mM EDTA and 1% normal rabbit serum) were incubated for 24 h at 32 °C before the addition of 125I-labeled 32-kDa bFF inhibin, and the incubation was continued for a further 24 h at 32 °C. A second antibody (goat antirabbit γ-globulin serum; 1:100) was added, and after overnight incubation at 4 °C. The tubes were centrifuged at 3,000 rpm for 20 min. The supernatants were aspirated and the precipitates were counted. The sensitivity of this assay was 0.32 IU/ml, with an ED50 of 8.21 IU/ml. The intra- and interassay coefficients of variation were 4.8% (n=4) and 5.6% (n=4), respectively.

FSH and LH: Serum levels of FSH and LH were measured in duplicate by an immunoradiometric method (SPAC-S LH kit, SPAC-S FSH kit, Daiichi Radioisotope Laboratory, Tokyo, Japan). The limit of detectability of these assays was 0.1 mIU/ml. The intra- and interassay coefficients of variation were 2.5%-5.3% and 2.1%-4.2% for LH, and 1.5%-4.6% and 2.0%-2.9% for FSH, respectively [14].

Testosterone: Serum levels of testosterone were measured by RIA with a TOTAL TESTOSTERONE kit supplied by Diagnostic Products Corporation (LA, USA). The limit of detectability of this assay is 0.04 ng/ml on a 95% confidence limit of the mean for zero standard [15].

**Statistical analyses**

Statistical analyses were performed with the StatFlex statistical program for an NEC computer (1990, ViewFlex Corporation, Japan). Mann-Whitney test was used to determine differences between groups. A P value <0.05 was considered significantly different.

**Results**

Table 1 shows all parameters measured in patients with cryptorchidism after orchidopexy and in healthy men. FSH was extremely high at 20 mIU/ml or above in 3 of the 17 patients. The inhibin level was 6.47 ± 2.19 IU/ml (mean ± SEM) in these 3 patients but 14.31 ± 3.96 IU/ml in the other 14 patients. The inhibin levels were significantly lower in the high-FSH group than in the normal-FSH group (P<0.01). The LH levels were

<table>
<thead>
<tr>
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<th>FSH mIU/ml</th>
<th>Inhibin IU/ml</th>
<th>LH mIU/ml</th>
<th>T ng/ml</th>
<th>Sperm count × 10⁶/ml</th>
<th>Total vol. ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cryptorchidism</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>High FSH</td>
<td>3</td>
<td>46.27 ± 10.81</td>
<td>6.47 ± 2.19</td>
<td>18.87 ± 9.17</td>
<td>3.33 ± 1.41</td>
<td>0</td>
</tr>
<tr>
<td>Normal FSH</td>
<td>14</td>
<td>8.36 ± 2.95</td>
<td>14.31 ± 3.96</td>
<td>5.21 ± 2.45</td>
<td>5.47 ± 1.43</td>
<td>41.05 ± 41.22</td>
</tr>
<tr>
<td>Healthy men</td>
<td>26</td>
<td>4.57 ± 2.66</td>
<td>14.75 ± 4.84</td>
<td>4.32 ± 1.55</td>
<td>5.15 ± 1.56</td>
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Values are shown as the mean ± SEM. **P<0.01, *P<0.05 vs. a Normal FSH group.**
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significantly higher in the high-FSH group than those in the normal-FSH group (P<0.05). But there was no significant difference between the high-FSH group and the normal-FSH group in the testosterone level. All three patients with high FSH levels had a reduced inhibin level. In 2 of the three patients, a decrease in T and an increase in LH were noted, but both were normal in one of them. All of the 3 patients had azoospermia and small testicular volume.

None of the healthy volunteers had an abnormally high FSH level, and their FSH and inhibin levels were 4.57 ± 2.66 mIU/ml and 14.75 ± 4.84 IU/ml, respectively.

Discussion

Follow-up of patients after orchidopexy revealed very high FSH levels in 3 patients, all of whom had a reduced inhibin level and azoospermia. However, T and LH were normal in one of them. These findings suggest that FSH is regulated by inhibin by a negative feedback mechanism.

The history of inhibin is long. In 1932, McCullagh suggested that a non-steroidal factor derived from the testis inhibits FSH, and termed it inhibin [1]. In 1985, inhibin was successfully purified from porcine and bovine ovarian follicular fluid [3-7]. Inhibin is a protein hormone with a molecular weight of 32,000 and is secreted from the Sertoli cells of the testis, granulosa cells of the ovary, corpora lutea, and the placenta. Two precursors, namely α subunit and β subunit, form a dimer, which is then processed into mature inhibin. Inhibin is classified into inhibin A and inhibin B according to the difference in the β subunit, but both inhibin classes are considered to have the same biological activity of suppressing FSH [3-7]. The presence of inhibin was thus confirmed, and methods for its microassay have been developed [10-12].

In some studies, however, no correlation was noted between inhibin and FSH by RIA techniques. de Kretser (1989) measured serum inhibin and FSH in various patients with primary testicular disorders by RIA and reported no correlation between the two hormone levels [16]. Thereafter, the dual control theory that feedback regulation of FSH secretion is based on coordinated actions of testosterone and inhibin was proposed. One reason why no correlation was observed between the inhibin and FSH levels in biological samples is considered to be related to problems with assay technique [17, 18]. By current assay methods, dimeric inhibin cannot be distinguished from assay technique [α subunit]. The inhibin antibody used for the assay is targeted to α chains and cross-reacts with various α chains. Therefore free α chains with no biological activity are also measured as inhibin if they are present in blood. Robertson et al. reported an α-subunit-derived protein, termed protαC, which lacked inhibin bioactivity also reacted in the RIA system [17].

Our assay also measures free α chains without inhibin activity as well as active inhibin. Our anti-inhibin antibody showed 50% cross-reactivity with bovine inhibin monomer. But if immunoreactive inhibin is low, bioactive inhibin is also expected to be low. It is probably for this reason that the bioactive inhibin level was low in all patients who had very high FSH levels after orchidopexy.

Testosterone as well as inhibin is considered to be involved in the suppression of FSH [16, 19]. This possibility was not excluded by our finding that LH was increased and T was reduced in 2 of the 3 patients who had high FSH values. However, both T and LH were normal in one patient. The high FSH level may therefore have been caused by a decrease in the blood inhibin level secondary to Sertoli cell dysfunction.

In primates, immunological neutralization of circulating inhibin has been shown experimentally to cause a selective increase in FSH [20]. MacNaughton et al. reported decreases in inhibin, no marked changes in testosterone, and increases in FSH with age also in males [21]. In addition, the present study revealed an association between low inhibin levels and high FSH levels in patients after orchidopexy. FSH secretion and inhibin secretion are therefore considered to be negatively correlated in males.

In conclusion, endocrinological evaluation of 17 patients after orchidopexy revealed very high FSH levels in 3 patients, all of whom had low immunoreactive inhibin levels and azoospermia. T and LH were normal in one of them. Despite problems with the inhibin assay, the high FSH levels are considered to be due to decreases in the blood inhibin level secondary to Sertoli cell dysfunction. These findings suggest that inhibin plays an important role in the suppression of FSH secretion at
least in some patients after orchidopexy.

References